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Changes in the volatile profile of Pinot noir wines caused by Patagonian *Lactobacillus plantarum* and *Oenococcus oeni* strains

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ABSTRACT

The ability of Patagonian *L. plantarum* and *O. oeni* strains to change the volatile profile of a sterile Pinot noir wine was studied through fermentation assays, at laboratory scale. Two strains of each LAB species were selected based on data regarding to their ability to survive in wine and to consume L-malic acid. Both *O. oeni* strains but only one *L. plantarum* (UNQLp 11) strain were able to remain viable, consuming L-malic acid through the fermentation assay with a concomitant increase of L-lactic acid. The volatile profile of Pinot noir wine, before and after LAB inoculation, was measured by using HS-SPME gas chromatography technique. This analysis showed that alcohols were the main volatile compounds after alcoholic fermentation and that after fermentation with the selected LAB strains, a decrease in the volatile alcohols concentration and an increase in the volatile esters concentration could be observed. The *O. oeni* UNQOe 73.2 strain produced the most notable change in the volatile profile, with the production of some important odorant esters at higher concentration, compared to *O. oeni* UNQOe 31b strain. Although, *L. plantarum* UNQLp 11 strain showed a better performance in the consumption of L-malic acid, this strain had a low capacity to modify the volatile compounds profile after incubation in red wine. The results found in the present work showed that different strains selected as potential malolactic starters could have different behavior when are incubated in real wine. Although *L. plantarum* UNQLp 11 strain showed a good consumption of L-malic acid, the *O. oeni* UNQOe 73.2 strain exhibited superior capacity to improve the flavor of wine due to its esterase activity that produce an increase of fruity and creamy odorants.

1. Introduction

Oenococcus oeni is the major bacterial species found in wines during spontaneous malolactic fermentation (MLF) due to high tolerance to harsh wine conditions (low pH, high ethanol and SO_2 concentrations, low nutrients, and low temperatures) (Wibowo, Eschenbruch, Davis, Fleet, & Lee, 1985). However, *O. oeni* can also be detected with other LAB, mainly *Lactobacillus* spp., and in particular *Lactobacillus plantarum* species (Lonvaud-Funel, 1999, Lerm, Engelbrecht, & du Toit, 2011, Bravo-Ferrada et al., 2013, Valdés La Hens, Bravo-Ferrada, Delfederico, Caballero, & Semorile, 2015,). In the last four decades, the use of malolactic starter cultures has become widespread to control MLF process, and several starter of *O. oeni* and few of *L. plantarum* strains are commercially available (Lerm et al., 2011). MLF generally occurs after alcoholic fermentation (AF) produced by yeast, and it consists in the conversion of L-malic acid to L-lactic acid, resulting in a decrease of titrable acidity, and a small increase in the pH. MLF also leads to enhanced microbial stability and is usually believed to improve the complexity of wine aroma (Cappello, Zapparoli, Logrieco, & Bartowsky, 2017; Iorizzo et al., 2016; Liu, 2002). Several organic acids, in addition to L-malic acid, could be metabolized by LAB during MLF, such as, acetic, citric and tartaric acids. The balance of organic acids has a strong impact on wine taste, being the total consumption of L-malic acid the premise to reduce acidity and astringency of wines (Volschenk, van Vuuren, & Viljoen-Bloom, 2006). On the other hand, some strains of *O. oeni* and *L. plantarum* are able to produce other enzymatic reactions that modify the wine aroma profile (Cappello et al., 2017; Tristezza et al., 2016). Although the presence of a broad range of enzymes in wine LAB have been documented (glycosidases, esterases, phenolic acid decarboxylases, citrate lyases) (Liu, 2002; Ugliano, Genovese, & Moio,

Abbreviations: LAB, lactic acid bacteria; MLF, malolactic fermentation; AF, alcoholic fermentations; MAC, malic acid consumption; HS-SPME, headspace solid phase microextraction

