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Influence of skin maceration and *Torulaspora delbrueckii* inoculation on white wine production: changes in fermentation dynamics and wine composition

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ABSTRACT

Innovative approaches in white wine production, including extended skin contact and malolactic fermentation (MLF), are gaining attention for their potential to enhance wine complexity. This study examines how skin contact during alcoholic fermentation (AF), fermentation temperature, and yeast inoculation strategies impact white wine composition, focusing on *Torulaspora delbrueckii* (Td) behavior.

Physicochemical analyses confirmed spontaneous MLF in Td-fermented wines, linked to indigenous *Oenococcus oeni*. Metabolomic and volatile analysis, through PCA and ASCA, revealed that vinification style was the primary driver of wine composition, specially influencing phenolic acids and Krebs cycle metabolites. Skin-fermented wines showed higher levels of phenolic compounds, especially at higher temperatures. Td fermentation reduced hydroxycinnamic acids, likely improving MLF compatibility. Notably, 2-isopropylmalic acid (2-IPMA) was detected, with higher concentrations in skin-fermented wines at elevated temperatures.

Td-fermented wines underwent spontaneous MLF, progressing faster than in controls, likely due to reduced medium-chain fatty acids and increased mannoproteins. These findings highlight the potential of Td to facilitate MLF by improving the biochemical environment for *O. oeni*, although spontaneous MLF requires careful management. Nineteen *O. oeni* genotypic profiles were identified, with the M2 profile persisting through AF and spontaneous MLF, and the C5 profile predominating in wines inoculated with OoVP41. Sensory analysis revealed that wines fermented with Td exhibited distinct aromas, such as tropical, pear, and terpenic notes, especially in skin-fermented wines.

This work underscores how specific winemaking strategies can modulate fermentation dynamics and wine composition, offering new insights into the role of *T. delbrueckii* in skin-fermented and white wine production.

1. Introduction

The vinification of white wines offers a wide range of possibilities, ranging from fresh and young styles to the aromatic expression of ageing wines. Among the oenological techniques available in white wine vinification, controlled skin contact represents a deliberate strategy employed to modulate the wine's phenolic composition, aromatic profile and overall complexity. In so-called orange wines, also called skin-fermented, contact with the skins lasts until the end of alcoholic fermentation (AF) (Kemp et al., 2021, pp. 339–354; Lorteau, 2018) whereas the traditional approach only involves brief contact with the skins for hours or days.

There is a growing consumer appreciation for orange wines (Bene & Kállay, 2019) because this extended skin contact results in a deeper colour and a more textured and tannic profile compared to traditional white wines (Beara et al., 2024). It also enables the expression of varietal

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components concentrated in grape skins and seeds, which accentuates the distinctive characteristics of the wine (Singleton et al., 1975). Despite these wines becoming widely popular in recent decades and spreading through many winemaking regions, their origin can be traced back 8000 years to the Republic of Georgia (Glonti, 2001). Although the tradition of skin-fermented white wines dates back thousands of years, their formal regulation remains limited, with only a few regions defining specific criteria (Lorteau, 2018). The production of orange wines offers winemakers the opportunity to experiment with different grape varieties and achieve a distinct phenolic and flavour profile (Beara et al., 2024; Bestulić et al., 2022; Maante-Kuljus et al., 2024; Mezey et al., 2025). Thus, aromatic grape varieties such as Riesling, Gewürztraminer or Muscat are suitable due to the high levels of free and glycosidically bound compounds present in their skins (Kemp et al., 2021, pp. 339–354; Sokolowsky et al., 2013).

Malolactic fermentation (MLF), traditionally uncommon in white wines to preserve acidity and freshness, except for certain Chardonnay wines (Semon et al., 2001), is gaining relevance as demand for more complex wines and climate change drive its use, especially in cooler regions with naturally higher acidity.

