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ARTICLE

Metabolomics Reveals Alterations in Both Primary and Secondary Metabolites by Wine Bacteria

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Lactic acid bacteria (LAB) were isolated from Korean Meoru (*Vitis coigneties*) wine and identified as *Lactobacillus plantarum* meoru0711 (KACC 91436C). The fermentative behavior and metabolic effects of *L. plantarum* during malolactic fermentation (MLF) were compared with those of the commercial *Oenococcus oeni* strain through ^{1}H NMR- and GC-based metabolic profiling. Twenty-two primary metabolites of amino acids, carbohydrates, and organic acids, and 55 secondary metabolites of volatile compounds were identified in wines by ^{1}H nuclear magnetic resonance (NMR) spectroscopy and gas chromatography (GC), respectively. Principal component analysis (PCA) revealed that malolactic (ML)-fermented and non-ML-fermented wines, and wines ML-fermented with *O. oeni* and *L. plantarum* were clearly differentiated. Both the primary and secondary metabolites were responsible for these differentiations. Compared to non-MLF wines, MLF wines were characterized by increased levels of primary metabolites such as lactic acid, phenylalanine, uracil, ornithine, alanine, threonine, leucine, isoleucine, and valine with decreased levels of monosaccharides, glycerol, malic, and citric acids. In addition, higher levels of secondary metabolites such as butanal, ethyl isobutylate, isobutanol, isoamyl acetate, 2-butanoate ethyl ester, isoamyl alcohol, ethyl hexanoate, glycine, acetic acid, and benzaldehyde characterized the MLF wine. Higher levels of primary metabolites such as tyrosine, monosaccharides, glycerol, alanine, 2,3-butanediol, valine, and leucine, and of secondary metabolites such as propyl acetate, isobutanol, isoamyl acetate, 1-butanol, ethyl hexanoate, prenyl alcohol, glycine, 2-hexen-1-ol, ethyl octanoate, acetic acid, benzaldehyde, and butyric, together with lower levels of lactic acid, were observed in the wines fermented by *L. plantarum* compared with those by *O. oeni*. This present study demonstrates that different genera of LAB affect both the primary and second metabolites in wine. Moreover, metabolomics with multivariate statistical analysis provide insight into wine fermentation.

KEYWORDS: Wine; NMR; GC; metabolites; volatile compound; lactic acid bacteria

INTRODUCTION

Metabolites are a starting material, an intermediate, or an end product of metabolism. Typically, a primary metabolite is directly involved in normal growth, development, and reproduction, while a secondary metabolite is not directly involved in those processes but rather has an important ecological function. Metabolomics is the systematic and comprehensive study of chemical processes involving metabolites. Metabolomics mainly focuses on the comprehensive and quantitative profiling of metabolites in a biological system (*1*). A number of analytical methods, such as NMR, GC-MS, LC-MS, and CE-MS, have been applied for metabolic profiling (*2*). Recently, metabolomic studies have been undertaken in food and nutrition fields (*3*). However, there are only a few studies of metabolomics in fermented foods such as wine.

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Wine is a microbial product that contains numerous metabolites. Many factors affect wine metabolites. The terroir is an elementary factor that affects the chemical composition of the grapes, and physical fermentative conditions such as temperature, fermentation period, and enology techniques are also important factors (*4*). However, most metabolites in wine are produced during fermentation by microorganisms. Winemaking involves two different fermentation processes: alcoholic fermentation with yeast such as *Saccharomyces cerevisiae* and malolactic fermentation (MLF) with lactic acid bacteria (LAB) such as *Oenococcus oeni* or *Lactobacillus plantarum*. NMR-based metabolomics for monitoring wine fermentation and evaluating the fermentative characteristics of yeast strains have been reported, and the metabolites were found to depend on yeast strains (*5*, *6*)

LAB mainly convert L-malic acid into L-lactic acid, which is the predominant compound of MLF wines. This biological deacidification results in an increase in pH and a reduction in perceived wine acidity. In addition to the deacidification during MLF, LAB produce other compounds such as acetate and CO_2, and wine

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flavor is affected by their metabolism. Therefore, the LAB is an important factor in winemaking because different LAB induce different metabolic characteristics. Although the deacidification is still the major reason for MLF, many winemakers consider it a means for adjusting wine flavor. A number of studies have reported flavor modification in wines during MLF (*7*−*11*). MLF is also performed to reduce the incidence of vegetal notes and to accentuate fruit flavor in wines (*12*).

Only some strains of LAB, *Oenococcus*, *Lactobacillus*, and *Pediococcus*, are resistant to the severe conditions in wine such as low pH, high SO_2 concentration, and high ethanol levels. The LAB in grape, must, and wines have been characterized in numerous studies (*13*−*15*). Fermentative byproducts in wine depend on the strain of LAB and affect the wine flavor as well as organoleptic and other qualities. López et al. (*16*) reported that *O. oeni* strains account for the most predominant species in spontaneous MLF wines. Furthermore, Plessis et al. (*17*) isolated 22 strains of *O. oeni*, 8 strains of *Lactobacillus brevis*, 8 strains of *Lactobacillus paracasei*, and 6 strains of *L. plantarum* from brandy base wines with no addition of sulfur dioxide, demonstrating that not only *Leuconostoc oenos* but also other LAB species are involved in spontaneous MLF of wine. Guerzoni et al. (*18*) reported that the isolated *L. plantarum* showed better growth performance than *Leuconostoc oenos* under various conditions with respect to the effects of chemico-physical factors on the growth and malolactic activity of LAB. The occurrence of spontaneous MLF is very common in all wine-producing areas but is considered a negative feature with respect to wine qualities, such as flavor; thus, MLF must be controlled.

Korean Meoru (*Vitis coigneties*) wine contains many polyphenols including phenolic acid, flavonoid, and resveratrol, and has a high acidity due to the intrinsically high level of malic acid in Meoru grapes (*19*−*22*). Also, because of the high levels of malic acid in Meoru, the MLF process is essential for making wine with Meoru grapes. Recently, on the basis of ^{1}H NMR and GC-MS-based metabolomic studies, we reported that *O. oeni* strains contributed to variations in the secondary metabolites rather than the primary metabolites in wine during MLF (*23*). In the present study, we isolated LAB from Meoru wine undergoing spontaneous MLF and identified the isolated LAB through sequence analyses of 16S rRNA and the internal transcribed spacer (ITS) region. Furthermore, we characterized and compared the fermentative behaviors of the identified LAB with that of commercial *O. oeni* in MLF Meoru wine through ^{1}H NMR- and GC-MS-based metabolic profiling.

MATERIALS AND METHODS

Vinification. Meoru (*Vitis coignetiae*), Korean wild grapes, were harvested in 2008 from the region around Young-weol, Kyung-buk, in South Korea. Fifty kilograms of grapes was destemmed and crushed. Sugar content of the must was adjusted to 22 °Brix with sucrose. The must was transferred into a 50-L plastic tank for alcoholic fermentation, which was carried out with activated *Saccharomyces cerevisiae* D-47 (ICV/D-47, Lalvin, Canada) at 25 °C for 14 days, following the addition of 100 ppm of $K_2S_2O_5$ to avoid a spontaneous MLF. After completion of the alcoholic fermentation, the occurrence of MLF was checked by analyses of malic and lactic acid. After pressing, the wine was transferred into nine 5-L glass carboys and then inoculated with the isolated and commercial LAB for MLF.

Lactic Acid Bacteria Isolation. To obtain bacterial debris from the wine samples, ten milliliters of Meoru wine, which was vinified in 2007 and in which MLF occurred spontaneously, was centrifuged at 1,000*g* and 4 °C for 10 min. Pellets were collected, diluted in 0.85% sterile saline solution and then plated onto MRS agar (contains 0.005% bromocresol purple). Plates were incubated at 28 °C for 48 h. After the incubation, yellowish colonies were selected for evaluation in LAB pure culture.

Species Identification of LAB Isolates. LAB species were identified with the API 50 CHL kit and 16S rRNA sequencing. Working cultures were streaked on MRS agar and grown for 72 h at 30 °C. Genomic DNA was extracted from a single colony using the *Insta* Gene Matrix (BioRad Laboratories) according to the supplier's instruction. The DNAs obtained were stored at −20 °C until use. The identity of the isolated species was confirmed by species-specific polymerase chain reaction (PCR) with the primers On1 (5′-AGAGTTTGATCMTGGCTCAG-3′) and On2 (5′-TACGGHTACCTTGTTACGACTT-3′), which were used to amplify the *mle* gene (1,492 bp). The amplified fragments were purified using the high Pure PCR product purification Kit (Roche) according to the manufacturer's instructions. The fragments were sequenced and compared with sequences in GenBank using the BLAST search program (NCBI).

LAB Culture Preparation for Malolactic Fermentation. Commercial *O. oeni* and isolated LAB were grown in Rogosa medium (20% unpreserved apple juice, 20 g/L trypton, 5 g/L pepton, 5 g/L glucose, 5 g/L yeast extract, and 0.005% Tween-80) for activation. Each activated LAB was incubated in a mixture of medium and Meoru juice (1:1) at 25 °C for 24 h to obtain the desired biomass of 3×10^8 cfu/mL. The wine base cultures were inoculated at 2% concentration into each Meoru wine for MLF.

Organic Acid Analysis. pH and total acidity of wine were determined with a pH meter (Orion 3star, Thermo Scientific, USA) and sodium hydroxide titration, respectively. The Gilson HPLC series was used for organic acid analysis. Meoru wines were filtered through a 0.45-μm membrane filter and injected directly on the Prevail organic acid column (250 mm × 4.6 mm, Alltech, USA). The injection volume of the sample was 20 μL. The mobile phase, 25 mM KH_2PO_4 (pH 2.5 by phosphoric acid), was used at a flow rate of 1.0 mL/min, and UV detection was carried out at 210 nm.