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2003, Matthews et al., 2004, Grimaldi, Bartowsky, & Jiranek, 2005a, 2005b), information on the role of these bacterial enzyme activities including their potential use in winemaking is still limited (Cappello et al., 2017). Glycosidase activities that can affect wine aroma have been detected in wine strains of *Oenococcus*, *Lactobacillus* and *Pediococcus* (Grimaldi et al., 2005a, 2005b). Numerous potential glycosidases have been identified by genome sequence analysis of *O. oeni* strains (Borneman, Bartowsky, McCarthy, & Chambers, 2010), and a gene coding for a β-glucosidase enzyme has been detected in several LAB strains (Spano et al., 2005). The first study on the expression of an *O. oeni* β-glucosidase gene (*hgl*) under winemaking conditions was carried out by Olguín, Alegret, Bordons, and Reguant (2011), highlighting that the factors that influence the gene expression are ethanol concentration, wine matrix, and also the strain involved. On the other hand, changes in ester concentrations have a potential to influence wine quality (Sumby, Grbin, & Jiranek, 2010; Swiegers, Bartowsky, Henschke, & Pretorius, 2005), being responsible for the desirable fruity aroma of young wines. Recent studies demonstrated that wine LAB exhibit two different ester-synthesizing activities that may increase the ethyl ester content thus modulating the fruity-berry character of red wines (Costello, Siebert, Solomon, & Bartowsky, 2013).

In wine, the citrate utilization leads to the production of compounds such as diacetyl, acetoin, butanediol, and acetate which are important contributors to wine aroma and complexity (Bartowsky, E. J.& Henschke, P. A., 2004; Olguín, Bordons, & Reguant, 2009). Citrate lyase is a key enzyme of citrate metabolism since it cleaves citrate into acetate and oxaloacetate, and this latter is decarboxylate to pyruvate, leading onto the formation of diacetyl, which can be further metabolized through to 2,3-butanediol. The organoleptic impact of diacetyl in wine has been debated for many years. Wine tasters generally agree that diacetyl content must not exceed 5–6 mg/L (Davis, Wibowo, Eschenbruch, Lee, & Fleet, 1985), although it depends on the characteristics of each wine (Martineau & Henick-Kling, 1995). Below that level it is considered to contribute to the wine's bouquet, while higher concentrations have a negative impact. For these reasons, the gene encoding the citrate lyase enzyme (*citE*) has been investigated in various wine LAB species (Mills, Rawsthorne, Parker, Tamir, & Makarova, 2005).

Other important volatile compounds found in wines are phenols. The production of volatile phenols in wine are usually associated with *Dekkera bruxellensis* spoilage (Ribéreau-Gayon, Dubourdieu, Donèche, & Lonvaud-Funel, 2006). However, some *Lactobacillus* species have also been shown to be able to produce these compounds, but in lower quantities (Couto, Campos, Figueiredo, & Hogg, 2006). The conversion of phenolic compounds to vanillin (a very powerful aroma compound) by wine LAB has been demonstrated for *Lactobacillus* spp. by Bloem, Bertrand, Lonvaud-Funel, and de Revel (2007). One of the enzymes involved in the production of these compounds is the phenolic acid decarboxylase (PAD), and the presence of a gene encoding for PAD (*pad* gene) in *L. plantarum* and *O. oeni* was reported by several authors (Mtshali, Divol, van Rensburg, & du Toit, 2010, Mtshali, 2011, Lerm et al., 2011).

Argentinean North Patagonia is one of the southernmost wine-growing regions of the world that has optimal agro-ecological conditions for high quality viticulture, in which the Pinot noir varietal has found the optimal conditions to express its full oenological potential (Crisóstomo, 2007). Although the flavor of Pinot noir wine could vary among wine regions, and according to winemaking practices, in general it has a fruity and spicy bouquet (Feng, Skinkis, & Qian, 2017; Girard, Kopp, Reynolds, & Cliff, 1997; Guinard & Cliff, 1987). In Patagonian red wines, MLF occurs spontaneously and randomly, mainly by action of native *L. plantarum* and *O. oeni* strains (Valdés La Hens et al., 2015). In order to avoid delay and spoilage during this process, the use of malolactic starter cultures is an option. However, the commercial cultures are formulated with strains from other wine-growing regions and their use could negatively affect the properties of wine *terroir* (Bokulich, Thorngate, Richardson, & Mills, 2014; González-Arenzana, López, Santamaría, Tenorio, & López-Alfaro, 2012). The selection of autochthonous strains, best adapted to regional winemaking conditions, is desirable. In previous works, we have isolated and characterized, several strains of *L. plantarum* and *O. oeni* from Patagonian Pinot noir wines. Studies of tolerance to wine stress factors in wine-like medium or in sterile wine, as well as the existence of some enzymatic activities and the screening of aroma related genes, allowed us to select the best candidate strains to formulate regional starter cultures for MLF (Bravo-Ferrada et al., 2013; Bravo-Ferrada et al., 2016; Brizuela et al., 2017; Valdés La Hens et al., 2015). The presence of a β-glucosidase gene, and β-glucosidase activity was verified in selected *L. plantarum* and *O. oeni* strains (Bravo-Ferrada et al., 2013; Brizuela et al., 2017). A putative *pad* gene was also analyzed in some Patagonian *O. oeni* and *L. plantarum* strains (Brizuela et al., 2017), although this gene was only found in *L. plantarum* strains. The existence of a citrate lyase complex gene was also screened and reported (Brizuela et al., 2017). This gene was found in all Patagonian *O. oeni* strains analyzed, but only in some *L. plantarum* strains. However, the potential of these Patagonian LAB strains to produce changes in the profile of compounds related to aroma and flavor of regional red wines has not been studied yet.

