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Acetaldehyde Released by *Lactobacillus plantarum* Enhances Accumulation of Pyranoanthocyanins in Wine during Malolactic Fermentation

Shaoyang Wang[1,2,†], Siyu Li[3,†], Hongfei Zhao[1], Pan Gu[1], Yuqi Chen[1], Bolin Zhang[1], Baoqing Zhu[1]*

[1] Department of Food Science, Beijing Key Laboratory of Forestry Food Processing and Safety, College of Biological Sciences and Biotechnology, Beijing Forestry University, Beijing 100083, P.R. China

[2] Centre for Nutrition and Food Sciences, Queensland Alliance for Agriculture and Food Innovation, University of Queensland, P.O. Box 156, Archerfield BC, QLD 4108, Australia

[3] Center for Viticulture and Enology, College of Food Science & Nutritional Engineering, China Agricultural University, Beijing 100083, P.R. China

† These authors contributed equally to this work.

* To whom correspondence should be addressed:

Baoqing Zhu, Tel/Fax: +86-10-6233-8221; Email: zhubaoqing@bjfu.edu.cn

ABSTRACT

This study investigated the evolution of acetaldehyde and pyranoanthocyanins in wine during malolactic fermentation, and further evaluated the correlation between acetaldehyde and pyranoanthocyanins. Cabernet Gernischt wine after alcoholic fermentation was inoculated with four lactic acid bacteria strains. Malolactic fermentation kinetics and wine characteristics were compared. Results showed these strains exhibited different kinetics on wine malolactic fermentation. Wine with *Lactobacillus plantarum* had lower reducing sugar, total acid, and yellowness. *Lactobacillus plantarum* elevated the level of acetaldehyde in wine model medium and wine during malolactic fermentation. Malolactic fermentation using *Lactobacillus plantarum* significantly increased the concentration of pyranoanthocyanins, whereas *O. oeni* strain reduced the level of pyranoanthocyanins in wine. Polymerized anthocyanins percentage in wine was significantly enhanced after fermentation with *Lactobacillus plantarum*. Principal component analysis indicated that the characteristics of these strains inoculated wines after malolactic fermentation were segregated. The findings from this study could provide useful information on the wine color improvement through malolactic fermentation with suitable lactic acid bacteria strains.

Keywords: *Lactobacillus plantarum*; acetaldehyde; pyranoanthocyanins; malolactic fermentation; wine color

1. Introduction

Malolactic fermentation is an important fermentation process that is normally applied in almost all red wines after alcoholic fermentation (Sumby, Grbin, & Jiranek, 2014). Generally, malolactic fermentation converts malic acid into lactic acid under the activity of lactic acid bacteria, which can reduce the acidity of wine. Malic acid is an important nutrient for the proliferation of microorganisms in wine. The consumption of malic acid during malolactic fermentation can lower the risk of the wine spoilage. More importantly, the conversion of malic acid to lactic acid during wine malolactic fermentation could significantly improve the palatability of wine (Burns, Osborne, & Edwards, 2011; Sumby et al., 2014). In addition, aroma precursors in wine can be further hydrolyzed into free-form volatiles under malolactic fermentation (Bartowsky & Borneman, 2011; Knoll et al., 2011; Lonvaud-Funel, 1999). It has been reported that different bacteria strains exhibited different abilities of hydrolyzing aroma precursors, which could result in a significant difference on the wine overall aroma after malolactic fermentation (Boulton, Singleton, Bisson, & Kunkee, 1999; Capaldo, Walker, Ford, & Jiranek, 2011; Iorizzo et al., 2016; Pozo-Bayón et al., 2005). In previous studies, different strains of lactic acid bacteria (*Oenococcus*, *Lactobacillus*, and *Pediococcus*) were found in wine during malolactic fermentation (Pozo-Bayón et al., 2005; Sumby et al., 2014). For example, *Oenococcus oeni* possesses high tolerance on acid and ethanol conditions (Iorizzo et al., 2016; Pozo-Bayón et al., 2005). Therefore, it is normally inoculated to the harsh wine system with low pH and high ethanol level (López, Núñez, Lozano, & Larrea, 2008). *Lactobacillus plantarum* exhibits the similar tolerant capacity on ethanol and acidity as *O. oeni* (Berbegal et al., 2016; Cappello, Zapparoli, Logrieco, & Bartowsky, 2017; Iorizzo et al., 2016; Pozo-Bayón et al., 2005). More importantly, *L. plantarum* can release more enzymes during malolactic fermentation, which could favor the yield of free volatiles in wine (Iorizzo et al., 2016).

Phenolic compounds are important compounds in wine that essentially determine the organoleptic properties of wine, including appearance, bitterness, and astringency (Gao et al., 2015; Li, He, Zhu, Wang, & Duan, 2016). According to their structure, phenolic compounds can be classified into non-anthocyanin phenolic compounds and anthocyanins (Gao et al., 2015). Phenolic compounds are mainly extracted from grape into wine during crushing, pressing and maceration (He et al., 2012a; Monagas & Bartolomé, 2009), whereas their composition in wine can be further altered through the metabolism by microorganisms during wine fermentation process (Hernandez et al., 2007; Li, Liu, Pan, Shi, & Duan, 2015). For instance, anthocyanins are mainly responsible for red wine color, undergo a series of reactions happen to anthocyanins during alcoholic and malolactic fermentation, which could further alter their structures and thus influence wine color (Burns et al., 2011). For instance, pyranoanthocyanins can be synthesized from anthocyanins and small microbial metabolites, such as acetaldehydes, pyruvic acid, or acetone (Burtch, Mansfield, & Manns, 2017; Morata, Calderón, González, Gómez-Cordovés, & Suárez, 2007). This can stabilize anthocyanins and improve the appearance of wine (He et al., 2012b).

Yeasts metabolism in wine during alcoholic fermentation is a major route to produce acetaldehyde in wine (Osborne, Mira de Orduña, Pilone, & Liu, 2000). Acetaldehyde can be further consumed in wine during malolactic fermentation (such as *Lactobacillus brevis* and *Oenococcus oeni*), which limited its conjugation with anthocyanins in wine (Liu & Pilone, 2000; Osborne et al., 2000). As a result, a color loss has been normally found in wine after malolactic fermenation (Burns & Osborne, 2015). It has been reported that some lactic acid bacteria strains, such as *Lactobacilli*, *Leuconostocs*, *Pediococci* and *Streptococcus* spp., have ability to produce acetaldehyde (Chaves et al., 2002; Liu & Pilone, 2000). We hypothesized that wine malolactic fermentation using these lactic acid bacteria could increase the

accumulation of acetaldehyde, which could further stabilize anthocyanins in wine after fermentation. To this end, we applied malolactic fermentation to Cabernet Gernischt wine using four strains of lactic acid bacteria, and the evolution of acetaldehyde and anthocyanins in wine were compared. The findings from this study could provide useful information on color improvements of wine through selecting suitable lactic acid bacteria strains during wine malolactic fermentation.

2. Materials and Methods

2.1 Starter Culture Preparation

Four strains of lactic acid bacteria were selected in the present study for wine malolactic fermentation. Two *L. plantarum* strains, including *L. plantarum* Lp39 (CICC6240) and C8-1 (CICC23138), were received from the China Center of Industrial Culture Collection (Beijing, China). Two commercial *O. oeni* strains (Viniflora® Oenos and CiNe) were purchased from Chr. Hansen (Hoersholmm, Denmark). Preparation of the starter culture followed the published methods with some modifications (Lerm, Engelbrecht, & du Toit, 2011). In brief, the *L. plantarum* strains were cultured in the De Man, Rogosa and Sharpe agar (MRS) medium at 37^{o}C, whereas the MRS medium supplemented with 20% (v/v) preservative-free tomato juice was used to culture the *O. oeni* strains at 23^{o}C. A preliminary study was conducted to determine the growth curve of each strain under the culture condition. Specially for *L. plantarum*, after 8 h of growing, the culture was centrifuged at 6,000 rpm for 10 min and washed by normal saline for 2 times, then transferred into the adaptation media which were evenly mixed with MRS and wine model medium or wine (to be detailed in 2.2 and 2.3 hereinafter). Afterwards, the adaptation media were consecutively incubated at 37^{o}C for 8 h. At the end of incubation, the correspondences between

absorbance and cell density of *L. plantarum* were determined, then the cultures were washed twice again by normal saline before inoculation.