Microorganisms play a crucial role in the production of white wine, and non-*Saccharomyces* yeast strains have gained significant attention for their potential to enhance the sensory characteristics of wines. Non-*Saccharomyces* strains are particularly useful for improving the varietal aromatic profile of wines, which is highly important for white wines (Canonico et al., 2019; Jolly et al., 2014). Among them *Saccharomyces*, *Torulaspora delbrueckii* is an interesting choice because it enhances the aroma profile of white wines (Azzolini et al., 2015; Velázquez et al., 2015)*T. delbrueckii* contributing to the improvement of MLF performance via the lactic acid bacteria (LAB) *O. oeni* (Balmaseda et al., 2023; Ruiz-de-Villa, Poblet, et al., 2023c). Despite this, there is very limited knowledge of how *T. delbrueckii* interacts with *O. oeni* in skin-fermented white wines, where the biochemical environment differs markedly from conventional vinifications. This represents a critical gap, as these interactions may determine both the success of MLF and the resulting sensory profile.

Given the limited literature on skin-fermented or orange wines and MLF in white wines, this research introduces a novel perspective on the use of *T. delbrueckii* in skin-fermented white wines and its impact on the MLF. The present study addresses this gap by performing a sequential inoculation approach using *T. delbrueckii* and *S. cerevisiae* to optimise the fermentation performance of AF and MLF and enhance the overall organoleptic profile of the wines under two fermentation temperatures. This study aims to investigate the influence of *T. delbrueckii* sequential inoculation, vinification style, and fermentation temperature on fermentation performance, metabolomic and volatile composition, and sensory properties of white wines, with particular emphasis on their impact on malolactic fermentation dynamics. By integrating microbiological, metabolomic, and sensory analyses, this work provides new insights into how *T. delbrueckii* may shape both fermentation outcomes and wine composition in skin-fermented white wines.

2. Materials and methods

2.1. Alcoholic fermentation

Grapes of Muscat of Alexandria cultivar (*Vitis vinifera* L.) were sourced from the *Mas dels Frares* experimental winery of *Rovira i Virgili* University, located within the Tarragona denomination of qualified origin (DOC Tarragona), and all processing and AF occurred at the same winery. The AF was performed in 10 L food-grade plastic tanks. Half of the grapes were pressed, clarified with Lallzyme-C-Max pectinases (Lallemand Inc., Montreal, Canada) at 4 °C without any addition of sulphur dioxide, and vinified as a traditional white wine (W). Conversely, the other half of the grapes were vinified as skin-fermented wine/orange wine (S), with the grape skins during maceration (Table 1).

Table 1
Conditions tested in the study.

	Temperature	Vinification	Yeast
H-S-Sc	High (25 °C)	Skin-fermented wine	*S. cerevisiae*
H-S-Td	High (25 °C)	Skin-fermented wine	*T. delbrueckii* + *S. cerevisiae*
L-S-Sc	Low (16 °C)	Skin-fermented wine	*S. cerevisiae*
L-S-Td	Low (16 °C)	Skin-fermented wine	*T. delbrueckii* + *S. cerevisiae*
H-W-Sc	High (25 °C)	White wine	*S. cerevisiae*
H-W-Td	High (25 °C)	White wine	*T. delbrueckii* + *S. cerevisiae*
L-W-Sc	Low (16 °C)	White wine	*S. cerevisiae*
L-W-Td	Low (16 °C)	White wine	*T. delbrueckii* + *S. cerevisiae*

Two AF temperature conditions were tested: 25 °C and 16 °C. Two microbiological treatments were implemented: a control condition using *S. cerevisiae* QA23 (Sc, from Lallemand S.A.,), and a sequential condition involving the inoculation of *T. delbrueckii* Prelude Viniflora (TdP) (Chr. Hansen Holding AS, Hoersholm, Denmark) followed by Sc after a four-day interval, according to Ruiz-de-Villa et al. (2023c). Both active dry yeasts were rehydrated following the instructions provided by the manufacturer to achieve an initial population of $2{\cdot}10^6$ cells/mL. Strains Sc and TdP were rehydrated for 30 min at 37 °C and 30 °C, respectively. Three biological replicates for each condition were performed.

