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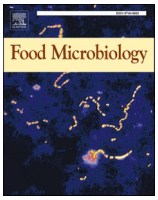

Screening of *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* strains in relation to their effect on malolactic fermentation

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ABSTRACT

The use of *Torulaspora delbrueckii* in the alcoholic fermentation (AF) of grape must is increasingly studied and used in the wine industry. In addition to the organoleptic improvement of wines, the synergy of this yeast species with the lactic acid bacterium *Oenococcus oeni* is an interesting field of study. In this work, 60 strain combinations were compared: 3 strains of *Saccharomyces cerevisiae* (Sc) and 4 strains of *Torulaspora delbrueckii* (Td) in sequential AF, and four strains of *O. oeni* (Oo) in malolactic fermentation (MLF). The objective was to describe the positive or negative relationships of these strains with the aim of finding the combination that ensures better MLF performance. In addition, a new synthetic grape must has been developed that allows the success of AF and subsequent MLF. Under these conditions, the Sc-K1 strain would be unsuitable for carrying out MLF unless there is prior inoculation with Td-Prelude, Td-Viniferm or Td-Zymaflore always with the Oo-VP41 combination. However, from all the trials performed, it appears that the combinations of sequential AF with Td-Prelude and Sc-QA23 or Sc-CLOS, followed by MLF with Oo-VP41, reflected a positive effect of *T. delbrueckii* compared to inoculation of Sc alone, such as a reduction in L-malic consumption time. In conclusion, the obtained results highlight the relevance of strain selection and yeast-LAB strain compatibility in wine fermentations. The study also reveals the positive effect on MLF of some *T. delbrueckii* strains.

1. Introduction

Winemaking is particularly influenced by microorganisms, which are responsible in a large part for the quality of the final product. Yeasts transform grape must sugars (glucose and fructose) into ethanol, carrying out alcoholic fermentation (AF), which is the main reaction of wine production (Ribéreau-Gayon, 2006).

The other highlighted biochemical transformation is the malolactic fermentation (MLF), which is desired to happen in a wide range of wines, such as red wines and some specific white and *rosé* wines. In this case, lactic acid bacteria (LAB) decarboxylate L-malic acid into L-lactic acid. Apart from deacidification, MLF assures microbiological stability and improves organoleptic characteristics (Chambers and Pretorius, 2010; Paramithiotis et al., 2022). In addition, there are other interesting microbiological reactions such as those involved in secondary aromas, i.e., the production of ethyl esters or higher alcohols during fermentation (Belda et al., 2017; Carpena et al., 2021).

Saccharomyces cerevisiae is the species that mainly conducts AF of grape must due to its good fermentation ability (Ribéreau-Gayon, 2006). Nevertheless, there are a large number of yeast species with useful properties that are included as non-*Saccharomyces* yeasts. For many years, they have been considered undesirable microorganisms, but their use has increased (Jolly et al., 2014). Because they are present in grape and winery microbiota, they play an important role in spontaneous fermentation, giving varietal character to wines (Rossouw and Bauer, 2016). Some of the most relevant species are *Hanseniaspora uvarum, Starmerella bacillaris, Lachancea thermotolerans, Metschnikowia pulcherrima, Torulaspora delbrueckii* and *Zygosaccharomyces bailii* (Beltran et al., 2002; Du Plessis et al., 2019; Fairbairn et al., 2021; Jolly et al., 2014). Most of these non-*Saccharomyces* enhance the aromatic profile of wines thanks to their enzymatic activities that release volatile compounds from their precursors (Belda et al., 2017; Carrau and Henschke, 2021;

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Padilla et al., 2016; Russo et al., 2020). Furthermore, some species have shown other advantages, such as the capacity to reduce ethanol content (Hranilovic et al., 2020; Zhu et al., 2021). Some of them are available as starter cultures and are also used by oenologists in the first stages of fermentation. *T. delbrueckii*, *M. pulcherrima* and *L. thermotolerans* are the most common ones and distributed as commercial products.

Recent studies show the synergy between non-*Saccharomyces* and *Oenococcus oeni,* the LAB species mainly responsible for MLF (Arnink and Henick-Kling, 2005; Ferrando et al., 2020; Paramithiotis et al., 2022). Specifically, several studies have revealed that wines fermented with *T. delbrueckii* can improve MLF performance and consequently the final product (Balmaseda et al., 2021d). Therefore, *T. delbrueckii* is a species with potential applications, for example, advantageous changes in organoleptic profiles of red wines, increasing their aromatic characteristics, complexity, mouthfeel and colour (Balmaseda et al., 2021a; Ramírez et al., 2016; Zhang et al., 2021).

Despite this improvement in wine quality linked to the use of non-*Saccharomyces* species, it is necessary to check the compatibility between strains of non-*Saccharomyces*, *S. cerevisiae* and *O. oeni*. Sometimes there may be a stimulatory effect between the yeast-bacteria strain combination, but there may also be complex inhibitions specific for each strain blend. Generally, non-*Saccharomyces* yeasts influence LAB development and consequently, the MLF, in a species and strain dependent manner (Alexandre et al., 2004; Du Plessis et al., 2017; Englezos et al., 2022; Russo et al., 2020; Torres-Guardado et al., 2022). The interactions between *Saccharomyces*, non-*Saccharomyces* and *O. oeni* are also dependent on the inoculation regimes of yeast and bacteria. Although here we have considered only the most usual way of performing MLF after AF, there is an emerging trend of co-inoculating bacteria with yeasts, and not only *O. oeni* but also other LAB (Bartowsky et al., 2015; Englezos et al., 2022).

The aim of this work was to study the potential suitability or incompatibility of a wide range of yeast-bacteria combinations in a synthetic grape must to perform AF followed by complete MLF to confirm the advantages of *T. delbrueckii*.

2. Materials and methods

2.1. Microorganism strains

Seven yeast strains were used to carry out AF. Three *S. cerevisiae* strains from Lallemand Inc. (Montreal, Canada): Lalvin-QA23 (Sc-QA), Lalvin-CLOS (Sc-CL) and ICV K1 Marquée (Sc-K1). Even though Sc-K1 is not a strain recommended for MLF by the manufacturer (Lallemand Inc., n. d.), it was selected with the aim of observing whether its combination with *T. delbrueckii* improves the MLF performance.

In addition, four *T. delbrueckii* strains were tested: *Biodiva TD291* (Lallemand Inc., Montreal, Canada) (Td-B), Viniflora Prelude (Chr. Hansen Holding AS, Hoersholm, Denmark) (Td-P), NSA1 Viniferm NSDT (Agrovin, Spain) (Td-V) and Zymaflore Alpha (Agrovin S. A, Spain) (Td-Z). Yeast inocula were prepared from active dry yeast (ADY) for both species after rehydration according to the manufacturer's instructions, 37 °C and 30 °C for *Saccharomyces* and *Torulaspora* species respectively.

Regarding *O. oeni* strains, Lalvin VP41 (Lallemand Inc., Montreal Canada) (VP41), Viniflora CH11 (Chr. Hansen Holding AS, Hoersholm, Denmark), 1Pw13 (own collection, CECT 8893) and PSU-1 (ATCC BAA-331) were used for MLF. LAB strains were preserved in MRS plates. MRS medium was supplemented with 4 g/L D, L-malic acid and 5 g/L fructose at pH 5. Then, isolated colonies from these plates were grown in MRS broth at 27 °C in a 10% CO_2 atmosphere. The inocula (2×10^7 cells/mL) were obtained from a pre-culture in the final phase of exponential growth cultivated in 50 mL of the same MRS medium under the same conditions for 3 days at 28 °C.

2.2. Alcoholic fermentation

A new synthetic must was developed in order to perform MLF after AF. Since studies which work with both, AF and MLF, usually tested fermentations in natural must (Ferrando et al., 2020; Martín-García et al., 2020). AF was performed in triplicate with a new synthetic must (110 g/L glucose, 110 g/L fructose, 5 g/L L-tartaric acid, 2 g/L L-malic acid, 0.5 g/L citric acid, 1.7 g/L yeast nitrogen base w/o amino acids and ammonium sulphate, 50 mg/L ammonium chloride and 1.505 g/L amino acid stock (Table SD1) with a total nitrogen content of 243 mg N per L at pH 3.5. The composition of this must is similar to that of natural must but without either lipid factors or phenolic compounds. Bottles of 500 mL were used for the fermentation of 450 mL at 22 °C with agitation at 120 rpm, using a cap with two valves that allows carbon dioxide to escape and sample extraction.

For the sequential fermentation, the four strains of *T. delbrueckii* were separately inoculated, followed by *S. cerevisiae* inoculation after 48 h. Fermentation with the three *S. cerevisiae* strains were used as a control. So, the number of different AF was four *T. delbrueckii* strains by three *S. cerevisiae*, plus the controls with only *S. cerevisiae* strains, *id est*, a total of 15 obtained wines. Each one was performed in triplicate. The initial population inoculated was 2.5×10^6 cells/mL for both species.