2.2 Malolactic Fermentation in Wine Model Medium

Wine model medium was prepared according to a published study with minor modifications (Ugliano, Genovese, & Moio, 2003). The wine model medium consisted of 12.0% (v/v) ethanol, 5.0 g/L tartaric acid, 3.5 g/L malic acid, 2.0 g/L glucose, 2.0 g/L fructose, 0.2 g/L NaCl, 1.0 g/L $(NH_4)_2SO_4$, 2.0 g/L K_2HPO_4, 0.2 g/L $MgSO_5 \cdot 7H_2O$, 0.05 g/L $MnSO_4$, and 2.0 g/L yeast extract. The pH of the wine model medium was adjusted to 3.5 using potassium hydroxide. Afterwards, 0.1 g/L natamycin (purity of 95%, Lifecome Biochemistry Co., Ltd., Pucheng County, Fujian, China) was supplemented into the medium to inhibit the growth of the film yeast. The activated *O. oeni* and adapted *L. plantarum* starter cultures were directly inoculated to the wine model medium with a final density of 10^8 CFU/mL (the final density was calculated according to the correspondence between absorbance, cell density and culturing time that determined in adaptation medium) in a 100-mL capacity Erlenmeyer flask under anaerobic environment (23^{o}C) for the malolactic fermentation according a published method (Iorizzo et al., 2016). The wine model medium was sampled at 0 (before malolactic fermentation), 2, 4, 8, and 12 days. The level of malic acid, lactic acid, and acetaldehyde were measured. Fermentation was performed in duplicate.

2.3 Malolactic Fermentation in Wines

Ripened Cabernet Gernischt grapes (*Vitis vinifera* L.) from 10-year grape vines were harvested in a vineyard at the Huailai region (Hebei, China) in 2016. After harvest, the grapes were immediately

transported to the enology center at the Beijing Forestry University (Beijing, China) for wine fermentation. The grapes had 225 g/L sugar content and 5.64 g/L total acid. The grapes (150 kg) were crushed, destemmed, and placed in a 200-L capacity temperature-controlled fermentation vessel supplied with 50 mg/L SO_2 and 20 g/L Zym Color Plus pectinase (Enartis, Tracete, Italy). After 24 hours, the commercial yeasts (ES488, Enartis, Spain) were added to the vessel to start alcoholic fermentation. The alcoholic fermentation was conducted at 18-25^{o}C for 10-11 days. After the alcoholic fermentation, the wine was separated from the grape pomace and then filtered through 0.22 μm filters. The wine sample after the alcoholic fermentation had a 12.5% (v/v) alcohol level.

The starter culture (10^8 CFU/mL) prepared with the same method hereinbefore was inoculated to the wine in a 200-mL Erlenmeyer flask under an anaerobic condition (23^{o}C) to initiate malolactic fermentation. Malolactic fermentation for each strain was conducted in duplicate. During the malolactic fermentation, the wine was sampled every 2 days to measure the strain colony and malic acid-to-lactic acid conversion rate. The *L. plantarum* C8-1, *L. plantarum* Lp39, *O. oeni* Oenos, and *O. oeni* CiNe took 14, 10, 4 and 6 days respectively to fully consume malic acid. Afterwards, the malolactic fermentation was terminated by removing the bacteria through centrifuging the wine sample at 6,000 rpm for 10 min. The resultant wine sample was stored at -20^{o}C for further analysis.

2.4 Malic Acid-to-Lactic Acid Conversion

The level of malic acid and lactic acid in the wine model medium or the wine sample were measured using a published method (Wei et al., 2014). A Shimadzu LC-20AT HPLC system (Shimadzu Corp., Kyoto, Japan) equipped with a Venusil ASB C18 column (Agela Technologies Co., Ltd., Tianjin, China) was used to analyze malic acid and lactic acid. The column was eluted with formic acid: methanol: water (0.1:

3.0: 96.9, v/v/v) for 20 min with a flow rate of 0.5 mL/min. The sample was filtered through 0.45 µm filters and the injection volume was 20 µL. The wavelength on SPD-M20A detector (Shimadzu Corp., Kyoto, Japan) was 210 nm. The external standard malic and lactic acid were used for the quantitation.

2.5 Physiochemical Index and Color Attributes of Wine

The physiochemical properties of the wine samples, including pH, total acid, reducing sugar, and volatile acid, were measured according to the National Standard Method GBT 15038-2006. CIELab assay was used to evaluate the color attributes of the wine (Liu et al., 2015). The color attributes were calculated using the equations below,

$$L^* = 116(Y/Y_0)^{1/3} - 16$$

$$a^* = 500[(X/X_0)^{1/3} - (Y/Y_0)^{1/3}]$$

$$b^* = 200[(Y/Y_0)^{1/3} - (Z/Z_0)^{1/3}]$$

$$C^* = (a^{*2}+b^{*2})^{1/2}$$

$$h^* = \arctan(b^*/a^*)$$

$$\Delta E = [(L^* - L_0^*)_2 + (a^* - a_0^*)_2 + (b^* - b_0^*)^2]^{1/2}$$

$$X = 14.172T_{440} + 28.583T_{530} + 52.727T_{600}$$

$$Y = 9.005T_{440} + 62.965T_{530} + 28.168T_{600} - 0.063$$

$$Z = 94.708T_{440} + 15.889T_{530} - 5.233T_{600} + 1.777$$

Where the constant $X_0 = 97.29$, $Y_0 = 100$ and $Z_0 = 116.14$, whereas L_0^*, a_0^*, and b_0^* represented the color attributes of the wine before the malolactic fermentation.

2.6 Anthocyanins

2.6.1 Anthocyanins composition

The analysis of anthocyanins followed a published method (Li, He, Zhu, Xing, et al., 2016). An Agilent series 1200 HPLC system equipped with an Agilent Poroshell 120 EC-C18 column (150 × 2.1 mm, 2.7 µm) was used to separate anthocyanins in the wines (Agilent Technologies, Santa Clara, CA, USA). The mobile phase consisted of (A) 0.1% formic acid (v/v) in water and (B) 0.1% formic acid (v/v) in acetonitrile: methanol (50:50, v/v). The elution was programmed as follows: 10%B to 46%B in 28 min, 46%B to 10%B in 1 min, and then 10%B for 5 min for re-equilibration of the column. The flow rate was 0.4 mL/min and the column was maintained at 55^{o}C. The wine sample (1.0 mL) was filtered through a 0.45 µm inorganic PES membrane, and the injection volume was 20 µL. Anthocyanins in the wine sample were identified using Agilent 6410 Triple-Quadrupole (QqQ) mass spectrometry (Agilent Technologies, Santa Clara, CA, USA). The spray voltage was set at 4 kV with the gas temperature of 350^{o}C, gas flow rate of 12 L/h and nebulizer pressure of 35 psi. Multiple reactions monitoring mode was used to identify anthocyanins based on their featured mass ion transition. Delphinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, and malvidin-3-*O*-glucoside were used as the external standard to quantify the anthocyanins in the wine sample.

