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DOI:10.5344/ajev.2019.19061

Impact of *Saccharomyces cerevisiae* strain selection on malolactic fermentation by *Lactobacillus plantarum* and *Oenococcus oeni*

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Abstract: Nowadays, the simultaneous inoculation of yeast and lactic acid bacteria (LAB) is considered a state-of-the-art strategy to reduce overall vinification time and improve microbiological stability of wines. This inoculation protocol drew interest as to how the selection of yeast and LAB strains could modulate malic acid consumption rate and wine composition. The study presented here addresses the impact of combining *Saccharomyces cerevisiae* strains (with different fermentation rates and nutrition demands) with *Lactobacillus plantarum* and *Oenococcus oeni* strains on malic acid consumption and the production of metabolites. *S. cerevisiae* strains in pure culture fermentations without LAB inoculation exhibited different patterns of malic acid consumption rate and metabolites production. Simultaneous *S. cerevisiae* and LAB inoculation influenced the kinetics of lactic acid production and titratable acidity content in a manner dependent on the selected LAB strain. The wines undergoing MLF with *L. plantarum* ML Prime™ finished faster and contained higher levels of L-lactic acid, compared to the respective wines inoculated with *O. oeni* Lalvin® VP41™, however the degree of acidification depended on the *S. cerevisiae* strain used to conduct the alcoholic fermentation. This study reveals new knowledge about the use of *L. plantarum* in winemaking and shows the effect of *S. cerevisiae* strains with different enological characteristics, accompanied by LAB or without LAB co-inoculation, on wine composition.

Key words: *Saccharomyces cerevisiae*, *Lactobacillus plantarum*, *Oenococcus oeni*, malolactic fermentation, interactions, color

Introduction

Malolactic fermentation (MLF) is a secondary fermentation carried out in most red grape wines (Sumby et al. 2010, Knoll et al. 2011), MLF is considered an important process in the winemaking industry for three reasons: (a) deacidification, (b) aroma and flavor modification and (c) microbial stability (Bauer and Dicks 2004, Swiegers et al. 2005, Cappello et al. 2017). It is conducted by lactic acid bacteria (LAB) and may occur spontaneously or be induced by inoculation of autochthonous or commercial strains (Sumby et al. 2014, Bartowsky et al. 2015, Lucio et al. 2017). Spontaneous MLF is the result of indigenous LAB strains, and its success depends greatly on grape sanitary conditions and physicochemical characteristics of musts and wines (Ruiz et al. 2010). Spontaneous MLF represent an unpredictable situation, the main risks are slow progression of MLF and potentially incomplete consumption of malic acid and production of high amounts of undesirable compounds (Bauer and Dicks 2004, Bartowsky et al. 2015). The use of LAB starter cultures together with nitrogen management can ensure more rapid onset and completion of MLF, reduce the potential spoilage by microorganisms and lead to overall more predictable MLF (Liu et al. 2017, Sumby et al. 2019). Since the introduction of these starter cultures, there has been considerable research to determine the optimal time point for inoculation in order to enhance MLF efficiency (Rosi et al. 2003, Abrahamse and Bartowsky 2012). LAB starter cultures could be co-inoculated with yeasts (at the beginning of alcoholic fermentation (AF)) or sequentially inoculated (at the end of AF) (Bartowsky et al. 2015, Sumby et al. 2019). However, due to the highly selective environment of wines, MLF remains difficult to accomplish, especially when LAB are sequentially inoculated, mainly due to the presence of high levels of inhibitory metabolites (mainly ethanol, sulphur dioxide (SO_2) and pH) (Bartowsky et al. 2015, Bartle et al. 2019, Sumby et al. 2019). To this end, in order to encourage MLF, wines have to be kept under conditions that may increase the risk of spoilage by other microorganisms (Ribéreau-Gayon et al. 2000). Simultaneous inoculation of yeasts and LAB starter cultures has gained attention in the recent years, since both AF and MLF are completed early and the wine can immediately be racked, stabilized and filtered for further storage or bottling, thus increasing microbial stability (Abrahamse and Bartowsky 2012). However, the application of this inoculation protocol poses risks, such as the presence of antagonistic interactions between yeasts and LAB, stuck AF before sugar depletion and production of excessive amounts of acetic acid under certain environmental and physicochemical conditions (Sumby et al. 2014, Bartowsky et al. 2015).

In the last decades, several studies reported the interactions between yeasts-LAB, and may range from inhibitory, to neutral, to stimulatory (Liu et al. 2017, Bartle et al. 2019). Most of these studies, have demonstrated that the type and degree of interactions is dependent upon several factors: (a) the physicochemical composition of the medium, (b) the uptake and release of nutrients by yeasts, (c) the ability of the yeasts to produce metabolites (such as ethanol, SO_2, medium-chain fatty acids and antibacterial proteins/peptides) able to inhibit or stimulate the growth of LAB (Tonon and Lonvaud-Funel 2000, Terrade and Mira de Orduña 2009, Liu et al. 2017, Balmaseda et al. 2018, Bartle et al. 2019). In addition, such studies have also highlighted the complexity of these interactions, showing that the same yeast strain may stimulate or inhibit different LAB strains under wine making conditions (Larsen et al. 2003, Arnink and Henick-Kling 2005).

In the present study, we performed MLF combining commercial *Lactobacillus plantarum* and *Oenococcus oeni* strains with five commercial *Saccharomyces cerevisiae* strains at the beginning of AF in order to evaluate the impact of their interactions on chemical and phenolic composition of the wines. *S. cerevisiae* strains were carefully chosen, with the intention of covering a wide range of fermentation rates and nutrition demands.

Materials and Methods

Strains. Five *S. cerevisiae* strains and two LAB species namely *O. oeni* and *L. plantarum* were used in this study (Table 1). All strains are commercially available as pure freeze-dried cultures and were obtained from Lallemand Inc. (Montreal, Canada).