With this background, the aim of this work was to investigate the ability of previously selected strains of *L. plantarum* and *O. oeni* to modify the volatile compounds profile after inoculation in a sterile Pinot noir (Bravo-Ferrada et al., 2013; Bravo-Ferrada et al., 2016; Bravo-Ferrada, Tymczyszyn, Gómez-Zavaglia, & Semorile, 2014, Brizuela et al., 2017). For this purpose, changes in wine volatile compounds (alcohols, esters, terpenoids, etc.), and in the concentration of some organic acids (mainly L-malic acid) were analyzed before and after inoculation of the sterile wine with these strains.

2. Materials and methods

2.1. Cell acclimation

Bacterial cells in early stationary phase (approximately 10^9 CFU/mL) were collected by centrifugation at 5000 rpm for 10 min and suspended in the same volume of a modified acclimation medium (50 g/L MRS, 40 g/L D(−) fructose, 20 g/L D (−) glucose, 4 g/L L-malate, 1 g/L Tween 80, and 0.1 mg/L pyridoxine, adjusted to pH 4.6) supplemented with 6% v/v ethanol (Bravo-Ferrada et al., 2014).Cultures were incubated during 48 h at 21 °C according to Brizuela et al., 2017.

2.2. Fermentation assays

Two strains of each LAB species (*Lactobacillus plantarum* UNQLp 11, *Lactobacillus plantarum* UNQLp 155, *Oenococcus oeni* UNQOe 31b, *Oenococcus oeni* UNQOe 73.2) were selected for carrying out fermentation assays, at laboratory scale, in sterile Pinot noir wine (at final AF stage). These strains were isolated from Patagonian Pinot noir wines (vintages 2008 and 2014) and selected according to their oenological properties (Bravo-Ferrada et al., 2016, 2013; Brizuela et al., 2017). A volume of 400 mL of wine (14.5% v/v ethanol, pH 3.82, < 2.00 g/L residual sugars, 2 g/L-malic acid, 96 mg/L total SO_2, total acidity of 3.98 g/L) was sterilized by filtration through 0.2 μm pore size (Sartorius Stedim Biotech GmbH, Göttingen, Germany). Acclimated cells were harvested by centrifugation and inoculated (~5×10^7 CFU/mL) in 80 mL of wine. Incubations were performed at 21 °C during 20 days, without shaking.

2.3. Cell viability and L-malic acid consumption

Viable cells were determined by plating on MRS or MLO agar, as appropriate, sampled at days 0, 5, 10, 15 and 20. Remaining L-malic acid was measured with an L-malic acid enzymatic kit (L-Malic Acid Enology enzymatic kit, BioSystems SA, Barcelona, Spain).

An exponential one-phase decay equation model was used for fitting the performed MAC kinetic by the different strains assayed. The equation for this model was obtained by the GraphPad Prism® software and it is:

$$[MA_t] = ([MA_0] - [MA_i])\mathbf{e}^{-\mathbf{Kt}} + [MA_i] \quad (1)$$

where $[MA_t]$ is the L-malic acid concentration at time = t, $[MA_0]$ is the initial concentration of L-malic acid (which was 2 g/L in the wine used), $[MA_i]$ is the L-malic acid concentration at infinite time and K is the rate constant.

2.4. Organic acid concentrations

Tartaric, citric and L-lactic acid concentrations were measured at day 20 with the Enology BioSystems kits (Tartaric acid, Citric Acid, and Lactic acid, BioSystems SA, Barcelona, Spain).