2.6.2 Free, copigmented, and polymerized anthocyanins

The percentage of the free, copigmented, and polymerized anthocyanins in wine were calculated using a published method (Boulton, 1996). The wine sample was adjusted to pH 3.6 using 0.1 M hydrochloric acid or sodium hydroxide. Afterwards, 4 mL of the wine sample was mixed with 40 µL of 10% (v/v) acetaldehyde solution. The resultant mixture was incubated in the darkness for 45 min. The absorbance (A_{acet}) of the mixture was measured at 520 nm. Similarly, 4 mL of the pH adjusted wine was

mixed with 320 µL 5% (v/v) sulfurous acid solution. The mixture was incubated in the darkness for 45 min, and then the absorbance (A_{SO2}) was measured at 520 nm. The model wine solution (consisting of 0.125 g potassium hydrogen tartrate, 6 mL anhydrous, and 44 mL de-ionized water with pH adjusted to 3.6) was diluted 20 times and the absorbance (A_{20}) of the diluted model wine solution was measured at 520 nm. The proportion of the free, copigmented, and polymerized anthocyanins were calculated using the equations below,

Free anthocyanins % = $(A_{20}-A_{SO2}) / A_{acet} \times 100\%$

Copigmented anthocyanins % = $(A_{acet}-A_{20}) / A_{acet} \times 100\%$

Polymerized anthocyanins % = $A_{SO2} / A_{acet} \times 100\%$

2.7 Acetaldehyde

The analysis of the total and free acetaldehyde in the wine model medium and wine samples followed a published method with minor modifications (Wang, 2014). For the total acetaldehyde, the wine sample (500 µL) was mixed with 200 µL of 4 M NaOH solution. The resultant mixture was kept in the darkness for 10 min, and then mixed with 500 µL of acetonitrile, 150 µL of 25% (v/v) sulfuric acid, and 200 µL of the 2,4-dinitrophenyl hydrazine (DNPH) solution (5 g/L in acetonitrile with 12% (v/v) sulfuric acid). Afterwards, the mixture was vortexed for 3 min, and then incubated at 37^{o}C for 1 hour in the darkness for the derivatization of acetaldehyde. Subsequently, the mixture was filtered through a 0.45 µm nylon membrane prior to HPLC analysis. For the free acetaldehyde, the wine sample (500 µL) was mixed with 500 µL of acetonitrile, 150 µL of 25% (v/v) sulfuric acid, and 200 µL of the DNPH solution. The resultant mixture was kept in the darkness for 1 hour at 37^{o}C. Afterwards, the mixture was filtered

through the 0.45 µm nylon membrane for HPLC analysis. A Shimadzu LC-20AT HPLC system coupled with a SPD-M20A detector was used for the analysis of acetaldehyde (Shimadzu Corp., Kyoto, Japan) on a Venusil ASB C18 column (Agela Technologies Co., Ltd., Tianjin, China). The mobile phase consisted of (A) acetonitrile and (B) 25 mM sodium acetate (pH 4.5). The elution gradient was as follows: 0 min, 50%B; 0-15 min, 40%B; 15-20 min, 50%B. The column was maintained at 40^{o}C. The flow rate was set at 1 mL/min, and the wavelength on the SPD-M20A detector was 365 nm. The injection volume was 20 µL. External acetaldehyde dissolved in ethanol:water (12: 88, v/v) solution was used to quantify the total and free acetaldehyde.

2.8 Statistical Analysis

Data were expressed as the mean ± standard deviation of duplicate tests. Analysis of variance (ANOVA) was used to investigate the significance among the means under Tukey's multiple range test at a 0.05 significant level (SPSS Inc., Chicago, Ill., USA). Heatmap clustering analysis was used to investigate the correlation of anthocyanins composition and acetaldehyde in wine before and after the malolactic fermentation, whereas principal component analysis was carried out to differentiate the similarity of these wines.

3. Results and Discussion

3.1 Malolactic Fermentation in Wine Model Medium by Different Strains

The malolactic fermentation in the wine model medium by these four lactic acid bacteria strains were conducted to investigate if these strains possessed different fermentation kinetics on the consumption of malic acid and the formation of acetaldehyde. Except for the *L. plantarum* Lp39, the other 3 strains

consumed half amount of the malic acid level in the wine model medium after 8 days of the malolactic fermentation. These 2 *L. plantarum* strains did not completely convert malic acid into lactic acid untill 12 days of the malolactic fermentation (**Figure 1a**). Meanwhile, lactic acid in the *O. oeni* strains inoculated wine model medium was gradually released during the fermentation process, whereas a dramatic accumulation of lactic acid was observed in the medium with *L. plantarum* (**Figure 1b**). After the fermentation, the lactic acid level in the *L. plantarum* strains inoculated medium appeared to be about 3 times higher than that in the *O. oeni* strains medium. In this case in the wine model medium the *L. plantarum* strains showed higher lactic acid production potential than *O. oeni.* This might be because when MLF is complete, the remaining lactic acid bacteria, such as *Lactobacillus*, are still able to metabolize residual sugar to produce lactic acid (du Toit, Engelbrecht, Lerm, & Krieger-Weber, 2011).

It has been reported that acetaldehyde was yielded in wine during wine alcoholic fermentation, and the yielded acetaldehyde can be further metabolized by lactic acid bacteria (such as *O. oeni*) during malolactic fermentation (Chaves et al., 2002; Liu & Pilone, 2000; Osborne et al., 2000). In the present study, the wine model medium contained 4.56 mg/L acetaldehyde before the inoculation of the strain, which might be brought by the yeast extract in the medium. The *O. oeni* strains increased the level of acetaldehyde in the medium at the beginning of the malolactic fermentation (**Figure 1c**). However, a dramatic decrease on the acetaldehyde level was observed after 4 days of the malolactic fermentation in the *O. oeni* strains treated medium. Our results were consistent with the previous reports (Osborne, Dubé Morneau, & Mira de Orduña, 2006; Osborne et al., 2000). In the wine model medium treated by *L. plantarum*, a continuous accumulation of acetaldehyde was observed along with the fermentation. At the end of the fermentation, the acetaldehyde concentration in the *L. plantarum* strains inoculated

medium was about 2-3 times higher than that in the medium with *O. oeni*. The change trend differences of acetaldehyde between *O. oeni* and *L. plantarum* was probably because *O. oeni* owns more active acetaldehyde consumption rate than *L. plantarum* strains, whereas *L. plantarum* might have the ability to produce acetaldehyde in wine during malolactic fermentation when the sufficient energy source was present in the matrix (Burns & Osborne, 2015). We hypothesized that in the presence of anthocyanins, and due to the microbial activities, such an increase on the acetaldehyde level might favor the production of pyranoanthocyanins, which could improve the stability of anthocyanins in wine to maintain the color attributes of wine after malolactic fermentation. In order to verify our hypothesis, the Cabernet Gernischt wine after the alcoholic fermentation was inoculated with these strains and their malolactic fermentations were compared.

3.2 Malolactic Fermentation in Cabernet Gernischt Wine by Different Strains

In **Table 1**, basic malolactic fermentation indexes of these four strains are compared, and it is shown that these four strains showed same impacts on the change trends of pH, total acid, reducing sugar and volatile acidity respectively. The malolactic fermentation resulted in an elevation on the pH value of the wine samples from 3.46 to above 3.60. Meanwhile, the total acid content in the wines was significantly reduced. The fermentation by these strains also led to a significant decrease on the reducing sugar content in the wine (**Table 1**). The fermentation duration in the wine by the *L. plantarum* C8-1 strain was the longest, which resulted in the wine with the lowest reducing sugar. It has been reported that long malolactic fermentation process could trigger the conversion of reducing sugar to volatile acids, which might lead to wine spoilage (Lerm, 2010). However, the volatile acid level

in *L. plantarum* C8-1 fermented wine was significant lower than others, even in wines fermented by *O. oeni strains*. This could reduce the risk of the wine spoilage.

