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# Sequential inoculation of *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* in rosé wines enhances malolactic fermentation and potentially improves colour stability

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ABSTRACT

In rosé wines, colour influences consumer preferences and oenological trends. In this study, we propose an alternative approach employing *Torulaspora delbrueckii* (Td) as a biotechnological tool for colour modulation, coupled with its potential to enhance malolactic fermentation (MLF), necessary in certain rosé wines. Sequential alcoholic fermentations (AF) were conducted using Cabernet Sauvignon grape must, with inoculations of *Torulaspora delbrueckii* (Td) and *Saccharomyces cerevisiae* (Sc). Subsequently, different strains of Oenococcus oeni were employed to carry out MLF on the resulting rosé wines, allowing us to assess the impact of Td strains under these conditions. The formation of anthocyanins and pyranoanthocyanins depended on the specific strains of Td used, resulting in wines with varying hues, ranging from a more yellowish tone (Td + ScQ) to a bluish tone (Td + ScK1). Additionally, the performance of MLF displayed a positive synergy between Td and *Oenococcus oeni*, with outcomes varying depending on the strain combinations employed. In conclusion, this research highlights the potential of reducing total anthocyanins while maintaining a higher proportion of pyranoanthocyanins, offering an interesting technique for rosé wines with a lighter color intensity. This approach aligns well with evolving oenological trends, as certain regions prefer such rosé wine type

## 1. Introduction

The presence of microbiota in grapes and the cellar environment plays a significant role in the final complexity of wine (Belda et al., 2017; Beltran, et al., 2008). In recent years, studies have investigated the use of diverse microbiota, especially non-*Saccharomyces* yeasts, in alcoholic fermentation (AF) (Jolly et al., 2014; Padilla et al., 2016). Currently, there is significant interest in using non-*Saccharomyces* yeasts as starter cultures together with *Saccharomyces cerevisiae* because their interesting organoleptic characteristics and technological aspects, such as an improvement on foam of sparkling wines (González-Royo et al., 2015; Vejarano & Gil-Calderón, 2021; Viana et al., 2008). It is essential to discuss *Torulaspora delbrueckii* because of its relevance in biotechnology as it is commercially available and used in the fermentation of wine, beer or bread dough, having good resistance to osmotic stress and freezing (Fernandes et al., 2021). Studies have stated that utilizing *T. delbrueckii* can lead to significant changes in the characteristics of the final product, as the production of acetic acid is generally reduced and the ethanol content in wine can be lowered, which may be desirable in certain contexts (Benito, 2018). Furthermore, due to the high enzymatic activity exhibited by some strains of this species, the concentration of some volatile compounds in wine can be increased (Azzolini et al., 2015; Carpena et al., 2021; Renault et al., 2015). For example, a high production of total esters and other volatile compounds has been reported (Balmaseda et al., 2021a; Renault et al., 2015), although this aroma modulation depends on the specific strain and the population of *T. delbrueckii* and *S. cerevisiae* present (Renault et al., 2015). While most research has focused on aroma modification, *T. delbrueckii* has been shown to exhibit a significant impact on the colour of wine. Studies have demonstrated that in presence of *T. delbrueckii* the anthocyanin

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concentration increases in red wines (Balmaseda, et al., 2021a; Escribano-Viana et al., 2019; Minnaar et al., 2018). All these effects could be interesting in rosé wines. Although the technological process for *rosé* wines is similar to that for white wines, achieving the desired colour is crucial for these wines. Different nuances and intensities can be achieved by adjusting the process of skin maceration for red grape varieties; for example, for pale *rosé* wines, shorter maceration times are needed, which can result in low release of aromas (Ribéreau-Gayon et al., 2006).

In general, rosé wines are known for their freshness. However, some of these wines must undergo malolactic fermentation (MLF) to achieve greater complexity. This can be a challenging process with *rosé* wines due to the amount of sulphur dioxide necessary to protect their colour. Some studies have shown that *T. delbrueckii* can promote MLF, since the presence of this specie is related with more suitable conditions after AF for the development of *O. oeni,* even in red wines (Balmaseda et al., 2021a). This effect could be particularly useful for rosé wines, and several studies have demonstrated the potential of *T. delbrueckii* in this regard (Balmaseda, et al., 2021c; Ruiz-de-Villa et al., 2023).

The aim of this study was to investigate the potential of *T. delbrueckii* in improving Cabernet Sauvignon rosé wines in several ways. Specifically, the use of *T. delbrueckii* was proposed as a method to modulate colour, preserve aroma, and improve MLF. To achieve this goal, various strains of *S. cerevisiae*, *T. delbrueckii*, and *O. oeni* were tested with the aim of identifying the optimal combination to achieve the aforementioned improvements. From an industrial perspective, the results of this study could have significant implications for the production of high-quality rosé wines.

## 2. Materials and methods

### 2.1. Microorganism strains and inocula

The following *S. cerevisiae* strains were used in this work: Lalvin-QA23 (ScQ) and ICV K1 Marquée (ScK1) from Lallemand S.A. (Montreal, Canada). For sequential fermentations, the following strains of *T. delbrueckii* were also tested: *Biodiva TD291* (TdB, Lallemand S.A.) and Vinoflora Prelude (TdB, Chr. Hansen Holding AS, Hoersholm, Denmark). The inocula were prepared from dry active yeast as recommended by the manufacturers for 30 min at 37 °C for *S cerevisiae* strains and 30 °C for *T. delbrueckii* strains. Fermentation flasks were inoculated with an initial population of $2{\cdot}10^6$ cells/mL.

Regarding MLF, the following strains of *O. oeni* strains were used: Lalvin VP41 (Oo-VP41, Lallemand S.A.), Viniflora CH11 (Oo-CH11, Chr. Hansen Holding AS), 1Pw13 (Oo-1Pw13, own collection), and PSU-1 (Oo-PSU-1, American Type Culture Collection BAA- 331). These strains were replicated from isolated colonies and grown in MRS broth (Difco Laboratories, Detroit, MI, USA) medium (De Man et al., 1960) modified following the procedure described in Margalef-Català et al. (2017) at pH 5 supplemented with 4 g/L DL-malic acid (Sigma–Aldrich, Barcelona, Spain) and 5 g/L D-fructose (Panreac, Barcelona, Spain). Then, the inocula were prepared from a preculture in 50 mL of modified MRS until the final phase of exponential growth. The inoculation volume was calculated with growth curves, depending on the strain. The growing conditions were 27 °C in a 10% $CO_2$ atmosphere. *O. oeni* populations were determined by plating on modified MRS plates, containing 2% (w/v) agar (Panreac) and supplemented with 100 mL/L of centrifuged tomato juice (Aliada, Madrid, Spain), 100 mg/L of nystatin (Panreac) to avoid yeast growth and 25 mg/L of sodium azide (Bio-Sciences, St. Louis MO, USA) to prevent acetic acid bacteria growth.