^{1}H NMR Spectroscopic Analysis. One milliliter of wine was lyophilized in a 1-mL eppendorf tube and dissolved in 99.9% deuterium oxide (400 μL, D_2O), mixed with 400 mM oxalate buffer (140 μL, pH 4.0) and 5 mM 3-(triethylsilyl) [2,2,3,3-$^2H_4$] propionate (60 μL, TSP, 97%), and then centrifuged at 13,000 rpm for 10 min. Supernatants (550 μL) were transferred into 5-mm NMR tubes. D_2O and TSP provided a field frequency lock and a chemical shift reference (^{1}H, δ 0.00), respectively. ^{1}H NMR spectra were acquired on a Varian INOVA-600 MHz NMR spectrometer (Varian Inc., Palo Alto, CA) operating at 599.84 MHz ^{1}H frequency and a temperature of 298 K, using a triple resonance 5-mm HCN salt-tolerant cold probe. A NOESYPRESAT pulse sequence was applied to suppress the residual water signal. For each sample, 16 transients were collected into 32 K data points using a spectral width of 9615.4 Hz with a relaxation delay of 1.5 s, an acquisition time of 4.00 s, and a mixing time of 400 ms. A 0.3-Hz line-broadening function was applied to all spectra prior to Fourier Transformation (FT).

Volatile Compounds Analysis. A headspace solid-phase microextraction (SPME) method was utilized to prepare samples for GC-MS analysis. The carboxen/PDMS (CAR/PDMS)-fused silica 75-μm fiber (Supelco, Bellefont, PA, USA) was selected for the entire range of volatile and polar compounds according to Setkova et al. (*24*). Glass screw cap vials with polytetrafluoroethylene (PTFE)/silicone septa (20 mm) were obtained from Agilent Technologies. The SPME experiments were optimized using 2 mL of Meoru wine and 0.5 g of NaCl in a 20-mL vial. The vial was soaked in water in a beaker on a hot plate with a magnetic stirrer; the water was heated to 40 °C. The wine was agitated at 500 rpm using a tiny magnetic bar in the vial. The wine sample was incubated for 5 min, and the NaCl dissolved completely within 5 min. One microliter of 3-octanol solution (8.22×10^{-4} g/mL in methanol) was added to the wine in the vial as an internal standard prior to sample incubation. The extraction time was 2 min.

Gas chromatograph 7890 (Agilent Technologies, Palo Alto, CA, USA) coupled to mass spectrometer 5975C (Agilent Technologies) was used to analyze volatile compounds. A DB-WAXetr (Agilent 122−7322, 30 m × 250 μm × 0.25 μm) column was used for GC analysis. Thermal desorption into the GC injector was carried out for 3 min at 300 °C. The splitless injection mode was applied, and helium gas was used as the carrier gas with a constant flow rate of 1.0 mL/min. The GC oven temperature was set at 40 °C for 5 min and increased at a rate of 3 °C/min to 80 °C, at 4 °C/min to 180 °C, and at 5 °C/min to 210 °C. The mass spectrometer (MS) was operated in electron impact (EI) mode (70 eV). Data acquisition was performed in full scan mode for m/z 50 to 650 with a scan time of 2.9 s.

Table 1. Biochemical Substrate Assimilation Profiles (API 50 CHL gallery) of LAB Isolated from Meoru Wine[a]

	API CHL		API CHL		API CHL
0. control	−	17. inositol	−	34. melezitose	+
1. glycerol	−	18. mannitol	+	35. D-raffinose	+
2. ertythritol	−	19. sorbitol	+	36. amidon	−
3. D-arabinose	−	20. α-ethyl-D-mannoside	−	37. glycogene	−
4. L-arabinose	+	21. α-methyl-D-glucoside	+	38. xylitol	−
5. ribose	+	22. *N*-acetyl glucosamine	+	39. β-gentiobiose	+
6. D-xylose	−	23. amygdaline	+	40. D-turanose	+
7. L-xylose	−	24. arbutine	+	41. D-lyxose	−
8. adonitol	−	25. esculine	+	42. D-tagatose	−
9. β-methyl-xyloside	−	26. salicino	+	43. D-fucose	−
10. galactose	+	27. cellobiose	+	44. L-fucose	−
11. D-glucose	+	28. maltose	+	45. D-arabitol	−
12. D-fructose	+	29. lactose	+	46. L-arabitol	−
13. D-mannose	+	30. melibiose	+	47. gluconate	+
14. L-sorbose	−	31. saccharose	+	48. two keto-gluconate	−
15. rhamnose	+	32. trehalose	+	49. five keto-gluconate	−
16. dulcitol	−	33. inuline	−		

[a] Incubated at 37 °C for 24 h.

Multivariate Data Analysis. All NMR spectra were phased and baseline corrected with VnmrJ software 2.1B (Varian Inc., Palo Alto, CA) and then converted to ASCII format. The ASCII format files were imported into MATLAB (R2008a, Mathworks, Inc.). Probabilistic quotient normalization of the spectra using the median spectrum to estimate the most probable quotient was carried out (*25*), and the spectra were aligned by the recursive segment-wise peak alignment (RSPA) method to reduce variability in the peak positions (*26*).

The regions corresponding to water (4.6−4.8 ppm), residual ethanol (1.15−1.20 and 3.59−3.72 ppm), and TSP (−0.5−0.7 ppm) were removed prior to normalization and spectral alignment. Furthermore, succinate peaks (2.61−2.66 ppm) were excluded prior to multivariate statistical analysis because they were not aligned properly. The changes in succinate levels were evaluated by calculation of individual integral area after spectral normalization.

The resulting data sets were then imported into SIMCA-P version 12.0 (Umetrics, Umeå, Sweden) for multivariate statistical analysis. Signal assignment for representative samples was facilitated by two-dimensional (2D) total correlation spectroscopy (TOCSY), heteronuclear multiple bond correlation (HMBC), heteronuclear single quantum correlation (HSQC), spiking experiments, and comparisons to literature (*7*, *8*).

Selected GC/MS peaks were identified by comparing the mass spectra and the retention index of the peaks with those from the National Institute of Standards and Technology (NIST) mass spectral library (Wiley registry). The signal-to-noise threshold level was set at 17 for the selection of major volatile compounds. All selected GC peaks were integrated and normalized to integral peak area of the internal standard. The normalized peaks were imported into SIMCA-P software for multivariate statistical analysis.

The mean-centered (for NMR data) and scaling (for GC-MS data) methods were applied for all multivariate analysis by SIMCA-P version 12.0 (Umetrics, Sweden). Principal components analysis (PCA), an unsupervised pattern recognition method, was initially performed to examine intrinsic variation in the data set. A supervised pattern recognition method, orthogonal projection on latent structure discriminant analysis (OPLS-DA), was used to extract maximum information on discriminant compounds from the data. OPLS-DA provides a way to remove systematic variation from an input data set X (compounds) not correlated to the response set Y (spectral intensities in NMR spectra and spectral areas in the GC chromatogram) (*14*). Hotelling's *T*2 region, shown as an ellipse in the scores plots, defines the 95% confidence interval of the modeled variation (*15*). The quality of the models is described by Rx^2 and Q^2 values. Rx^2 is defined as the proportion of variance in the data explained by the models and indicates goodness of fit, and Q^2 is defined as the proportion of variance in the data predictable by the model and indicates predictability.

Statistical Analysis. The statistical analysis system (SAS package ver. 9.20) was used for data analysis. Significance of differences in organic acid content by HPLC and peak areas of volatile compounds by GC/MS was analyzed by ANOVA and Duncan's multiple range test. The paired *t*-test was performed for volatile compound analysis in pairs of MLF wines and non-MLF and of wines fermented by *O. oeni* and by *L. plantarum* meoru0711.

Chemicals. All chemical reagents were of analytical grade. All organic acid standards, D_2O (99.9%), and TSP (97%) were purchased from Sigma (St. Louis, MO, USA). 3-Octanol (99%) was obtained from Aldrich (Milwaukee, MI, USA).

RESULTS

Species Identification of LAB Isolated from Meoru Wine. To identify the species of LAB isolated from Meoru wine, API 50 CHL and cellular fatty acid composition analysis were applied. **Table 1** shows that biochemical substrate assimilation profiles of the isolate were mostly related to *Lactobacillus plantarum* (99.3%). Moreover, the isolate was also identified as *L. plantarum* through cellular fatty acid composition (data not shown). Genotypic identification of the LAB was performed by amplification of its 16S rRNA sequence; in this analysis, the isolate was also closely related to various *L. plantarum* (99%) strains without any other comparable *Lactobacillus* strains. **Figure 1** shows the phylogenic analysis of 16S rRNA of the isolated *L. plantarum*. The isolate was named *L. plantarum* meoru0711 (KACC 91436C). *L. plantarum* meoru0711 did not produce CO_2 from glucose (data not shown) and, thus, was also classified as a homofermentative strain.