Samples were taken every two days, and the fermentation kinetics were monitored by measuring the density of a centrifuged sample with an electronic densimeter (Densito 30PX Portable Density Metre (Mettler Toledo, Spain). The end of AF was established when the sugar concentration was lower than 2 g/L using the Miura One Multianalyzer (TDI, Barcelona, Spain).

2.3. Malolactic fermentation

Once AF was finished, wines were centrifuged at 3730 g for 15 min at 4 °C and filtered with 0.22 μm filters (Merck, Germany). Biological triplicates were mixed to avoid differences in MLF. The 15 resulting wines were distributed in 50 mL tubes to perform the MLF. Four strains of *O. oeni* were inoculated in a population of 2×10^7 cells/mL, obtaining a total of 60 combinations of yeast-LAB, fermented in triplicate. Anaerobic and static conditions at 20 °C were used to perform the MLF. Consumption of L-malic acid was monitored by measuring its concentration every day using Miura One Multianalyzer (TDI, Barcelona, Spain). The MLF was considered completed with a concentration of L-malic acid <0.1 g/L. No supplementation with L-malic acid was performed before MLF. Viable populations were analysed to check the populations and verify the AF and MLF inocula by plating serial dilutions in YPD and MRS media, respectively.

2.4. Analyses of metabolites

Wines after AF and MLF were analysed to calculate the concentration of organic acids and other metabolites. Acetic acid and L-malic acid were quantified with a Miura One Multianalyzer (TDI, Barcelona, Spain). Citric acid, glycerol and ethanol were determined using an Agilent 1100 HPLC (Agilent Technologies, Germany) (Quirós et al., 2014). The wine samples were filtered with 0.22 μm pore filters before injection (Agilent Technologies). The HPLC had a Hi-Plex H (300 mm × 7.7 mm) column inside a 1260 MCT (Infinity II Multicolumn Thermostat). The column conditions were 60 °C for 30 min, and the mobile phase was 5 mM H_2SO_4 at a flow rate of 0.6 mL/min. Finally, the chromatograph was equipped with two detectors, an MWC detector (G1365B multi-wavelength detector) and an RID detector (1260 Infinity II refractive index detector) (Agilent Technologies).

The following analysis was performed only in wines after AF. Succinic acid was analysed by an enzymatic kit with microplates (Megazyme, Wicklow, Ireland). The content of mannoproteins after AF was determined as equivalents of mannose using a D-mannose and D-glucose enzymatic assay kit (Megazyme). Mannoprotein precipitation and acid

hydrolysis to release mannose from mannoproteins were performed following the procedure described in Balmaseda, et al. (2021b). The variations in acetic, succinic and L-malic acids as well as glycerol and mannoproteins in wines are calculated as follows: [Cmetabolite $_{seq.\ FA}$ - Cmetabolite $_{control.\ FA}$/Cmetabolite $_{control.\ FA}$]* 100, where Cmetabolite $_{seq.\ FA}$ is the metabolite concentration in the sequential FA and Cmetabolite $_{control.\ FA}$, the metabolite concentration in the control wine for each sequential combination and *S. cerevisiae* strains.

Finally, the volatile compounds were liquid/liquid extracted with 400 μL of dichloromethane in presence of 2.5 g $(NH_4)_2SO_4$ using 4-methyl-2-penthanol (0.8 g/L) and heptanoic acid (0.7 g/L) as internal standards, following Ortega et al. (2001) modified by (Balmaseda et al., 2021a). All reagents were analytical grade from Sigma-Aldrich (Barcelona, Spain). The organic phase was extracted and 2 μL was injected in split mode (10:1, 30 mL/min) into a gas chromatograph (Agilent Technologies, Germany) with a FFAP column of 30 m × 0.25 mm × 0.25 μm. All aromatic volatile compounds were identified and quantified by comparison with standards. They included fusel alcohols (1-butanol, isoamyl alcohol, 1-pentanol, *cis*-3-hexen-1-ol, 2-phenylethanol), their corresponding acetate esters (isobutyl acetate, isoamyl acetate, hexyl acetate, 2-phenyletanol acetate), other alcohols (2-butanol, isopropanol), short-chain fatty acids (propionic, isobutyric, butyric, 3-methyl butanoic and valeric acids), medium-chain fatty acids (hexanoic, octanoic, decanoic and dodecanoic acids) and their corresponding ethylic esters of C6, C8, C10 and C12.

2.5. Statistical analysis

All alcoholic and malolactic fermentations were performed using three independent biological samples. These data were statistically analysed using ANOVA and Tukey test analyses performed by XLSTAT 2020.2.3 software (Addinsoft, Paris, France). The statistical significance level was considered at p-value <0.05. The heat map was created to examine the correlation between metabolites and samples (conditions). All data used to construct the heat map were centred and reduced. The clusters were constructed from centred and reduced data by using XLSTAT 2020.2.3 software. In addition, multiple linear regressions were performed for each *O. oeni* strain using an optimization index (OI) as the dependent variable and the mean values of the families of volatile compounds, acetic acid, succinic acid and mannoproteins, as independent variables. Briefly, the OI was calculated from MLF data values, using the means of total MLF time, malic acid consumption rate and percentage of MLF completion. For each parameter, the OI was calculated as follows: the maximum value for a parameter was considered as 1 (x/x), if it had a positive effect on FML performance, or 0 (1 x/x) whether it had a negative effect OI was calculated, based on (Borrull et al., 2016).

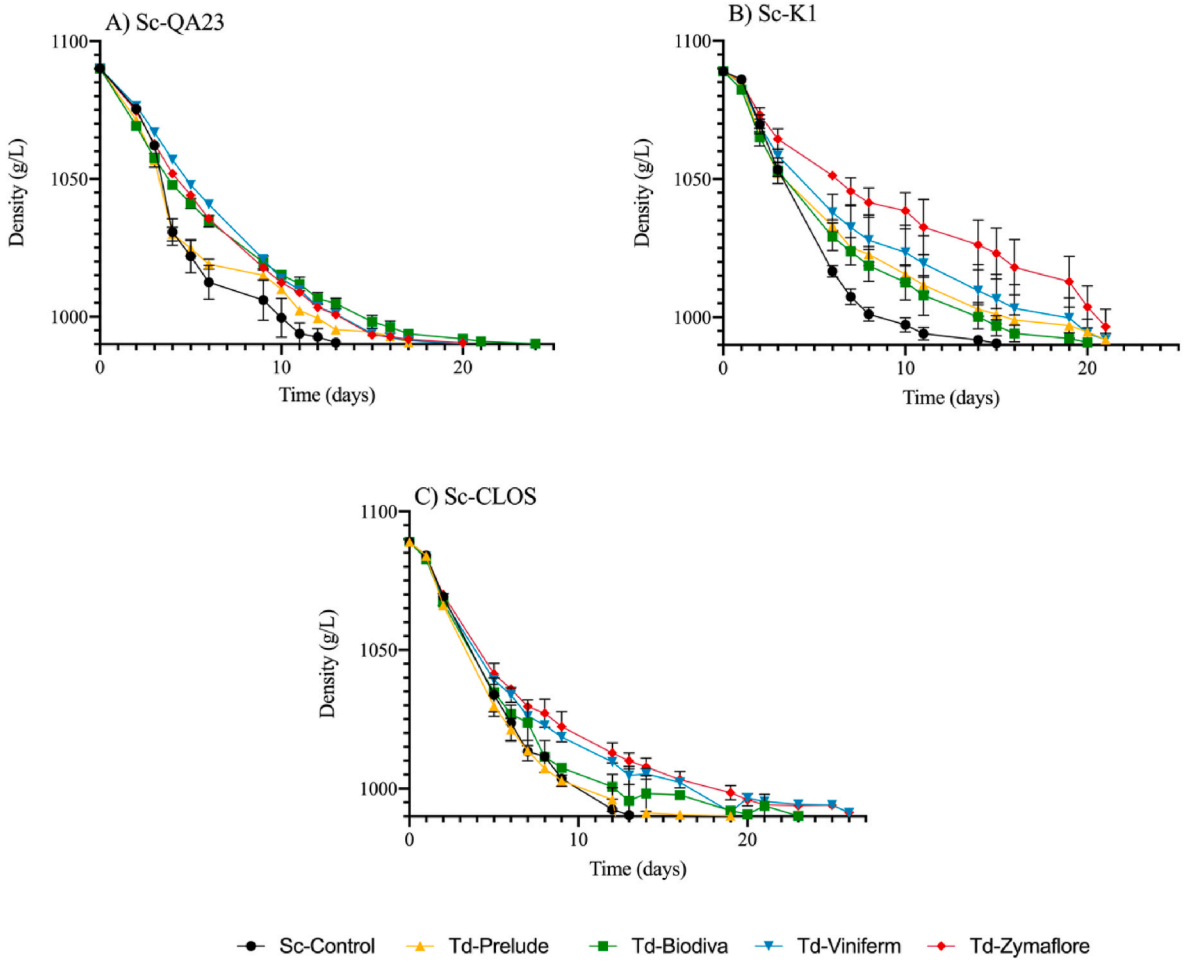

Fig. 1. Evolution of alcoholic fermentation kinetics: AF with the different *S. cerevisiae* strains (Sc): A) Sc-QA23, B) Sc-K1, C) Sc-CLOS. Sc-Control, Td-Biodiva, Td-Prelude, Td-Viniferm and Td-Zymaflore correspond to *S. cerevisiae* control fermentations and *T. delbrueckii* (Biodiva, Prelude, Viniferm and Zymaflore) + *S. cerevisiae* sequential fermentations. ● Sc-Control, ■ Td-Biodiva, ▲ Td-Prelude, ▼ Td-Viniferm and ♦ Td-Zymaflore.