Must preparation. Barbera grapes were harvested, destemmed, crushed and 30 mg/L of SO_2 were added in the yielded must. A cold maceration was performed at 5 ^oC for 72 hours to promote color extraction (Boulton et al. 1996) and subsequently the grape juice was separated from the solid parts using a stainless-steel sieve, cooled down and frozen at -20 ^oC until use. The racked grape juice had a sugar content of 226 g/L, a total acidity of 7.15 g/L as tartaric acid, a pH of 3.32, a YAN of 260 mg/L (composed of 75 mg/L of ammonium and 185 mg/L of amino acids) and 1.85 g/L of malic acid.

Fermentation trials. Fifteen sets of fermentations (in duplicate), consisting of inoculating each *S. cerevisiae* strain in pure culture and combining each *S. cerevisiae* strain with *L. plantarum* ML Prime™ or *O. oeni* Lalvin® VP41™, were performed. LAB species were inoculated 24 h after *S. cerevisiae* inoculation. Fermentations were performed in 1 L sterile glass bottles, containing 900 mL of Barbera grape must. The absence of indigenous yeast and LAB populations prior inoculation was checked by plate counts using appropriate culture media (Englezos et al. 2019). Yeast and LAB inocula were prepared according to manufacturer's recommendations using a dose of 20 g/L for *S. cerevisiae* strains, 1 g/hL for *O. oeni* Lalvin® VP41™ and 10 g/hL for *L. plantarum* ML Prime®. Organic nitrogen (Fermaid O, Lallemand Inc.) was added at a dose of 0.2 g/L (corresponds to 8 mg/L of YAN) together with yeast inoculum and when yeasts consumed about 30 % of the total sugars. Bottles were closed with sterile airlocks containing sterile paraffin oil to allow CO_2 to escape from the fermenting must. Fermentations were performed at 23 ± 2 ^oC and considered finished when sugars and malic acid concentration were below 2.0 and 0.2 g/L, respectively.

Standard chemical parameters. Sugars, glycerol, ethanol, acetic, L-malic and D/L-lactic acid concentrations were determined during (0, 2, 4 and 7 days) and at the end of AF and MLF by enzymatic kits (Megazyme International, Wicklow, Ireland). Must and wine parameters like total acidity (expressed as g/L of tartaric acid) (Method OIV-MA-AS313-01), pH (Method OIV-MA-BS-13), volatile acidity (expressed as g/L of acetic acid) (Method OIV-MA-AS313-02), free and total SO_2 (Method OIV-MA-AS323-04B) were determined according to the official protocols of the International Organization of Vine and Wine (OIV 2015). Total YAN (ammonium and amino acids) was analysed in the must before the alcoholic fermentation using enzymatic test kits (Megazyme International).

Color analysis and phenolic profile of wines. The wine chromatic characteristics was assessed spectrophotometrically according to the OIV reference method (OIV 2015). These are colour intensity and CIELab space parameters: lightness (L*), red/green values (a*), blue/yellow (b*) and their derived magnitude hue angle (H*). The spectrophotometric measurements were carried out using 2 mm path cuvettes and absorbance values were recorded over the range of 380 – 780 nm wavelength at 5 nm intervals using an UV-1400 spectrophotometer (Shimazdu Corporation, Kyoto, Japan). The phenolic composition of wines was determined by several spectrophotometric indices using the above-mentioned spectrophotometer and the protocols described by Rolle et al. (2018): absorbance at 280 nm (as A280), total anthocyanins (as mg/L of malvidin-3-glucoside chloride) and total flavonoids (as mg/L of (+)-catechin). Total phenols were determined by the reduction of phosphotungstic and phosphomolybdic acids (Folin–Ciocalteu reagent) to blue pigments by phenolic substances in alkaline solution (Singleton and Rossi 1965). The concentrations of flavonoids and anthocyanins were determined after dilution with ethanol/water/HCl (37%) (70:30:1) (Rolle et al. 2018).

Statistical analysis. Analysis of variance (ANOVA) was used to determine differences between inoculation protocols. The ANOVA was performed by using IBM SPSS Statistics software package (Version 19.0; IBM Corp., Armonk, NY), while a Tukey-b post hoc multiple comparison was performed using 95% confidence interval. The effect and interaction of *S. cerevisiae* strains and LAB species were analyzed by factorial ANOVA.

Results and discussion

Metabolites evolution during fermentation. The evolution of sugars, malic and lactic acid during AF and MLF are shown in Figure 1 (A-O). Regardless of the inoculation protocol and combination of strains used, all fermentations, completed sugar consumption (< 2.0 g/L) in 7 days. *S. cerevisiae* strains exhibited quite similar sugar consumption rate in pure AF, except strains Lalvin ICV® D254 and Lalvin ICVK1 which consumed sugar faster during the first 4 days of AF, than the other strains. In AF without LAB inoculation, malic acid concentration decreased up to 30 % and 50 % at the end of fermentation with Lalvin ICV® D254 and Lalvin® 71B™, respectively. This is in line with general observations that *S. cerevisiae* is capable of consuming small amounts of malic acid, but the concentrations are considered very low compared to LAB (Husnik et al. 2006, Rezdepovic et al. 2003). Malic acid passes through the yeast cellular membrane by simple diffusion (Pretorius 2000) where it is metabolized mainly to ethanol through the malo-ethanolic pathway (Main et al. 2007). In the present study, the ability *S. cerevisiae* strains to degrade malic acid could be attributed to the efficient transport of dicarboxylic acid, as well as the efficacy of the intracellular malic enzyme (Ansanay et al. 1996). Wines fermented with the other *S. cerevisiae* strains, consumed lower levels of malic acid during the first 4 days of AF and then a slight final increase was seen at the end of AF. This increase may be explained by the formation of malic acid as secondary metabolite of the tricarboxylic acid pathway, as previously demonstrated by a previous study using synthetic must without malic acid or due to the ability of cells to adsorb and release further malic acid (Fatichenti et al. 1984, Yeramian et al. 2007).