Development of MLF in Meoru Wine. Table 2 shows the organic acid composition and total acidity of Meoru juice and wines before and after MLF. High levels of malic acid, which would contribute the high total acidity, were observed in Meoru juice. Wines in which MLF was induced with isolated *L. plantarum* meoru0711 and commercial *O. oeni* after alcoholic fermentation showed almost complete conversion of malic acid into lactic acid. Tartaric acid was markedly decreased after alcoholic and ML fermentation. In addition to precipitation of tartaric acid during the winemaking process, microorganisms can also contribute to the reduction of tartaric acid levels. Among the many strains of LAB, only *L. brevis* and *L. plantarum* have been identified as being able to utilize L-(+)-tartaric acid (*27*). Wibowo et al. (*28*) also reported tartaric acid utilization that ranged from 3% to 30% in wines with pH > 3.50 during MLF. However, there were no significant differences in the tartaric acid content among all wines, including non-MLF wines, in the present study. It is,

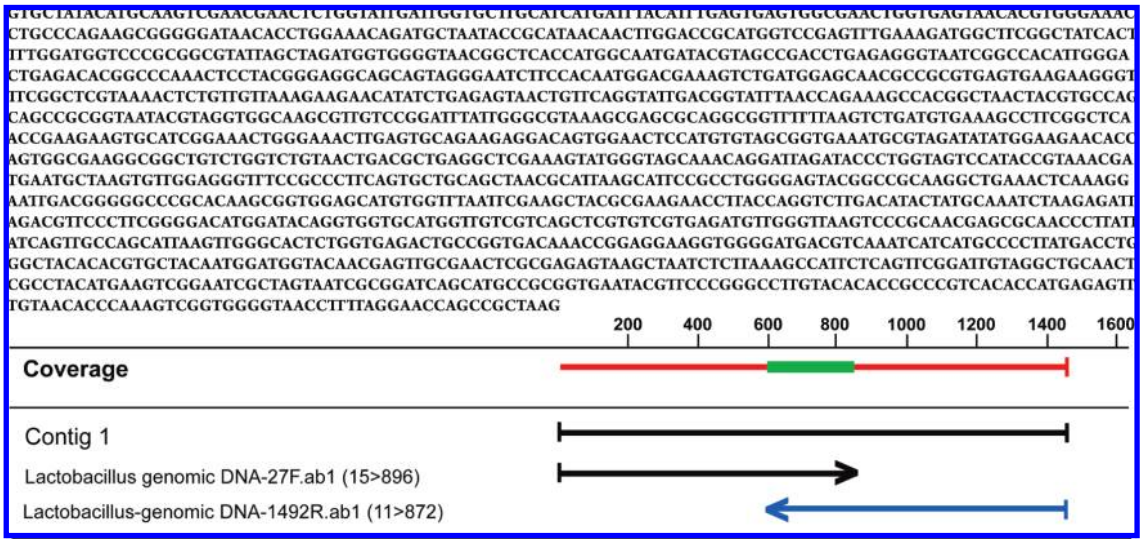

Figure 1. Sequencing analysis of the 16s RNA of LAB isolated from Meoru.

Table 2. Total Acidity and Organic Acid Composition (mg/L)[a]

				organic acids				
		pH	total acidity	oxalic	tartaric	malic***	lactic***	acetic***
	Meoru juice	3.32	16.7	546.8 ± 1.18	4869.5 ± 480.0	8926.0 ± 64.7	318.3 ± 39.4	295.5 ± 56.9
	L. plantarum	3.82	6.6	162.0 ± 1.4	1885.2 ± 11.1	109.0 ± 22.1 b	6748.7 ± 105.4 a (2.51 ± 0.03 b)[b]	1852.7 ± 70.5 b
after MLF	*O. oeni*	3.80	6.7	161.9 ± 5.9	1909.5 ± 36.2	91.0 ± 15.6 b	6518.2 ± 84.5 a (2.84 ± 0.02 a)	2550.7 ± 62.4 a
	non MLF	3.40	9.9	176.9 ± 8.7	2004.3 ± 86.6	7037.2 ± 234.2 a	1806.2 ± 128.5 b (0.18 ± 0.01 c)	1129.0 ± 12.1 c

[a] Means ± SD. Values with different letters are significantly different by Duncan's multiple range test at $p < 0.001$. Concentrations of organic acids were determined by HPLC. [b] Lactic acid levels in parentheses are expressed as the integral area of individual NMR spectra from 1.31 to 1.42 ppm (area values of $\times 10^{-5}$).

therefore, likely that precipitation during vinification, including fermentation, aging, pressing, and racking procedures, contributed to the variations in tartaric acid levels rather than its utilization by LAB. The lowest acetic acid contents were observed in non-MLF wine, whereas levels of acetic acid were higher in the wines fermented with *O. oeni* compared to those with *L. plantarum* meoru0711.

Primary Metabolic Changes in Wines by ^{1}H NMR Spectroscopy. Representative ^{1}H NMR spectra of Meoru wines fermented with *O. oeni* (**A**) and *L. plantarum* meoru0711 (**B**) and of non-MLF wine (**C**) following alcoholic fermentation with *S. cerevisiae* are shown in **Figure 2**. Twenty-two primary metabolites, including malic acid, lactic acid, acetic acid, succinic acid, 2,3-butanediol (2,3-BD), proline, leucine, isoleucine, valine, alanine, threonine, arginine, tyrosine, phenylalanine, γ-aminobutyric acid (GABA), choline, ornithine, glutamine, glycerol, monosaccharide, and an unknown compound, were identified by ^{1}H NMR spectroscopy. We defined the metabolites detected by NMR as the primary metabolites in the present study. To investigate significant differences in the primary metabolites, PCA analysis was applied to all Meoru wines. The PCA score plot showed clear differentiation between non-MLF wine and induced MLF wines, demonstrating that significant changes in the primary metabolites occurred during MLF and were dependent on LAB genera (**Figure 3**).

Figure 4 represents PCA score (**A** and **C**) and loading (**B** and **D**) plots derived from ^{1}H NMR spectra of wines, showing a clear differentiation between non-MLF wine and MLF wine induced with *O. oeni* (**A**) and between MLF wines with *O. oeni* and *L. plantarum* meoru0711 (**C**). The differentiation between non-MLF wine and MLF wine induced with *O. oeni* was characterized by the first principal component (PC1) in the PCA score plot with high goodness of fit and predictability as indicated by R_x^2 and Q^2 values of 0.995 and 0.991, respectively (**Figure 4A**). Moreover, different ML-fermentative behaviors of two LAB genera on the primary metabolites were also significant in the PCA model with an R_x^2 value of 0.798 and aQ^2 value of 0.485, respectively, indicating the dependence of the primary metabolites on LAB genera (**Figure 4C**). This dependence of the primary metabolites on LAB genera was interesting because there were no significant differences in the primary metabolites among different *O. oeni* strains, as reported in our previous study (*23*). To identify the primary metabolites responsible for the differentiations in the PCA score plots, complementary PCA loading plots were generated.

The upper and lower sections represent higher and lower levels, respectively, of the metabolites in the group of interest than in another group. Increased levels of lactic acid, phenylalanine, uracil, ornithine, alanine, threonine, leucine, isoleucine, and valine together with decreased levels of monosaccharides, glycerol, malic, and citric acids in MLF wines mainly contributed to the separation of MLF wine from non-MLF wine as shown in the PCA loading plot (**Figure 4B**). Higher levels of tyrosine, monosaccharide, glycerol, alanine, 2,3-BD, valine, and leucine, together with lower levels of lactic acid, were observed in the wines induced with *L. plantarum* meoru0711 compared to those in the wines with *O. oeni*. (**Figure 4D**). Moreover, we calculated succinic acid levels by the integral area of individual NMR spectra. The succinic acid levels were decreased after MLF with *O. oeni* ($p < 0.01$) and not significantly different between MLF wines with *O. oeni* and *L. plantarum* moeru0711 ($p < 0.01$).

Changes in Volatile Compounds in MLF Wines by GC-MS. A typical GC chromatogram obtained from all Meoru wines using the headspace SPME method is shown in **Figure 5**. Fifty-five volatile compounds were identified and summarized in **Table 3**. Nineteen volatile compounds had significantly different values of mean area according to MLF performance and different LAB

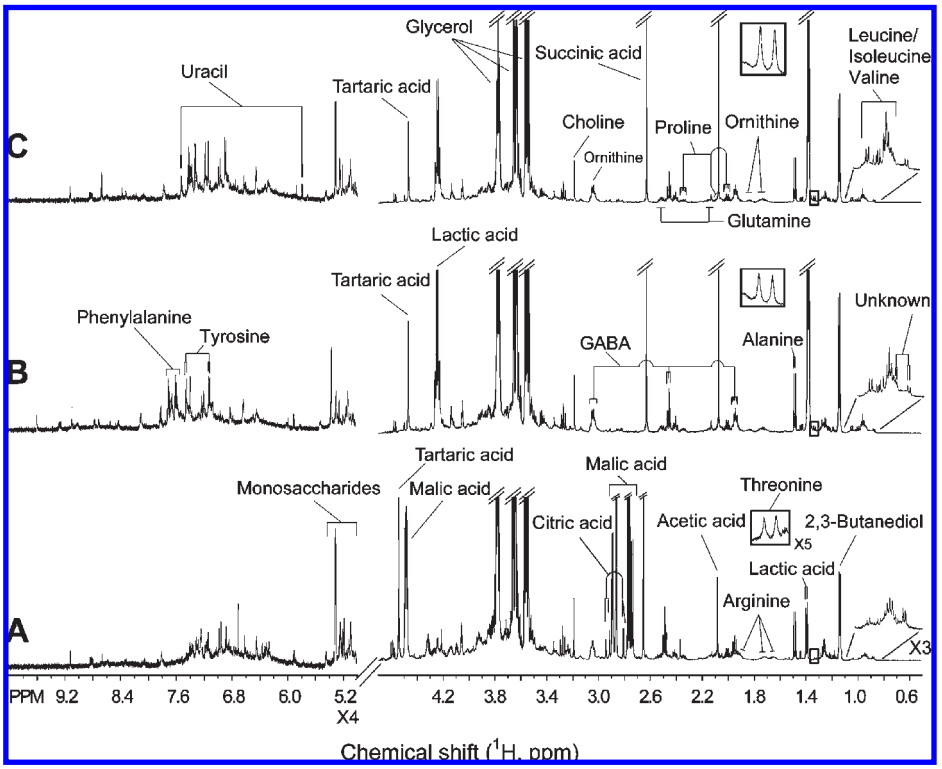

Figure 2. Representative ^{1}H NMR spectra of Meoru wines fermented with *L. plantarum* meoru0711 (**B**) and *O. oeni* (**C**), following alcoholic fermentation with *S. cerevisiae*. The spectrum of **A** represents wine fermented only with *S. cerevisiae* without MLF.