Growth pattern of these strains during the wine malolactic fermentation appeared to be different (**Figure 2**). For example, the cell population of *O. oeni* Oenos increased during the first 2 days of the fermentation, followed by a density decrease (**Figure 2a**). However, the population density of the other strains exhibited a decrease during the fermentation. At the end of the fermentation, these wines contained the cell density above 10^7 CFU/mL except for the *L. plantarum* C8-1 strain treated wine. Malic acid was consumed mush faster in the wine with the *O. oeni* strains and *L. plantarum* Lp39. However, the conversion process of malic acid in the wine with *L. plantarum* C8-1 was much slower (**Figure 2b**). The accumulation of lactic acid in the wine was accompanied with the consumption of malic acid during the wine malolactic fermentation (**Figure 2c**). A dramatic increase on the lactic acid level was observed in the *O. oeni* strains and *L. plantarum* Lp39 strain inoculated wine during the first 4 days of the fermentation. Afterwards, its level remained the similar by the end of the fermentation in these wines. A gradual accumulation of lactic acid happened in the wine fermented with *L. plantarum* C8-1, and its concentration kept increasing at the end of the fermentation. A previously published study has reported that *L. plantarum* had a slower malolactic rate than the *O. oeni* strains (Lerm, Engelbrecht, & du Toit, 2011). The viability of lactic acid bacteria to complete malolactic fermentation in wine has been suggested to be above 10^6 CFU/mL (Fugelsang & Edwards, 2006), and the cell density can be significantly enhanced during malolactic fermentation (Pozo-Bayón et al., 2005; Sun, Gong, Liu, & Jin, 2016). However, inoculation of the extremely high cell density in wine could result in a significant reduction on the cell population in wine during malolactic fermentation due to the limited nutrients (Fugelsang & Edwards, 2006). The cell density of these strains decreased after the malolactic

fermentation, especially in the *L. plantarum* strains. This indicated that *L. plantarum* might either need a higher nutrition demand or possessed a poorer resistant capacity to the wine environment than the *O. oeni* strains during the malolactic fermentation process. Our result indicated that the conversion of malic acid to lactic acid in wine was highly linked to the cell density in the wine during the fermentation.

The total and the free acetaldehyde concentration in the wine before the malolactic fermentation were 13.48 and 1.86 mg/L, respectively (**Figure 2d** and **2e**). The inoculation of *O. oeni* Oenos did not result in an increase on the total or free acetaldehyde concentration in the wine during the fermentation process. However, the malolactic fermentation with the other strains led to an alteration on the total and free acetaldehyde concentration in the wines. For example, an initial increase and then a decrease on the total acetaldehyde level were observed in the wine with *O. oeni* CiNe during the fermentation, whereas the free acetaldehyde concentration continued to increase in this strain treated wine. In the wine with the *L. plantarum* Lp39 strain, a dramatic increase on the concentration of the total and free acetaldehyde was observed at the beginning of the fermentation, followed by a concentration fluctuation. After 8 days of the fermentation, another significant accumulation of the total and free acetaldehyde was found in this wine sample. The wine with *L. plantarum* C8-1 during the fermentation from 0 to 10 days exhibited a continuous elevation on the total and free acetaldehyde concentration in the wine. Afterwards, a dramatic reduction on the level of the total and free acetaldehyde was observed in this wine.

Acetaldehyde has been considered an important by-product through lactic acid bacteria metabolism since these small molecular compounds could improve the flavor of dairy foods (Chaves et al., 2002). In

wine, alcoholic fermentation normally results in the release of acetaldehydes under the activity of yeasts, and acetaldehydes normally are consumed in wine during malolactic fermentation since alcohol dehydrogenase released by lactic acid bacteria such as *O. oeni* has been reported to take charge of the acetaldehyde degradation (Chaves et al., 2002; Tong et al., 2012). Our results were consistent with these reports. The degradation of acetaldehydes limits the conjugation between anthocyanins and acetaldehyde in wine, which could weaken the color of wine after malolactic fermentation. However, in our research, we find that *L. plantarum* helped accumulating acetaldehyde in the wine during the fermentation, which might enhance the color stabilization in wine through sufficient reaction between anthocyanins and acetaldehyde. It should be aware that the wine with *L. plantarum* C8-1 decreased the level of acetaldehyde at the late stage of the fermentation in the present study (**Figure 2d** and **2e**). However, the final acetaldehyde level was found to be depended on the polymerized anthocyanin level in the wine.

3.3 Anthocyanins and Color Attributes in Wine by Different Strains

It has been reported that β-glycosidase in wine environment could result in the degradation of anthocyanins during malolactic fermentation (Burns et al., 2011; Capaldo, Walker, Ford, & Jiranek, 2011). On the other side, studies also suggested that acetaldehyde, acetone, acetoin, oxalacetic acid, acetoacetic acid, and diacetyl were produced during alcoholic fermentation with the help of microbial activity, and these molecules can covalently bind with free or acylated anthocyanins to yield corresponding pyranoanthocyanins (He et al., 2012b). For example, Romero and Bakker (2000) reported that a reaction between anthocyanin and the added pyruvic acid resulted in the formation of pyranoanthocyanins (vitisin A) in a model wine solution. Morata et al. (2007) suggested that more

stable pyranoanthocyanins were found in red wine during storage after supplementing wine with pyruvid acid and acetaldehyde. Burns and Osborne (2015) have also reported that adding exogenous acetaldehyde in wine during malolactic fermentation could enhance wine color and increase the level of polymeric pigments.

In the present study, a total of 26 anthocyanins were detected, including 5 monomer anthocyanins, 5 acetyl anthocyanins, 5 coumaroyl anthocyanins, and 11 pyranoanthocyanins (**Table 2**). By comparing the compositions and concentrations of anthocyanins in different wine samples before and after malolactic fermentation, a significant decrease on the total anthocyanins concentration in these wines was found except for the wine with *O. oeni* CiNe. Additionally, the content of the total anthocyanins in the *L. plantarum* strains inoculated wines appeared to be lower than that in the wine with *O. oeni*. The monomer anthocyanins, acetyl anthocyanins, and coumaroyl anthocyanins decreased on their concentration in the wine after the fermentation. The total pyranoanthocyanins concentration in the wines before the fermentation was 13.39 mg/L. The malolactic fermentation with *L. plantarum* C8-1 and Lp39 resulted in a significant increase on the total pyranoanthocyanins concentration in the wine (24.03 mg/L and 51.40 mg/L, respectively), whereas a dramatic content reduction was observed in the *O. oeni* Oenos and CiNe treated wines (**Figure 3**). Vitisin B, an acetaldehyde adduct of malvidin-3-*O*-glucoside, was also detected in these wine samples. However, it appeared not to be a predominant pyranoanthocyanin in these wine samples except for that fermented with the *L. plantarum* Lp39 strain. Such variations on the concentration of vitisin B in these wine samples might be because these strains exhibit different activities on the cycloaddition and oxidation of malvidin-3-O-glucoside to yield vitisin B (Bakker & Timberlake, 1997; Fulcrand, Benabdeljalil, Rigaud, Cheynier, & Moutounet, 1998; Li & Duan, 2018; Lu & Foo, 2001; Morata et al., 2016).