The two LAB species exhibited different evolution patterns of malic and lactic acid in the co-inoculated musts with *S. cerevisiae* strains (Figure 1). The inoculation of LAB strains did not influence sugar consumption by *S. cerevisiae*. About 3 days after LAB inoculation were necessary to successfully complete MLF by *L. plantarum*, independently of the *S. cerevisiae* strain used. The use of *L. plantarum* ML PrimeTM was notable for the early start of the malic acid consumption over AF, as seen by the fact that consumption of malic acid began immediately after its inoculation. In particular, malic acid concentration ranged from 0.5 to 0.9 g/L after 1 day from *L. plantarum* inoculation and reduced to 0.1 – 0.3 after 3 days. As a result, a sharp increase of lactic acid was observed during this time. This finding is in accordance with a previous study that demonstrated successful and fast MLF after co-inoculation of *L. plantarum* and *S. cerevisiae* (Lucio et al., 2018). The faster malic acid consumption by *L. plantarum* compared to *O. oeni* could also be explained by the higher inoculation rate of the first (10 g/hL versus 1 g/hL). Among wines that underwent MLF, *S. cerevisiae* Uvaferm® VRBTM and *L. plantarum* ML PrimeTM produced more lactic acid than the other wines, while the couples with Lalvin ICV® D254 and Lalvin® 71BTM achieved the lowest levels of lactic acid concentration.

In contrast, consumption of malic acid by *O. oeni* Lalvin® VP41TM was generally slow during the first 3 days following its inoculation. It is worth noticing that the evolution of malic acid was very close to that observed in AF without LAB inoculation during this time interval. Malic acid consumption had a steep increase from day 4 of AF and onwards and was totally consumed on day 7. As expected, production of lactic acid by *O. oeni* in co-inoculated wines was generally quite slow during the first 4 days after yeast inoculation (production ranged from 0.4 – 0.5 g/L) and a sharp increase was evident from day 4 of AF and onwards. *S. cerevisiae* strain choice influenced greatly the final concentration of lactic acid concentration at the end of AF. As for *L. plantarum*, lactic acid production by *O. oeni* was significantly influenced by the strain of *S. cerevisiae* used. Comparing the two LAB strains tested here, it should be highlighted that *L. plantarum* ML Prime™, showed a shorter lag phase, compared to *O. oeni* Lalvin® VP41™, since malic acid concentration dropped to very low levels (0.1 – 0.3 g/L) in the first 3 days after LAB inoculation . Early start of MLF gives LAB an advantage in colonizing must and is significant from a technological point of view because of the shorter time required to conclude the total vinification process and the early microbiological stability conferred on wines (Bauer and Dicks 2004). The use of *L. plantarum* poses a distinct advantage over the *O. oeni* starter culture. The different pairs of *S. cerevisiae* and LAB showed different patterns of inhibition and stimulation of MLF depending on *S. cerevisiae* strain and LAB species chosen, in agreement with Lucio et al. 2018. These results demonstrate that the same yeast strains could inhibit or stimulate different LAB strains, in agreement with Nehme et al. (2008).

Analytical parameters of wines. The analytical parameters of wines are reported in Table 2. All fermentations ended up with residual sugar content of less than 2.0 g/L. The ethanol concentration ranged from 12.9 to 13.1 % (v/v). Volatile acidity ranged from 0.21 to 0.42 g/L (expressed as acetic acid) in all wines that underwent MLF. Considering that during AF, the *S. cerevisiae* strains, produced from 0.24 to 0.34 g/L of acetic acid, it could be assumed that neither LAB strains produced significant levels of acetic acid from sugars and citric acid. These results are in agreement with previous studies, that demonstrated that simultaneous inoculation of yeasts and LAB does not necessarily lead to excessive production of metabolites such as acetic acid (Bartowsky et al. 2015). However, *S. cerevisiae* strain selection in pure AF significantly influenced acetic acid production, since wines fermented with Lalvin® 71B™ produced the highest concentration (0.35 g/L) and Lalvin™

ICV® K1® the lowest (0.24 g/L).

SO_2 is considered one of the inhibitory factors to LAB growth (Sumby et al. 2019); its concentration is generally associated with the yeast strain performing AF and the must or wine composition. In the present study, SO_2 production was not influenced by the *S. cerevisiae* strain, since all wines produced from the different inoculation protocols gave values no greater than 5 mg/L of free SO_2 (data not shown) and between 18-24 mg/L of total SO_2, well below the concentration of 15 mg/L of free SO_2 and 100 mg/L of total SO_2, which were found to inhibit or limit LAB growth (Bauer and Dicks 2009, Sumby et al. 2019). The pH of wines ranged from 3.18 to 3.24, and a significant increase (from 0.03 to 0.07 units) was observed in wines that underwent MLF. On the other hand, MLF resulted in an average decrease of titratable acidity (expressed as g/L of tartaric acid) of 0.6 g/L, compared to respective control wines. Generally, wines that underwent MLF using *L. plantarum* ML Prime™ had higher acidity levels compared to the respective wines inoculated with *O. oeni* Lalvin® VP41™. This observation was notable when comparing pairs Lalvin® 71B™ - *O. oeni* Lalvin® VP41™ and Lalvin® 71B™ – *L. plantarum* ML Prime™; a 0.4 g/L increase in titratable acidity was registered. The smallest difference in titratable acidity production was observed in MLF using Lalvin™ ICV® K1® paired with LAB (ML Prime™ 8.0 g/L and Lalvin® VP41™ 7.9 g/L). Among wines without MLF, *S. cerevisiae* Lalvin™ ICV®K1® produced the highest levels of total acidity (8.8 g/L), while using Lalvin® 71B™ (7.1 g/L) produced the lowest value for this parameter. The same trend was also observed in wines that underwent MLF with the above-mentioned *S. cerevisiae* strains indicating the ability of yeast strains to modulate total acidity in a strain-dependent manner. This finding agrees with those of Lucio et al. (2018) and indicate that choosing appropriate *S. cerevisiae* and LAB strains to conduct co-inoculated MLF could be considered as a smart strategy to solve problems associated with climate change, such as increased titratable acidity in wines from warm-climate regions (Mira de Orduna 2010).