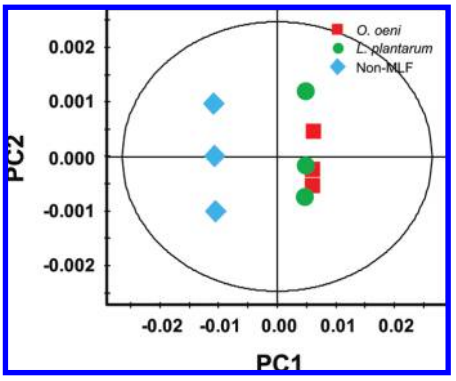

Figure 3. Principal component analysis (PCA) score plot derived from ^{1}H NMR spectra of all wines, demonstrating that LAB genera contribute to variation in primary metabolites of wines as well as large variation during MLF and non-MLF.

genera. We defined the volatile compounds observed by GC-MS as the secondary metabolites. PCA analysis was applied to investigate the differences in the volatile compounds in wines (**Figure 6**). PCA score plot shows the differentiation among all wines with R_x^2 value of 0.892 and Q^2 value of 0.334, indicating significant differences in the secondary metabolites among the wines.

To understand the effects of MLF and LAB genera on variations in the volatile compounds, the OPLS-DA model was applied. **Figure 7** represents OPLS-DA score plots (**A** and **C**) derived from the GC-MS data set of wines, demonstrating a clear discrimination between non-MLF wines and MLF wines induced with *O. oeni* (**A**) and between MLF wines with *O. oeni* and *L. plantarum* meoru0711 (**C**), with high values of R_x^2 and Q^2 of 0.954 and 0.998 and of 0.701 and 0.885, respectively. OPLS-DA scatter loading *S*-plots were also generated to identify the secondary metabolites discriminating the wines. Moreover, the significances of the discriminatory secondary metabolites were confirmed by a pairwise comparison with the paired *t*-test. The secondary metabolites with open symbols are significantly different ($p < 0.05$), whereas closed symbols represent no statistical significance, as illustrated in **Figure 7B** and **D**.

The OPLS-DA scatter loading *S*-plot revealed that butanal, ethyl isobutylate, isobutanol, isoamyl acetate, 2-butanoate ethyl ester, isoamyl alcohol, ethyl hexanoate, aminoacetic acid, acetic acid, and benzaldehyde were responsible for discriminating MLF wines induced with *O. Oeni* from non-MLF wines (**Figure 7B**). Of these metabolites, the levels of ethyl isobutyrate, 2-butanoate methyl ester, isoamyl acetate, glycine, 2-hexen-1-ol, and acetic acid were increased after MLF, whereas the levels of butanal, propyl acetate, isobutanol, ethyl hexanoate, benzaldehyde, and isoamylalcohol were higher in non-MLF wines. Moreover, levels of propyl acetate, isobutanol, 1-butanol, ethyl hexanoate, prenyl alcohol, ethyl octanoate, benzaldehyde, and butyric acid were higher, while levels of 2-hexen-1-ol, acetic acid, glycine, and isoamyl acetate were lower in MLF wines with *L. plantarum* meoru0711 than in those with *O. oeni* as shown in the OPLS-DA loading *S*-plot (**Figure 7D**).

DISCUSSION

We isolated LAB from Korean Meoru wine undergoing spontaneous MLF and identified *L. plantarum* meoru0711 through genetic-based analysis. We then compared its ML-fermentative behaviors with those of a commercial *O. oeni* strain through ^{1}H NMR- and GC-MS-based metabolic profiling. The metabolic pathways and relationships of the primary and secondary metabolites produced during MLF and by the lactic acid bacteria are summarized in **Figure 8**.

Sugar Metabolism. Sugar and acetic acid metabolism are considered to characterize the fermentative behavior because sugar and acetic acid are consumed and produced by LAB, respectively, during MLF in wine. Citric acid is also utilized by

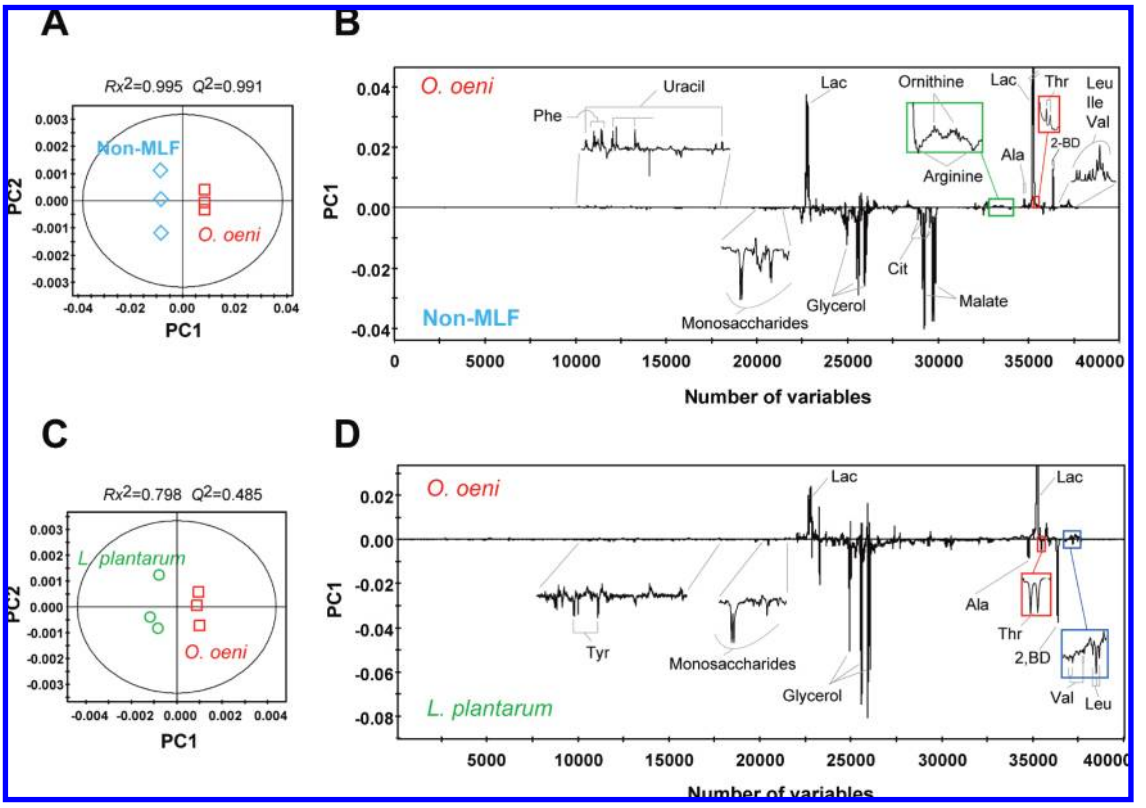

Figure 4. PCA score (**A** and **C**) and loading (**B** and **D**) plots derived from the ^{1}H NMR wine spectra, demonstrating the differentiation between non-MLF wine and MLF wine induced with *O. oeni* (**B**) and between MLF wines with *O. oeni* and *L. plantarum* meoru0711 (**D**) following alcoholic fermentation. Leu, leucine; Ile, isoleucine; Val, valine; 2,3-BD, 2,3-butanediol; Thr, threonine; Lac, lactate; Ala, alanine; Tyr, tyrosine; Phe, phenylalanine.

LAB, resulting in increases of acetic acid levels during MLF. These results are consistent with acetic acid production from citric acid and sugar metabolism by LAB during MLF, following alcoholic fermentation with wine yeast (*29*). The major residual sugars in wine after the completion of alcoholic fermentation are glucose and fructose and range from 10 g/L to less than 0.5 g/L, depending on the wine style (*30*). There are two main glucose metabolic pathways for LAB: the homolactic-fermentative (EMP) and heterolactic-fermentative (6-phosphogluconate/phosphoketolase; 6-PG/PK) pathways (*31*). The EMP pathway results almost exclusively in the production of lactic acid as an end-product and indicates homofermentation, whereas the 6-PG/PK pathway results in other end-products, such as ethanol, acetic acid, and CO_2 as well as lactic acid, indicating heterofermentation (*31*). It is, therefore, possible to divide LAB into two groups, homo- and heterofermentative, according to their sugar metabolic characteristics. *L. plantarum* and few other species are obligatorily homofermentative, whereas *Leuconostoc* and *Oenococcus* are heterofermentative. Some *Lactobacillus* and *Pediococcus* are also facultatively heterofermentative. In the present study, markedly lower concentrations of acetic acid were observed in MLF wines with isolated *L. plantarum* meoru0711 than in those with commercial *O. oeni*, demonstrating that *L. plantarum* meoru0711 has homofermentative characteristics and does not produce CO_2.

Lactic Acid. Lactic acid is mainly produced from the conversion of malic acid by LAB. As expected, malic acid was completely converted to lactic acid by *L. plantarum* meoru0711 and *O. oeni* (**Figures 2** and **4**).

Levels of lactic acid in wines with *L. plantarum* meoru0711 and *O. oeni* observed by NMR analysis were not consistent with the levels measured by HPLC: there were no significant differences in lactic acid levels calculated by HPLC data between the two wines, although lactic acid levels were significantly higher in MLF wines with *O. oeni* than in those with *L. plantarum* meoru0711 by NMR (**Table 2**). In fact, both sides of the liquid chromatogram corresponding to lactic acid overlapped with the chromatograms of unknown compounds during HPLC analysis. This may cause subtle differences in the calculations of lactic acid concentration by HPLC. However, there were no doubts in calculating the levels of lactic acid by NMR spectroscopy because the NMR peaks corresponding to lactic acid did not overlap with neighboring peaks.

Citric acid is a major organic acid in must and wines with concentrations ranging from 0.1 to 0.7 g/L and serves as a substrate in the formation of diacetyl, acetoin, and acetic acid (*12*). Numerous studies have reported citric acid degradation during MLF by LAB, consistent with the results in the present study. However, *Oenococcus* strain vinibacti111 does not metabolize citric acid, as reported in our previous study (*23*).