The monitoring of AF progress involved daily density measurements using a Densito 30PX portable density metre (Mettler, Toledo, Spain). AF was considered complete when the concentration of the reductive sugars reached less than 2 g/L according to a Y15 Enzymatic Auto-analyzer (Biosystems S.A., Barcelona, Spain). To evaluate population dynamics, YPD agar (Panreac Química SLU, Castellar del Vallés, Spain) was used for total yeast assessment, and the selective medium Lysine (BD Difco, Massachusetts, USA) was used to evaluate non-*Saccharomyces* yeasts. Yeast species were identified based on the amplicon size of the ITS-5.8 S rDNA region (Esteve-Zarzoso et al., 1999). Twenty colonies were isolated before Td or Sc inoculation and from wine at the end of AF.

2.2. General parameters

The pH of the wines was measured using a Crison Micro pH 2002 pH meter (Hach Lange Spain, l'Hospitalet, Spain). Acetic acid, L-malic acid, L-lactic acid, D-lactic acid, residual glucose and fructose were analysed using a Y15 Enzymatic Autoanalyzer.

To estimate the amount of mannoproteins, 10 mL of sample was first precipitated with 95 % (v/v) ethanol. Acid hydrolysis of the precipitate with 5 M sulfuric acid in water at 90 °C was performed, which resulted in the release of mannose. This was analysed following a previously outlined procedure (Balmaseda, Rozès, Bordons, & Reguant, 2021). The quantification of mannose equivalents was performed using a D-mannose and D-glucose enzymatic assay kit provided by Megazyme (Wicklow, Ireland).

For the analysis of citric acid, succinic acid, glycerol and ethanol, an Agilent 1100 HPLC system (Agilent Technologies, Waldbronn, Germany) was used according to the method described by Quirós et al. (2014). The samples were filtered through 0.22-μm pore filters from Merck. The HPLC system consisted of a Hi-Plex H column (300 mm × 7.7 mm) housed within a 1260 MCT (Infinity II Multicolumn Thermostat), and it included two detectors: an MWC detector (multiwavelength detector, Agilent Technologies) and an RID detector (1260 Infinity II refractive index detector, Agilent Technologies).

2.3. Volatile composition

The volatile compounds after AF were analysed following the procedure outlined in Ruiz-de-Villa et al. (2023c). Briefly, the samples underwent a pre-treatment process involving liquid-liquid extraction using 500 μL of methyl tert-butyl ether/hexane (1:1) and 100 μL of orthophosphoric acid/water (1:3) with 5 mL of wine. For this analysis,

25 μL of three internal standards were used (octanol-3, 1.98 g/L; heptanoic acid, 3.33 g/L; and heptadecanoic acid, 1.03 g/L). The mixture was stirred for 2 min then centrifuged at 5,200 × *g* for 5 min at room temperature. The upper organic phase was injected into a gas chromatograph with flame ionisation detection (GC-FID, Agilent Technologies). GC-FID analysis was performed under the following chromatographic conditions: an injection volume of 2 μL in splitless injection mode; an inlet temperature of 250 °C; a detector temperature of 250 °C; and an HP-FFAP column (30 m × 250 μm, 0.25 μm, Agilent Technologies). Concentrations were quantified using known external standards and calibration curves.

Volatile compounds identified based on retention times. Identified compounds were categorized into chemical groups including fusel alcohols, their acetates, ethyl esters of fatty acids, short-chain fatty acids (SCFAs), medium-chain fatty acids (MCFAs), and long-chain fatty acids (LCFAs).

2.4. Metabolomic analysis of wine samples

Wine metabolites were extracted using an organic solvent and then derivatized to make them volatile, which allowed their identification using GC–MS (Agilent Technologies), according to Garcia-Viñola et al. (2024). The metabolites were identified using the NIST17 library and confirmed via their own library (injection of pure standards). The results were normalized by calculating the ratio of the area of each identified metabolite to the area of the internal standard (tridecanoic acid). Each biological replicate was analysed.

2.5. Sensory analysis

A trained tasting panel comprised of 12 tasters performed a blind triangle test and a descriptive sensory analysis. The purpose of the triangle test was to identify any discernible differences between the wines (after AF) produced using different microbiological treatments: Sc alone versus the sequential inoculation of TdP and Sc. Following the triangle test, a descriptive sensory analysis was performed, where the tasters evaluated the intensity of five specific attributes using a numerical scale ranging from 0 to 5. These attributes included pear, tropical and grass aromas, acidity, bitterness, and global perception.