Malic and lactic acid composition of the wines at the end of the vinification period are presented in Table 3. Wines that underwent MLF completed malic acid consumption, while wines without LAB consumed malic acid in a *S. cerevisiae* strain-dependent way. Control wines with *S. cerevisiae* Lalvin® 71BTM and Lalvin ICV® D254, produced the highest reduction of malic acid (- 0.95 g/L, 53% reduction for Lalvin® 71BTM and -0.59 g/L, 34% reduction for Lalvin ICV® D254) while the wine fermented with LalvinTM ICV® K1® had the lowest malic acid reduction registered in wines without MLF (- 0.08 g/L, -4% reduction). *S. cerevisiae* strains Uvaferm® VRBTM and Lalvin® EC1118TM consumed medium levels of malic acid, since the consumption of this organic acid ranged from 0.27 - 0.32 g/L (15% and 18% reduction). The decrease in malic acid concentration also correlated with the decrease in total acidity (Table 2). In the wines fermented Lalvin® 71BTM and Lalvin ICV® D254, total acidity ranged from 7.1 to 7.7 g/L while in the rest of wines ranged from 8.0 to 8.8 g/L . These differences were also reflected in the pH of the wines. Wines fermented with Lalvin® 71BTM and Lalvin ICV® D254 had the highest pH values (3.18 to 3.21) whereas the other wines had the lowest pH values (3.12-3.17). Consequently, we can hypothesize that malic acid was converted into ethanol through malo-ethanolic fermentation (Main et al. 2007).

Concerning lactic acid production, AF without LAB contained up to 0.3 g/L regardless of the *S. cerevisiae* strain used to conduct AF, indicating that malic acid was not transformed in L-lactic acid and MLF was not performed. On the contrary, wines that underwent MLF contained significantly higher levels of L-lactic acid (1.2 - 1.9 g/L). Among wines produced by co-inoculation of yeast and *L. plantarum* ML PrimeTM, the pairs with LalvinTM ICV® K1® (1.5 g/L) and Uvaferm® VRBTM (1.5 g/L) contained the highest level of L-lactic acid, while the pairs with Lalvin ICV® D254 (1.1 g/L) and Lalvin® 71BTM (1.0

g/L) the lowest values. A significant decrease in L-lactic acid values was seen for wines that underwent MLF with *O. oeni* Lalvin® VP41TM, independently of the *S. cerevisiae* strain used, compared to the respective MLF with *L. plantarum* ML PrimeTM. This difference could be explained by the early start of MLF by *L. plantarum* compared to *O. oeni*, preventing *S. cerevisiae* to metabolize malic acid. Concerning co-inoculated wines with Lalvin® VP41TM, the pairs with LalvinTM ICV® K1® (1.26 g/L) and Uvaferm® VRBTM (1.12 g/L) accounted for significantly higher concentration, while the pair with Lalvin® 71BTM showed the lowest value (0.65 g/L), compared to the other couples with Lalvin® VP41TM.

D- and L- lactic acid isomers were measured to identify the consumption of sugars by *L. plantarum* and *O. oeni*. From the literature, it is known that *L. plantarum* forms D,L - lactic acid and only L-lactic acid from malic acid, while under certain conditions *O. oeni* forms D-lactic acid from sugars and only L-lactic acid from malic acid (du Toit et al. 2011). On average, MLF wines with Lalvin® VP41TM had a D- lactic acid of 0.3 and L-lactic acid of 1.02 g/L, MLF wines with ML PrimeTM had a D- lactic acid of 0.38 and L-lactic acid of 1.28 g/L. The higher concentration of L-lactic acid in wines that co-inoculated with *L. plantarum* ML PrimeTM, could be explained by the early start of MLF compared to *O. oeni*. Low levels of malic acid in the medium, due to prompt MLF by *L. plantarum,* may prevent S. *cerevisiae* from utilizing it. Furthermore, the presence of D-lactic acid in the wines that underwent MLF (especially those with ML PrimeTM) could be explained by the fact that part of this organic acid derives from the degradation of sugars. Therefore, a corresponding portion of L-lactic may result from this pathway (du Toit et al. 2011). However, the consumption of sugars is safe as no acetic acid is produced when *L. plantarum* consumes hexoses (du Toit et al. 2011).

Color is an important parameter in red wine and is greatly influenced by numerous chemical and microbial factors, including phenolic substances, pH, free SO_2 concentration, yeast, and LAB metabolites (Ribéreau-Gayon et al. 2000). Table 4 shows the composition of the phenolic substances and chromatic characteristics of the wines, produced form the different inoculation protocols. As can be seen, total anthocyanins varied between 153 and 280 mg/L and intensity ranged from 2.61 to 3.07 at the end of AF and MLF. *S. cerevisiae* strain choice had a great impact on these parameters. Wines produced from *S. cerevisiae* Lalvin ICV® D254 with or without the inoculation of LAB, generally had the lowest concentrations of total anthocyanins (153 – 163 mg/L and intensity 1.03 – 1.12, compared to other wines. The addition of LAB n’tn’t influence total anthocyanins. Only one exception was found: the induction of MLF in must fermented by Lalvin™ ICV® K1® retains higher levels of total anthocyanin content of wines (from 269 to 281 mg/L), possibly by preventing oxidation. Concerning total phenolic content (A_{280}) and total flavonoids, the wines produced by pure AF with Lalvin™ ICV® K1® and Lalvin® 71B™ accounted for significantly higher concentration of these parameters compared to the other wines that did not undergo MLF. Additionally, the presence of LAB did not influence significantly the concentration of these parameters, with the exception of total phenolic content, which was found to be higher in wines produced by simultaneous inoculation of Lalvin ICV® D254 with *O. oeni* Lalvin® VP41™ compared to the respective wine produced by *L. plantarum* ML Prime™ and that produced without LAB inoculation.