### 2.2. Fermentation conditions

Must from a Cabernet Sauvignon grape variety (*Vitis vinifera* L.) was used for all fermentations. Grapes were harvested and processed in the experimental winery *Mas dels Frares* of *Rovira i Virgili* University (41°08′44.1″N 1°11′51.0″E), which belongs to the AOC Tarragona, during the 2022 vintage harvest. Before clarification at 7 °C without any addition of oenological products, sulphur dioxide at 50 mg/L was added in the grape must and macerated for 2 h at 25 °C to extract the colour. After that, the clear must was treated for 24 h with 1 mL/L dimethyl dicarbonate (Fisher Scientific, Hampton, USA) to eliminate the largest possible population of microorganisms present and to be able to better evaluate how the strains inoculated behave. No nutrient supplementation in the form of added nitrogen or thiamine was used in the fermentations. Alcoholic fermentation (AF) was performed in 500 mL bottles filled with 450 mL of must at 22 °C. The bottle was closed using a system of two valves, allowing sample extraction to be performed and carbon dioxide to be released. The initial parameters of grape must was as follows: density 1102.4 g/L, pH 3.6, titratable acidity 3.75 g tartaric acid/L, 48 mg N/L α-amino nitrogen (NOPA) and 17 mg N/L $NH_4$.

The following groups were utilized in triplicate: control ScQ, control ScK1, sequential TdB + ScQ, sequential TdP + ScQ, sequential TdB + ScK1 and sequential TdP + ScK1. These sequential fermentations were inoculated first with *T. delbrueckii,* and after 48 h of AF with *S. cerevisiae*, in an initial population of $2{\cdot}10^6$ cells/mL, a viable inoculum population was determined by plating a 1:10 serial dilution in YPD agar (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, 17 g/L agar, Panreac).

AF monitoring was performed every day by measuring the density of the centrifuged samples using an electronic densimeter (Densito 30PX Portable Density Metre (Mettler Toledo, Barcelona, Spain). AF was estimated to end when the glucose/fructose concentration was below 2 g/L, analysed enzymatically using a Y15 Enzymatic Autoanalyzer (Biosystems S. A, Barcelona, Spain).

Prior to MLF, wines were stabilized for 4 days at 4 °C. Then, the wines were centrifuged at $3000{\times}g$ for 15 min at 4 °C and sterilized by filtration with a 0.22 μm membrane (Merck, Germany). With the objective of reducing variability, triplicates were mixed and divided again for each condition. The final concentration of L-malic acid was corrected to obtain an initial concentration of 2 g/L, and the pH was adjusted before L-malic acid was added. At this time, MLF was performed in small volumes of 50 mL at 20 °C in anaerobic and static conditions. Each *O. oeni* strain was inoculated to reach an initial population of $2{\cdot}10^7$ cells/mL. The consumption of L-malic acid was measured daily up to a concentration lower than 0.1 g/L using the Y15 Enzymatic Autoanalyzer (Biosystems).

### 2.3. Calculating the area under the curve (AUC)

To evaluate the performance of fermentation, the area under the curve (AUC) was measured by analysing the decrease in density during AF and consumption of L-malic acid during MLF. This approach allowed us to assess fermentation performance independent of total fermentation times and kinetics. The AUCs were calculated by integrating the density decrease during AF or L-malic acid consumption during MLF between two consecutive time points according to García-Ríos and Guillamón (2019). The formula used for the calculation was the sum of consecutive AUCs, which was obtained by summing the areas of consecutive data points as follows: Σ [((d2+d1)/2) * (t2-t1) + …. + ((dn + dn-1)/2) * (tn-tn-1)], where d1, d2, …dn-1, dn represent the densities or L-malic acid values at times 1, 2, n-1, and n, respectively.

### 2.4. Physico-chemical parameter analysis

Citric acid, glycerol and ethanol were determined by using an Agilent 1100 HPLC (Agilent Technologies, Waldbronn, Germany) according to Quirós et al. (2014). Wine samples were filtered with 0.22 μm pore filters (Merck) before injection. The HPLC was equipped with a Hi-Plex H column (300 mm × 7.7 mm) inside a 1260 MCT (Infinity II Multicolumn Thermostat) with two detectors, an MWC detector (Multiwavelength detector, Agilent Technologies) and an RID detector (1260 Infinity II refractive index detector, Agilent Technologies). The column temperature was maintained at 60 °C for a 30 min run time, and the mobile

phase was 5 mM $H_2SO_4$ with a flow rate of 0.6 ml $min^{-1}$. The injection volume was 10 μL.

In addition, acetic acid, L-malic acid, α-amino nitrogen, $NH_4$, pyruvic acid and acetaldehyde were enzymatically measured by a Y15 Enzymatic Autoanalyzer (Biosystems). Succinic acid was also analysed by an enzymatic method with microplates (Megazyme, Wicklow, Ireland) using the POLARstar Omega (BMG LABTECH, Ortenberg, Germany).

To estimate the mannoproteins present, mannoprotein precipitation was first performed with 95% ethanol from *rosé* wines, and then acid hydrolysis at 90 °C was performed, which led to the release of mannose. The released mannose was analysed by following the procedure described in Balmaseda et al. (2021b). Then, the equivalents of mannose were quantified with a D-mannose and D-glucose enzymatic assay kit (Megazyme, Ireland).

Finally, a Crison micro pH 2002 pH meter (Hach Lange Spain, Barcelona, Spain) was used to determine the pH of the wines.

### 2.5. Volatile composition

The volatile composition of the wines after AF was analysed. The pretreatment of the samples consisted of a liquid/liquid extraction with a methyl *tert*-butyl ether/hexane mixture (1/1). The internal standards (Sigma-Aldrich) used were octanol-3 (1.98 g/L), heptanoic acid (3.33 g/L) and heptadecanoic acid (1.03 g/L), which were added to 5 mL of wine. The organic phase was injected into a GC-FID chromatograph (Agilent Technologies). The chromatographic conditions were as follows: injection volume, 2 μL; injection mode, spitless; inlet and detector temperatures, 250 °C; and column, HP-FFAP (30 m × 250 μm 0.25 μm, Agilent). Helium gas was used as the carrier gas at a flow of 1.5 mL $min^{-1}$. The oven temperature was set initially at 50 °C and programmed to 220 °C at a range of 4 °C $min^{-1}$ with holding time at this final temperature of 20 min. The concentrations of the volatile compounds were calculated from known external standards by calibration curves. The volatile compounds identified were acetates of fusel alcohols (AFA): isobutyl acetate, isoamyl acetate, 2-phenylethaol acetate); fusel alcohols (FA): amyl and isoamyl alcohols, hexanol, *cis*3-hexenol, 2-phenylethanol); ethyl esters of fatty acids (EEFA): ethyl butanoic, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, ethyl lactate and diethyl succinate); short-chain fatty acids (SCFA): propanoic, butanoic (butyric acid), isobutyric (2-methylpropanoic acid), pentanoic (valeric acid) and isovaleric acids (3-methylbutanoic acid)); medium-chain fatty acids (MCFA): hexanoic, octanoic, decanoic and dodecanoic acids) and long-chain fatty acids (LCFA): tetradecanoic (myristic acid), hexadecanoic (palmitic acid), 9-hexadecenoic (palmitoleic acid), octadecanoic (stearic acid), 9-octadecenoic (oleic acid) and 9,12-octadecadienoic acid (linoleic acid)).

### 2.6. Colour and anthocyanin analysis

CIELab coordinates were determined as described Ayala et al. (1997). The colorimetric coordinates hue (H*), lightness (L*) and chroma (C*) were calculated with MSCV software (http://www.unizar.es/). The colour intensity (CI) was calculated from the sum of absorbances at 420, 520 and 620 nm and measured in a spectrophotometer using a method described by Glories (1984). The limit for determining chromatic characteristics according to CIELab (OIV, 2006), is for Clarity (L*) from 0 to 100 and Tone (Hue, H*) is from 0 to 360°, and Chroma (C*) has no interval.