2.5. Headspace solid phase microextraction (HS-SPME)

HS-SPME was employed for volatile compounds sampling following the protocols previously described (Rodríguez-Bencomo et al., 2011), with some modifications. Briefly, 8 mL of wine or hydroalcoholic solution containing the aroma compounds were placed in a 20 mL headspace vial with 40 µl of the three internal standards (3-octanol, methylnonanoate, and 3,4-dimethylphenol), and sealed with a TFE/silicone septum (Supelco, Bellefonte, PA). Samples were left in a water bath at 40 °C for 5 min before the extraction. The extraction was performed with the exposure of a StableFlex 85 µm carboxen–polydimethylsiloxane, CAR–DVB-PDMS fiber (Supelco) to the headspace of the sample for 10 min at 40 °C and under constant stirring (500 rpm). After the extraction, the fiber was removed from the sample vial and desorbed in the GC injector port in splitless mode for 80 min. Six levels of concentration of each aroma compound (2,10, 100, 500, 1000, 5000 µg L^{-1}), covering the concentration ranges expected in wines, were tested in duplicate. Prior to use, the fiber was conditioned following the supplier's recommendation.

2.6. Gas chromatography–mass spectrometry analysis

An Agilent 7890A GC system (Agilent, Palo Alto, CA), with asplit/splitless injector and interfaced with an Agilent 5975 N mass spectrometer, was used for samples analysis. The injector was set at 250 °C. The AgilentMSD ChemStation Software (D.01.02 16 version) was used to control the system. Volatile compounds were separated on a DB-Wax polar capillary column (60 m × 0.25 mm i.d. × 0.50 µm film thickness) from Agilent (J&W Scientific, Folsom, USA). Helium was the carrier gas at a flow rate of 1 mL min^{-1}. The oven temperature was programmed as follows: an initial temperature of 40 °C, which was maintained during 5 min, and then increased to 240 °C (4 °C/min), which was kept for 15 min. For the MS system, the temperatures of the transfer line, quadrupole and ionization source were 270, 150 and 230 °C respectively; electron impact mass spectra were recorded at 70 Ev ionization voltages and the ionization current was 10 A. The acquisitions were performed in scan (from 35 to 450 amu) and SIM modes. Peak identification was carried out by analogy of mass spectra with those of the mass library (Wiley 6.0 and NIST 2.0), and with those from reference compounds analyzed in the same conditions that wine samples. Quantitative data were obtained by calculating the relative peak area (or TIC signal) in relation to that of the three internal standards used (3-octanol, methylnonanoate and 3,4-dimethylphenol), depending on the volatile compound. Calibration curves of each compound were performed using a hydroalcoholic solution (pH 3.6, 14% v/v ethanol) spiked with the commercial pure reference compounds at six levels of concentration (2, 10, 100, 500, 1000, 5000 µg L^{-1}) covering the concentration ranges expected in wine and tested in duplicate.

The aroma standard solutions for the calibration curve were prepared in HPLC grade absolute ethanol supplied by Merck. The 51 compounds used were: butyl acetate(123-86-4), ethyl hexanoate (123-66-0), ethyl decanoate (110-38-3) and vanillin (121-33-5) from Merck (Darmstadt, Germany); isobutyl acetate (110-19-0), ethylbutanoate (105-54-4), isopentyl acetate (123-92-2), hexyl acetate(142-92-7), 1-hexanol (111-27-3), cis-3-hexen-1-ol (928-96-1), ethyl octanoate (106-32-1), furfural (98-01-1), linalool (78-70-6), γ-butyrolactone (96-48-0), diethyl succinate (123-25-1), α-terpineol (98-55-5), β-damascenone (23726-91-2), 2-phenylethyl acetate (103-45-7), geraniol (106-24-1), guaiacol (90-05-01), whiskey lactone (39212-23-2), α-ionone (79-77-6) and eugenol (97-53-0) from Sigma-Aldrich; hexanoic acid (142-62-1), and decanoic acid (334-48-5) from Scharlau (Barcelona, Spain) and 4-ethyl guaiacol (2785-89-9) from Lancaster (Eastgate, White Lund, Morecambe, England); α-pinene, β-pinene, limonene, terpinen-4-ol, β-citronellol, nerol, 5-methylfurfural, furfuryl alcohol, benzyl alcohol, β-phenylethyl alcohol, decanoic acid,2,3-butanodione, ethyl propanoate, 1-butanol, ethyl 2-methylbutirate, trans-3-hexen-1-ol, β-ionone, γ-nonalactone, ethyl cinnamate, 4-ethylphenol, 2-methoxy-4-vinylphenol, 2,6-dimethylphenol, methyl vanillate, ethyl vanillate, acetovanillone, ethyl dodecanoate from Sigma-Aldrich. These compounds were selected for their important role in wine aroma, being representative of the wine volatile profile. The aroma standards were purer than 98%. All the solutions were stored at 4 °C.

2.7. Reproducibility of the results

Fermentation assays were carried out using three independent bacterial cultures and all the experiments were carried out on duplicate. The statistical analyses were carried out using GraphPad Prism 5 software (GraphPad Software Inc., San Diego, CA, 2007). Means were compared by one-way ANOVA, and if $p < 0.05$ the difference was considered statistically significant.