Amino Acids. Increased levels of branched chain amino acids (BCAAs), such as leucine, isoleucine, and valine, were observed in MLF wines in the present study. Pozo-Bayón et al. (*32*) reported that total amino acid content were increased in MLF wines induced by both *O. oeni* and *L. plantarum* strains. *O. oeni* strains produce extracellular peptidases or proteases that metabolize peptides and proteins to release amino acids, resulting in increases in the total amino acid content (*33*). Moreover, BCAAs are synthesized from threonine and pyruvate by α-acetolactate synthase (α-ALS) (*34*). α-ALS functions in two metabolic pathways: catabolic α-ALS, which catalyzes the formation of acetolactate, is involved in the 2,3-butanediol pathway, while anabolic α-ALS contributes to the biosynthesis of BCAAs (*35*, *36*). Therefore, it is likely that increased levels of BCAAs after MLF are due to the activation of anabolic α-ALS as well as proteases in LAB, suggesting that the activities of α-ALS and

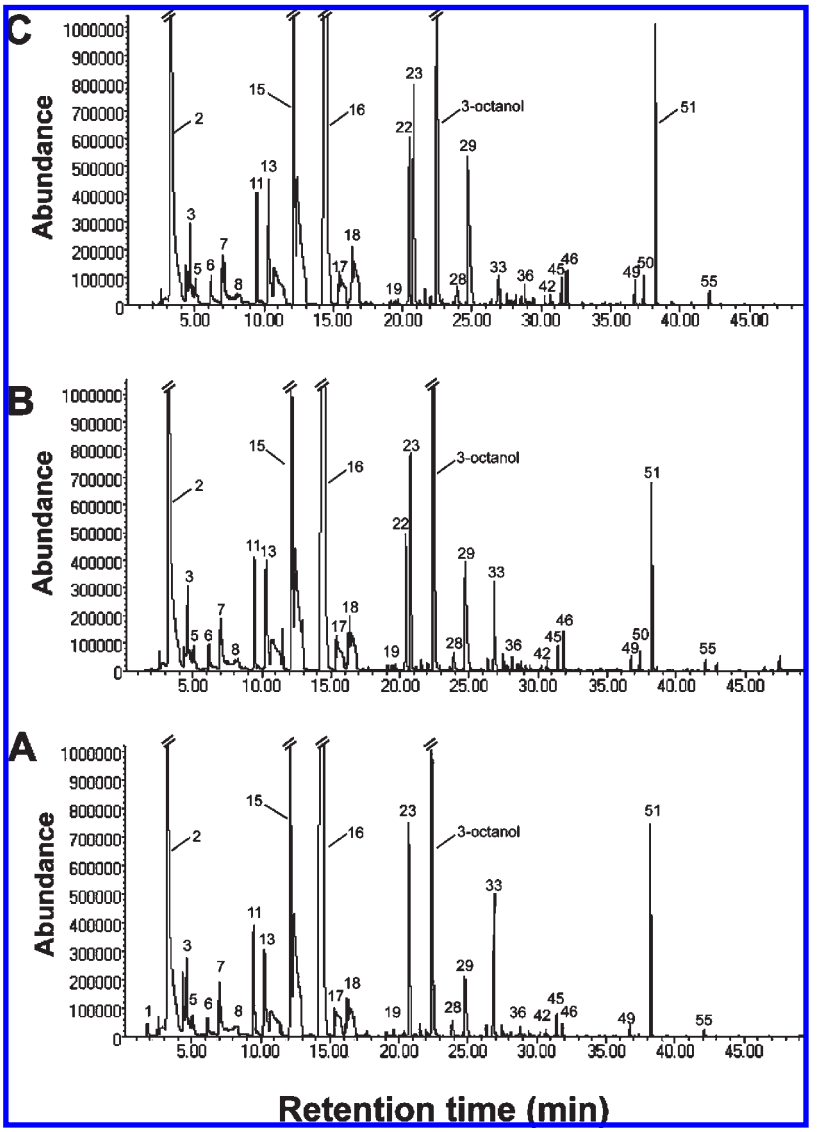

Figure 5. Representative GC chromatogram of Meoru wines fermented with *L. plantarum* meoru0711 (**B**) and *O. oeni* (**C**), following alcoholic fermentation with *S. cerevisiae*. The spectrum of **A** represents wine fermented only with *S. cerevisiae* without MLF. Peak numbers correspond to volatile compounds in **Table 3**. 3-Octanol represents the internal standard.

proteases of *L. plantarum moeru0711* are stronger than those of *O. oeni* because the levels of BCAAs and 2,3-butanediol were higher in MLF wine with *L. plantarum* meoru0711 than that in MLF wine with *O. oeni* (**Figure 4D**). Markedly increased levels of alanine in MLF wines, especially in those induced with *L. plantarum* meoru0711, were probably synthesized from pyruvate, consistent with reports that alanine levels were increased in MLF wines (*6*, *32*).

In general, significant L-arginine content is found in grape juice and wine, and LAB metabolizes arginine during MLF through the arginine deiminase (ADI) pathway, which leads to the formation of ornithine, citrulline, ammonia, ATP, and CO_2 (*37*, *38*). There are two different points of view about arginine metabolism in cellular growth and production of undesirable compounds by LAB. Its metabolism can be taken advantage of as an additional energy source and, thus, increases the viability of *O. oeni* (*39*, *40*). However, citrulline produced from arginine is the precursor of the carcinogen ethyl carbamate through the ADI pathway. Moreover, formation of ammonia can be undesirable because it will likely increase wine pH, causing the wine to become more susceptible to spoilage organisms (*31*). Numerous studies have demonstrated a correlation between citrulline excretion and ethyl carbamate formation in wine (*41*−*43*). Therefore, some researchers have tried to reduce citrulline levels by controlling arginine metabolism of LAB to prevent ethyl carbamate production in wine (*38*, *44*). Although the homofermentative *Lactobacillus* and *Pediococcus* strains are unable to catabolize arginine (*38*, *41*), arginine degradation in homofermentative LAB from other sources has been reported (*45*, *46*). Therefore, lower levels of arginine together with higher levels of ornithine in MLF wine compared to those in non-MLF wine reveal that *O. oeni* and *L. plantarum* meoru0711 metabolize arginine through the ADI pathway. However, no significant differentiation between *O. oeni* and *L. plantarum* meoru0711 in terms of ornithine levels was observed, and citrulline was not detected.

Glycerol and 2,3-BD. Glycerol, a colorless, odorless polyol, is a major byproduct of alcoholic fermentation. Its high viscosity is related to heavy mouth-feel in wine (*47*). LAB modulates the concentration of glycerol. The metabolism of glycerol by LAB has not been described but may induce wine spoilage through acrolein production. Greater acrolein synthesis from glycerol is found in red wine than in white. It is also likely that acrolein

Table 3. Volatile Compounds in Meoru Wines (Peak Area: TIC × 10^5)[a]

no	compounds	class[b]	odor	t_R(s)[c]	LAB		control
					L. plantarum	*O. Oeni*	no MLF
1	butanal**[d]	Ad	distinct buttery	1.8			25.2 a
2	ethyl acetate	Es	fruity	3.2	2720.4	2739.7	2727.2
3	ethyl propanoate	Es	sweet fruit	4.6	177.4	133.1	157.1
4	ethyl isobutylate*	Es	fruity	4.7	51.7 a	50.1 a	16.4 b
5	propyl acetate	Es		5.0	68.8	44.2	61.5
6	isobutyl acetate	Es		6.1	66.6	76.3	61.7
7	1-propanol	Al	fruit alcohol	7.0	198.3	187.9	199.1
8	unknown 1	Un			37.1	25.3	42.8
9	unknown 2	Un			49.8	31.7	43.2
10	ethyl butyrate	Es		7.4	29.8	26.7	29.5
11	isobutanol**	Al		9.5	225.9 a	197.8 b	235.3 a
12	2-butenoate methyl ester	Es		9.7	8.5	21.1	
13	isoamyl acetate*	Es	banana, pear	10.2	532.8 b	690.4 a	582.3 b
14	1-butanol***	Al	fusel, spirituous	11.6	63.6 a	27.4 b	19.0 c
15	2-butanoate ethyl ester	Es	sweet cheese	12.1	1.7	1.6	1.8
16	isoamylalcohol*	Al	nail polish	14.5	5929.0 b	5868.6 b	6228.4 a
17	ethyl hexanoate*	Es	green apple	15.3	241.8 a	192.2 b	235.1 a
18	ethenybenzene	Ot		16.2	404.7	420.5	407.7
19	isohexanol	Al		19.0	8.3	7.8	7.7
20	prenyl alcohol***	Al		19.4	10.3 a	7.8b	4.6 c
21	3-methyl-1-pentanol	Al		19.6	12.4	12.9	11.5
22	glycine***	Ac		20.4	244.5 b	311.1 a	14.6 c
23	1-hexanol	Al	fatty/fruity	20.7	435.8	419.1	474.5
24	3-hexen-1-ol	Al	herbaceous	21.1	21.0	20.0	20.9
25	3-etoxy-1-propanol	AL		21.5	20.6	20.3	22.5
26	2-hexen-1-ol***	AL	herbaceous	22.8	9.9 b	15.8 a	
27	3-hydroxy-3-methyl-butyric acid ethyl ester***	Es	fruity	23.6	6.8 a	5.6 a	
28	ethyl octanoate***	Es	sweet soap	23.9	52.2 a	39.6 b	45.2 ab
29	acetic acid***	Ac	vinegary	24.7	381.1 b	538.5 a	153.4 c
30	2-ethyl-1-hexanol*	Al		25.8	7.6 a	4.6 ab	1.3 b
31	unknown 3	Un			6.6	7.1	9.3
32	unknown 4	Un			4.4	3.2	7.7
33	benzaldehyde***	Ad	almond	26.9	142.2 a	85.6 b	213.8 a
34	pentyl prolanoate	Es		27.5	25.4	24.8	19.4
35	propanoic acid	Ac	vinegar	27.8	4.8	4.9	4.2
36	1-octanol***	Al		28.1	17.9 a	16.1 a	6.1 b
37	isopentyl hexanoate**	Es		28.5	12.1 a	11.9 a	
38	isobutyric acid	Ac		28.7	20.3	27.0	20.3
39	unknown 5	Un		28.9	2.3	2.2	7.1
40	unknown 6	Un		29.1	11.0	9.6	9.5
41	4-terpineol	Tr		29.4	8.6	7.4	9.4
42	γ-butyrolactone	Ot		30.2	12.2	14.9	8.9
43	butyric acid*	Ac	buttery	30.6	13.3 a	9.8 b	10.2 b
44	phenyl acetaldehyde	Ad		30.7	5.9	6.8	5.8
45	ethyl benzoate	Es		31.4	31.9	33.5	33.5
46	diethyl succinate*	Es	faint, pleasant	31.8	70.8 a	66.3 a	53.8 b
47	pentanoic acid	Ac		33.8	27.4	20.7	22.1
48	methyl salicylate***	Es		34.5	6.0 a	6.2 a	
49	hexanoic acid	Ac	pineapple	36.7	26.4	23.4	26.3
50	benzyl alcohol	Al		37.3	24.9	22.6	7.6
51	2-phenethy alcohol	Al		38.2	252.0	307.7	273.8
52	heptanoic acid	Ac	floral	39.4	3.0	3.1	4.0
53	phenol	Ph	medicinal	40.7	5.3	3.4	3.7
54	γ-aminobutyrolactam	Ot		41.1	2.8	1.6	1.1
55	octanoic acid	Ac	currant-like	42.1	17.3	20.7	17.1