Regarding CIELab parameters (Table 4), the maximum values of hue (H*) were obtained using Lalvin ICV® D254 and Uvaferm® VRB™ (only for *L. plantarum* ML Prime™) paired with LAB, compared to the other wines. Wine lightness (L*) increased significantly in wines produced by *S. cerevisiae* Lalvin ICV® D254, compared to those produced from the other pairs, while LAB choice had no impact on this parameter. An opposite tendency was reported for the red/green color component (a*). In this case, wines produced from the above-mentioned *S. cerevisiae* strain produced the lowest values, while

LAB choice had a great impact in wines that were co-inoculated with Lalvin® EC1118TM and Lalvin® 71BTM. Finally, the yellow/blue component (b*), is significantly influenced by the specific couple yeast-LAB. It is worth noticing that the greatest color differences were found in wines co-inoculated with Lalvin® EC1118TM and *O. oeni* Lalvin® VP41TM, since they had the lowest levels of h* and b* and the highest levels of a*, compared to the other wines. Many studies demonstrated a decrease in red wine color, mainly due to the increase of pH after MLF (Bartowsky et al. 2015). Other studies have demonstrated that *O. oeni* may impact the compounds involved in wine color, due to the production of metabolites like pyruvic acid and acetaldehyde (Osborne and Edwards, 2006). The results of this study, indicated that phenolic substances and chromatic characteristics of wines that underwent MLF did not differ significantly from those that underwent only AF and, therefore, the chromatic characteristics at the end of vinification are dependent on the combination of *S. cerevisiae* and LAB.

Conclusion

The data supports the use of *L. plantarum* and *O. oeni* as early partners of *S. cerevisiae*, but at the same time the specific combination of *S. cerevisiae* with *O. oeni* and *L. plantarum* is important. In particular, we have demonstrated that *L. plantarum* completed MLF faster compared to *O. oeni* (probably due to the higher inoculation rate and shorter lag phase), however the concentration of lactic acid depended on the *S. cerevisiae* strain used to perform alcoholic fermentation. This data contributes to further understanding of yeast-LAB interactions during co-inoculated MLF and allows for better management of the specific metabolites to enhance wine quality.

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Table 1 Origin of the five *S. cerevisiae* and two lactic acid bacteria strains used in this study.

Strain	Species	Origin	Fermentation rate [b]	Nutrition demands [b]
Lalvin ICV® D254	*Saccharomyces cerevisiae*	Lallemand[a]	Moderate	Moderate
Lalvin® 71B™	*Saccharomyces cerevisiae*	Lallemand	Moderate	Low
Uvaferm® VRB™	*Saccharomyces cerevisiae*	Lallemand	Moderate	Moderate
Lalvin® EC1118™	*Saccharomyces cerevisiae*	Lallemand	Fast	Low
Lalvin™ ICV® K1®	*Saccharomyces cerevisiae*	Lallemand	Fast	Moderate
Lalvin® VP41™	*Oenococcus oeni*	Lallemand	/	Low
ML Prime™	*Lactobacillus plantarum*	Lallemand	/	Very low

[a] Lallemand Inc. (Montreal, Canada), [b] (http://www. lallemandwine.com)

Table 2 Chemical analysis of wines following alcoholic and malolactic fermentation.

S. cerevisiae	LAB	Residual sugars (g/L)	Acetic acid (g/L)	Ethanol (% v/v)	pH	TA (g/L)
Lalvin ICV® D254	*L. plantarum*$_{\alpha}$	0.8 ± 0.2	0.35 ± 0.03	12.9 ± 0.1 A	3.21 ± 0.01 b,ABC	7.7 ± 0.4 AB
	O. oeni$_{\beta}$	0.7 ± 0.1 B	0.34 ± 0.07	12.9 ± 0.2	3.21 ± 0.01 b,B	7.2 ± 0.1 B
	/$_{\gamma}$	0.7 ± 0.3 B	0.30 ± 0.02 AB	13.2 ± 0.3	3.18 ± 0.01 a,C	7.7 ± 0.4 AB
Lalvin® 71B™	*L. plantarum*	0.8 ± 0.1 a	0.35 ± 0.07	13.0 ± 0.1 B	3.23 ± 0.01 b,C	7.1 ± 0.1 b,A
	O. oeni	1.0 ± 0.1 b,C	0.42 ± 0.02	13.0 ± 0.1	3.24 ± 0.01 b,C	6.7 ± 0.1 a,A
	/	0.9 ± 0.1 b,C	0.35 ± 0.02 B	13.0 ± 0.1	3.21 ± 0.02 a,D	7.1 ± 0.1 b,A
Uvaferm® VRB™	*L. plantarum*	0.5 ± 0.1 a	0.3 ± 0.04	13.1 ± 0.1 C	3.18 ± 0.02 b,A	7.9 ± 0.1 ab,AB
	O. oeni	0.5 ± 0.2 a,A	0.36 ± 0.02	13.2 ± 0.1	3.19 ± 0.01 b,AB	7.5 ± 0.1 a,C
	/	0.6 ± 0.1 b,A	0.27 ± 0.03 AB	13.1 ± 0.2	3.12 ± 0.01 a,A	8.4 ± 0.3 b,BC
Lalvin® EC1118™	*L. plantarum*	0.5 ± 0.1	0.21 ± 0.02	13.1 ± 0.1 a,C	3.21 ± 0.01 b,BC	7.4 ± 0.2 a,AB
	O. oeni	0.6 ± 0.2 AB	0.33 ± 0.02	13.1 ± 0.1a	3.21 ± 0.01 b,B	7.1 ± 0.1 a,B
	/	0.6 ± 0.1 A	0.28 ± 0.04 AB	13.2 ± 0.1b	3.17 ± 0.01 a,C	8.0 ± 0.1 b,BC
Lalvin™ ICV® K1®	*L. plantarum*	0.6 ± 0.1	0.26 ± 0.01	13.1 ± 0.2 C	3.20 ± 0.01 b,AB	8.0 ± 0.1a,B
	O. oeni	0.7 ± 0.1 B	0.31 ± 0.05	13.1 ± 0.1	3.18 ± 0.01 b,A	7.9 ± 0.01 a,D
	/	0.6 ± 0.2 A	0.24 ± 0.01 A	13.1 ± 0.1	3.14 ± 0.01 a,B	8.8 ± 0.01 b,C
Statistical differences						
S. cerevisiae strain effect in wine (Sign$_{\delta}$)	*L. plantarum*	NS	NS	**	**	*
	O. oeni	***	NS	NS	**	***
	/	***	*	NS	***	**
LAB species effect in wine (Sign$_{\varepsilon}$)	Lalvin ICV® D254	NS	NS	NS	*	NS
	Lalvin® 71B™	*	NS	NS	*	**
	Uvaferm® VRB™	*	NS	NS	***	*
	Lalvin® EC1118™	NS	NS	**	*	*
	Lalvin™ ICV® K1®	NS	NS	NS	**	***