Color attributes play an essential role in affecting the appearance of wine (Burns & Osborne, 2015; Romero & Bakker, 2000). The wine with *L. plantarum* Lp39 exhibited a similar L* value before and after the malolactic fermentation, whereas an increase on the value of L* was observed in the other strains inoculated wines after the fermentation (**Table 1**). These indicated that the wine with *L. plantarum* Lp39 exhibited stronger color intensity. Although the wines with *L. plantarum* C8-1 and Lp39 experienced much longer fermentation periods, they showed a decreased b* and H* values after the fermentation, whereas wines fermented with *O. oeni* exhibit oppositely. This indicated that yellowness of wines with *L. plantarum* decreased, which was a symbol of enhanced color quality in red wine. The malolactic fermentation also reduced the redness (a* value) chroma (C* value) in all the wines. Although a lower level of the total anthocyanin was found in the wines with the *L. plantarum* strains, the a* values of these wines fermented by the *O. oeni* and *L. plantarum* strains were very close. It has been known that the accumulation of pyranoanthocyanins could result in a deeper red color in wine than the anthocyanidin-3-*O*-glucoside accumulation (Pissarra, Mateus, Rivas-Gonzalo, Santos Buelga, & De Freitas, 2003). We speculated that the high level of pyranoanthocyanins, especially the most abundant acetaldehyde adduct of malvidin-3-*O*-acetylglucoside, helped maintain the redness in the wines with *L. plantarum*, although a level decrease on the total anthocyanin took place in the wines.

It has been known that in addition to the pyranoanthocyanins' resistance against SO_2 blenching and greater stability in a wide range of pH conditions (Li et al., 2015), adding exogenous acetaldehyde to stabilize anthocyanins in wines during malolactic fermentation could raise a legal question (Liu & Pilone, 2000). In this case, the results from this study indicated that the *L. plantarum* strains could yield acetaldehyde in wine during malolactic fermentation, which could help prevent the color loss of wine during malolactic fermentation without supplementing the exogeneous acetaldehyde.

As for the existing states of anthocyanins, malolactic fermentation with these strains did not significantly alter the percentage of the copigmented anthocyanins in the wine (**Table 2**). The percentages of the free anthocyanins in the wines fermented with *L. plantarum* were to a large extent reduced after the fermentation, whereas the wine with *O. oeni* Oenos and CiNe showed higher percentage of the free anthocyanins than the wine before the fermentation. Wines fermented with the *L. plantarum* C8-1 and Lp39 strains led to a significant increase on the polymerized anthocyanins percentage. It has been reported that the polymerized anthocyanins can be formed between anthocyanins and other big molecules in wine (Burns & Osborne, 2015; Romero & Bakker, 2000), and acetaldehyde can indirectly facilitate the formation of the pigmented complexes, such as ethyl linked anthocyanin-proanthocyanin and hydroxylethyl-anthocyanin-ethyl-proanthocyanidin condensation products (Li & Sun, 2017). In the present study, acetaldehyde was consumed during 10 to 14 days of the malolactic fermentation in the wine with the C8-1 strain. Meanwhile, the polymerized anthocyanins appeared to exhibit the highest percentage in that wine. These indicated that acetaldehyde played an important role in forming the polymerized colorants. These polymerized anthocyanins exhibit more stable structure, which could delay the discoloration of anthocyanin chromophore (Burtch et al., 2017). These indicated that *L. plantarum* strains could also stabilize the wine color by increasing the formation of the polymerized anthocyanins.

3.4 Principal Component Analysis

Principal component analysis was conducted to investigate the similarity of these wines with these strains using wine physicochemical indexes, anthocyanins, acetaldehydes, and color attributes as variables (**Figure 4**). The first and second principle component (PC1 and PC2) represented 71.0% and

19.1% of the total variance, respectively. The polymerized anthocyanins, acetaldehyde, and pyranoanthocyanins were positioned at the positive scale of the PC1 and the negative part of the PC2. Non-pyranoanthocyanins were pointed at the negative section of the PC1. The L* value and copigmented anthocyanins were located at the positive scale of the PC2, whereas volatile acid and color difference (ΔE) were positioned at the positive scale of both PC1 and PC2. The total acid, reducing sugar, C*, and a* were located at the negative part of PC1 and PC2. Regarding these wine samples, the wine before the malolactic fermentation was found at the negative position of PC1 and PC2. After the fermentation, these wines were segregated from each other in the plot, indicating that these wines exhibited different characteristics. For example, the wine with *L. plantarum* C8-1 and Lp39 were positioned at the positive scale of the PC1, but exhibited the opposite values on the PC2. Such segregation might be mainly attributed to higher acetaldehyde and pyranoanthocyanins level and lower ΔE* value in the *L. plantarum* Lp39 treated wine. The *O. oeni* Oenos and CiNe treated wines were positioned at the positive PC1 scale but negative section of the PC2, which resulted from their high level of non-pyranoanthocyanins and the absence of pyranoanthocyanins.

4. Conclusions

In conclusion, malolactic fermentation of Cabernet Gernischt wine by these strains were different. *L. plantarum* C8-1 and Lp39 treated wines showed lower reducing sugar, total acid, and b* value. *L. plantarum* inoculated wine after malolactic fermentation showed higher level of pyranoanthocyanins, whereas malolactic fermentation with *O. oeni* strains resulted in a significant concentration decrease on pyranoanthocyanins in wine. *L. plantarum* strains elevated the accumulation of acetaldehyde in wine model medium and wine during malolactic fermentation. Principal component analysis revealed

that these wines exhibited different characteristics after malolactic fermentation. *L. plantarum* appeared to facilitate the formation of acetaldehyde during malolactic fermentation, which could further favor the accumulation of pyranoanthocyanins in wine to improve the wine color.

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6. Declarations of Interest

The authors declare no conflict of interest.

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Figure legends

Figure 1. Malic acid consumption **(a)**, lactic acid production **(b)**, and acetaldehyde production **(c)** of different lactic acid bacteria strains in wine model medium.

Figure 2. Growing kinetics **(a)**, malic acid consumption **(b)**, lactic acid production **(c),** total acetaldehyde production **(d),** and free acetaldehyde production **(e)** of wine during malolactic fermentation by four strains of lactic acid bacteria.

Figure 3. HPLC-MS chromatographs (Extracted MRM) of acetaldehyde adduct of petunidin-3-*O*-glucoside **(a)**, acetaldehyde adduct of petunidin-3-*O*-acetylglucoside **(b)**, acetaldehyde adduct of malvidin-3-*O*-glucoside **(c)**, acetaldehyde adduct of malvidin-3-*O*-acetylglucoside **(d)**, and acetaldehyde adduct of malvidin-3-*O*-coumaroylglucoside **(e)** in wine after malolactic fermentation by *L. plantarum* C8-1 (—), Lp39 (—), and *O. oeni* Oenos (—), CiNe (—), appended with chemical structure and MS/MS spectrum of individual anthocyanin composition. "—" represents the origin wine before malolactic fermentation.

Figure 4. Principal component analysis of wine components after malolactic fermentation by four strains of lactic acid bacteria. Acet, acetaldehyde adduct; Dp, delphinidin-3-*O*-; Cy, cyanidin-3-*O*-; Pt, petunidin-3-*O*-; Pe, peonidin-3-*O*-; Mv, malvidin-3-*O*-; -ace, acetyl; -cou, -coumaroyl; -g, glucoside; Before MLF, wine before malolactic fermentation; C8-1, Lp39, Oenos, CiNe, wine malolactic fermented by *L. plantarum* C8-1, Lp39, and *O. oeni* Oenos, CiNe.