[a] Data expressed as relative mean peak area (TICX10^6) with respect to the area of internal standard. [b] Compound classes are abbreviated as Ad, aldehyde; Es, ester; Al, alcohol; Ac, acid; Ph, phenol; Ot, other; and Un, unknown. [c] Compounds are reported in order of retention time. [d] Values with different letters are significantly different by Duncan's multiple range test at ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$.

enhances the bitterness of the wine by reacting with phenolic hydroxyl groups (*27*). In the present study, glycerol was still present in the non-MLF wine, while glycerol was degraded by LAB during MLF. Also, higher levels of glycerol in wine induced with *L. plantarum* meoru0711 than in that with *O. oeni* revealed lower glycerol utilization by *L. plantarum* meoru0711. This indicated that *L. plantarum* meoru0711 may produce wine with less bitterness than *O. oeni*.

The most predominant diol in wine is 2,3-butanediol (2,3-BD). 2,3-BD is produced from pyruvic acid through acetoin during MLF and alcoholic fermentation (*48*). The reduction of acetoin leads to the production of 2-BD, and this reaction is reversible.

During alcoholic fermentation, yeasts produce diacetyl, which is rapidly converted to acetoin and 2,3-BD. Acetoin and diacetyl are strong buttery smelling compounds. Thus, high concentrations of these compounds have a negative effect on wine. LAB metabolizes citric acid to higher quantities of these carbonyl compounds than yeast. As expected, 2,3-BD levels were increased after MLF (**Figure 4**). 2,3-BD is produced from glycerol with intermediates of pyruvate, acetoin, and diacetyl. Although glycerol was higher in ML-fermented wines with *L. plantarum* meoru0711 than in those with *O. oeni*, higher levels of 2,3-BD were observed in wines with *L. plantarum* meoru0711. This indicated that *L. plantarum* meoru0711 had stronger enzymatic activities related to the metabolism of pyruvate, acetoin, and diacetyl to 2,3-BD, compared to that of *O. oeni*.

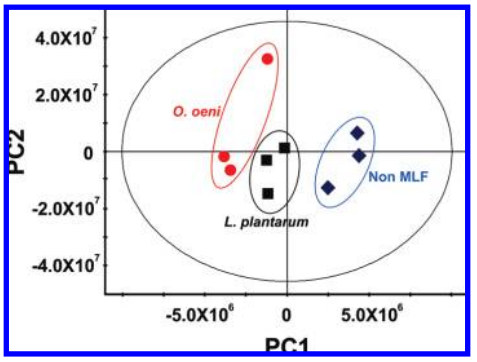

Figure 6. PCA score plots derived from volatile compounds on GC-MS chromatograms of all wines, demonstrating that MLF induces variation in volatile compounds, and LAB genera contribute to the variation.

Higher Alcohols. Although the grape is a main source of wine flavor and aroma, many flavors are produced by yeast and LAB in wine during fermentation. In particular, flavor modification in wine during MLF depends on the activities of esterases and glycosidic enzymes in LAB (*49*, *50*).

Higher alcohols, such as 1-propanol, isobutanol, and isoamyl alcohol, are the largest group of aroma compounds in alcoholic beverages (*51*). They are produced from decarboxylation and deamination of amino acids, as well as from sugar metabolism, and have both positive and negative effects on wine flavor (*52*). Many factors influence the final concentration of higher alcohols in alcoholic beverages. In wine, viticultural conditions, yeast, and LAB strains, and the other winemaking conditions contribute to variations in higher alcohol profiles. The amino acid content of the grape berry is also an important factor that influences higher alcohol production in wine. Branched-chain higher alcohols, including isoamyl alcohol, active amyl alcohol, and isobutanol, are synthesized during fermentation through the Ehrlich pathway, which involves degradation of BCAAs such as leucine, isoleucine, and valine (*51*). Increases in isoamyl alcohol and isobutanol levels together with decreased levels of BCAAs after MLF in wines may result from higher alcohol metabolism from BCAAs, but the mechanism is unclear (*53*). Maicas et al. (*11*) reported that concentrations of isobutanol, 1-propanol, 1-butanol, and isoamyl alcohol were dependent on the LAB strain. Ugliano et al. (*7*) reported that slight increases of higher alcohols, including 1-hexanol, 1-heptanol, 3-methyl-1-pentanol and 1-octen-3-ol, were observed after MLF. Pozo-Bayón et al. (*32*) also reported that most higher alcohols increased during MLF. It is well known that MLF does not affect those higher alcohol concentrations directly. However, some studies have reported

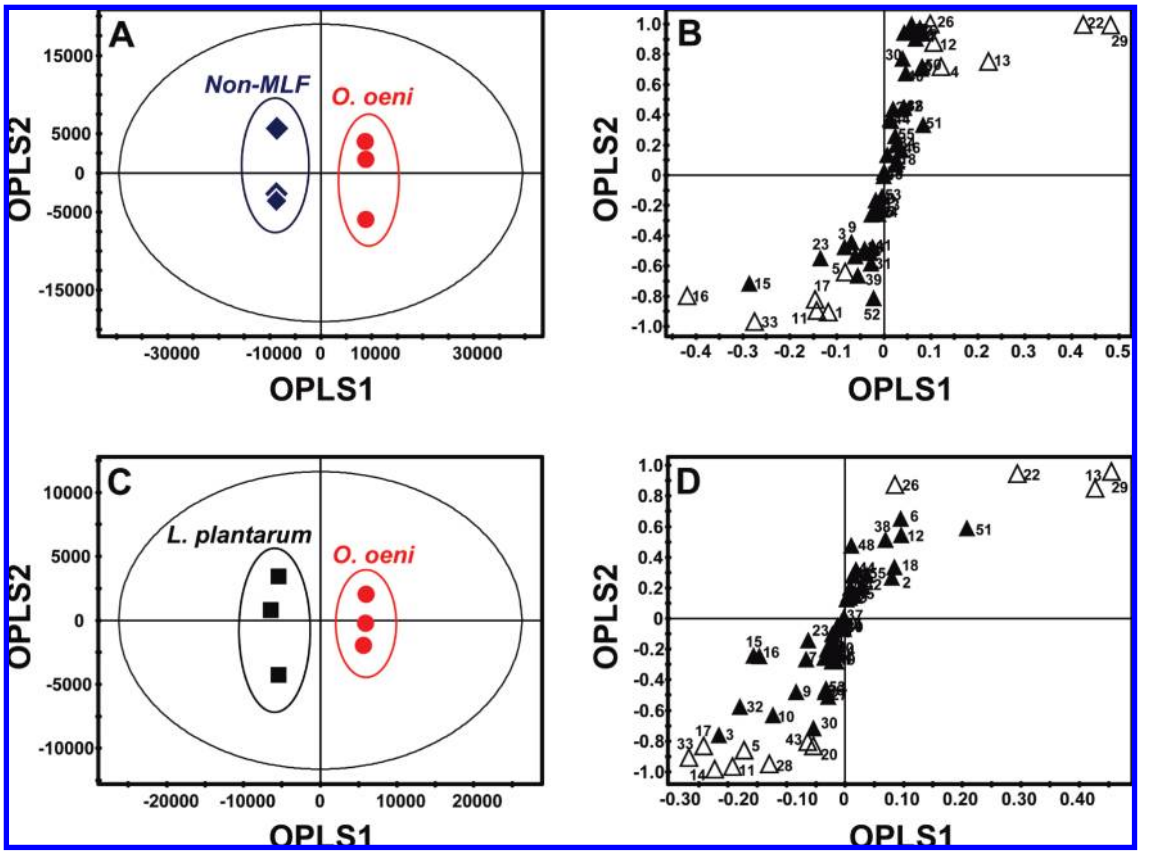

Figure 7. OPLS-DA score (**A** and **C**) and loading (**B** and **D**) *S*-plots demonstrating the differentiation in the volatile compounds between non-MLF wine and MLF wine induced with *O. oeni* (**B**) and between MLF wines with *O. oeni* and *L. plantarum* meoru0711 (**D**) following alcoholic fermentation. Numbers in panels **B** and **D** correspond to the volatile compounds in **Table 3**. Volatile compounds with open symbols are significantly different at $p < 0.05$.