2.6. Malolactic fermentation

The AF replicates of each condition were combined and stored at 4 °C for one week to allow the wines to stabilise. The final concentration of L-malic acid was adjusted to achieve 2 g/L, and the pH was adjusted at 3.5 prior its addition. MLF was performed in a 500 mL glass container at 20 °C under anaerobic and static conditions. L-malic acid was monitored daily using a Y15 enzymatic autoanalyzer, until its concentration dropped below 0.1 g/L, when MLF was considered complete,.

MLF was carried out in three different inoculation conditions. One of them without inoculated strains, spontaneous MLF, and two inoculated with *O. oeni* strains Lalvin VP41 (Oo-VP41) from Lallemand SA, and PSU-1 (ATCC BAA-331), respectively, to achieve an initial population of $2{\cdot}10^7$ cells/mL. The strains were pre-cultured in MRS broth medium (Difco Laboratories, Detroit, MI, USA) supplemented with 4 g/L DL-malic acid (Sigma–Aldrich, Barcelona, Spain) and 5 g/L D-fructose (Panreac) at pH 5.0 at 28 °C in a 10 % CO_2 atmosphere, following the procedure described in Margalef-Català et al. (2017). Populations of *O. oeni* were monitored by plating on modified MRS media, which included 100 mL/L centrifuged tomato juice (Aliada, Madrid, Spain), 100 mg/L nystatin (Panreac), and 25 mg/L sodium azide (BioSciences, St. Louis MO, USA) (Margalef-Català et al., 2017), at 28 °C in a 10 % CO_2 atmosphere.

2.7. Lactic acid bacteria identification

Regarding LAB, random colonies from the inoculated MLF wines and 20 from the spontaneous MLF wines were isolated. DNA from the colonies was extracted with a High Pure PCR Template Preparation Kit (Roche, Barcelona, Spain). The LAB isolates were analysed using species-specific PCR targeting *O. oeni* (Zapparoli et al., 1998). *O. oeni* isolates were typed using multilocus variable number tandem repeat (VNTR) (Claisse & Lonvaud-Funel, 2014).

2.8. Statistical analysis

To ensure the consistency of the results, the AF and MLF processes were performed in biological triplicates. For statistical analyses of the obtained data, ANOVA and Tukey's test were used, using XLSTAT 2022.2.3 software (Addinsoft, Paris, France). A *p*-value threshold less than 0.05 was chosen to determine statistical significance. To process the sensory analysis data, Panel Check software (V1.February 4, 2012) was used.

Principal component analysis (PCA) was also performed to determine the relationships between the main metabolites analysed and the wine conditions. The main metabolites used were (the numbers correspond to both the order of retention time in the GC analysis and the PCA figure legend): 4, lactic acid; 12, pyruvic acid; 20, succinic acid monoethyl ester; 32, malic acid 1-ethyl ester; 33, malic acid 4-ethyl ester; 39, tyrosol; 40, 2-iisopropylmalic acid; 43, tartaric acid ethyl ester; Krebs, sum of organic acids in Krebs's cycle; and phenols, sum of phenolic acids. A 3-way ANOVA was performed to determine the effects of the three factors used in the experimental design and their interactions (yeast, type of vinification and fermentation temperature) on the families of metabolites, such as Krebs organic acids, total phenolic acids or ethyl esters of organic acids.

ANOVA-simultaneous component analysis (ASCA) was used to decompose the sources of variability affecting the volatile and metabolomic data. ASCA is a multivariate extension of ANOVA, which decomposes the variation in the data into the main effects and their binary combinations obtained from a predefined experimental design (Smilde et al., 2005). The present study considered three variability factors: yeast species, vinification style, fermentation temperature, and the binary interactions between these factors. A permutation test of 10000 iterations was performed for each ASCA model to assess the significance of each factor (a *p* value threshold of less than 0.05 indicated that the factor was significant) (Bertinetto et al., 2020). To perform this analysis Matlab 2023b (Mathworks Inc., Natick, MA, USA) and PLS_Toolbox version 9.0 for MATLAB (Eigenvector Inc, Manson, WA, USA) software was used.