3. Results and discussion

Two *L. plantarum* (UNQLp 11, UNQLp 155) and two *O. oeni* (UNQOe 31b, UNQOe 73.2) Patagonian strains were previously characterized as potential candidates to formulate malolactic starter cultures due to their oenological properties (Bravo-Ferrada et al., 2013; Bravo-Ferrada et al., 2016; Brizuela et al., 2017).

Fermentation assays were performed with cultures of these strains previously acclimated, according to Materials and Methods section. Fig. 1 shows the evolution of the viable cell number and the L-malic acid consumption (MAC) kinetics. The number of viable cells of both *O. oeni* strains and the *L. plantarum* UNQLp 11 strain remained relatively invariable during the 20 days of incubation, and these three strains were able to consume L-malic acid, being its final concentration lower than 0.46 mg/L (Fig. 1). In contrast, a drastic loss of cell viability and a low consume of L-malic acid was observed in the UNQLp 155 strain. This strain was selected because it showed potential as a good candidate to formulate a malolactic starter culture, although the previous studies were carried out only in a synthetic wine (ethanol 14% v/v, pH 3.5) (Bravo-Ferrada et al., 2013, 2014; Bravo-Ferrada, Gonçalves et al., 2015; Bravo-Ferrada, Brizuela et al., 2015). However, the results now obtained demonstrated that the performance of this strain in sterile red wine was significantly lower than the other strains assayed. This could be due to the presence of several stress factors such as ethanol, low pH, SO_2, among others, which have a synergistic effect causing bacterial damage, leading to viability loss.

Table 1 shows the concentration of L-malic, L-lactic, tartaric and citric acids after 20 days of incubation of inoculated sterile wine, compared to sterile wine not inoculated with LAB strains. The *L. plantarum* UNQLp 155 strain was not included in this analysis due to the loss of viability showed in Fig. 1. A notable increase in L-lactic acid concentration was observed, which would correspond to decarboxylation of L-malic acid during fermentation assays. In addition, no significant

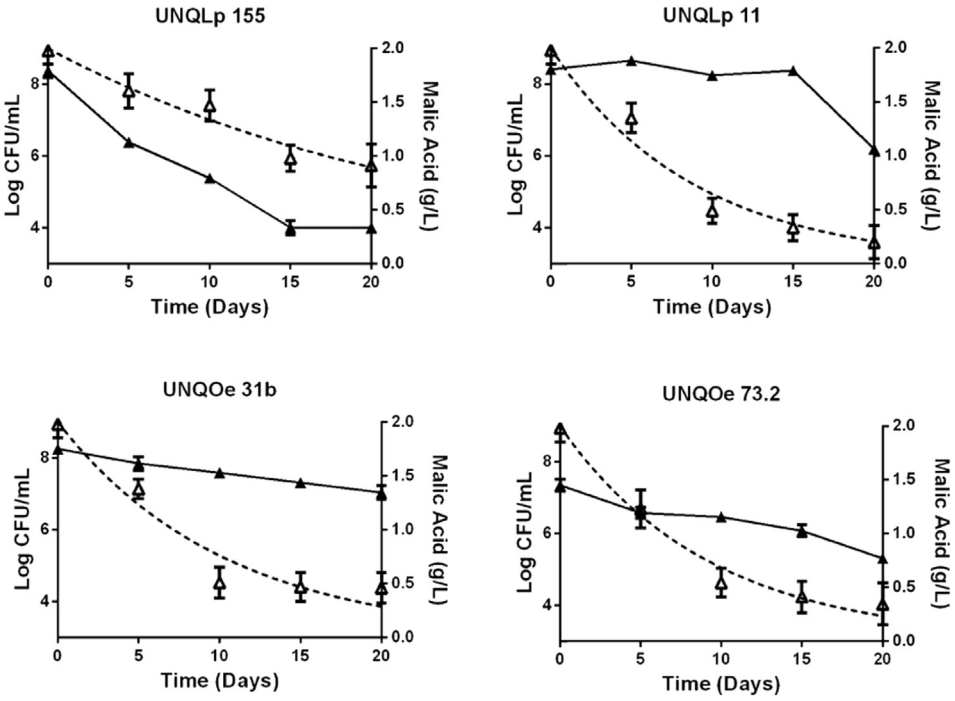

Fig. 1. Viable cell counts and L-malic acid consumption after inoculation of *L. plantarum* UNQLp 155, *L. plantarum* UNQLp 11, *O. oeni* UNQOe 31b or *O. oeni* UNQOe 73.2 strains in sterile Pinot noir wine incubated at 21 °C during 20 days. Bacterial cultures were previously acclimated in the presence of ethanol 6% v/v at 21 °C. (▲) denotes log CFU/mL and (Δ) represents L-malic acid concentration. Dashed line represents the fit according exponential one-phase decay.