All data are expressed as average value ± standard deviation of two independent experiments. Sign$_{\delta,\varepsilon}$: *, **, *** and NS indicate significance at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant respectively between the wines produced. TA: titratable acidity expressed as g/L of tartaric acid. $_{\alpha}$ Malolactic fermentation with *Lactobacillus plantarum* ML Prime™, $_{\beta}$ Malolactic fermentation with *Oenococcus oeni* Lalvin® VP41™, $_{\gamma}$ Fermentation without LAB inoculum, Latin letters indicate the statistical differences between LAB using the same *S. cerevisiae* strain ($Sign_{\delta}$), Upper Latin letters indicate statistical differences between *S. cerevisiae* strains using the same LAB ($Sign_{\varepsilon}$).

Table 3 Chemical analysis of wines following alcoholic and malolactic fermentation.

S. cerevisiae	LAB	Malic acid (g/L)	L -lactic acid (g/L)	D-lactic acid (g/L)	Lactic acid (g/L)
Lalvin ICV® D254	*L. plantarum* $_{\alpha}$	0.11 ± 0.01 a	1.10 ± 0.10 c,A	0.31 ± 0.12 b,A	1.40 ± 0.22 c,A
	O. oeni $_{\beta}$	0.14 ± 0.11 a,A	0.91 ± 0.14 b,B	0.33 ± 0.10 ab	1.20 ± 0.12 b,B
	/ $_{\gamma}$	1.32 ± 0.16 b,B	0.02 ± 0.11 a	0.20 ± 0.11 a	0.21 ± 0.11 a
Lalvin® 71B™	*L. plantarum*	0.12 ± 0.11 a	1.01 ± 0.10 c,A	0.40 ± 0.13 b,B	1.42 ± 0.14 c,A
	O. oeni	0.15 ± 0.12 a,A	0.72 ± 0.12 b,A	0.31 ± 0.11 a	1.00 ± 0.11 b,A
	/	0.91 ± 0.02 b,A	0.02 ± 0.14 a	0.33 ± 0.12 a	0.32 ± 0.14 a
Uvaferm® VRB™	*L. plantarum*	0.13 ± 0.10 a	1.54 ± 0.11 c,C	0.40 ± 0.02 b,B	1.80 ± 0.10 c,C
	O. oeni	0.11 ± 0.04 a,A	1.14 ± 0.21 b,C	0.31 ± 0.10 ab	1.40 ± 0.12 b,C
	/	1.54 ± 0.11 b,C	0.02 ± 0.14 a	0.36 ± 0.11 a	0.32 ± 0.22 a
Lalvin® EC1118™	*L. plantarum*	0.10 ± 0.10 a	1.37 ± 0.10 c,B	0.41 ± 0.12 b,B	1.7 0± 0.12 c,B
	O. oeni	0.12 ± 0.11 a,B	1.10 ± 0.11 b,C	0.32 ± 0.11 ab	1.41 ± 0.10 b,C
	/	1.61 ± 0.12 b,C	0.03 ± 0.10 a	0.20 ± 0.11 a	0.32 ± 0.14 a
Lalvin™ ICV® K1®	*L. plantarum*	0.01 ± 0.02 a	1.51 ± 0.21 c,C	0.42 ± 0.11 B	1.92 ± 0.13 c,C
	O. oeni	0.05 ± 0.12 a,B	1.31 ± 0.01 b,D	0.34 ± 0.11	1.50 ± 0.20 b,D
	/	1.80 ± 0.11 b,C	0.04 ± 0.10 a	0.33 ± 0.10	0.31 ± 0.11 a
Statistical differences (Sign)					
S. cerevisiae strain effect in wine (Sign $_{\delta}$)	*L. plantarum*	*	***	*	***
	O. oeni	***	***	NS	***
	/	***	NS	NS	NS
LAB species effect in wine (Sign $_{\varepsilon}$)	Lalvin ICV® D254	***	***	*	***
	Lalvin® 71B™	***	***	**	***
	Uvaferm® VRB™	***	***	*	***
	Lalvin® EC1118™	***	***	*	***
	Lalvin™ ICV® K1®	***	***	NS	***

All data are expressed as average value ± standard deviation of two independent experiments. Sign $_{\delta,\varepsilon}$: *, **, *** and NS indicate significance at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant respectively between the wines produced. TA: titratable acidity expressed as g/L of tartaric acid. $_{\alpha}$ Malolactic fermentation with *Lactobacillus plantarum* ML Prime™, $_{\beta}$ Malolactic fermentation with *Oenococcus oeni* Lalvin® VP41™, $_{\gamma}$ Fermentation without LAB inoculum, Latin letters indicate the statistical differences between LAB using the same *S. cerevisiae* strain (Sign$_δ$), Upper Latin letters indicate statistical differences between *S. cerevisiae* strains using the same LAB (Sign$_ε$).