Figure 1

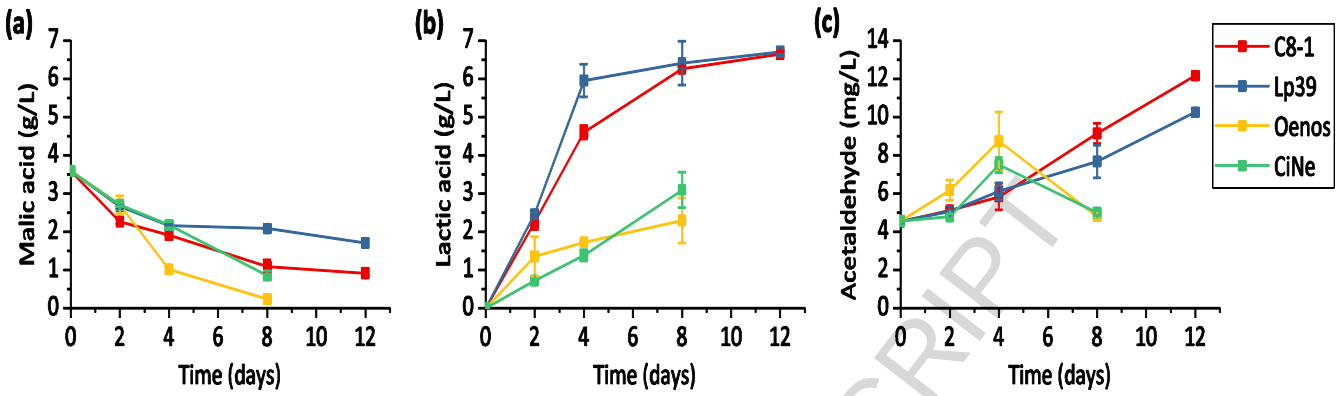


Figure 2

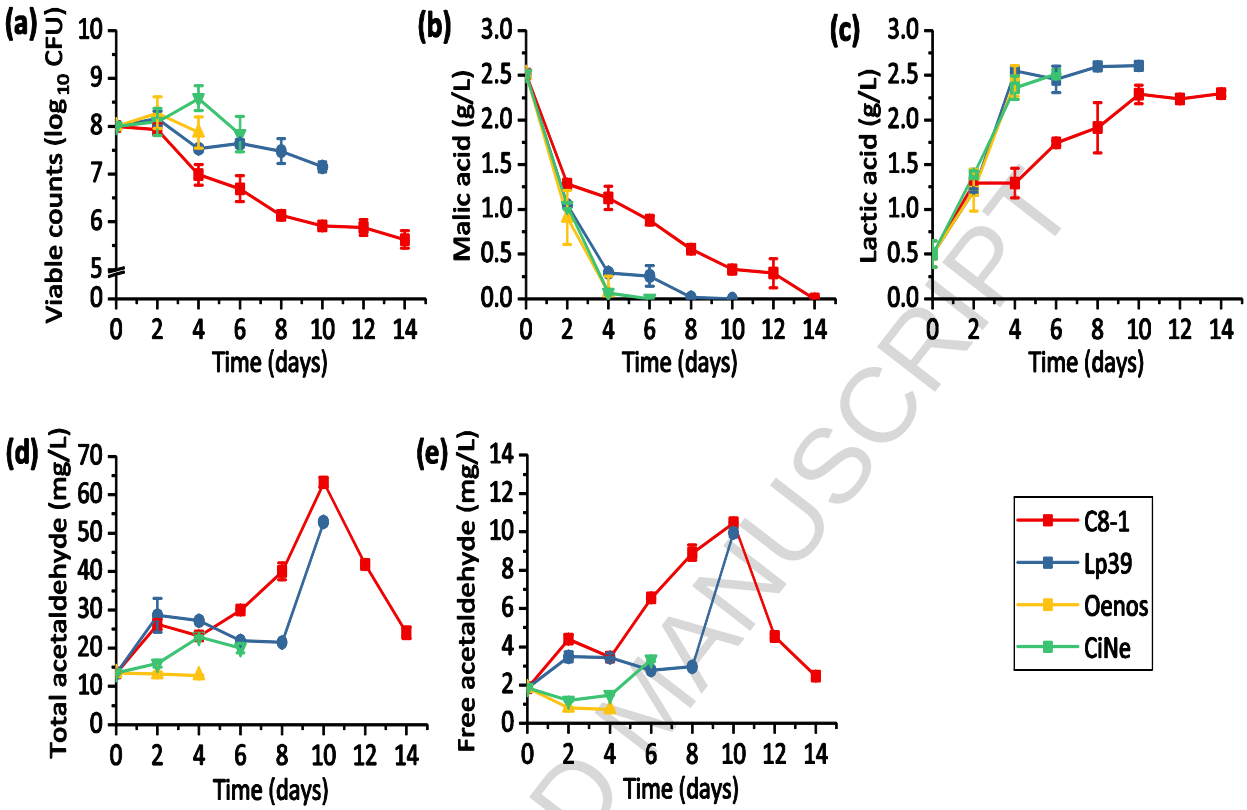


Figure 3

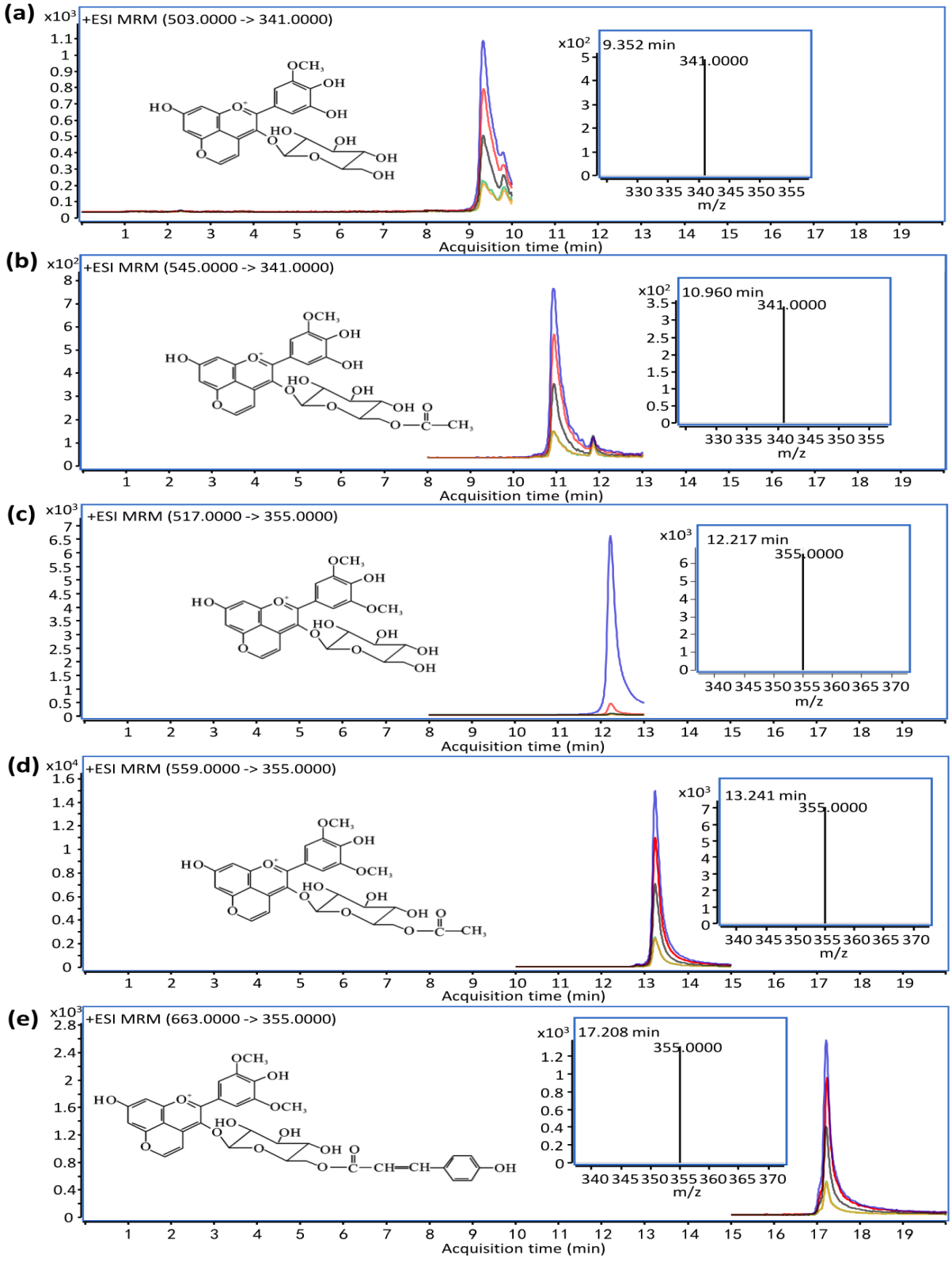


Figure 4

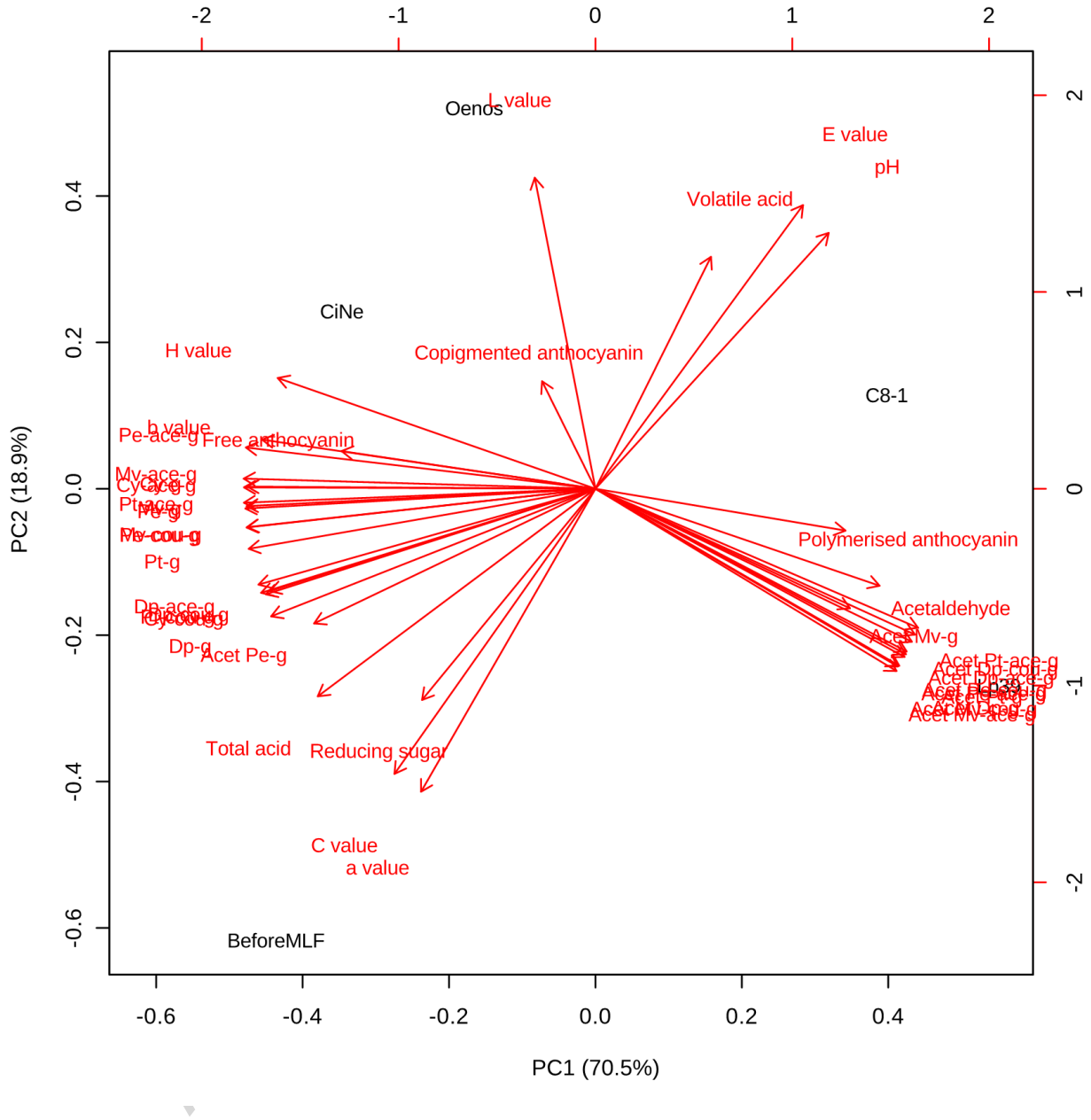


Table 1. Physiochemical indexes and color parameters of wine after malolactic fermentation by different strains of lactic acid bacteria.

	Before Malolactic Fermentation	*L. plantarum* C8-1	*L. plantarum* Lp39	*O. oeni* Oenos	*O. oeni* CiNe
pH	3.46±0.00 A	3.64±0.01 C	3.64±0.01 C	3.64±0.00 C	3.60±0.01 B
Total acid	4.28±0.04 E	2.59±0.01 A	2.97±0.06 B	3.15±0.02 C	3.37±0.02 D
Reducing sugar	5.21±0.59 C	2.84±0.64 A	3.93±0.51 AB	4.15±0.18 BC	3.27±0.26 AB
Volatile acid	0.14±0.01 A	0.21±0.00 B	0.31±0.01 D	0.36±0.03 E	0.24±0.00 C
Color parameters					
L*	66.96±0.15 A	69.64±0.22 C	66.26±0.11 A	70.86±0.32 D	68.74±0.44 B
a*	43.17±0.20 C	35.83±0.19 A	38.52±0.13 B	36.09±0.61 A	38.27±0.56 B
b*	6.75±0.09 D	3.42±0.06 B	2.57±0.08 A	5.78±0.31 C	8.33±0.27 E
C*	43.69±0.20 C	36.00±0.19 A	38.61±0.13 B	36.55±0.65 A	39.16±0.61 B
H*	8.89±0.11 C	5.45±0.09 B	3.82±0.11 A	9.10±0.33 C	12.29±0.22 D
ΔE*	—	8.49±0.46 C	6.29±0.30 B	8.14±0.81 C	5.46±0.64 B

Data are expressed as mean ± standard deviation of duplicate tests; different letters in the same row indicate significant difference at $p<0.05$.

Table 2. Anthocyanin composition and percentage of copigmented, free, polymerised anthocyanin in wine after malolactic fermentation by different strains of lactic acid bacteria.