3. Results and discussion

3.1. Sequential alcoholic fermentation

3.1.1. Fermentation kinetics

AFs were performed in the new synthetic must at 22 °C. All the strains showed common viable populations for alcoholic fermentation, starting from the desired initial population. Both control and sequential fermentations finished the AF up to a concentration lower than 2 g/L of sugars in a range of 12–25 days (Table SD2). Despite that, the first 48 h of fermentation with *T. delbrueckii* started a slightly faster consumption of sugars than the controls, mainly in Sc-QA23 and Sc-K1 AF (Fig. 1). There were some differences between controls: AF with Sc-QA23 lasted 13 days (Fig. 1A), AF with Sc-CLOS lasted 13 days (Fig. 1C), and AF with Sc-K1 lasted 15 days (Fig. 1B). The total time of AF increased in sequential fermentations. According to the literature, the slower kinetics in sequential fermentations may be due to nutritional competition between non-*Saccharomyces* and *S. cerevisiae* (Balmaseda et al., 2018; Belda et al., 2015; Martín-García et al., 2020; Romano et al., 2003). Additionally, depending on the combination of *T. delbrueckii* and *S. cerevisiae* strains, differences were observed between the four *T. delbrueckii* strains (Fig. 1, Table SD2). Td-Prelude was the fastest in almost every combination, especially with Sc-QA23, with a final time of 17 days. However, in the Sc-K1 batch, Td-Biodiva finished one day before Td-Prelude. In addition, of note, the fermentation kinetics of Sc-K1 showed a lower slope and consequently a lower consumption rate of sugars, specifically during sequential fermentation with Td-Zymaflore (Fig. 1B).

Consumption of 50% total sugars occurred in the first three, four and five days depending on the type of yeast inoculation (Table SD2), probably because the speed slows down due to an increase in alcohol concentration and a decrease in nutrients (Ribéreau-Gayon, 2006).

3.1.2. Oenological parameters after AF

Several metabolites were analysed after AF with the aim of describing the obtained wine and observing the potential advantages in MLF. Regarding organic acids, there were no substantial changes between the conditions (Table SD2). However, there were differences in relation to the formation of some products depending on the combination *T. delbrueckii/S. cerevisiae* strains (Fig. 2). The presence of Td-Biodiva strains induced less acetic acid formation regardless of the strains of *S. cerevisiae* used (Fig. 2A). Moreover, in Sc-QA23 wines, a reduction in acetic acid production was observed under these conditions when *S. cerevisiae* was inoculated after two days of growth of the *T. delbrueckii* strains. With the other *S. cerevisiae* strains (K1 and CLOS), acetic acid production was dependent on the *T. delbrueckii* strain. The presence of *Torulaspora* strains generally induced less acetic acid formation regardless of the strains of *S. cerevisiae* used (Fig. 2A). The formation of acetic acid as well as glycerol in wine mainly depends on the initial sugar level due to the adaptation mechanism of the *Saccharomyces* strains to a medium with a high sugar concentration (Ribéreau-Gayon , 2006). Sequential inoculation or co-inoculation of *T. delbrueckii* strains with *Saccharomyces* is generally well known to decrease the acetic acid level of natural grape must (red or white) regardless of fermentation types. As reported by several authors, *T. delbrueckii* can go from 0.05 g/L (Balmaseda et al., 2021a, 2021b, 2021c, 2021d) to 0.51 g/L (Canonico et al., 2019), or even 1.6 g/L in a synthetic medium (Contreras et al., 2015).

Normally, the formations of glycerol and ethanol by *Saccharomyces* come respectively from glyceropyruvic and alcoholic fermentations, which influences the yield of the two products in AF (Ribéreau-Gayon ., 2006). In our study, we can observe that strains of *Torulaspora* had lower glycerol concentrations than those obtained by *S. cerevisiae* monocultures (Fig. 2B). Nevertheless, Td-Biodiva seemed to behave differently compared to other *T. delbrueckii* strains in the presence of the Sc-QA23 strain because the glycerol content decreased, whereas it increased in the presence of Td-Viniferm and Td-Zymaflore. Glycerol values were reasonable (5–8 g/L) (Ribéreau-Gayon, 2006), except for monoculture Sc-K1, which showed an overproduction around 10 g/L. Nevertheless, those differences in glycerol could not be related to ethanol reduction (Table SD2) as reported by (Zhu et al. (2020). However, ethanol values were similar under all conditions, near 12% (v/v) without a significant variation.

When a molecule of glycerol is formed, a molecule of pyruvate

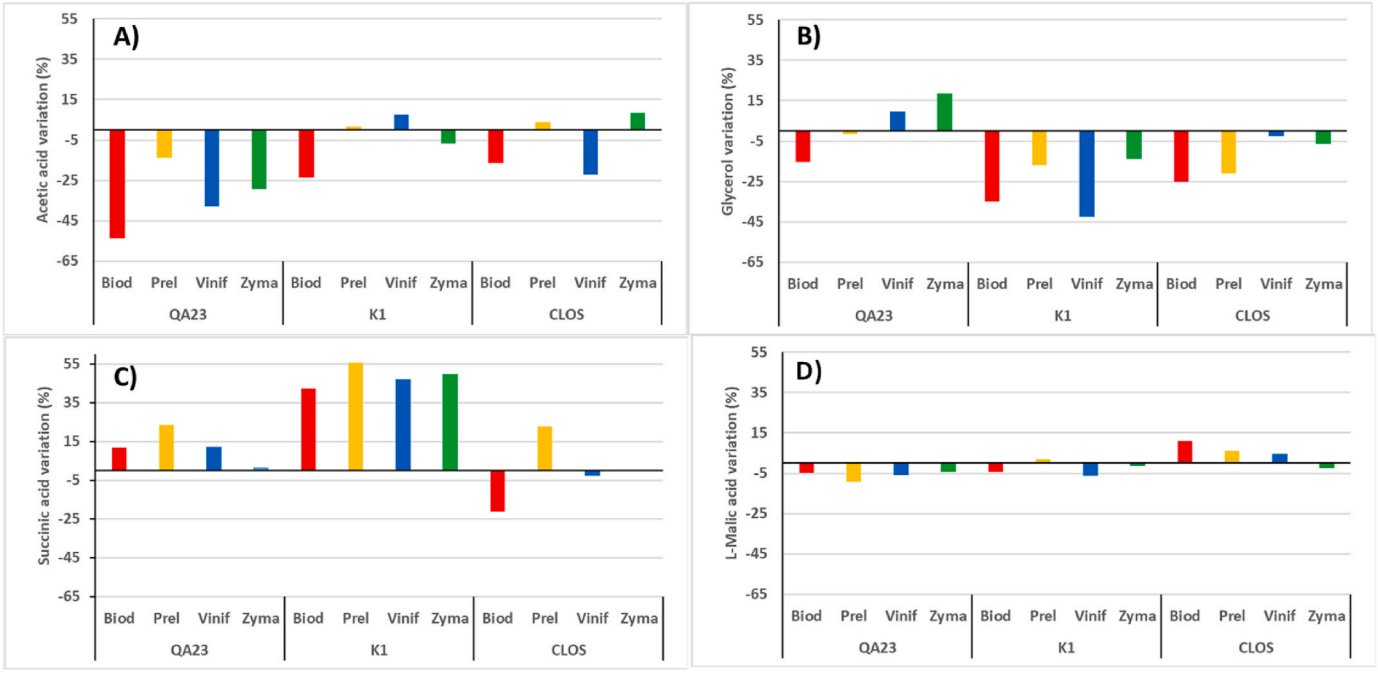

Fig. 2. Variation in the production of acetic acid (A), glycerol (B), succinic acid (C) and L-malic acid (D) at the end of alcoholic fermentation by the *T. delbrueckii/S. cerevisiae* combination compared to the corresponding *S. cerevisiae*. B, P, V and Z correspond to the *T. delbrueckii* strain: Td-Biodiva, Td-Prelude, Td-Viniferm and Td-Zymaflore, respectively. The values expressed the average of three biological replicates for each condition.

cannot be transformed into ethanol after its decarboxylation to ethanal. In AF condition, this pyruvate molecule enters the incomplete citric acid cycle (inactivation of succinodehydrogenase) which leads to the formation of succinic acid (Ribéreau-Gayon et al., 2006). Regarding this by-product of AF, which is described in the literature as a competitive inhibitor of MLF (Lonvaud-Funel et al., 1988; Caridi and Corte, 1997), no significant difference was observed in the final content of the wines (Table SD2). However, depending on the strains used for the sequential inoculation, some variations were observed (Fig. 2C). In fact, the greatest increase in succinic acid compared to the control wines was detected for the Sc-K1 strains.