Table 4 Chromatic characteristics of wines following alcoholic and malolactic fermentation.

S. cerevisiae	LAB	Total anthocyanin [mg/L malvidin-3-glucoside chloride]	Total phenol [mg/L (+)-catechin]	A 280	Color intensity (optical path 10mm)	Color hue	L*	a*	b*
Lalvin ICV® D254	*L. plantarum*[α]	159 ± 4 A	390 ± 2 a	15.9 ± 0.1	2.61 ± 0.03 A	1.03 ± 0.03 C	52.6 ± 0.6 B	45.5 ± 0.6 A	34.3 ± 0.6 AB
	O. oeni[β]	163 ± 15 A	407 ± 6 b	16.4 ± 0.6	2.41 ± 0.17 A	1.12 ± 0.07 C	56.5 ± 2.6 B	44.7 ± 2.6 A	37.1 ± 0.4 D
	/[γ]	153 ± 4 A	388 ± 4 a,A	15.6 ± 0.4 AB	2.45 ± 0.11 A	1.09 ± 0.04 B	55.8 ± 1.4 B	45.7 ± 1.0 A	36.8 ± 1.1 B
Lalvin® 71B™	*L. plantarum*	235 ± 13 B	426 ± 9	15.3 ± 0.4	2.98 ± 0.06 B	0.68 ± 0.02 AB	47.6 ± 0.5 A	57.6 ± 0.9 b,BC	28.7 ± 0.2 A
	O. oeni	260 ± 30 B	434 ± 42	16.0 ± 1.2	2.82 ± 0.08 AB	0.71 ± 0.01 AB	48.8 ± 0.8 A	55.9 ± 0.5 ab,B	28.0 ± 0.4 B
	/	211 ± 7 B	396 ± 6 A	15.3 ± 0.1 A	2.79 ± 0.03 B	0.75 ± 0.03 A	49.5 ± 0.1 A	54.7 ± 0.5 a,B	29.6 ± 1.3 A
Uvaferm® VRB™	*L. plantarum*	230 ± 1 B	417 ± 43	18.6 ± 3.3	3.05 ± 0.09 B	0.81 ± 0.02 C	46.0 ± 0.1 A	54.4 ± 1.5 B	31.9 ± 0.4 AB
	O. oeni	256 ± 11 B	453 ± 28	17.5 ± 0.1	3.23 ± 0.14 B	0.75 ± 0.03 B	45.4 ± 0.7 A	56.8 ± 1.9 BC	32.5 ± 1.2 C
	/	241 ± 20 BC	426 ± 4 B	17.2 ± 0.1 C	3.12 ± 0.05 C	0.74 ± 0.01 A	46.6 ± 0.6 A	57.4 ± 0.3 BC	31.9 ± 0.3 AB
Lalvin® EC1118™	*L. plantarum*	234 ± 8 B	430 ± 7	16.5 ± 0.3	3.16 ± 0.06 B	0.77 ± 0.01 c,AB	46.9 ± 0.6 A	56.5 ± 0.1 a,BC	35.2 ± 0.3 c,B
	O. oeni	279 ± 16 B	459 ± 16	16.8 ± 0.1	3.07 ± 0.1 B	0.60 ± 0.01 a,A	48.4 ± 0.8 A	61.9 ± 0.4 c,C	28.9 ± 0.7 a,B
	/	247 ± 9 BC	439 ± 5 B	16.4 ± 0.2 B	3.05 ± 0.11 BC	0.70 ± 0.01 b,A	48.1 ± 1.2 A	58.9 ± 0.4 b,C	32.4 ± 0.3 b,AB
Lalvin™ ICV® K1®	*L. plantarum*	281 ± 2 bC	449 ± 2	16.3 ± 0.1	2.99 ± 0.09 B	0.65 ± 0.08 A	48.3 ± 1.3 A	60.3 ± 2.1 C	28.4 ± 3.6 A
	O. oeni	280 ± 2 bB	453 ± 22	16.4 ± 0.2	2.8 ± 0.07 AB	0.61 ± 0.01 A	50.1 ± 0.8 A	61.1 ± 0.3 BC	24.7 ± 1.1 A
	/	269 ± 1 a,C	435 ± 7 B	16.4 ± 0.1 B	2.85 ± 0.04 BC	0.64 ± 0.06 A	49.6 ± 0.8 A	60.4 ± 1.9 C	27.0 ± 2.7 A
Statistical differences (Sign)									
	L. plantarum	***	NS	NS	**	**	**	***	*