Compounds (mg/L)	Before Malolactic Fermentation	*L. plantarum* C8-1	*L. plantarum* Lp39	*O. oeni* Oenos	*O. oeni* CiNe
Monomer anthocyanins					
Delphinidin-3-*O*-glucoside	10.08±0.00 D	6.27±0.07 AB	5.70±0.15 A	7.18±0.80 B	8.61±0.11 C
Cyanidin-3-*O*-glucoside	0.35±0.01 B	0.26±0.00 A	0.25±0.00 A	0.33±0.01 B	0.33±0.01 B
Petunidin-3-*O*-glucoside	43.33±0.53 C	29.23±0.20 A	27.61±0.70 A	36.10±4.03 B	40.06±0.11 BC
Peonidin-3-*O*-glucoside	6.22±0.09 C	4.37±0.05 A	4.22±0.09 A	5.50±0.45 B	6.10±0.06 C
Malvidin-3-*O*-glucoside	278.18±2.97 C	219.54±0.94 A	210.43±3.01 A	252.37±18.59 B	276.78±0.60 C
Total monomer anthocyanins	338.16±3.61 C	259.67±1.26 A	248.22±3.95 A	301.49±23.88 B	331.88±0.87 C
Acetyl anthocyanins					
Dp-3-*O*-acetylglucoside	4.07±0.04 D	2.83±0.04 A	2.63±0.07 A	3.27±0.26 B	3.64±0.03 C
Cy-3-*O*-acetylglucoside	0.71±0.01 C	0.53±0.00 A	0.51±0.01 A	0.66±0.04 B	0.69±0.00 BC
Pt-3-*O*-acetylglucoside	16.34±0.25 C	11.36±0.07 A	10.34±0.22 A	14.37±0.94 B	15.67±0.12 C
Pe-3-*O*-acetylglucoside	6.24±0.09 B	4.74±0.03 A	4.31±0.09 A	5.96±0.35 B	6.25±0.00 B
Mv-3-*O*-acetylglucoside	92.97±1.16 C	69.20±0.22 A	65.45±1.25 A	86.52±3.83 B	91.12±0.60 BC
Total acetyl anthocyanins	120.33±1.54 C	88.67±0.31 A	83.25±1.63 A	110.77±5.42 B	117.38±0.69 BC
Coumaroyl anthocyanins					
Dp-3-*O*-coumaroylglucoside (*cis+trans*)	1.59±0.04 D	0.97±0.06 B	0.81±0.03 A	1.20±0.05 C	1.24±0.05 C
Cy-3-*O*-coumaroylglucoside (*cis+trans*)	0.50±0.01 D	0.36±0.01 AB	0.33±0.03 A	0.41±0.03 BC	0.43±0.01 C