The content of free and acylated anthocyanins as well as pyranoanthocyanins was analysed with an Agilent 1200 series liquid chromatograph (HPLC–diode array detection) using an Agilent Zorbax Eclipse XDB-C18 column (4.6, 250 mmx 5 mm, Agilent Technologies) with the procedure described by (Gil et al., 2012). An external malvidin-3-monoglucoside calibration curve was used to determine phenolic compounds in mg/L.

### 2.7. Statistical analysis

To ensure the reliability of the results, all assays were performed in triplicate. The data obtained were subjected to statistical analysis using ANOVA and Tukey's HSD test (honestly significant difference) with XLSTAT 2020.2.3 software (Addinsoft, Paris, France). A p value of less than 0.05 was considered statistically significant. A principal component analysis (PCA) was also carried out to describe the relationship between colour and the content of phenolic compounds in *rosé* wine according to AF conditions.

## 3. Results and discussion

### 3.1. Alcoholic fermentation

The yeast combinations used in this study were selected based on a previous investigation (Ruiz-de-Villa et al., 2023), which aimed to identify the most effective strains to promote malolactic fermentation (MLF). The results showed that TdP + ScQ was the most efficient for MLF performance, followed by TdB and NSA1 Viniferm NSDT (Td-Viniferm, Agrovin, Spain). As a result, Td-P and TdB were chosen for this study. Two strains of *S. cerevisiae*, ScQ and ScK1, were also used. ScK1 did not perform well in MLF when synthetic grape must was applied, but we wanted to test it with real grape must.

Therefore, six different yeast combinations were tested. Due to the low yeast assimilable nitrogen (YAN) concentration in the must, the alcoholic fermentations lasted for several days (Fig. SD1). No nitrogen was added to the fermentation process, and no additional nutrients were introduced to evaluate their effects under less favourable conditions. This approach prevented fermentation from occurring too rapidly; thus, the potential of the yeast combinations could be more comprehensively examined.

The results of this study demonstrate that the AF duration was significantly shorter for *S. cerevisiae* strains compared to sequential fermentations. Specifically, the AF duration for ScQ and ScK1 was 17 and 19 days, respectively, while sequential fermentation with TdP and ScK1 took a maximum of 25 days (Fig. SD1). These findings align with previous studies, which indicate that *T. delbrueckii* has a significant nutrient requirement that restricts the subsequent fermentation activity of *S. cerevisiae*; as a result, competition occurs between the two yeast species (Belda et al., 2015; Romano et al., 2003; Ruiz-de-Villa et al., 2023). In Table 1, the areas under the curves (AUCs) are compared for each fermentation condition. The results showed that ScQ achieves a significantly faster fermentation rate than that of ScK1. Additionally, differences were observed with and without *T. delbrueckii*. In general, the sequential fermentations were slower except for TdB with ScK1, as the fermentation rate was faster even though the process lasted longer.

### 3.2. Physico-chemical parameters after alcoholic fermentation

The wines obtained from different fermentation conditions were analysed, and the levels of general parameters were compared, including the levels of organic acids, ethanol, and glycerol; equivalents of mannose; and pH (Table 1).

The results indicate that the levels of citric acid, succinic acid or L-malic acid did not show significant differences among wines under these fermentation conditions. However, regarding the values of acetic acid were significant changes, it is worth noting that high concentrations are a remarkable defect in wines. The use of TdP resulted in a significant decrease in the levels of acetic acid compared to that of the Sc Control condition (Table 1). Interestingly, a decrease in acetic acid was also observed when TdB was used in combination with ScQ but not with ScK1. Taillandier et al. (2014) and Ruiz de Villa et al. (2023) described sequential fermentations with some strains of *T. delbrueckii,* and *S. cerevisiae* produced less acetic acid in synthetic media. For natural must, an increase in volatile acidity has been reported in red wines,

**Table 1**
Principal parameters analysed from alcoholic fermentation. ScQ and ScK1 correspond to the pure fermentation with *S. cerevisiae* QA23 and *S. cerevisiae* K1, respectively; TdB + ScQ and TdP + ScQ correspond to sequential fermentations with *S. cerevisiae* QA23 and *T. delbrueckii* Biodiva or *T. delbrueckii* Prelude, respectively; TdB + ScK1 and TdP + ScK1: correspond to sequential fermentations with *S. cerevisiae* K1 and *T. delbrueckii* Biodiva or *T. delbrueckii* Prelude, respectively. AUC, Area Under the Curve. Lowercase letters indicate a significant difference between *S. cerevisiae* strains at $p < 0.05$; Capital letters indicate a significant difference between *S. cerevisiae*, *T. delbrueckii* Biodiva and *T. delbrueckii* Prelude at $p < 0.05$. Mean ± standard deviation (n = 3).

| | AUC | Malic acid (g/L) | Citric acid (g/L) | Succinic acid (g/L) | Acetic acid (g/L) | Ethanol (% vol) | Glycerol (g/L) | Eq-Mannose (mg/L) |
|---|---|---|---|---|---|---|---|---|
| ScQA23 | 113.3 ± 1.1 $^{aB}$ | 1.73 ± 0.03 $^{aA}$ | 0.15 ± 0.16 $^{aA}$ | 0.19 ± 0.06 $^{aA}$ | 0.41 ± 0.04 $^{aB}$ | 12.24 ± 0.01 $^{bAB}$ | 9.14 ± 0.14 $^{aA}$ | 89.7 ± 18.5 $^{aA}$ |
| TdB + ScQ | 107.8 ± 1.8 $^{aA}$ | 1.75 ± 0.02 $^{aA}$ | 0.24 ± 0.06 $^{aA}$ | 0.22 ± 0.05 $^{aA}$ | 0.28 ± 0.04 $^{aB}$ | 12.66 ± 0.19 $^{bA}$ | 11.33 1.06 $^{aB}$ | 217.1 ± 40.6 $^{aB}$ |
| TdP + ScQ | 104.2 ± 2.4 $^{aB}$ | 1.66 ± 0.05 $^{aA}$ | 0.19 ± 0.03 $^{aA}$ | 0.25 ± 0.1 3$^{aA}$ | 0.14 ± 0.05 $^{aA}$ | 13.16 ± 0.17 $^{bB}$ | 9.66 ± 0.77 $^{aA}$ | 224.2 ± 1.4 $^{aC}$ |
| ScK1 | 139.6 ± 0.9 $^{bB}$ | 1.74 ± 0.06 $^{aA}$ | 0.36 ± 0.07 $^{aA}$ | 0.20 ± 0.05 $^{aA}$ | 0.26 ± 0.06 $^{aB}$ | 12.75 ± 0.06 $^{aAB}$ | 9.80 ± 0.28 $^{aA}$ | 85.2 ± 14.9 $^{aA}$ |
| TdB + ScK1 | 137.5 ± 2.9 $^{bA}$ | 1.63 ± 0.03 $^{aA}$ | 0.16 ± 0.06 $^{aA}$ | 0.19 ± 0.06 $^{aA}$ | 0.29 ± 0.08 $^{aB}$ | 11.12 ± 0.58 $^{aA}$ | 10.06 ± 0.53 $^{aB}$ | 173.0 ± 9.2 $^{aB}$ |
| TdP + ScK1 | 148.2 ± 1.2 $^{bB}$ | 1.73 ± 0.04 $^{aA}$ | 0.17 ± 0.11 $^{aA}$ | 0.20 ± 0.04 $^{aA}$ | 0.15 ± 0.07 $^{aA}$ | 12.24 ± 0.47 $^{aB}$ | 9.04 ± 0.44 $^{aA}$ | 245.3 ± 24.9 $^{aC}$ |

while a decrease has been observed in white wines (Balmaseda, et al., 2021a; Balmaseda et al., 2021c; Oliveira & Ferreira, 2019). In *rosé* wines, a slight decrease in volatile acidity has been described (Muñoz-Redondo et al., 2021). These findings suggest that the impact of *T. delbrueckii* on acetic acid in wines depends on the strain and type of vinification, as previously described in the literature.