differences in the concentration of citric and tartaric acids were observed (Table 1). The decrease of L-malic acid concentration, with an increase in the L-lactic acid concentration, has a positive impact in wine flavor, due to the reduction of titratable acidity, making a smoother mouthfeel (Volschenk et al., 2006).

The HS-SPME gas chromatography analysis allowed us to monitor, in fermented wine samples, the presence of 22 volatile compounds (Table 2). Several differences in the volatile compounds profile, before and after fermentation with LAB strains, were observed. Alcohols and esters were the main volatile compounds which evidenced changes due to wine fermentation with LAB strains. In Table 2, it can be seen that alcohols were the main aroma compounds of Pinot noir wine samples (final of AF). As was reported by other authors (Grimaldi et al., 2005a, 2005b; Matthews et al., 2004; Pozo-Bayón et al., 2005), wine inoculation with LAB strains produce changes in its volatile profile. After fermentation with UNQLp 11, UNQOe 31b, and UNQOe 73.2 strains, it was observed a decrease in alcohols concentration together with an increase in esters concentration, whose contribution to the fruity aroma in Pinot noir wine is known (Costello, Francis, & Bartowsky, 2012; Feng et al., 2017; Girard et al., 1997; Guinard & Cliff, 1987). In particular, LAB inoculation significantly decreases the concentration of β-phenyl ethyl alcohol to levels below the sensory threshold (14 μg/L) (Ferreira, López, & Cacho, 2000), likely reducing undesirable herbal flavor associated to this compound. On the other hand, it was also seen a desirable reduction in furfural content (Table 2), which might contribute to reduce the "caramel-like" flavor notes (Hale, Mccafferty, Larmie, Newton, & Swan, 1999).

The UNQOe 73.2 strain was able to produce the greatest change on wine aroma compounds (Table 2). This strain caused an increase in the concentration of some esters with high impact in wine aroma, for instance isobutyl acetate, isoamyl acetate, hexyl acetate, ethyl octanoate, and ethyl decanoate, which have been related with some fruity notes, such as banana, strawberry and pear (Peinado, Moreno, Medina, & Mauricio, 2004; Sumby et al., 2010; Tomasino et al., 2015). A similar volatile pattern was observed in wine inoculated with *O. oeni* UNQOe 31b strain, which produced an increase in total esters concentration (with the exception of isoamyl acetate), but a decrease in the content of the remaining aroma compounds (Table 2). Evidence of esterase activity in *O. oeni* strains was demonstrated through several reports that showed changes of wine volatile compounds after MLF conducted by these strains (Costello et al., 2012). Significant differences in some ethyl esters contents have been found between wines fermented by *O. oeni* or by *L. plantarum* strains (Lee, Hong, & Lee, 2009; Pozo-Bayón et al., 2005).

On the other hand, both Patagonian *O. oeni* strains were able to increase the ethyl succinate concentration in wines. The presence of this compound is frequent after the barrel storage of wine and contributes to its volume and roundness, giving it creamy and coffee notes (Tristezza et al., 2016).

In wines inoculated with the UNQLp 11 strain, a decrease in the content of volatile compounds was observed, but level of total esters showed an increase with respect to initial wine sample (Table 2), demonstrating that this *L. plantarum* strain possesses a certain level of esterase activity. Previous reports have shown the presence of genes coding esterases in wine *L. plantarum* strains (Liu, 2002; Matthews et al., 2004). Studies performed by Mtshali et al. (2010) on *Lactobacillus*

Table 1
Concentrations of L-malic acid, L-lactic acid, tartaric acid, and citric acid measured after 20 days of wine incubation.

LAB strains	Malic acid (g/L)	Tartaric acid (g/L)	Citric acid (mg/L)	Lactic acid (g/L)
Control	1.98 ± 0.12	2.56 ± 0.31	0.11 ± 0.03	1.03 ± 0.07
UNQLp11	0.20 ± 0.15 (£)	1.55 ± 0.16	0.15 ± 0.04	2.53 ± 0.09*
UNQOe31b	0.46 ± 0.14 (£)	2.06 ± 0.26	0.07 ± 0.09	2.53 ± 0.05*
UNQOe73.2	0.34 ± 0.19 (£)	2.04 ± 0.36	0.15 ± 0.07	2.86 ± 0.10*

(£) Significantly lower respect to control before fermentation with LAB strains, (*) Significantly higher respect to control before fermentation with LAB strains. No significant difference in the tartaric and citric acid concentration was observed.