3. Results and discussion

The design of the present study was intended to reflect realistic winemaking conditions. Muscat of Alexandria grapes, a cultivar commonly used for aromatic white wines, were selected, and fermentations were carried out with widely available commercial yeast strains under controlled inoculation protocols. These choices replicate common winery practices.

3.1. Alcoholic fermentation

Successful completion of the AF was observed across all conditions, as depicted in Figure SD1. However, noteworthy variations were observed between the different experimental setups. Sequential fermentations exhibited longer durations compared to the control group (Ruiz-de-Villa et al., 2023a). This disparity can be attributed to the presence of the two different yeast species, which compete for essential nutrients (Roca-Mesa et al., 2020). Notably, the difference between sequential and control fermentations became more pronounced in the

case of white wine at higher temperatures (H-W-Sc compared to H-W-Td). Skin-fermented wines initiated AF earlier, regardless of the yeast combination or temperature (Figure SD1). In contrast, white wines experienced a lag phase during the initial three days of fermentation, which indicated a slower start. This difference may be due to the presence of lipids in the grape skins, which enhanced yeast fermentative activity (Le Fur et al., 1994; Santos et al., 2011; Tumanov et al., 2015). Some fatty acids (FAs), such as linoleic acid, which can be assimilated by yeast (Thurston et al., 1981), increase yeast viability (Beltran et al., 2008). Sterols, such as β-sitosterol, which is the major phytosterol of grapes, may also be incorporated by yeast for growth (Luparia et al., 2004).

The fermentations at high temperature had shorter durations compared to lower temperature. This observation is consistent with the literature, which suggests that 25 °C is the optimal temperature for *S. cerevisiae* (Heard & Fleet, 1988). Therefore, fermentation at lower temperatures progresses more slowly due to the more stressful conditions.

Regarding yeast populations, grapes must reach a total yeast concentration of 8.4×10^4 CFU/mL before inoculation. For the sequential wines, *T. delbrueckii* represented greater than 80 % of the total yeast population before inoculation with *S. cerevisiae* and remained at 30 % after AF. Notably, the percentage of *T. delbrueckii* present was greater in orange and white wines at lower temperatures compared to higher temperatures. This result indicates that lower temperatures provide a more favourable environment for the persistence of *T. delbrueckii*. Previous studies also showed that *S. cerevisiae* had advantages over non-*Saccharomyces* yeasts, such as *T. delbrueckii*, at higher temperatures (Salvadó et al., 2011). Interestingly, in this study, *T. delbrueckii* maintained a high population at the end of the AF under all vinification conditions, as was previously described (Roca-Mesa et al., 2020; Ruiz--de-Villa, Poblet, et al., 2023c).

3.2. Physicochemical parameters after alcoholic fermentation

3.2.1. General oenological parameters

Various physicochemical parameters were analysed following the completion of AF, as shown in Table 2. Spontaneous MLF was observed under specific conditions (L-S-TD, H-W-Td and L-W-Td), which indicated the influence of *T. delbrueckii* regardless of the temperature or type of vinification. This is suported by the consumption of L-malic acid consumption and L-lactic acid production (Table 1). Therefore, a slight increase in pH was produced. However, MLF started during AF mainly without grape skins as L-malic acid consumption was lower in the presence of grape skins. Certain factors in grape skins likely delay MLF in sequential fermentations. D-lactic acid concentration varied between conditions, with the highest found in H-W-Td, followed by L-S-Td wine. Additionally, a significant ($p < 0.05$) reduction in citric acid was observed in L-S-Td wines compared to condition L-S-Sc, possibly owing also to LAB metabolism. These results highlighted the presence of autochthonous LAB in the initial grape must in a population enough to carry on spontaneously MLF.

The glycerol concentration increase in skin-fermented wines in presence of *T. delbrueckii* (H-S-Td and L-S-Td in Table 2), as previously reported (Mezey et al., 2025), specially low-temperature fermentation. This increase was previously attributed to the heightened glycerol-pyruvic pathway activity of *T. delbrueckii*, although strain-specific effects have also been observed (Loira et al., 2012). Skin-fermented wines revealed a lower trend in ethanol concentrations for the *S. cerevisiae* fermentations to the white fermentations. Bene and Kállay (2019) also reported lower levels of alcohol in traditional Qvevri wine (skin-fermented white wine in an amphora) than in traditional white wine vinification. However, with skin maceration, sequential inoculation with T. delbrueckii produced more ethanol than *S. cerevisiae* alone, while in white wines, the opposite was observed.