The ethanol content in wines produced using the different strains of *S. cerevisiae* varied significantly, and ScK1 wines exhibited lower ethanol values than those of ScQ wines. Additionally, the use of TdB in conjunction with ScK1 resulted in a significant decrease in alcohol content. Although the decrease of ethanol has been linked to the use of some *T. delbrueckii* strains (Zhu et al., 2020), the extent of this decrease can vary depending on factors such as the strain, must and winemaking conditions (Balmaseda, et al., 2021c). It could be interesting since it in rosé wine, lower values are related to more balanced wines as well as the current trend to reduce the alcohol content which is increasing due to climate change. The glycerol content was found to be significantly higher in wines produced using TdB. This trend may be related to the decrease in ethanol content observed when this strain was used in combination with ScK1, although the development of the glycerol-pyruvic pathway has been described as strain-dependent (Benito, 2018). For the pH, no significant differences were found (Table 3) with 2 days of *T. delbrueckii* contact. Regarding equivalents of mannose, there were significant differences among wines fermented with *S. cerevisiae* and sequential fermentations, in which the concentration was significantly higher, as previously described (Belda et al., 2015; Ruiz-de-Villa et al., 2023). In addition, TdP showed the highest concentrations of equivalents of mannose, since this strain is an overproducer of mannoproteins (Benito, 2018).

### 3.3. Volatile composition

Different volatile compounds were detected (Table 2), including fusel alcohols (isoamyl alcohol, 1-hexanol and 2-phenyl ethanol), short-chain fatty acids (SCFAs) (butyric acid, isobutyric acid and valeric acid), medium-chain fatty acids (MCFAs) (octanoic acid and decanoic acid),

**Table 3**
Colour parameters in wines after alcoholic fermentations. ScQ and ScK1 correspond to the pure fermentation with *S. cerevisiae* QA23 and *S. cerevisiae* K1, respectively; TdB + ScQ and TdP + ScQ correspond to sequential fermentations with *S. cerevisiae* QA23 and *T. delbrueckii* Biodiva or *T. delbrueckii* Prelude, respectively; TdB + ScK1 and TdP + ScK1: correspond to sequential fermentations with *S. cerevisiae* K1 and *T. delbrueckii* Biodiva or *T. delbrueckii* Prelude, respectively. All data is expressed as the mean of three biological replicates ± standard deviation, Different lowercase letters indicate the existence of significant difference between the samples ($p < 0.05$).

| | CI | H* | L* | C* | pH |
|---|---|---|---|---|---|
| ScQ | 3.98 ± 0.01 $^{b}$ | 28.09 ± 1.15 $^{ab}$ | 41.07 ± 1.02 $^{ab}$ | 67.19 ± 2.15 $^{abc}$ | 3.53 ± 0.03 $^{a}$ |
| TdB + ScQ | 3.41 ± 0.28 $^{a}$ | 36.94 ± 2.25 $^{c}$ | 45.10 ± 3.37 $^{bc}$ | 73.51 ± 0.88 $^{cd}$ | 3.51 ± 0.01 $^{a}$ |
| TdP + ScQ | 3.46 ± 0.11$^{a}$ | 32.77 ± 1.84 $^{bc}$ | 45.13 ± 1.86 $^{bc}$ | 70.96 ± 0.86 $^{cd}$ | 3.49 ± 0.03 $^{a}$ |
| ScK1 | 4.28 ± 0.51$^{b}$ | 32.93 ± 0.62 $^{bc}$ | 36.57 ± 4.13 $^{a}$ | 69.95 ± 1.12 $^{bcd}$ | 3.56 ± 0.05 $^{a}$ |
| TdB + ScK1 | 3.14 ± 0.59 $^{a}$ | 24.13 ± 6.13 $^{a}$ | 46.27 ± 4.31$^{bc}$ | 67.06 ± 1.72 $^{ab}$ | 3.50 ± 0.03 $^{a}$ |
| TdP + ScK1 | 2.99 ± 0.03 $^{a}$ | 26.38 ± 5.48 $^{ab}$ | 48.10 ± 1.41$^{c}$ | 65.91 ± 2.27 $^{a}$ | 3.54 ± 0.02 $^{a}$ |

LCFAs (myristic acid, palmitic acid and stearic acid), fusel alcohol acetates (isobutyl acetate, isoamyl acetate, hexyl acetate, 2-phenyl ethanol acetate), and ethyl esters of FA (ethyl butanoate, ethyl hexanoate and ethyl dodecanoate). It has been shown that *T. delbrueckii* lead to higher concentrations of fusel alcohols, especially TdB; compared to wines fermented only with *S. cerevisiae*, wines produced with *T. delbrueckii* generated significantly higher concentrations of isoamyl alcohol. The high values of fusel alcohols with this species have already been reported in other studies (Azzolini et al., 2015; Belda et al., 2017; Benito, 2018; Muñoz-Redondo et al., 2021; Ruiz-de-Villa et al., 2023). However, other studies have reported a decrease in fusel alcohols in the presence of *T. delbrueckii* (Belda et al., 2017), which may be related to the regulation of the Ehrlich pathway responsible for the production of these

**Table 2**
Volatile compounds (mg/L) analysed from alcoholic fermentation. Sum of Fusel alcohol acetates (isobutyl acetate, isoamyl acetate and 2-phenylethanol acetate), Sum of Ethyl esters of FA (ethyl butanoate, ethyl hexanoate, ethyl octanoate and ethyl dodecanoate), Sum of Fusel alcohols (isoamyl alcohol, 1-hexanol, *cis*-3-hexen-1-ol, 2-phenylethanol), Sum of SCFA (propionic, butyric and valeric acids), Sum of MCFA (octanoic and decanoic acids), Sum of LCFA (myristic, palmitic and stearic acids). ScQ and ScK1 correspond to the pure fermentation with *S. cerevisiae* QA23 and *S. cerevisiae* K1, respectively; TdB + ScQ and TdP + ScQ correspond to sequential fermentations with *S. cerevisiae* QA23 and *T. delbrueckii* Biodiva or *T. delbrueckii* Prelude, respectively; TdB + ScK1 and TdP + ScK1: correspond to sequential fermentations with *S. cerevisiae* K1 and *T. delbrueckii* Biodiva or *T. delbrueckii* Prelude, respectively. Mean ± standard deviation (n = 3). Lowercase letters indicate a significant difference between *S. cerevisiae* strains at $p < 0.05$.