Table 2
Volatile compounds content (mg/L) in wine before and after (20 days) inoculation of LAB strains.

Aromatic compounds (mg/L)	Initial wine	*L. plantarum*	*O. oeni*	
		UNQLp11	UNQOe 31b	UNQOe 73.2
Alcohols				
3-methyl-1-butanol	0.89 ± 0.20	1.23 ± 0.28	1.09 ± 0.21	1.39 ± 0.12
1-butanol	1.06 ± 0.12	1.90 ± 0.63	2.12 ± 0.09	1.01 ± 0.07
1-hexanol	1.36 ± 0.05	1.47 ± 0.07	1.27 ± 0.01	1.96 ± 0.01 (*)
Benzyl alcohol	0.84 ± 0.03	0.96 ± 0.06	0.64 ± 0.01 (£)	1.16 ± 0.02 (*)
β-phenylethyl alcohol	18.86 ± 0.80	5.68 ± 1.07 (£)	11.52 ± 0.61 (£)	5.43 ± 0.66 (£)
% total alcohols	93.2 ± 1.0	89.8 ± 1.0 (£)	90.9 ± 0.9 (£)	77.4 ± 1.2 (£)
Esters				
Ethylpropanoate	0.10 ± 0.03	0.08 ± 0.03	0.05 ± 0.07	n.d.
Isobutylacetate	n.d.	n.d.	n.d.	0.04 ± 0.01 (*)
Ethylbutirate	0.13 ± 0.02	0.14 ± 0.01	0.14 ± 0.02	0.14 ± 0.02
Isoamyl acetate	0.47 ± 0.02	0.44 ± 0.03	0.31 ± 0.01 (£)	0.64 ± 0.01 (*)
Ethyl hexanoate	0.32 ± 0.01	0.12 ± 0.01	0.07 ± 0.01	0.21 ± 0.01
Hexyl acetate	0.004 ± 0.002	0.001 ± 0.001	0.018 ± 0.020 (*)	0.018 ± 0.023 (*)
Ethyl octanoate	0.067 ± 0.003	0.034 ± 0.006 (£)	0.045 ± 0.0.002 (£)	0.100 ± 0.009 (*)
Ethyl decanoate	0.013 ± 0.001	0.015 ± 0.001	0.018 ± 0.001 (*)	0.026 ± 0.001 (*)
Diethyl succinate	0.43 ± 0.03	0.35 ± 0.04	0.85 ± 0.01 (*)	1.94 ± 0.01 (*)
β-phenyl ethyl acetate	0.046 ± 0.006	0.038 ± 0.003	0.144 ± 0.016	0.046 ± 0.001
Ethyldodecanoate	0.0025 ± 0.0028	0.0024 ± 0.0001	0.0021 ± 0.0003	0.0036 ± 0.0006 (*)
% total esters	6.4 ± 0.5	9.7 ± 0.6 (*)	9.0 ± 0.7 (*)	20.7 ± 1.0 (*)
Terpens				
β-citronelol	0.026 ± 0.002	n.d.	n.d.	n.d.
Volatile phenols				
4-ethyl guaiacol	0.003 ± 0.001	0.001 ± 0.001	n.d.	n.d.
4-ethyl phenol	n.d.	0.008 ± 0.001 (£)	n.d.	n.d.
Other compounds				
2,3-butanodione	n.d.	n.d.	n.d.	1.15 ± 0.06 (*)
γ-butyrolactone	10.32 ± 0.28	7.58 ± 0.52	6.12 ± 0.01	12.64 ± 0.02
Furfural	0.061 ± 0.001	0.049 ± 0.003 (£)	0.018 ± 0.001 (£)	0.022 ± 0.001 (£)

(*): Significantly higher respect to control without inoculation with LAB ($P < 0.05$), (£): Significantly lower respect to control without inoculation with LAB ($P < 0.05$). n.d.: not detected.

strains from South African grape and wine samples, demonstrated that a 60% of tested strains possessed genes coding for esterases, although enzyme activity was not measured, suggesting that these strains may be involved in ester synthesis and / or hydrolysis, and contributing to wine aroma and flavor (Liu, 2002; Matthews et al., 2004).