The greatest incidence of the *S. cerevisiae/T. delbrueckii* was noted in the variation in malic acid consumption (Fig. 2D). The L-malic acid concentration decreased from the initial value of 2 g/L, particularly in Sc-QA23 fermentations, due to the partial consumption by yeast (10–25%) (Ribéreau-Gayon, 2006). Lower L-malic acid consumption was achieved in Sc-K1 fermentations in comparison with Sc-QA23 and Sc-CLOS fermentations. Nevertheless, it was decided to start the MLF with the remaining L-malic acid and not to increase it to the initial concentration, reproducing real winemaking.

Finally, citric acid consumption was generally higher in Td-Prelude fermentations than in other sequential fermentations (Table SD2). Furthermore, this consumption for the Td-Prelude strains was significantly different from that for all *Saccharomyces* strains and Td-Viniferm strains (Fig. SD6).

Mannoproteins are released in wine during the autolysis of yeasts (Guilloux-Benatier et al., 1995). A higher quantity of these compounds was related to a better performance of MLF because *O. oeni* is able to hydrolyse them and metabolise some of the resultant products such as mannose (Alexandre et al., 2004; Balmaseda et al., 2021c). Recent studies have shown that non-*Saccharomyces* species increase the release of mannoproteins (González-Royo et al., 2015). Sequential fermentations with *T. delbrueckii* had significantly higher equivalent mannose values than controls (Fig. 3), especially the Td-Prelude and Td-Viniferm strains.

Concerning the volatile compounds, there were few significant differences between the conditions (Table SD3). However, a trend regarding medium chain fatty acids (MCFAs) was observed. Wines fermented only with *S. cerevisiae* (control fermentations) showed higher values than wines from sequential AF with *T. delbrueckii*. This trend is clearly higher in the Sc-K1 wines. This reduction of MCFA in fermentations performed by non-*Saccharomyces* has been previously shown in other studies (Balmaseda et al., 2021a) and could be related to their binding to mannoproteins (Guilloux-Benatier et al., 1995). In contrast, the corresponding ethyl esters of MCFA were more highly produced by some wines fermented in sequential AF. This fact confirmed the modulation of aroma produced by non-*Saccharomyces* (Padilla et al., 2016). This favourable effect was demonstrated for ethyl octanoate, which increased with *T. delbrueckii* (Viana et al., 2008); in this case, Td-Viniferm showed a tendency towards higher concentrations (Table SD3). Anyway, there is clearly a strain-specificity of non-*Saccharomyces* strains in shaping the aromatic characteristics of wine (Zhang et al., 2021).

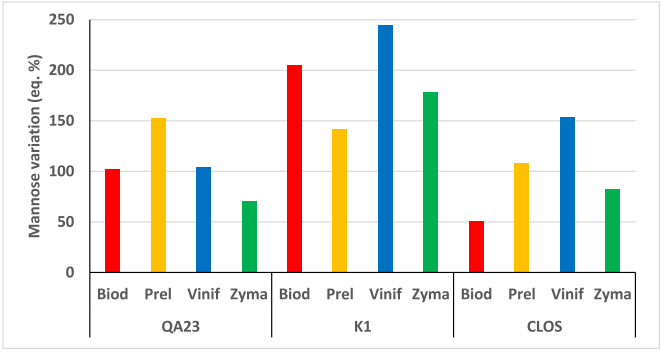

Fig. 3. Variation of mannoproteins (equivalent mannose) at the end of alcoholic fermentation according to the *Saccharomyces cerevisiae* strain used in sequential inoculation respect to *S. cerevisiae* strain alone. Sc and Td correspond to wines fermented with *S. cerevisiae* and *T. delbrueckii*, respectively. B, P, V and Z correspond to the *T. delbrueckii* strain: Td-Biodiva, Td-Prelude, Td-Viniferm and Td-Zymaflore, respectively. The values expressed the average of three biological replicates for each condition.

All abovementioned differences in metabolic parameters among the different conditions are shown in a heatmap (Fig. 4). Fermentation was clustered into two groups according to the *S. cerevisiae* strain used: Sc-QA23 was separated from Sc-K1 and Sc-CLOS. Of note, relevant physiological differences have been found between strains *S. cerevisiae* QA23 and K1; thus, sometimes, Sc-QA23 is considered as *S. cerevisiae bayanus* and Sc-K1 as *S. cerevisiae cerevisiae* (Ruiz-de-Villa et al., 2022). Similarly, Sc-CLOS is considered as *S. cerevisiae* var. *cerevisiae* by the manufacturer.

3.2. Malolactic fermentation

3.2.1. Fermentation kinetics

After AF, all wines were inoculated with the four strains of *O. oeni,* and there were large differences in the behaviour of different combinations (Fig. 5). Of note that almost all MLFs reach the end, leaving the L-malic acid concentration below 0.1 g/L, except for some wines. Thus, it is possible to determine that this must is useful for both fermentations. The goal of using a synthetic must for both fermentations is to standardise the experimental conditions, obtaining more reproducibility compared to the natural musts used in other studies (Ferrando et al., 2020; Martín-García et al., 2020). There are very few studies regarding synthetic musts used for the whole process, AF plus MLF. However, some researchers developed a methodology to study the compatibility between yeasts and LAB (Costello et al., 2003).

A heat map (Fig. 6) was created to determine the most successful MLFs compared to control wines. However, some of the combinations did not finalize the process, resulting in a stuck MLF. For example, in this study, it was verified that the strain *S. cerevisiae* K1 induces a stop of the MLF. Nevertheless, the positive effect of *T. delbrueckii* becomes clear in this case; thus, the main or most of the sequential Sc-K1 improved the final L-malic acid consumption (Fig. 6). Indeed, this synergy was significant in the combination of Oo-VP41 with Td-Prelude, Td-Viniferm and Td-Zymaflore, and L-malic acid was completely consumed. In addition, Td-Prelude with Sc-QA23 and Sc-CLOS improved the performance of MLF with the four strains of *O. oeni* in relation to the control condition and the other *Td* strains. These data are consistent with those reported in the literature describing faster MLF in wines inoculated with *T. delbrueckii* and *S. cerevisiae* in comparison to the wines inoculated only with *S. cerevisiae* (Balmaseda et al., 2021d).

As mentioned above, wines fermented with *T. delbrueckii* showed higher concentrations of equivalent mannose than the control, which may be related to this MLF improvement.

The type of mannoproteins consumed is variable for *O. oeni* strains. It has been described that yeast mannoproteins up to 200 mg/L can positively influence the growth of many LAB and specially commercial mannoproteins of intermediate molecular weight (6–22 kD) activate growth of most of *O. oeni* strains in the presence of ethanol (Diez et al., 2010). In contrast, Sc-K1 fermentations were the ones with the highest mannoprotein values and in general showed negative MLF results. This indicates that there are other inhibiting factors that affected more in this condition.

MCFAs are one of these metabolites that could negatively influence MLFs, especially decanoic and dodecanoic acids, which have been reported to be inhibitors of *O. oeni* (Capucho & San Romao, 1994; Edwards and Beelman, 1987). MCFA content in wines are significantly different between *Saccharomyces* strains and some variations between *Torulaspora* strains were observed (Table SD3). In some wines, the lower presence of MCFA could be correlated with the improvement of the MLF, as in some