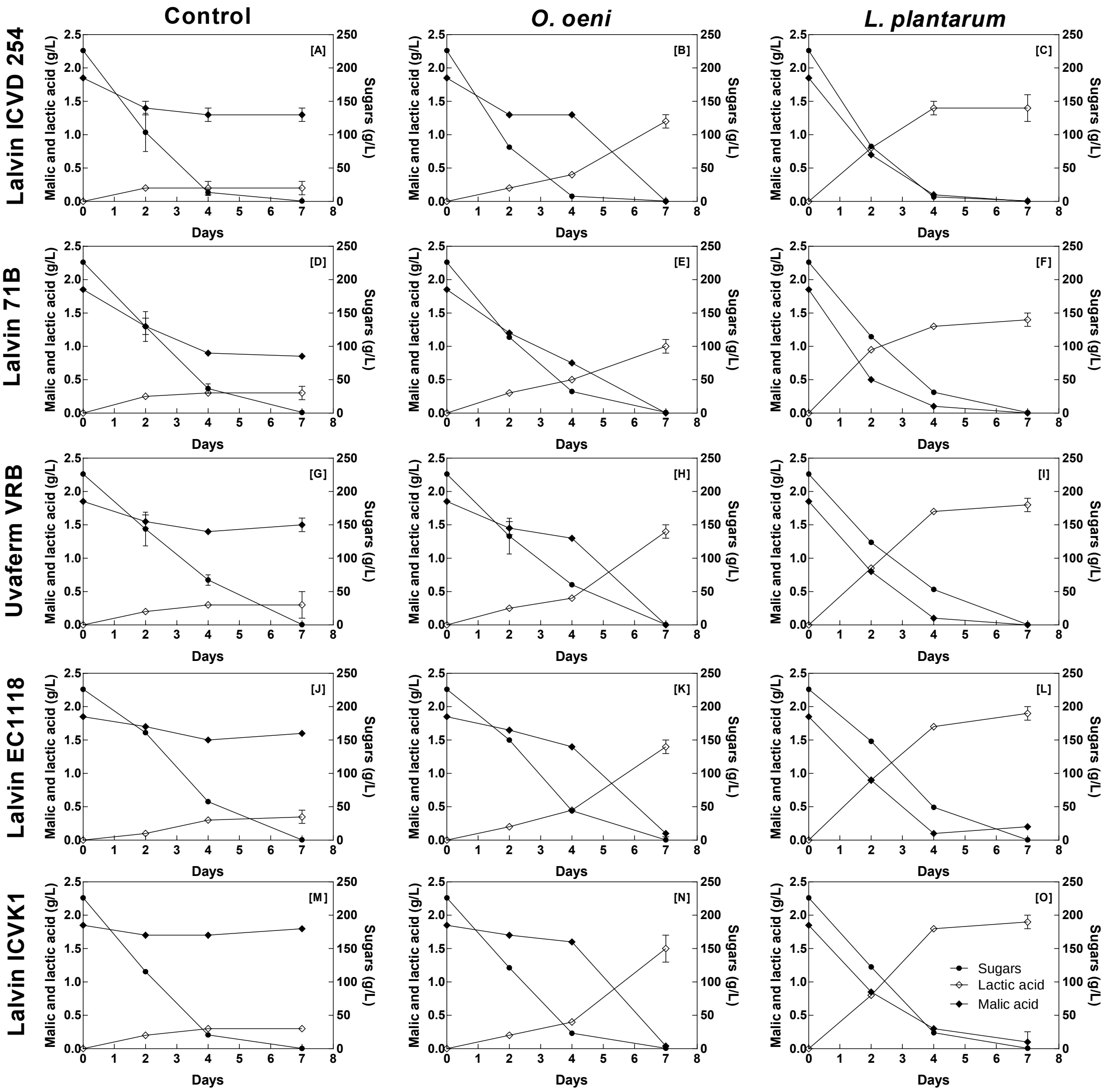
S. cerevisiae strain effect in wine (Sign$_{\delta}$)	*O. oeni*	**	NS	NS	**	***	**	***	***
	/	***	***	**	**	***	**	***	**
LAB species effect in wine (Sign$_{\varepsilon}$)	Lalvin ICV® D254	NS	*	NS	NS	NS	NS	NS	NS
	Lalvin® 71B™	NS	NS	NS	NS	NS	NS	*	NS
	Uvaferm® VRB™	NS	NS	NS	NS	NS	NS	NS	NS
	Lalvin® EC1118™	NS	NS	NS	NS	***	NS	**	**
	Lalvin™ ICV® K1®	**	NS	NS	NS	NS	NS	NS	NS

All data are expressed as average value ± standard deviation of two independent experiments. Sign$_{\delta,\varepsilon}$: *, **, *** and NS indicate significance at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant respectively between the wines produced. TA: titratable acidity expressed as g/L of tartaric acid. $_{\alpha}$ Malolactic fermentation with *Lactobacillus plantarum* ML Prime™, $_{\beta}$ Malolactic fermentation with *Oenococcus oeni* Lalvin® VP41™, $_{\gamma}$ Fermentation without LAB inoculum, Latin letters indicate the statistical differences between LAB using the same *S. cerevisiae* strain (Sign$_{\delta}$), Upper Latin letters indicate statistical differences between *S. cerevisiae* strains using the same LAB (Sign$_{\varepsilon}$). A280 absorbance at 280 nm, L*: luminosity; a*: red/green color component and b*: yellow/blue color component. ΔE* parameter was calculated considering average values of L*, a*, and b* color components, for each mixed fermentation sample with relation to the same variety pure fermentation sample.

Figure captions

Figure 1 Metabolites evolution (sugars, malic and lactic acid) during fermentation without LAB inoculation (left panel), fermentation with LAB inoculation *Oenococcus oeni* Lalvin® VP41TM (central panel) and *Lactobacillus plantarum* ML PrimeTM (right panel), using 5 different *S. cerevisiae* strains: *S. cerevisiae* Lalvin ICVD® D254 (A-C); *S. cerevisiae* Lalvin® 71BTM (D-F); *S. cerevisiae* Uvaferm® VRBTM (G-I); *S. cerevisiae* Lalvin® EC1118TM (J-L); *S. cerevisiae* LalvinTM ICV® K1® (M-O). Data are the mean ± standard deviations. Data are representative of two independent experiments

Figure 1


Control
O. oeni
L. plantarum
Lalvin ICVD 254
Lalvin 71B
Uvaferm VRB
Lalvin EC1118
Lalvin ICVK1
Malic and lactic acid (g/L)
Sugars (g/L)
Days
[A]
[B]
[C]
[D]
[E]
[F]
[G]
[H]
[I]
[J]
[K]
[L]
[M]
[N]
[O]
Sugars
Lactic acid
Malic acid