Only in wine sample inoculated with *L. plantarum* UNQLp 11 strain was possible to detect production of volatile phenols, particularly a low concentration of ethyl guaiacol, and an increase in the ethyl phenol level, respect to control wine sample. However, levels found for both volatile compounds were below their sensory threshold (Feng et al., 2017). The presence of both compounds has been related to the action of *Brettanomyces* yeast during AF, but also in some winemaking practices involving wine aging in oak barrel, and their presence is considered detrimental for wine aroma (Rayne & Eggers, 2007). Nevertheless, it has been shown that some *Lactobacillus* species are able to produce these compounds, but in smaller amounts (Couto et al., 2006).

The presence of a putative *pad* gene was detected in UNQLp 11 strain, but was not found in UNQOe 31b strain (Brizuela et al., 2017). Although the presence of this gene was not yet investigated in UNQOe 73.2 strain, it did not showed production of volatile phenols under the conditions assayed. Mtshali (2011) showed the presence of a putative *pad* gene in two *O. oeni* strains (out of 27 strains tested) from South African wines, and a comparative sequence analysis revealed that such genes shared a 74–80% identity to other closely related species of genera *Lactobacillus* and *Pediococcus*. In another study, the presence of *pad* gene was screened in 120 South African *Lactobacillus* strains (including *L. plantarum*), finding that > 70% possessed this gene (Mtshali et al., 2010). The presence or absence of *pad* gene in *L. plantarum* and *O. oeni* strains from South African wines was also reported by Lerm et al., 2011.

Although UNQLp 11 strain showed the presence of a putative *pad* gene, and was able to produce volatile phenols under the fermentation conditions used, no vanillin production was detected, and it may be due to the limit detection of the technique employed. In addition, this compound could be hardly extracted using a headspace sampling technique (Andújar-Ortiz, Moreno-Arribas, Martín-Álvarez, & Pozo-Bayón, 2009).

Of the assayed strains, only UNQOe 73.2 was able to produce diacetyl (2,3 butanodione), in a concentration capable to give wine a pleasant buttery smell (Bartowsky, E. J.& Henschke, P. A., 2004). It has been reported that some *L. plantarum* strains did not possess a citrate lyase complex genes (Mtshali, Divol, & du Toit, 2012; Mtshali et al., 2010), and it is feasible that wines undergo MLF guided by these strains will have very low diacetyl concentrations. Brizuela et al. (2017) demonstrated the presence of a citrate lyase complex in a high percentage of Patagonian *L. plantarum* (3 of 4) and *O. oeni* (4 of 4) strains. Olguín et al. (2009) investigated the expression of this gene in the *O. oeni* PSU-1 strain, to further understand the role of this metabolic route in the adaptation to wine environment and its impact on wine organoleptic qualities, revealing that LAB strains may have different metabolic traits. With respect to Patagonian LAB strains here analyzed, UNQOe 73.2 seems to be the most interesting in relation to the ability to produce diacetyl.

Although the three LAB strains were positive for the presence of a *bgl* (β-glucosidase) gene (Brizuela et al., 2017) or showed β-glucosidase activity (Bravo-Ferrada et al., 2016), no terpenes were observed in any of the fermentation assays performed. This could be due to deficiency in the extraction method used, or that gene expression was affected by abiotic stress factors during the fermentation assays performed (Grimaldi et al., 2005a, 2005b).

4. Conclusion

The results obtained in the present work showed that inoculation of

a sterile Pinot noir wine with Patagonian *L. plantarum* and *O. oeni* strains may have different effects on its wine volatile composition and its organic acid concentration. The four strains assayed were previously selected from > 150 isolations obtained from Pinot noir wines based on their oenological properties (Bravo-Ferrada et al., 2016, 2013, Brizuela et al., 2017, Valdés La Hens et al., 2015). *L. plantarum* UNQLp 155 strain showed a good performance in synthetic wine after acclimation, but it did not survive in sterile wine under the conditions assayed. In contrast, *O. oeni* UNQOe 73.2 strain, previously studied in synthetic wine, showed a good performance to consume L-malic acid and excellent ability to produce ester compounds. Although the strains UNQLp 11 and UNQOe 31b were previously studied in sterile wine and showed a good capacity to consume the L-malic acid, they showed a lower potential to reduce alcohols concentrations and to increase esters concentration than UNQOe 73.2. In addition, UNQOe 73.2 could produce an adequate level of diacetyl and a higher concentration of fruity esters, that are characteristics of Pinot noir wines. More studies at pilot level, as well as biomass production and cultures preservation, could be carried out with these strains for their future use as malolactic starter cultures at regional level.

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