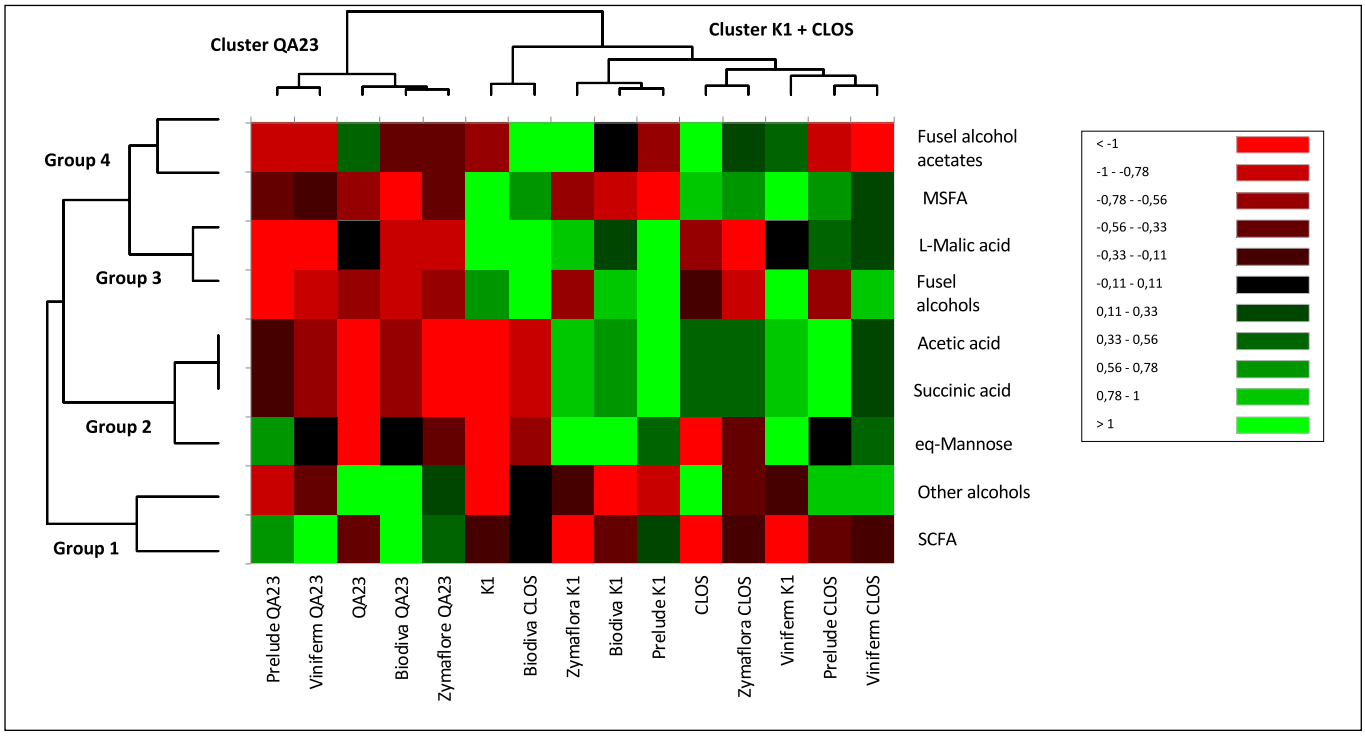

Fig. 4. Heat map of the strains used in this work in relation to the products of alcoholic fermentation (Green higher concentrations, red lower concentrations). Group 1: Short-Chain Fatty Acids + Other alcohols; Group 2: Mannoproteins + Succinic acid + Acetic acid; Group 3: Fusel alcohols + L-malic acid and Group 4. Medium-Chain Fatty Acids + Fusel alcohols acetates. QA23, K1 and CLOS correspond to the *S. cerevisae* strains. B, P, V and Z correspond to the *T. delbrueckii* strains: Td-Biodiva. Td-Prelude. Td-Viniferm and Td-Zymaflore, respectively. The values expressed the average of three biological replicates for each condition. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

of the sequential fermentations.

Looking at *O. oeni* strains, Oo-VP41 was the only strain, which assures the total consumption of L-malic acid in almost all combinations. This strain also improved the MLF duration in some cases; for example, MLF in Td-Prelude – Sc-QA23 with Oo-VP41 required three days while that with Oo-CH11 required 6 days (Table 1). In addition, it is important to highlight that this strain had a significantly better L-malic acid consumption rate than the other strains.

Apart from Oo-VP41, the other strain, which obtained favourable results, was Oo-CH11 and Oo-1Pw13, especially for Sc-CLOS wines. On the other hand, MLFs performed with Oo-PSU-1 were mostly incomplete with the lowest L-malic acid consumption rates. This behaviour was different compared to some results of MLF from natural must. For example, Balmaseda et al. (2021a) observed that in high polyphenolic wines, the strain Oo-PSU-1 showed a better MLF performance than Oo-CH11. The differences found with previous works can be explained by the fact that the behaviour of *O. oeni* strains depend on the specific wine conditions, due to the numerous factors affecting its survival in this harsh environment that can be very variable in wine.

3.2.2. *Wine composition after malolactic fermentation*

Regarding wine metabolites after MLF, it is worth noting that wines with Oo-VP41 had higher citric acid consumption, which is significantly different from Oo-1Pw13 (Table SD2). Moreover, the glycerol content was higher in Oo-VP41 MLF than in Oo-CH11 and Oo-1Pw13.

The difficulty in interpreting the results of MLFs led us to perform multiple linear regressions for each *O. oeni* strain used in this work.

For this, an optimization index (OI) was calculated from three MLF parameters as response variables (Table SD5) and parameters of the chemical composition of wines (SCFA, MCFA, fusel alcohols, fusel alcohol acetates, other alcohols, succinic acid, acetic acid, L-malic acid and mannoproteins) as explanatory variables. From a multiple linear regression calculated according to the best model, we were able to obtain information concerning the behaviour of each LAB strain under our MLF conditions.

As observed in Table 2 and in the results of MLF kinetics, *O. oeni* strains have different behaviours depending on the chemical composition of the wines. The variability of the response variable (OI) was explained by two explanatory variables for each strain, and it was 46, 61.5, 35.2 and 60.9% for the Oo-VP41, Oo-1Pw13, Oo-CH11 and Oo-PSU-1 strains, respectively. For all analyses of total variance, the p-value was less than 0.05 The results in Table 2 also showed that the OI of strain Oo-VP41 was explained by acetic acid and mannoproteins, that of strain Oo-1Pw13 by fusel alcohols and MCFA, that of Oo-CH11 by acetic acid and mannoproteins and that of Oo-PSU by other alcohols and mannoproteins.

Surprisingly, the strains Oo-VP41 and Oo-CH11 were found to be more accommodating to high acetic acid content (positive correlation, equation coefficient of 3.05 and 3.75 respectively) regardless of yeast species tested in fermentation, but negatively (equation coefficient of −5.3 and −4 respectively) related to mannoprotein level which however increased in sequential AF. Of these two variables, the concentration of mannoproteins most influenced the model obtained by this regression analysis. These wines were obtained by the fermentation of the *S. cerevisiae* K1 strain, which hypothetically could have caused a faster lysis of the *T. delbrueckii* strains during AF due to the production of K1 toxin, increasing the mannoprotein content. According to Fig. 3, this pattern could be explained by the fact that the more mannoproteins there are in the wines, the worse is the malolactic fermentation. Nonetheless, this negative correlation must be related to other yeast metabolites not studied in this work because in the literature, a high mannoprotein content is reported to be positive for MLF performance (Diez et al., 2010). For example, Rizk et al. (2018) described that *Saccharomyces cerevisiae* Uvaferm BDX produces nine peptides that are able to inhibit MLF in vitro. Thus, the toxins identified in Sc-K1 (Reiter et al., 2005) could have some implications in the inhibition of *O. oeni.*