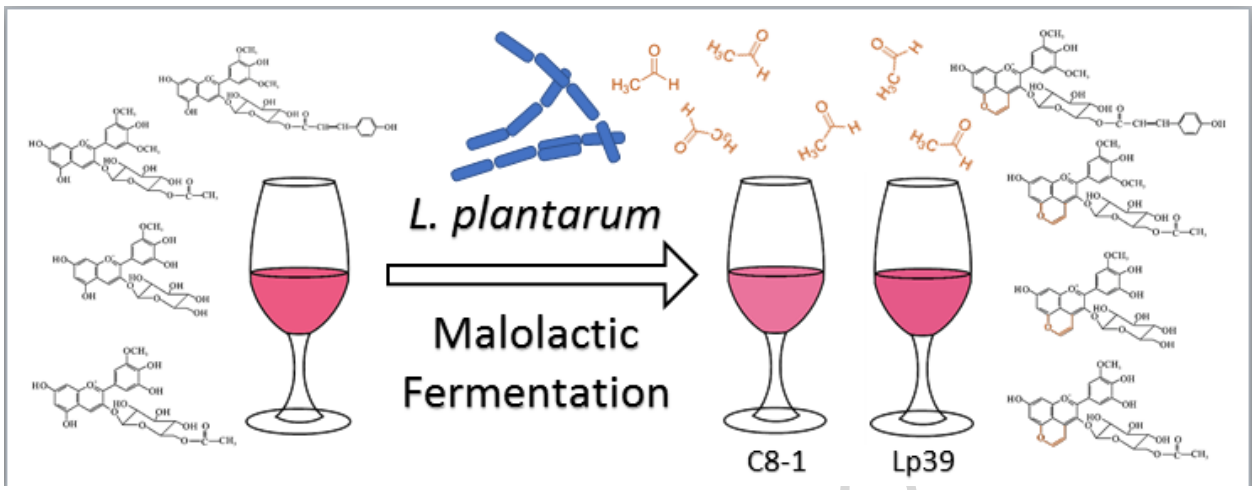
Pt-3-*O*-coumaroylglucoside (*cis+trans*)	5.33±0.07 E	3.16±0.02 B	2.83±0.19 A	3.88±0.11 C	4.55±0.09 D
Pe-3-*O*-coumaroylglucoside (*cis+trans*)	4.47±0.06 C	2.87±0.04 A	2.79±0.29 A	3.87±0.20 B	4.10±0.10 BC
Mv-3-*O*-coumaroylglucoside (*cis+trans*)	24.23±0.37 C	16.23±0.08 A	14.90±0.75 A	20.97±1.05 B	21.79±0.50 B
Total coumaroyl anthocyanins	36.11±0.40 C	23.60±0.04 A	21.66±1.23 A	30.32±1.44 B	32.11±0.63 B
Pyranoanthocyanins					
Acetaldehyde adduct of Mv-3-*O*-glucoside	0.44±0.02 A	1.44±0.04 B	20.14±0.12 C	0.33±0.00 A	0.39±0.00 A
Acetaldehyde adduct of Dp-3-*O*-glucoside	0.33±0.00 B	0.53±0.01 C	0.74±0.00 D	0.13±0.02 A	0.15±0.00 A
Acetaldehyde adduct of Pt-3-*O*-glucoside	1.14±0.05 B	2.33±0.02 C	3.44±0.02 D	0.16±0.06 A	0.17±0.01 A
Acetaldehyde adduct of Pe-3-*O*-glucoside	0.07±0.00 AB	0.06±0.00 A	0.06±0.00 A	0.06±0.00 AB	0.07±0.00 B
Acetaldehyde adduct of Dp-3-*O*-acetylglucoside	0.17±0.00 B	0.35±0.00 C	0.47±0.00 D	0.06±0.00 A	0.07±0.00 A
Acetaldehyde adduct of Pt-3-*O*-acetylglucoside	0.42±0.03 B	1.36±0.02 C	2.05±0.01 D	0.00±0.00 A	0.00±0.00 A
Acetaldehyde adduct of Pe-3-*O*-acetylglucoside	0.46±0.00 B	0.82±0.01 C	1.11±0.00 D	0.18±0.00 A	0.19±0.00 A
Acetaldehyde adduct of Mv-3-*O*-acetylglucoside	8.36±0.12 B	13.56±0.05 C	18.87±0.06 D	2.68±0.12 A	2.82±0.13 A
Acetaldehyde adduct of Dp-3-*O*-coumaroylglucoside	0.05±0.00 B	0.11±0.00 C	0.14±0.00 D	0.02±0.01 A	0.01±0.00 A
Acetaldehyde adduct of Pe-3-*O*-coumaroylglucoside	0.24±0.00 B	0.43±0.01 C	0.54±0.01 D	0.10±0.00 A	0.11±0.00 A
Acetaldehyde adduct of Mv-3-*O*-coumaroylglucoside	1.70±0.03 B	3.03±0.01 C	3.85±0.01 D	0.42±0.09 A	0.37±0.01 A
Total pyranoanthocyanins	13.39±0.17 B	24.03±0.02 C	51.40±0.18 D	4.14±0.28 A	4.34±0.13 A
Total anthocyanins	507.99±5.72 C	395.96±1.54	404.53±6.63	446.71±31.03	485.73±2.32

		A	A	B	C
Copigmented anthocyanins (%)	0.96±0.62 A	0.94±0.40 A	1.51±0.37 A	1.23±0.57 A	2.04±0.47 A
Free anthocyanins (%)	32.49±1.97 C	13.46±0.37 A	27.53±0.71 B	36.23±1.37 D	36.45±0.55 D
Polymerised anthocyanins (%)	66.55±1.38 B	85.60±0.32 D	70.97±0.34 C	62.54±0.86 A	61.51±0.51 A

Data are expressed as mean ± standard deviation of duplicate tests; different letters in the same row indicate significant difference at $p<0.05$.

Abbreviation: Dp, Delphinidin; Cy, Cyanidin; Pt, Petunidin; Pe, Peonidin; Mv, Malvidin.

Graphical abstract



Highlights:

- *L. plantarum* strains were capable to complete malolactic fermentation of wine
- Acetaldehyde was accumulated during malolactic fermentation by *L. plantarum*
- Pyranoanthocyanin level increased after malolactic fermentation by *L. plantarum*
- Polymerized anthocyanins increased after malolactic fermentation by *L. plantarum*
- Wine L* and a* were reduced after malolactic fermentation by *L. plantarum*