| | Fusel alcohols acetates | Ethyl esters of FA | Fusel alcohols | SCFA | MCFA | LCFA |
|---|---|---|---|---|---|---|
| ScQ | 1.23 ± 0.22$^{a}$ | 0.54 ± 0.20$^{a}$ | 776.12 ± 64.47$^{a}$ | 11.49 ± 2.02$^{bc}$ | 1.18 ± 0.52$^{b}$ | 1.96 ± 0.61$^{a}$ |
| TdB + ScQ | 1.90 ± 0.15$^{b}$ | 1.03 ± 0.38$^{a}$ | 1274.39 ± 69.60$^{c}$ | 12.62 ± 3.13$^{c}$ | 0.01 ± 0.01$^{a}$ | 2.58 ± 0.80$^{ab}$ |
| TdP + ScQ | 1.09 ± 0.31$^{a}$ | 0.55 ± 0.09$^{a}$ | 909.43 ± 29.26$^{ab}$ | 17.10 ± 3.78$^{cd}$ | 0.09 ± 0.03$^{a}$ | 3.80 ± 0.34$^{b}$ |
| ScK1 | 1.08 ± 0.24$^{a}$ | 0.80 ± 0.10$^{a}$ | 877.60 ± 78.68$^{ab}$ | 5.68 ± 2.75$^{ab}$ | 0.45 ± 0.05$^{a}$ | 2.46 ± 0.28$^{ab}$ |
| TdB + ScK1 | 2.29 ± 0.21$^{b}$ | 0.82 ± 0.22$^{a}$ | 1275.40 ± 34.36$^{c}$ | 2.08 ± 1.30$^{a}$ | 0.14 ± 0.02$^{a}$ | 3.07 ± 0.89$^{ab}$ |
| TdP + ScK1 | 2.03 ± 0.25$^{b}$ | 0.79 ± 0.13$^{a}$ | 1035.07 ± 65.82$^{b}$ | 20.00 ± 0.56$^{d}$ | 0.19 ± 0.03$^{a}$ | 2.60 ± 0.04$^{ab}$ |

compounds, which is complex and strain-dependent (Benito, 2018).

Moreover, the presence of *T. delbrueckii* led to an increasing trend in the concentrations of 2-phenylethanol. This combination of strains in sequential wines also exhibited significantly higher concentrations of fusel alcohol acetates, even though some authors described a decrease in their concentration with *T. delbrueckii* (Azzolini et al., 2015; Belda et al., 2017). This increase in some volatile compounds could be related to the high enzymatic activity of *T. delbrueckii* (Romano et al., 2003).

However, in regard to MCFAs, a decreasing trend was observed in the presence of *T. delbrueckii*. Wines fermented with *S. cerevisiae*, especially the strain ScQ, generated higher values of MCFAs than those fermented with *T. delbrueckii*, as reported by Balmaseda et al. (2021a) and Ruiz--de-Villa et al. (2023).

### 3.4. Anthocyanins, pyranoanthocyanins and colour parameters

Fig. 1 shows the total anthocyanin concentration (1A) and the pyranoanthocyanin concentration (1B) of the different wines after AF determined by HPLC. Those parameters were also analysed in wines after MLF; however, no significant differences of interest were observed (data not shown). Fig. 1 also shows the relative proportions, expressed as percentages (%), of different pigments detected in the different samples (1C). As expected, nonacylated anthocyanins were predominant, and malvidine-3-O-glucoside was the main anthocyanin detected (data not shown). Acylated anthocyanins were also detected, but only the acetylated forms were present in the wines. Among the acylated anthocyanins, malvidin-3-O-acetylglucoside practically monopolizes this category. These data agree with previous research indicating that acetylated anthocyanins were the predominant type among the acylated anthocyanins found in Cabernet wines (Gil et al., 2012; Gombau et al., 2020). The total anthocyanin concentration (Fig. 1A) was significantly higher in control samples fermented with pure cultures of *S. cerevisiae* (ScQ or ScK1) compared to corresponding wines fermented with sequential inoculation of *T. delbrueckii* strains (TdB + ScQ, TdP + ScQ, TdB + ScK1 and TdP + ScK1). Furthermore, it seems that wines fermented only with ScK1 showed higher anthocyanin concentrations than wines fermented only with Sc-Q, although these differences did not reach statistical significance. As previously discussed, all sequential fermentations showed significantly lower anthocyanin concentrations than those of their respective control wines. The concentrations decreased by approximately 50% on average compared to that of the controls; the only exception was TdB + ScK1, which showed a higher decrease of approximately 60%. This decrease in anthocyanin concentration could be attributed to different factors. On the one hand, literature has described that some *T. delbrueckii* strains could have high β-glucosidase activity (Maturano et al., 2012). Consequently, the presence of β-glucosidase activity in these *T. delbrueckii* strains may promote the formation of aglycones from anthocyanins, making these pigments more susceptible to oxidation (Vidana Gamage et al., 2022). It has been observed that the *T. delbrueckii* strains used in this study had a higher β-glucosidase activity than the *S. cerevisiae* strains tested (Fig. SD2).

On the other hand, anthocyanins can react with different compounds, such as ethanal, to form flavanol-ethyl-anthocyanin adducts (Es-Safi et al., 1999). Additionally, anthocyanins can react with ethanal, pyruvic acid and vinylphenols through cycloaddition reactions to form pyranoanthocyanins (Bakker & Timberlake, 1997; Schwarz et al., 2003). However, in this case, the last process is not responsible since no differences were found in pyranoanthocyanin concentrations among the different samples. Besides, pyruvic acid and acetaldehyde were analysed after AF but there were no significant differences that could be related to variations in anthocyanin concentrations (Table SD1). Another factor to consider is that the different yeast species or even yeast strains could show different capacities to adsorb pigments, such as anthocyanins (Morata et al., 2003; Tofalo et al., 2021). Moreover, the kinetics of all sequential fermentations were slower than those of pure *S. cerevisiae* fermentations (ScQ and ScK1) (Suppl. Fig. S1). Thus, it is worth noting that the risk of oxidation is higher when the fermentation is longer since the wines remain unprotected for a longer period without the addition of sulphur dioxide.