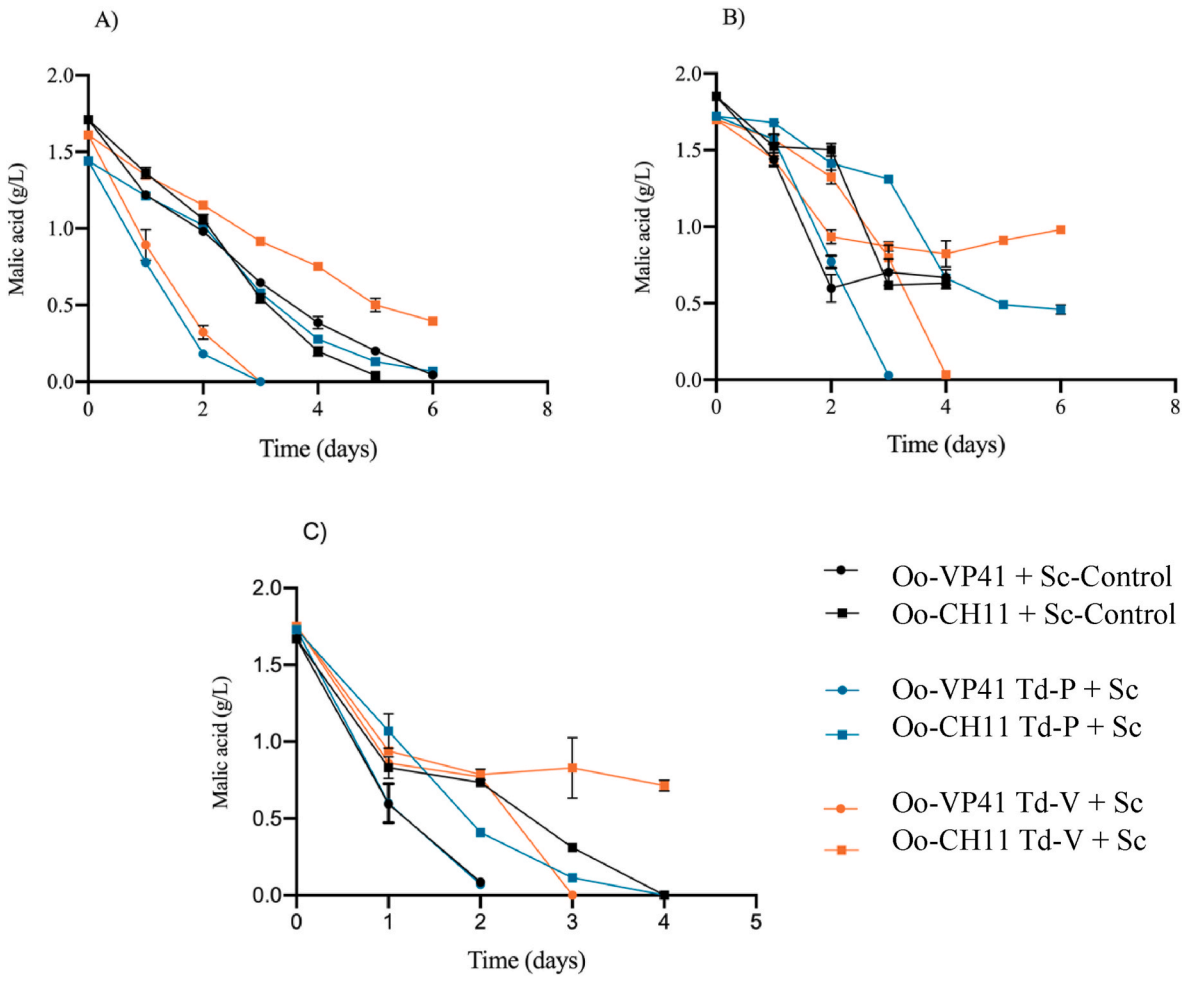

Fig. 5. Evolution of malolactic fermentation kinetics in some of the wines. A) Sc-QA23, B) Sc-K1 and C) Sc-CLOS correspond to wines fermented with *S. cerevisiae* QA23, K1 and CLOS in combination with *T. delbrueckii* strains: Td-Prelude and Td-Viniferm which correspond to P and V respectively. Oo-VP41 and Oo-CH11 corresponds to MLF performed with the *Oenococcus oeni* strains VP41 and CH11.

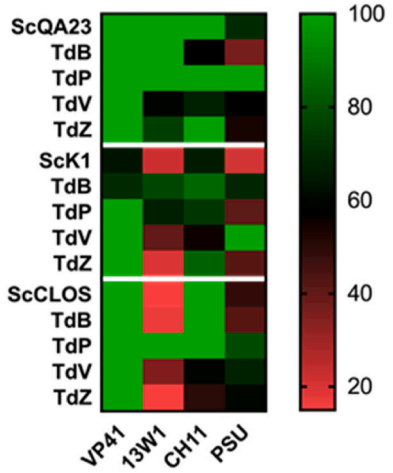

Fig. 6. Heat map of final L-malic consumption percentage in malolactic fermentations with all strain combinations (Green finished MLF. red unfinished MLF). Sc and Td correspond to wines fermented with *S. cerevisiae* and *T. delbrueckii*. respectively. B. P. V and Z correspond to the *T. delbrueckii* strain: Td-Biodiva, Td-Prelude, Td-Viniferm and Td-Zymaflore, respectively. VP41, 1Pw13, CH11 and PSU-1 are the used strains of *O. oeni*. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

In addition, the manufacturers of Lallemand Inc. do not recommend strain K1 for further MLF (Lallemand Inc., n. d.). Thus, from an industrial point of view, the use of this strain in AF may be an interesting option in some vinifications in which MLF is an undesirable process.

Regarding the performance of the strain Oo-1Pw13 in the same wines, the two explanatory variables are fusel alcohols (FA) and medium-chain fatty acids (MCFA) (Table 2). Both variables are negatively related to OI. This may indicate that the higher are the concentrations of FA and MCFA, the more difficult the MLF will be with this yeast combination. However, there is no description of an inhibition caused by these metabolites; therefore, they would not be the only ones producing this inhibition, as discussed above. In general, and this is confirmed by the results in Table SD3, the fewer higher alcohols there were in the wines, the more that 100% of MLF was observed.

The two explanatory variables of strain Oo-CH11 were acetic acid (AcAc) and mannoproteins (Mps) the first being the most influential statistical variable. Finally, the content of mannoproteins and other alcohols has a positive effect on the performance of the MLF produced by the Oo-PSU strain although the first one was statistically the best (Table 2). Indeed, for this strain, its behaviour in the presence of high levels of mannoproteins in the wines seems to improve, although only 20% (3/15 wines) of the MLFs can go to the end. Finally, based on the Type III sum of squares (Table 2), the following variables bring significant information to explain the variability of the OI for each LAB strains: acetic acid and mannoproteins for Oo-VP41, fusel alcohols for Oo-1PW13, acetic acid for Oo-CH11 and mannoproteins for Oo-PSU-1.

Table 1
L-malic acid consumption until stop or final of MLF (percentage and days); consumption rate (g/L·day). Mean ± standard deviation (n = 3). Sc correspond to wines fermented with *S. cerevisiae* and Td to wines fermented with a sequential fermentation of *T. delbrueckii* and *S.cerevisiae*,. B, P, V and Z correspond to the *T. delbrueckii* strains: Td-Biodiva, Td-Prelude, Td-Viniferm and Td-Zymaflore. respectively. Oo-VP41, Oo-1Pw13, Oo-CH11 and Oo-PSU-1 correspond to different *Oenococcus oeni* strains.

Strains	Final L-malic consumption (%)/(days)				Consumption rate (g/L·day)			
	Oo-VP41	Oo-1PW13	Oo-CH11	Oo-PSU-1	Oo-VP41	Oo-1PW13	Oo-CH11	Oo-PSU
Sc-QA23	100.00 (4)	100.00 (6)	100.00 (8)	70.37 (9)	0.33 ± 0.01	0.48 ± 0.03	0.38 ± 0.01	0.17 ± 0.00
Td-B	100.00 (4)	100.00 (5)	57.87 (5)	36.20 (4)	0.36 ± 0.02	0.33 ± 0.01	0.16 ± 0.04	0.10 ± 0.00
Td-P	100.00 (3)	100.00 (4)	100.00 (6)	100.00 (6)	0.63 ± 0.01	0.39 ± 0.02	0.29 ± 0.00	0.23 ± 0.01
Td-V	100.00 (3)	59.35 (4)	67.24 (6)	58.18 (6)	0.64 ± 0.02	0.28 ± 0.01	0.23 ± 0.00	0.18 ± 0.10
Td-Z	100.00 (3)	75.85 (5)	100.00 (5)	53.85 (6)	0.72 ± 0.03	0.37 ± 0.03	0.30 ± 0.01	0.15 ± 0.03
Sc-K1	63.84 (4)	22.04 (4)	66.09 (4)	20.84 (4)	0.63 ± 0.04	0.31 ± 0.03	0.17 ± 0.02	0.16 ± 0.09
Td-B	70.45 (4)	77.84 (5)	85.53 (5)	69.33 (4)	0.45 ± 0.06	0.44 ± 0.00	0.57 ± 0.06	0.51 ± 0.00
Td-P	100.00 (3)	66.74 (5)	73.43 (5)	40.46 (5)	0.47 ± 0.02	0.19 ± 0.03	0.14 ± 0.00	0.25 ± 0.03
Td-V	100.00 (4)	39.90 (6)	55.43 (5)	100.00 (6)	0.19 ± 0.02	0.18 ± 0.06	0.42 ± 0.01	0.31 ± 0.01
Td-Z	100.00 (3)	19.56 (3)	84.78 (4)	41.39 (4)	0.50 ± 0.00	0.30 ± 0.00	0.42 ± 0.09	0.38 ± 0.05
Sc-CLOS	100.00 (2)	14.97 (3)	100.00 (4)	48.50 (5)	0.84 ± 0.04	0.19 ± 0.03	0.33 ± 0.02	0.28 ± 0.09
Td-B	100.00 (3)	16.76 (3)	100.00 (5)	41.62 (5)	0.80 ± 0.06	0.20 ± 0.00	0.41 ± 0.06	0.27 ± 0.00
Td-P	100.00 (2)	100.00 (3)	100.00 (4)	78.61 (4)	0.88 ± 0.02	0.26 ± 0.03	0.56 ± 0.00	0.55 ± 0.03
Td-V	100.00 (3)	35.14 (4)	59.14 (4)	67.71 (4)	0.45 ± 0.02	0.32 ± 0.06	0.45 ± 0.01	0.45 ± 0.01
Td-Z	100.00 (3)	15.31 (3)	50.63 (3)	59.69 (5)	0.41 ± 0.00	0.23 ± 0.00	0.34 ± 0.09	0.40 ± 0.05

Table 2
Multiple linear regression results. R2. Coefficient of regression; Type III sum of squares analysis. variables providing significant information to explain the variability of the Total dependent variable; AcAc, acetic acid; Mps, Mannoproteins; FA, Fusel alcohols; FAA, Fusel alcohols acetates; SCFA, Short-chain fatty acids; MCFA, Medium-chain fatty acids; OAl. Other alcohols (2-butanol, isopropanol); Coef. Coefficient.