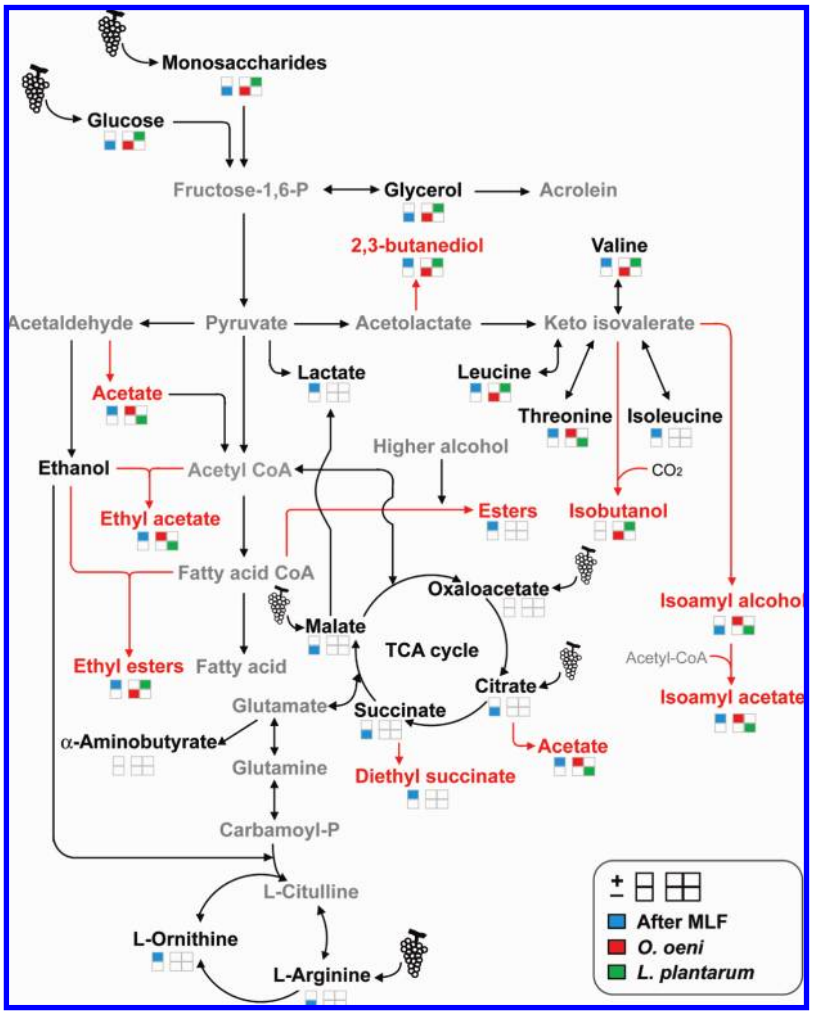

Figure 8. Schematic representation of metabolites produced during MLF. Metabolites observed in ^{1}H NMR spectroscopy and GC are black and red, respectively. Higher (+) and lower (−) levels of metabolites are marked in tops and bottoms of the indicative boxes, respectively. Blue in the indicative boxes represents changes in metabolites after MLF. Red and green denote the differences in metabolites between MLF wines with *O. oeni* and *L. plantarum* meoru0711.

higher alcohol changes during MLF (*7*, *11*, *54*). In the present study, levels of 1-butanol, prenyl alcohol, 2-hexen-1-ol, and 2-ethyl-1-hexanol were significantly increased after MLF. In addition, isobutanol, 1-butanol, and prenyl alcohol were more enhanced by *L. plantarum* meoru0711 than by *O. oeni*. Therefore, it is clear that different LAB genera contribute considerably to variations in higher alcohol profiles and concentrations in wines.

Esters. Esters, such as ethyl acetate and fatty acid ethyl esters with C_4 to C_{10}, are responsible for the fruity aroma of wines (*52*). The most abundant esters in wine are ethyl esters of organic acids, ethyl esters of fatty acids, and acetate esters. Esters are produced through lipid metabolism and acetyl CoA chemical esterification of alcohols and acids in yeast (*55*). Although yeast esterases have been studied (*56*), few studies have focused on the esterase activity of wine-associated LAB (*7*, *49*, *57*).

However, LAB esterases have been biologically characterized in the dairy industry, and these esterases contribute to the flavor development of foods such as cheeses (*58*). Maicas et al. (*11*) demonstrated that wine volatile compounds are noticeably changed during MLF. After MLF, fruity flavors derived from esters in wine are either reduced or enhanced (*9*, *10*, *54*, *59*−*63*).

These results indicate that the esterases of wine LAB are involved in both the synthesis and hydrolysis of esters. In the present study, ethyl isobutylate, 2-butenoate methyl ester, isoamyl acetate, 3-hydroxy-3-methyl-butyric acid ethyl ester, ethyl octanoate, and isopentyl hexanoate were enhanced by MLF (**Figure 7B**). In addition, higher levels of ethyl hexanoate, ethyl octanoate, and short-chained esters were observed in the wines ML-fermented with *L. plantarum* meoru0711 compared to those with *O. oeni*, indicating that esterification of fatty acids was dependent on the LAB genera. Matthews et al. (*64*) reported that wine-associated LAB, including *O. oeni* and *L. plantarum*, showed higher activity toward short-chained esters (C_2−C_8) than long-chained esters (C_{10}−C_{18}). The changes in ester concentrations following MLF may depend on ester metabolism by LAB and, thus, increase or decrease the quality of wine. However, further study of ester metabolism by LAB is needed to understand how wine flavors are modified during MLF.

Diethyl succinate is a wine ester that is produced from esterification of succinic acid. Diethyl succinate increases during wine aging (*12*) and accounts for a high percentage of wine flavor (*65*). Many researchers have found increased diethyl succinate concentrations in wines after MLF (*7*, *9*, *28*, *54*). Diethyl succinate contents in wine vary according to *O. oeni* strains (*23*). In the present study, increased levels in diethyl succinate after MLF revealed the esterification of succinate to diethyl succinate by LAB. In addition, no significant difference in diethyl succinate levels between MLF wines with *L. plantarum* meoru0711 and

O. oeni demonstrated that the esterification of succinate to diethyl succinate is not dependent on LAB genera.

Volatile Acids. Volatile organic acids are organic acids with short carbon chain length. The volatile acid content in wine usually ranges from 500 to 1000 mg/L, and acetic acid constitutes about 90% of this content (*55*). An increase in acetic acid is often observed after MLF due to citric acid metabolism by LAB. In the present study, the large amounts of acetic acid, 1852 mg/L for *L. plantarum* meoru071 and 2550 mg/L for *O. oeni*, might be due to the intrinsic property of Meoru grapes having high amounts of malic and citric acids compared to other grape varieties (*19*−*22*). Hexanoic acid and heptanoic acid are also produced as a result of fatty acid metabolism by yeast and bacteria.

In conclusion, we investigated the fermentative behaviors of different LAB genera through global metabolic profiling in wine. It was clear that *L. plantarum* and *O. oeni* affect both primary and secondary metabolites in Meoru wine. Moreover, the metabolomic approach coupled with multivariate statistical analysis provides insight into wine metabolism derived from LAB during fermentation or aging.

LITERATURE CITED

(1) Kell, D. B. Metabolomics and systems biology: making sense of the soup. *Curr. Opin. Microbiol.* **2004**, *7*, 296–307.

(2) Lenz, E. M.; Wilson, I. D. Analytical strategies in metabonomics. *J. Proteome Res.* **2007**, *6*, 443–458.

(3) Wishart, D. S. Metabolomics: applications to food science and nutrition research. *Trends Food Sci. Technol.* **2008**, *19*, 482–493.

(4) Van Leeuwen, C.; Friant, P.; Chone, X. Influence of climate, soil, and cultivar on terroir. *Am. J. Enol. Vitic.* **2004**, *55*, 207–217.

(5) Son, H. S.; Hwang, G. S.; Kim, K., M.; Kim, E. Y.; van den Berg, F.; Park, W. M.; Lee, C. H.; Hong, Y. S. ^{1}H NMR-based metabolomic approach for understanding the fermentation behaviors of wine yeast strains. *Anal. Chem.* **2009**, *81*, 1137–1145.

(6) Son, H. S.; Hwang, G. S.; Park, W. M.; Hong, Y. S.; Lee, C. H. Metabolomic characterization on malolactic fermentation and fermentative behaviors of wine yeasts in grape wine. *J. Agric. Food Chem.* **2009**, *57*, 4801–4809.

(7) 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.

(8) 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.

(9) du Plessis, H. W.; Steger, C. L. C.; du Toit, M.; Lambrechts, M. G. The occurrence of malolactic fermentation in brandy base wine and its influence on brandy quality. *J. Appl. Microbiol.* **2002**, *92*, 1005–1013.

(10) Herjavec, S.; Tupajic, P.; Majdak, A. Influence of malolactic fermentation on the quality of riesling wine. *Agric. Conspec. Sci.* **2001**, *66*, 59–64.

(11) 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.

(12) Jackson, R. S. *Wine Science*, 3rd ed. Academic Press: New York, 2008.

(13) Lafon-lafourcade, S.; Carre, E.; Ribereau-Gayon, P. Occurrence of lactic acid bacteria during the different stages of vinification and conservation of wines. *Appl. Environ. Microbiol.* **1983**, *46*, 873–880.

(14) Koh, S. K.; Lee, J. E.; Kim, H. W.; Kim, S. S.; Park, Y. K.; Park, Y. H.; Park, Y. H.; Koh, K. H. Identification and deacidification of lactic acid bacteria in Korean red wine. *Food Sci. Biotechnol.* **2004**, *13*, 96–99.

(15) Rodas, A. M.; Ferrer, S.; Pardo, I. 16S-ARDRA, a tool for identification of lactic acid bacteria isolated from grape must and wine. *Syst. Appl. Microbiol.* **2003**, *26*, 412–422.

(16) López, I.; Tenorio, C.; Zarazaga, M.; Dizy, M.; Torres, C.; Ruiz-Larrea, F. Evidence of mixed wild populations of *Oenococcus oeni* strains during wine spontaneous malolactic fermentations. *Eur. Food Res. Technol.* **2007**, *226*, 215–223.

(17) du Plessis, H. W.; Dicks, L. M. T.; Pretorius, I. S.; Lambrechts, M. G.; Toit, M. Identification of lactic acid bacteria isolated from South Africa brandy base wines. *Int. J. Food Microbiol.* **2004**, *91*, 19–29.

(18) Guerzoni, M. E.; Sinigaglia, M.; Gardini, F.; Ferruzzi, M.; Torriani, S. Effects of pH, temperature, ethanol and malate concentration on *Lactobacillus plantarum* and *Leuconostoc oenos*: Modeling of the malolactic activity. *Am. J. Enol. Vitic.* **1995**, *46*, 368–374.