Despite our results, some authors reported the opposite effect, as the total anthocyanins increased with sequential inoculation of *T. delbrueckii* in red wine (Balmaseda et al., 2021a; Chen et al., 2018; Escribano-Viana et al., 2019; Minnaar et al., 2018). However, it is important to note that these studies involved the production of red wine, while our study involved the production of rosé wine. This distinction leads to significant differences. First, in *rosé* winemaking, the contact time between the

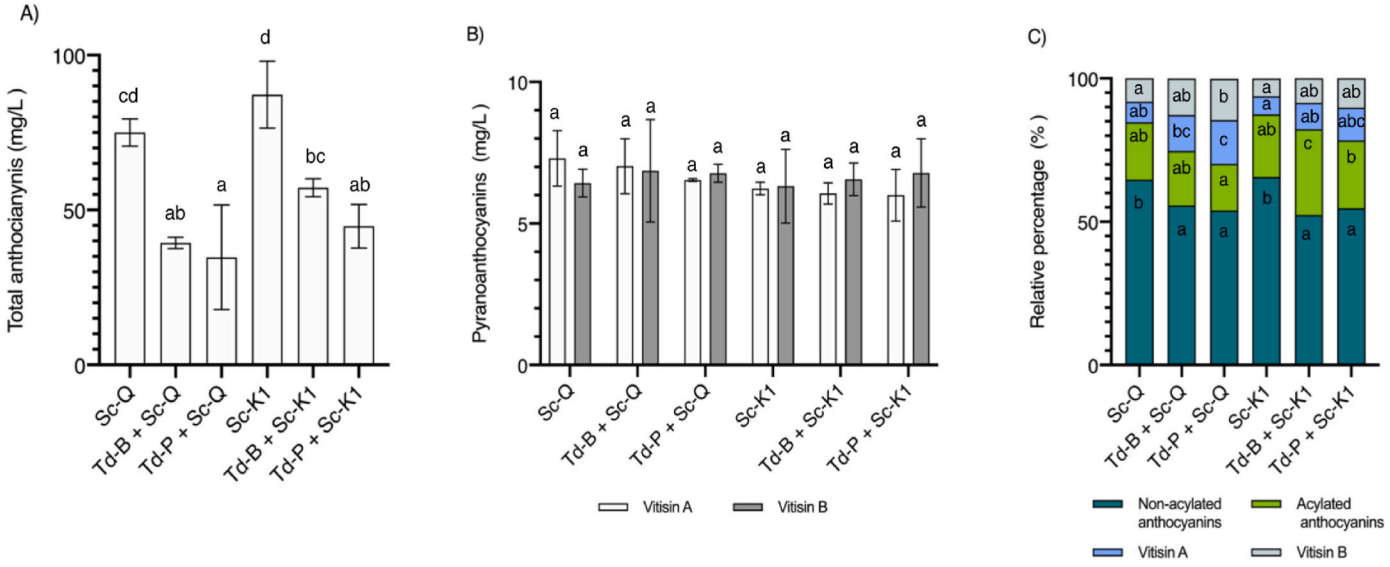



**Fig. 1.** Total anthocyanins (A) and pyranoanthocyanins (B) concentration at the end of the alcoholic fermentation, and (C) relative proportion of different pigments expressed as % (non-acylated anthocyanins, acylated anthocyanins and pyranoanhocyanins). ScQ and ScK1 correspond to the pure fermentation with *S. cerevisiae* QA23 and *S. cerevisiae* K1, respectively; TdB + ScQ and TdP + ScQ correspond to sequential fermentations with *S. cerevisiae* QA23 and *T. delbrueckii* Biodiva or *T. delbrueckii* Prelude, respectively; TdB + ScK1 and TdP + ScK1 correspond to sequential fermentations with *S. cerevisiae* K1 and *T. delbrueckii* Biodiva or *T. delbrueckii* Prelude, respectively The values are expressed as the mean of three biological replicates for each condition. Different lowercase letters indicate the existence of significant difference between the samples ($p < 0.05$).

grape skins and the juice is relatively short, resulting in a lower overall extraction of anthocyanins. Second, in rosé winemaking, yeast inoculation is performed after the maceration process, when the extraction of pigments from the grape skins has already been completed. In contrast, during the production of red wine, yeast inoculation occurs while grape skins are present, and maceration is prolonged for a much longer period. The presence of grape skins during fermentation serves as a source of anthocyanins, contributing to a potentially higher concentration of total anthocyanins in red wines (Gil et al., 2012).

Vitisin A and Vitisin B, as shown in Fig. 1B, were detected in all samples, and both are derived from malvidin-3-O-monoglucoside and malvidin-3-O-acetylmonoglucoside (He et al., 2012). However, Vitisin A and Vitisin B concentrations were statistically similar in all wines. Thus, the formation of pyranoanthocyanins was not influenced by the presence of *T. delbrueckii* strains and was also independent of the *S. cerevisiae* strain used. Nevertheless, as shown in Fig. 1C, some variations in the relative proportion of pyranoanthocyanins to total pigments (including anthocyanins and pyranoanthocyanins) were detected after AF. In this context, wines produced through sequential fermentation with ScQ (TdB + ScQ and TdP + ScQ) exhibited a higher proportion of pyranoanthocyanins than that of their control. This increase in pyranoanthocyanins can be attributed to the lower concentration of anthocyanins in these samples (Fig. 1A). A similar trend was observed in samples with sequential fermentation using the ScK1 strain and both *T. delbrueckii* strains, although these differences were not statistically significant. Moreover, the presence of the TdP strain leads to wines with a higher proportion of pyranoanthocyanins than those of wines fermented with the TdB strain.

Comparing the different fermentation conditions, the proportion of acylated anthocyanins was statistically similar in both wines fermented with pure cultures of *S. cerevisiae* (ScQ and ScK1). Regarding wines fermented with the ScK1 strain, the sequential inoculations showed a higher proportion of acylated anthocyanins and a lower proportion of nonacylated anthocyanins compared to that of the ScK1 control wine. Thus, the degradation of anthocyanins observed in TdB + ScK1 and TdP + ScK1 (Fig. 1A) was mainly attributed to nonacylated anthocyanidins. However, for sequential fermentation in the presence of ScQ and both *T. delbrueckii* strains, no significant differences were detected in the proportion of acylated anthocyanins. Therefore, the decrease in anthocyanin observed in these samples mainly resulted from the degradation of acylated and nonacylated anthocyanin.

Differences in the relative proportions of pyranoanthocyanins and acylated anthocyanins can lead to changes in wine colour. It has been reported that pyranoanthocyanins contribute more to a yellowish hue than anthocyanins (De Freitas & Mateus, 2011). These derived pigments are also less sensitive to pH changes and less prone to discolouration caused by the action of sulphur dioxide (Fulcrand et al., 1997). On the other hand, acetylated anthocyanins have been described to exhibit a higher bluish hue than that of their corresponding nonacetylated counterparts (De Villiers et al., 2004). Moreover, the structure of acylated anthocyanins is more resistant to nucleophilic attack by water, which results in the formation of hemiketal forms because their structure favours intrapigmentation phenomena (Trouillas et al., 2016; Vidana Gamage et al., 2022). All these phenomena collectively show the potential to significantly modulate the intensity and hue of wine colour.

Table 3 shows the colour parameters of the different wines. Some differences in colour parameters cannot be attributed to the pH since all the samples have similar values. Colour intensity (CI) was higher in conventional fermentations with ScK1 and ScQ strains. However, in all the wines produced through sequential fermentations, a lower colour intensity was observed, which could be attributed to the lower concentration of anthocyanins detected in these wines. There is limited research on the impact of *T. delbrueckii* on *rosé* wines. In a previous study, the sensory effects in *rosé* wines of *T. delbrueckii* as well as *Metschnikowia pulcherrima* were examined (Muñoz-Redondo et al., 2021). The same strain (Td-Biodiva) was employed and a significant decrease in CI was observed, even though the *S. cerevisiae* strain was different.

The CIELab coordinates L* (Lightness) and C* (Chroma) provide quantitative information about colour characteristics. In all sequential wines, an increase in L* values was detected in comparison with *S. cerevisiae* control wines, especially for ScK1 (Table 3). This finding aligns with the colour intensity (CI) results since L* is usually negatively correlated with CI. Therefore, sequential fermentations showed less intense colour. The C* values of sequential ScK1 wines (TdB + ScK1 and TdP + ScK1) decrease compared to that of the ScK1 control. However, in the ScK1 wines, the differences were more pronounced because the decrease in CI between the control and sequential wines was larger compared to that of the ScQ wines.