	Oenococcus oeni strains			
	Oo-VP41	Oo-1PW13	Oo-CH11	Oo-PSU-1
R^2	0.463	0.565	0.436	0.499
Adjusted R^2	0.373	0.492	0.341	0.415
p-value of variance	0.024	0.007	0.0032	0.016
Explanatory variables (p-value of type III sum of squares analysis)				
First variable	AcAc (0.017)	MCFA (0.163)	AcAc (0.010)	OAl (0.105)
Second variable	Mps (0.012)	FA (0.013)	Mps (0.068)	Mps (0.005)
Equation of the model				
Intercept	1.192	2.36	0.714	1.274
Coef first variable	3.050	−0.099	3.749	0.061
Coef. second variable	−5.275	−0.119	−4.003	5.136

Results of the linear regressions, although limited to the conditions of our study, show that for the same chemical composition of the wines, the LAB strains prepared under the same conditions are differently sensitive to the metabolites of the wine. Many factors and metabolites affect the compatibility between yeast and *O. oeni* strains. As we have seen, not only non-*Saccharomyces* species influence MLF (Balmaseda et al., 2018, 2021d; Ferrando et al., 2020), but *S. cerevisiae* strains can also inhibit or promote *O. oeni* development (Arnink and Henick-Kling, 2005; Comitini and Ciani, 2007; Osborne and Edwards, 2007). Thus, it becomes clear that a correct choice of the right yeast-bacteria starter culture combination is very important for a successful MLF.

4. Conclusion

In this study, fifteen alcoholic fermentations were combined with four strains of *O. oeni,* resulting in a total of 60 combinations. Most of them showed different behaviours depending on the *T. delbrueckii*, *S. cerevisiae* and *O. oeni* strains used. Wines fermented with Sc-K1 are unsuitable for performing MLF, which confirms the manufacturer's recommendation. However, this strain can be recommended for the fermentation of wines in which MLF is not desired. On the other hand, the positive effect of *T. delbrueckii* was highlighted in combinations of AF with Td-Prelude and Sc-QA23 or Sc-CLOS followed by MLF with *O. oeni* VP41. This blend assures the end of MLF as well as a good L-malic acid consumption rate. It is possible to conclude that the performance of MLF under these conditions depends on the strains of yeasts/lactic acid bacteria used. Other studies, such as the optimal moment of presence of *T. delbrueckii* in the must before inoculation with *S. cerevisiae,* will allow to determine the most favourable conditions for the realization of the MLF. This study also confirms that the sequential use of *Torulaspora* strains during AF induces a low level of acetic acid and MCFA in wines, negative factors for performing MLF. On the other hand, the content of MCFA ethyl esters and fusel alcohol acetates has increased, which makes it possible to obtain more aromatic wines.

Further studies should focus on *Torulaspora/Saccharomyces* combinations in order to know the effect of the *Saccharomyces* strain on the lysis of *Torulaspora* along the AF, which could induce a release of nitrogen or another nutrient favourable to the realization of the MLF.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2022.104212.

References

Alexandre, H., Costello, P.J., Remize, F., Guzzo, J., Guilloux-Benatier, M., 2004. *Saccharomyces cerevisiae-Oenococcus oeni* interactions in wine: current knowledge and perspectives. Int. J. Food Microbiol. 93, 141–154. https://doi.org/10.1016/j.ijfoodmicro.2003.10.013.

Arnink, K., Henick-Kling, T., 2005. Influence of *Saccharomyces cerevisiae* and *Oenococcus oeni* strains on successful malolactic conversion in wine. Am. J. Enol. Vitic. 56 (3), 228–237. https://doi.org/10.5344/ajev.2019.19061.

Balmaseda, A., Bordons, A., Reguant, C., Bautista-Gallego, J., 2018. Non-*Saccharomyces* in wine: effect upon *Oenococcus oeni* and malolactic fermentation. Front. Microbiol. 9, 534. https://doi.org/10.3389/fmicb.2018.00534.

Balmaseda, A., Aniballi, L., Rozès, N., Bordons, A., Reguant, C., 2021 b. Use of yeast mannoproteins by *Oenococcus oeni* during malolactic fermentation under different oenological conditions. Foods 10, 1540. https://doi.org/10.3390/foods10071540.

Balmaseda, A., Rozès, N., Bordons, A., Reguant, C., 2021a. *Torulaspora delbrueckii* promotes malolactic fermentation in high polyphenolic red wines. LWT 148. https://doi.org/10.1016/j.lwt.2021.111777.

Balmaseda, A., Rozès, N., Bordons, A., Reguant, C., 2021c. Simulated lees of different yeast species modify the performance of malolactic fermentation by *Oenococcus oeni* in wine-like medium. Food Microbiol. 99 https://doi.org/10.1016/j.fm.2021.103839.

Balmaseda, A., Rozès, N., Leal, M.Á., Bordons, A., Reguant, C., 2021d. Impact of changes in wine composition produced by non-*Saccharomyces* on malolactic fermentation. Int. J. Food Microbiol. 337 https://doi.org/10.1016/j.ijfoodmicro.2020.108954.

Bartowsky, E., Costello, P., Chambers, P., 2015. Emerging trends in MLF. Aust. J. Grape Wine Res. 21, 663–669. https://doi.org/10.1111/ajgw.12185.

Belda, I., Navascués, E., Marquina, D., Santos, A., Calderon, F., Benito, S., 2015. Dynamic analysis of physiological properties of *Torulaspora delbrueckii* in wine fermentations and its incidence on wine quality. Appl. Microbiol. Biotechnol. 99, 1911–1922. https://doi.org/10.1007/s00253-014-6197-2.

Belda, I., Ruiz, J., Esteban-Fernández, A., Navascués, E., Marquina, D., Santos, A., Moreno-Arribas, M.V., 2017. Microbial contribution to wine aroma and its intended use for Wine quality improvement. Molecules 22 (2), 1–29. https://doi.org/10.3390/molecules22020189.

Beltran, G., Torija, M.J., Novo, M., Ferrer, N., Poblet, M., Guillamón, J.M., Rozés, N., Mas, A., 2002. Analysis of yeast populations during alcoholic fermentation: a six-year follow-up study. Syst. Appl. Microbiol. 25 (2), 287–293. https://doi.org/10.1078/0723-2020-00097.

Borrull, A., López-Martínez, G., Miró-Abella, E., Salvadó, Z., Poblet, M., Cordero-Otero, R., Rozès, N., 2016. New insights into the physiological state of Saccharomyces cerevisiae during ethanol acclimation for producing sparkling wines. Food Microbiol. 4, 20–29. https://doi.org/10.1016/j.fm.2015.11.001.

Canonico, L., Solomon, M., Comitini, F., Ciani, M., Varela, C., 2019. Volatile profile of reduced alcohol wines fermented with selected non-*Saccharomyces* yeasts under different aeration conditions. Food Microbiol. 84, 103247 https://doi.org/10.1016/j.fm.2019.103247Capucho.

Caridi, A., Corte, V., 1997. Inhibition of malolactic fermentation by cryotolerant yeasts. Biotechnol. Lett. 19, 723–726. https://doi.org/10.1023/A:1018319705617.

Carpena, M., Fraga-Corral, M., Otero, P., Nogueira, R.A., Garcia-Oliveira, P., Prieto, M. A., Simal-Gandara, J., 2021. Secondary aroma: influence of wine microorganisms in their aroma profile. Foods 10, 51. https://doi.org/10.3390/foods10010051.

Carrau, F., Henschke, P.A., 2021. *Hanseniaspora vineae* and the concept of friendly yeasts to increase autochthonous wine flavor diversity. Front. Microbiol. 12, 51. https://doi.org/10.3389/fmicb.2021.702093.

Chambers, P.J., Pretorius, I.S., 2010. Fermenting knowledge: the history of winemaking, science and yeast research. EMBO Rep. 11 (12), 914–920. https://doi.org/10.1038/embor.2010.179.

Comitini, F., Ciani, M., 2007. The inhibitory activity of wine yeast starters on malolactic bacteria. Ann. Microbiol. 57 (1), 61–66. https://doi.org/10.1007/BF03175051.

Contreras, A., Hidalgo, C., Schmidt, S., Henschke, P.A., Curtin, C., Varela, C., 2015. The application of non-Saccharomyces yeast in fermentations with limited aeration as a strategy for the production of wine with reduced alcohol content. Int. J. Food Microbiol. 205, 7–15. https://doi.org/10.1016/j.ijfoodmicro.2015.03.027.

Costello, P.J., Henschke, P., Markides, J., A, J., 2003. Standardised methodology for testing malolactic bacteria and wine yeast compatibility. Aust. J. Grape Wine Res. 9, 127–137. https://doi.org/10.1111/j.1755-0238.2003.tb00263.x.

Diez, L., Guadalupe, Z., Ayestarán, B., Ruiz-Larrea, F., 2010. Effect of yeast mannoproteins and grape polysaccharides on the growth of wine lactic acid and acetic acid bacteria. J. Agric. Food Chem. 58 (13), 7731–7739. https://doi.org/10.1021/jf100199n.

Du Plessis, H.W., Du Toit, M., Hoff, J.W., Hart, R.S., Ndimba, B.K., Jolly, N.P., 2017. Characterisation of non-*Saccharomyces* yeasts using different methodologies and evaluation of their compatibility with malolactic fermentation. S. Afr. J. Enol. Vitic. 38, 46–63. https://doi.org/10.21548/38-1-819.