(19) Kim, S. K. Deacidification of new wild grape wine. *Korean J. Food Nutr.* **1996**, *9*, 265–270.

(20) Kim, S. Y.; Kim, S. K. Winemaking from new wild grape. *Korean J. Food Nutr.* **1997**, *10*, 254–262.

(21) Oh, Y. S.; Lee, J. H.; Yoon, S. H.; Oh, C. H.; Choi, D. S.; Choe, E.; Jung, M. Y. Characterization and quantification of anthocyanins in grape juices obtained from the grapes cultivated in Korea by HPLC/DAD, HPLC/MS, and HPLC/MS/MS. *J. Food Sci.* **2008**, *73*, C378–C389.

(22) Choi, S. Y.; Cho, H. S.; Kim, H. J.; Ryu, C., H.; Lee, J. O.; Sung, N. J. Physicochemical analysis and antioxidative effects of wild grape (*Vitis coignetiea*) juice and its wine. *Korean J. Food Nutr.* **2006**, *19*, 311–317.

(23) Lee, J. E.; Hong, Y. S.; Lee, C. H. Characterization of fermentative behaviors of lactic acid bacteria in grape wines through ^{1}H NMR- and GC-based metabolic profiling. *J. Agric. Food chem.* **2009**, *57*, 4810–4817.

(24) Setkova, L.; Risticevic, S. Pawliszyn., Rapid headspace solid-phase microextraction-gas chromatographic-time-flight mass spectrometric method for qualitative profiling of ice wine volatile fraction II. Classification of Canadian and Czech ice wines using statistical evaluation of the data. *J. Chromatogr., A* **2007**, *1147*, 224–240.

(25) Dieterle, F.; Ross, A.; Schlotterbeck, G.; Senn, H. Probabilistic quotient normalization as robust method to account for dilution of complex biological mixtures. Application in H-1 NMR metabonomics. *Anal. Chem.* **2006**, *78*, 4281–4290.

(26) Veselkov, K. A.; Lindon, J. C.; Ebbels, T. M. D.; Crockford, D.; Volynkin, V. V.; Holmes, E.; Davies, D. B.; Nicholson, J. K. Recursive segment-wise peak alignment of biological H-1 NMR spectra for improved metabolic biomarker recovery. *Anal. Chem.* **2009**, *81*, 56–66.

(27) Fugelsang, K. C. *Wine Microbiology*; Chapman & Hall: New York, 1997.

(28) Wibowo, D.; Eschenbruch, R.; Davis, C. R.; Fleet, G.; Lee, T. H. Occurrence and growth of lactic acid bacteria in wine: A review. *Am. J. Enol. Vitic.* **1985**, *36*, 302–313.

(29) Versari, A.; Parpinello, G. P.; Cattaneo, M. *Leuconostoc oenos* and malolactic fermentation in wine: A review. *J. Ind. Microbiol. Biotechnol.* **1999**, *23*, 447–455.

(30) Bauer, R.; Dicks, L. M. T. Control of malolactic fermentation in wine: A review. *S. Afr. J. Enol. Vitic.* **2004**, *25*, 74–88.

(31) Hornsey, I. *The Chemistry and Biology of Winemaking*; The Royal Society of Chemisty: Cambridge, U.K., 2007.

(32) Pozo-Bayón, M. A.; G-Alegria, E.; Polo, M. C.; Tenorio, C.; Martin-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.

(33) Alcaide-Hidalgo, J. M.; Moreno-Arribas, M. V.; Polo, M. C.; Pueyo, E. Partial characterization of peptides from red wines: Changes during malolactic fermentation and aging with lees. *Food Chem.* **2008**, *107*, 622–630.

(34) Umbarger, H. E. Amino acid biosynthesis and its regulation. *Annu. Rev. Biochem.* **1978**, *47*, 533–606.

(35) Benson, K. H.; Godon, J. J.; Renault, P.; Griffin, H. G.; Gasson, M. J. Effect of *ilv* B *N*-encoded acetolactate synthase expression on diacetyl production in *Lactococcus lactis*. *Appl. Microbiol. Biotechnol.* **1996**, *45*, 107–111.

(36) Cogan, T. M.; Fitzgerald, J.; Doonan, S. Acetolactate synthase of *Leuconostoc lactis* and its regulation of acetoin production. *J. Dairy Res.* **1984**, *51*, 597–604.

(37) Liu, S. Q.; Pritchard, G. G.; Hardman, M. J.; Pilone, G. J. Arginine catabolism in wine lactic acid bacteria: Is it via the arginine deiminase pathway or the arginase-urease pathway? *J. Appl. Bacteriol.* **1996**, *81*, 486–492.
(38) Liu, S. Q.; Pritchard, G. G.; Hardman, M. J.; Pilone, G. J. Occurrence of arginine deiminase pathway enzymes in arginine catabolism by wine lactic acid bacteria. *Appl. Environ. Microbiol.* **1995**, *61*, 310–316.
(39) Tonon, T.; Lonvaud-Funel, A. Metabolism of arginine and its positive effect on growth and revival of *Oenococcus oeni*. *J. Appl. Microbiol.* **2000**, *89*, 526–531.
(40) Tonon, T.; Lonvaud-Funel, A. Arginine metabolism by wine: *Lactobacilli* isolated from wine. *Food Microbiol.* **2002**, *19*, 451–461.
(41) Romero, S. V.; Reguant, C.; Bordons, A.; Masqué, M. C. Potential formation of ethyl carbamate in simulated wine inoculated with *Oenococcus oeni* and *Lactobacillus plantarum*. *Int. J. Food Sci. Technol.* **2009**, *44*, 1206–1213.
(42) Uthurry, C. A.; Suárez Lepe, J. A.; Lombardero, J.; García Del Hierro, J. R. Ethyl carbamate production by selected yeasts and lactic acid bacteria in red wine. *Food Chem.* **2006**, *94*, 262–270.
(43) Azevedo, Z.; Couto, J. A.; Hogg, T. Citrulline as the main precursor of ethyl carbamate in model fortified wines inoculated with *Lactobacillus hilgardii*: A marker of the levels in a spoiled fortified wine. *Lett. Appl. Microbiol.* **2002**, *34*, 32–36.
(44) Mira de Orduna, R.; Patchett, M. L.; Liu, S. Q.; Pilone, G. J. Growth and arginine metabolism of the wine lactic acid bacteria *Lactobacillus buchneri* and *Oenococcus oeni* at different pH values and arginine concentrations. *Appl. Environ. Microbiol.* **2001**, *67*, 1657–1662.
(45) Montel, M. C.; Champomier, M. C. Arginine catabolism in *Lactobacillus sake* isolated from meat. *Appl. Environ. Microbiol.* **1987**, *53*, 2683–2685.
(46) Jonsson, S.; Clausen, E.; Raa, J. Amino acid degradation by *Lactobacillus plantarum* strain from fish. *Syst. Appl. Microbiol.* **1983**, *4*, 148–154.
(47) Swiegers, J. H.; Bartowsky, E. J.; Henschke, P. A.; Pretorius, I. S. Yeast and bacterial modulation of wine aroma and flavour. *Aust. J. Grape Wine Res.* **2005**, *11*, 139–173.
(48) Moreno-Arribas, M. V.; Polo, M. C. *Wine Chemistry and Biochemistry*; Springer: New York, 2009.
(49) Matthews, A.; Grimaldi, A.; Walker, M.; Bartowsky, E.; Grbin, P.; Jiranek, V. Lactic acid bacteria as a potential source of enzymes for use in vinification. *Appl. Environ. Microbiol.* **2004**, *70*, 5715–5731.
(50) 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.
(51) Laskin, A. I.; Bennett, J. W.; Gadd, G. M. *Advances in Applied Microbiology*; Academic Press: New York, 2005.
(52) Ebeler, S. E. Analytical chemistry: unlocking the secrets of wine flavor. *Food Rev. Int.* **2001**, *17*, 45–64.
(53) Lilly, M.; Bauer, F. F.; Styger, G.; Lambrechts, M. G.; Pretorius, I. S. The effect of increased branched-chain amino acid transaminase activity in yeast on the production of higher alcohols and on the flavour profiles of wine and distillates. *FEMS Yeast Res.* **2006**, *6*, 726–743.
(54) Izquierdo Cañas, P. M.; Garcia Romero, E.; Gómez Alonso, S.; Palop Herreros, M. L. L. Changes in the aromatic composition of Tempranillo wines during spontaneous malolactic fermentation. *J. Food Compos. Anal.* **2008**, *21*, 724–730.
(55) Helmut, K.; Gottfried, U.; Jürgen, F. *Biology of Microorganisms on Grapes, in Must and in Wine*; Springer:New York, 2009.
(56) Swiegers, J. H.; Pretorius, I. S. Yeast modulation of wine flavor. *Adv. Appl. Microbiol.* **2005**, *57*, 131–175.
(57) Matthews, A.; Grbin, P.; Jiranek, V. A survey of lactic acid bacteria for enzymes of interest to oenology. *Aust. J. Grape Wine Res.* **2008**, *12*, 235–244.
(58) 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.
(59) Sauvageot, F.; Vivier, P. Effects of malolactic fermentation on sensory properties of four Burgundy wines. *Am. J. Enol. Vitic.* **1997**, *48*, 187–192.
(60) Gámbaro, A.; Boido, E.; Zlotejablko, 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.
(61) 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.
(62) Delaquis, P.; Cliff, M.; King, M.; Girard, B.; Hall, J.; Reynolds, A. Effect of two commercial malolactic cultures on the chemicals and sensory properties of chancellor wines vinified with different yeast and fermentation temperatures. *Am. J. Enol. Vitic.* **2000**, *51*, 42–48.
(63) Henick-Kling, T. Control of malolactic fermentation in wine: energetics, flavour modification and methods of starter culture preparation. *J. Appl. Bacteriol.* **1995**, *32*, 29–37.
(64) 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.
(65) Clarke, R. J.; Bakker, J. *Wine Flavour Chemistry*; Blackwell Publishing: Cambridge, MA, 2004.

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