When considering the CIELab coordinate h° (hue), which relates to the qualitative aspects of colour, the control samples (ScQ and ScK1) exhibited similar values. Therefore, both tested strains of *S. cerevisiae* did not have any significant effect on the hue of the wines when conventional inoculation was carried out. In contrast, sequential fermentations conducted with the ScQ strain and both *T. delbrueckii* strains (TdB + ScQ and TdP + ScQ) demonstrated significantly higher h° values compared to that of the control (ScQ). Thus, sequential fermentation with ScQ resulted in wines with more pronounced yellowish nuances. For sequential fermentation in the presence of ScK1, the h° values showed a decreasing trend compared to that of their control, indicating a less yellowish hue. One possible explanation is that these sequential fermentations (TdB + ScK1 and TdP + ScK1) did not exhibit statistically significant differences in the proportion of pyranoanthocyanins compared to the control (ScK1) (Fig. 1C).

A principal component analysis (PCA) was performed to clarify which factors contribute to the overall colour variation observed in rosé wines under these conditions (Fig. 2). The following parameters was used to perform the PCA: colour intensity, L*, C*, h°, total anthocyanins, relative proportion of nonacylated anthocyanins, relative proportion of acylated anthocyanins and relative proportions of pyranoanthocyanins. The first principal component (PC1) explains 52.67% of the variance, while the second (PC2) explains 34.69%; therefore, the combined variance explained by the first two components was 87.36%.

The loading variables presented in Fig. 2b indicates the contribution provided by the two components related to their length and direction. The loadings on PC1 are related to CI, anthocyanin total concentration and proportion of nonacylated anthocyanins and are directed towards the positive values (corresponding to the right in Fig. 2a), indicating that there was a correlation between these variables. In contrast, the L* coordinate, as it has opposite loadings on PC1, is directed towards the negative values being negatively correlated with the previous variables. This result was expected since the higher the CI of the wines was, the lower the L* value. Loading on PC1 separated samples into two clusters, control (ScQ and ScK1) in the positive values and sequential in the negative values. Therefore, it may be concluded that sequential fermentations, with the presence of *T. delbrueckii* strains, produced wines with lower anthocyanin concentrations and consequently, wines with less intense colours.

Moreover, the proportion of pyranoanthocyanins (vitisin A and vitisin B) was negatively correlated with the total anthocyanin concentration, as they had opposite loadings on PC1. Thus, according to our results, sequential fermentation with *T. delbrueckii* strains seems to promote the degradation of anthocyanins, which increased the relative proportion of pyranoanthocyanins in the final wines.

The loadings on PC2 explained the h° (hue) and the proportion of acylated anthocyanins. The loadings corresponding to the proportion of acylated anthocyanins are directed towards the positive values of this PC. In contrast, h° loading is directed towards the negative values. Moreover, the loadings corresponding to pyranoanthocyanins (Vitisin A and Vitisin B) correlated positively with h° loadings. Therefore, the h° becomes higher as the proportion of pyranoanthocyanins increases and the proportion of acylated anthocyanins decreases, indicating that the

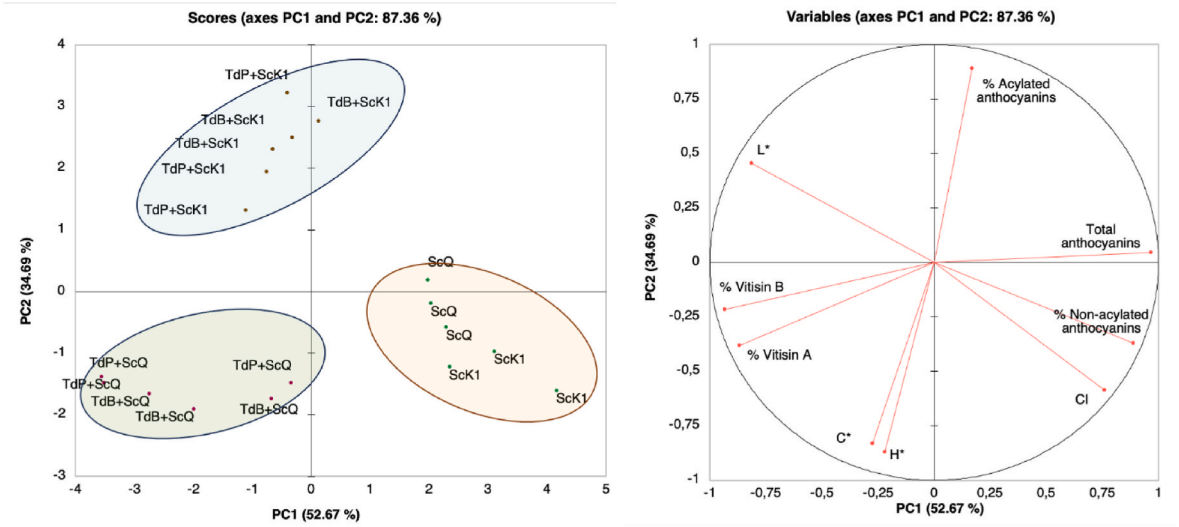



**Fig. 2.** Principal component analysis biplots built from the following loadings: total anthocyanins, acylated anthocyanins, non-acylated anthocyanins, CI, Vitisin A, Vitisin B, C*, L*, H* (b). The scores are the followings: ScQ and ScK1: correspond to the pure fermentation with *S. cerevisiae* QA23 and *S. cerevisiae* K1, respectively; TdB + ScQ and TdP + ScQ which correspond to sequential fermentations with *S. cerevisiae* QA23 and *T. delbrueckii* Biodiva or *T. delbrueckii* Prelude, respectively; TdB + ScK1 and TdP + ScK1: correspond to sequential fermentations with *S. cerevisiae* K1 and *T. delbrueckii* Biodiva or *T. delbrueckii* Prelude, respectively (a).

wines were more yellowish.

Thus, according to PC2, the sequential fermentations carried out with ScK1 (TdB + ScK1 and TdP + ScK1) were clustered as a different group from sequential fermentations carried out with ScQ (TdB + ScQ and TdP + ScQ) and control wines (ScQ and ScK1). This allowed us to separate the different sequential fermentations. In this sense, regardless of the *T. delbrueckii* strain, when sequential fermentation was carried out with ScQ, the produced wines contained a higher proportion of pyranoanthocyanins. However, when sequential fermentation was carried out with ScK1, the produced wines contained a higher proportion of nonacylated anthocyanins. This different pigment proportion led to wines with different H* values. In particular, the sequential fermentation of ScQ led to wines with a more yellowish hue, while the sequential fermentation with ScK1 led to wine with a more bluish hue.

### 3.5. Malolactic fermentations (MLF)

In terms of the MLF results, interesting differences were observed among the strains (Fig. 3). In these conditions for natural rosé, fermentation could not be completed by Oo1Pw13 (data not shown); previously, it had been observed that L-malic consumption by this strain was very slow (Balmaseda, et al., 2021a).