Du Plessis, H., Du Toit, M., Nieuwoudt, H., Van der Rijst, M., Hoff, J., Jolly, N., 2019. Modulation of wine flavor using *Hanseniaspora uvarum* in combination with different *Saccharomyces cerevisiae*, lactic acid bacteria strains and malolactic fermentation strategies. Fermentation 5 (3), 64. https://doi.org/10.3390/fermentation5030064.

Edwards, C.G., Beelman, R.B., 1987. Inhibition of the malolactic bacterium, *Leuconostoc oenos* (PSU-1), by decanoic acid and subsequent removal of the inhibition by yeast ghosts. Am. J. Enol. Vitic. 38, 239–242.

Englezos, V., Jolly, N.P., Di Gianvito, P., Rantsiou, K., Cocolin, L., 2022. Microbial interactions in winemaking: ecological aspects and effect on wine quality. Trends Food Sci. Technol. 127, 99–113. https://doi.org/10.1016/j.tifs.2022.06.015.

Fairbairn, S., Engelbrecht, L., Setati, M.E., du Toit, M., Bauer, F.F., Divol, B., Rossouw, D., 2021. Combinatorial analysis of population dynamics, metabolite levels and malolactic fermentation in *Saccharomyces cerevisiae/Lachancea thermotolerans* mixed fermentations. Food Microbiol. 96, 103712 https://doi.org/10.1016/j.fm.2020.103712.

Ferrando, N., Araque, I., Ortís, A., Thornes, G., Bautista-Gallego, J., Bordons, A., Reguant, C., 2020. Evaluating the effect of using non-*Saccharomyces* on *Oenococcus oeni* and wine malolactic fermentation. Food Res. Int. 138 https://doi.org/10.1016/j.foodres.2020.109779.

González-Royo, E., Pascual, O., Kontoudakis, N., Esteruelas, M., Esteve-Zarzoso, B., Mas, A., Canals, J.M., Zamora, F., 2015. Oenological consequences of sequential inoculation with non-*Saccharomyces* yeasts (*Torulaspora delbrueckii or Metschnikowia pulcherrima*) and *Saccharomyces cerevisiae* in base wine for sparkling wine production. Eur. Food Res. Technol. 240 (5), 999–1012. https://doi.org/10.1007/s00217-014-2404-8.

Guilloux-Benatier, M., Guerreau, J., Feuillat, M., 1995. Influence of initial colloid content on yeast macromolecule production and on the metabolism of wine microorganisms. Am. J. Enol. Vitic. 46 (4), 486–492.

Hranilovic, A., Gambetta, J.M., Jeffery, D.W., Grbin, P.R., Jiranek, V., 2020. Lower-alcohol wines produced by *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae* co-fermentations: the effect of sequential inoculation timing. Int. J. Food Microbiol. 329 https://doi.org/10.1016/j.ijfoodmicro.2020.108651.

Jolly, N.P., Varela, C., Pretorius, I.S., 2014. Not your ordinary yeast: non-*Saccharomyces* yeasts in wine production uncovered. FEMS Yeast Res. 14 (2), 215–237. https://doi.org/10.1111/1567-1364.12111.

Lonvaud-Funel, A., Joyeux, A., Desens, C., 1988. Inhibition of malolactic fermentation of wines by products of yeast metabolism. J. Sci. Food Agric. 44, 183–191. https://doi.org/10.1002/jsfa.2740440209.

Martín-García, A., Balmaseda, A., Bordons, A., Reguant, C., 2020. Effect of the inoculation strategy of non-*Saccharomyces* yeasts on wine malolactic fermentation. Oeno One 54 (1), 101–108. https://doi.org/10.20870/oeno-one.2020.54.1.2906.

Ortega, C., Lopez, R., Cacho, J., Ferreira, V., 2001. Fast analysis of important wine volatile compounds. Development and validation of a new method based on gas chromatographic-flame ionisation detection analysis of dichloromethane microextracts. J. Chromatogr. A 923, 205–214. https://doi.org/10.1016/s0021-9673(01)00972-4. www.elsevier.com/locate/chroma.

Osborne, J.P., Edwards, C.G., 2007. Inhibition of malolactic fermentation by a peptide produced by *Saccharomyces cerevisiae* during alcoholic fermentation. Int. J. Food Microbiol. 118 (1), 27–34. https://doi.org/10.1016/j.ijfoodmicro.2007.05.007.

Padilla, B., Gil, J.V., Manzanares, P., 2016. Past and future of non-*Saccharomyces* yeasts: from spoilage microorganisms to biotechnological tools for improving wine aroma complexity. Front. Microbiol. 7 https://doi.org/10.3389/fmicb.2016.00411.

Paramithiotis, S., Stasinou, V., Tzamourani, A., Kotseridis, Y., Dimopoulou, M., 2022. Malolactic fermentation—theoretical advances and practical considerations. Fermentation 8 (10), 521. https://doi.org/10.3390/fermentation8100521.

Quirós, M., Rojas, V., González, R., Morales, P., 2014. Selection of non-*Saccharomyces* yeast strains for reducing alcohol levels in wine by sugar respiration. Int. J. Food Microbiol. 181, 85–91. https://doi.org/10.1016/j.ijfoodmicro.2014.04.024.

Ramírez, M., Velázquez, R., Maqueda, M., Zamora, E., López-Piñeiro, A., Hernández, L. M., 2016. Influence of the dominance of must fermentation by *Torulaspora delbrueckii* on the malolactic fermentation and organoleptic quality of red table wine. Int. J. Food Microbiol. 238, 311–319. https://doi.org/10.1016/j.ijfoodmicro.2016.09.029.

Reiter, J., Herker, E., Madeo, F., Schmitt, M.J., 2005. Viral killer toxins induce caspase-mediated apoptosis in yeast. J. Cell Biol. 168 (3), 353–358. https://doi.org/10.1083/jcb.200408071.

Ribéreau-Gayon, P., 2006. Handbook of Enology, second ed. John Wiley & Sons Ltd, England.

Rizk, Z., Rayess, Y., Ghanem, C., Mathieu, F., Taillandier, P., Nehme, N., 2018. Identification of multiple-derived peptides produced by *Saccharomyces cerevisiae* involved in malolactic fermentation inhibition. FEMS Yeast Res. 18 (7) https://doi.org/10.1093/femsyr/foy080.

Romano, P., Fiore, C., Paraggio, M., Caruso, M., Capece, A., 2003. Function of yeast species and strains in wine flavour. Int. J. Food Microbiol. 86 (1–2), 169–180. https://doi.org/10.1016/S0168-1605(03)00290-3.

Rossouw, D., Bauer, F.F., 2016. Exploring the phenotypic space of non-*Saccharomyces* wine yeast biodiversity. Food Microbiol. 55, 32–46. https://doi.org/10.1016/j.fm.2015.11.017.

Ruiz-de-Villa, C., Poblet, M., Bordons, A., Reguant, C., Rozès, N., 2022. Differentiation of *Saccharomyces* species by lipid and metabolome profiles from a single colony. Food Microbiol. 103 https://doi.org/10.1016/j.fm.2021.103964.

Russo, P., Englezos, V., Capozzi, V., Pollon, M., Río Segade, S., Rantsiou, K., Spano, G., Cocolin, L., 2020. Effect of mixed fermentations with *Starmerella bacillaris* and *Saccharomyces cerevisiae* on management of malolactic fermentation. Food Res. Int. 134, 109246 https://doi.org/10.1016/j.foodres.2020.109246.

San Romão, I., M, V., 1994. Effect of ethanol and fatty acids on malolactic activity of Leuconostoc oenos. Appl. Microbiol. Biotechnol. 42, 391–395. https://doi.org/10.1007/BF00902747.

Torres-Guardado, R., Esteve-Zarzoso, B., Reguant, C., Bordons, A., 2022. Microbial interactions in alcoholic beverages. Int. Microbiol. 25 (1) https://doi.org/10.1007/s10123-021-00200-1.

Viana, F., Gil, J.V., Genovés, S., Vallés, S., Manzanares, P., 2008. Rational selection of non-Saccharomyces wine yeasts for mixed starters based on ester formation and enological traits. Food Microbiol. 25 (6), 778–785. https://doi.org/10.1016/j.fm.2008.04.015.

Zhang, B., Ivanova-Petropulos, V., Duan, C., Yan, G., 2021. Distinctive chemical and aromatic composition of red wines produced by *Saccharomyces cerevisiae* co-

fermentation with indigenous and commercial non-*Saccharomyces* strains. Food Biosci. 41, 100925 https://doi.org/10.1016/j.fbio.2021.100925.

Zhu, X., Navarro, Y., Mas, A., Torija, M.J., Beltran, G., 2020. A rapid method for selecting non-*Saccharomyces* strains with a low ethanol yield. Microorganisms 8 (5). https://doi.org/10.3390/microorganisms8050658.

Zhu, X., Torija, M.J., Mas, A., Beltran, G., Navarro, Y., 2021. Effect of a multistarter yeast inoculum on ethanol reduction and population dynamics in wine fermentation. Foods 10 (3). https://doi.org/10.3390/foods10030623.