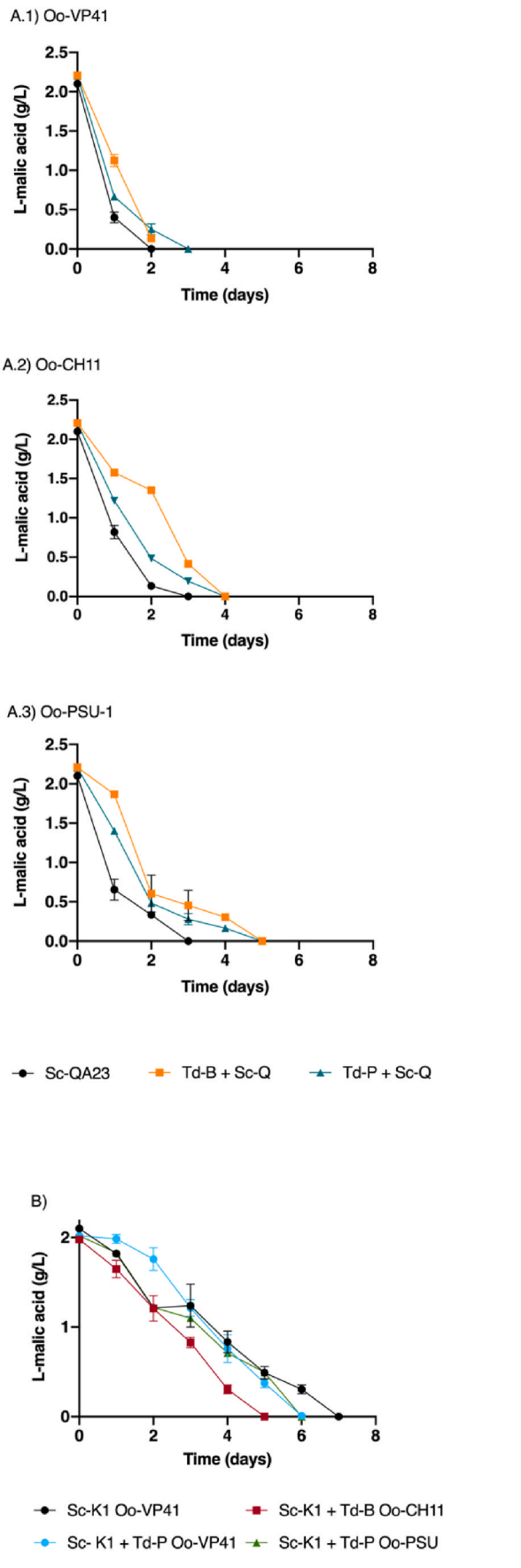
As it has been described, although ScK1 is not a *S. cerevisiae* strain recommended for MLF, the presence of *T. delbrueckii* during AF in combination with ScK1 can have a positive effect (Ruiz-de-Villa et al., 2023). Consequently, in this study the most interesting differences were found in the MLF of the ScK1 set, in which none of the control conditions could complete MLF, as expected. However, wine fermented with in presence of *T. delbrueckii* TdP completed MLF with three of the strains, OoVP41 (6 days), OoCH11 (6 days) and OoPSU-1 (7 days), and TdB with OoCH11 (5 days) (Fig. 3B).

Considering *O. oeni* strains, confirming previous results (Ruiz-de--Villa et al., 2023), OoVP41 showed excellent MLF performance. Wines fermented with ScQ finished the MLF when OoVP41 was used, and even for one condition of the ScK1 set, the wine fermented with TdP + ScK1 (Fig. 3A1 and B). Regarding ScQ wines, OoPSU-1 MLF managed to consume all L-malic acid only with TdB + ScQ and TdP + ScQ (5 days), contrary to the control condition (Fig. 3A2). These findings showed the beneficial effect of the synergy between *T. delbrueckii* and *O. oeni* described previously (Balmaseda et al., 2021a; Balmaseda et al., 2022; Ruiz-de-Villa et al., 2023). In this case, this improvement could be related to the higher content of mannoproteins (Table 1) in wines due to the presence of *T. delbrueckii* during the AF. In addition, a lower concentration of MCFAs was observed in sequential wines (Table 2), compounds that have been described as toxic to *O. oeni* (Capucho & San Romão, 1994) because the membrane of *O. oeni* is destabilized (Sereni et al., 2020). Even though the minimum concentrations reported of decanoic and dodecanoic acids with inhibitory effects in *O. oeni* were above 12.5 and 2.5 mg/L, respectively (Capucho & San Romão, 1994), these differences detected among conditions could have had a slight effect.

The positive synergy with these two species could have involved a decrease in pH or ethanol helping to ease the stressful environment for *O. oeni*; however, in our study, this behaviour was not observed. The decrease in polyphenolic compounds due to the presence of *T. delbrueckii* (Fig. 1) may have exert an effect since certain phenolic compounds are stressful for *O. oeni* (Bech-Terkilsen et al., 2020).

## 4. Conclusion

In conclusion, the use of *T. delbrueckii* in sequential fermentation with *S. cerevisiae* achieved promising results with rosé wines. Compared to control conditions, the presence of *T. delbrueckii* resulted in a significant colour change. Furthermore, sequential fermentations resulted in a decrease in anthocyanins and, consequently, a decrease in colour intensity. However, the behaviour varied depending on the *S. cerevisiae* strain used (ScQ or ScK1). Sequential fermentations with ScQ increase the proportion of pyranoanthocyanins, which could enhance colour stability, an essential characteristic for rosé wines. The decrease in total anthocyanins and colour intensity presents an interesting oenological tool for producing rosé wines with a lighter colour, which is currently highly sought after by consumers. Furthermore, the improvement in MLF performance is another valuable aspect of *T. delbrueckii*, although the performance is highly influenced by the specific strain combination.




*(caption on next column)*

**Fig. 3.** Consume of L-malic acid during MLF. ScQ and ScK1 correspond to the pure fermentation with *S. cerevisiae* QA23 and *S. cerevisiae* K1, respectively; TdB + ScQ and TdP + ScQ correspond to sequential fermentations with *S. cerevisiae* QA23 and *T. delbrueckii* Biodiva or *T. delbrueckii* Prelude, respectively; TdB + ScK1 and TdP + ScK1 correspond to sequential fermentations with *S. cerevisiae* K1 and *T. delbrueckii* Biodiva or *T. delbrueckii* Prelude, respectively. A) Corresponds to ended MLF of ScQ wines with: A.1) OoVP41 strain, A.2) OoCH11 strain and A.3) OoPSU-1 strain. B) Corresponds to ended MLF of ScK1 wines with OoVP41, OoCH11 and OoPSU-1 strains. All data are expressed as the mean of three biological replicates ±standard deviation.

## CRediT authorship contribution statement

**Candela Ruiz-de-Villa:** Investigation, Methodology, Original draft preparation. **Jordi Gombau:** Investigation, Methodology, Data curation. **Montse Poblet:** Investigation, Methodology, Data curation. **Albert Bordons:** Supervision, Reviewing &, Visualization. **Joan Miquel Canals:** Supervision, Reviewing. **Fernando Zamora:** Methodology, Original draft preparation. **Cristina Reguant:** Supervision, Writing – review & editing, Funding acquisition. **Nicolas Rozès:** Supervision, Writing – review & editing.

## Declaration of competing interest

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

## Data availability

No data was used for the research described in the article.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2023.115540.

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