Wines fermented with T. delbrueckii showed a significant increase in mannoproteins (Table 2), as also reported in previous studies (Balmaseda, Rozès, Leal, et al., 2021; Belda et al., 2016; Ruiz-de-Villa et al., 2023b). Among all conditions, L-S-Td had the highest level (210 mg/L), suggesting that T. delbrueckii and skin contact at low temperatures enhance mannoprotein release during fermentation.

3.2.2. Volatile composition of wines

The volatile composition of the wines after AF was analysed for each biological replicate. The identified volatile compounds were acetates of fusel alcohols, such as isobutyl acetate, isoamyl acetate, and 2-phenylethanol acetate, fusel alcohols, such as amyl alcohol, isoamyl alcohol, hexanol, cis-3-hexenol, and 2-phenylethanol, ethyl esters of fatty acids (FAs), such as ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, ethyl lactate, and diethyl succinate, short-chain fatty acids (SCFAs), such as propanoic acid, butyric acid, isobutyric acid (2-methylpropanoic acid), pentanoic acid (valeric acid), and isovaleric acid (3-methylbutanoic acid), medium-chain fatty acids (MCFA), such as hexanoic acid, octanoic acid, decanoic acid, and dodecanoic acid, and long-chain fatty acids (LCFAs), such as tetradecanoic acid (myristic acid), palmitic acid, palmitoleic acid, stearic acid, oleic acid, and linoleic acid. The most interesting differences were found between the types of vinification, e.g., skin-fermented wines and white wines, within volatile families (Table 3). The presence of skins was associated with significantly higher levels of fusel alcohols. In contrast, the traditional white wine vinification process exhibited elevated concentrations of esters of FAs and acetates of fusel alcohols.

Although few studies evaluated the impact of skin presence during AF in white wines, certain authors also observed an increase in fusel alcohols and a corresponding decrease in several ethyl esters when AF was performed in the presence of skins in red grape varieties (Herraiz

Table 2
Oenological parameters analysed. Wines: H corresponds to high temperature of fermentation, L corresponds to low temperature of fermentation, S correspond to wines fermented in presence of grape skins (Skin-fermented wines), W correspond to White wines with racking (White wines), Sc corresponds to fermentation solely with *S. cerevisiae* and Td corresponds to sequential fermentation with *T. delbrueckii* and *S. cerevisiae*. Different lowercase letters indicate the existence of significant difference between samples at the end of alcoholic fermentation (p-value <0.05). n.d correspond to non-detected values. All data is expressed as the arithmetic average of three biological replicates ± standard deviation (n = 3).

	H-S-Sc	H-S-Td	L-S-Sc	L-S-Td	H-W-Sc	H-W-Td	L-W-Sc	L-W-Td
Citric acid (g/L)	0.34 ± 0.02 ^{ab}	0.24 ± 0.04 ^{a}	0.50 ± 0.09 ^{b}	0.26 ± 0.03 ^{a}	0.29 ± 0.10 ^{a}	0.28 ± 0.03 ^{a}	0.29 ± 0.04 ^{a}	0.23 ± 0.01 ^{a}
Succinic acid (g/L)	0.47 ± 0.06 ^{a}	0.48 ± 0.06 ^{a}	0.51 ± 0.02 ^{a}	0.39 ± 0.03 ^{a}	0.46 ± 0.02 ^{a}	0.50 ± 0.01 ^{a}	0.48 ± 0.03 ^{a}	0.53 ± 0.14 ^{a}
Acetic acid (g/L	0.21 ± 0.01 ^{a}	0.18 ± 0.01 ^{a}	0.44 ± 0.02 ^{c}	0.26 ± 0.04 ^{ab}	0.25 ± 0.06 ^{ab}	0.34 ± 0.01 ^{bc}	0.41 ± 0.05 ^{c}	0.41 ± 0.08 ^{c}
L-malic acid (g/L)	1.35 ± 0.09 ^{c}	0.95 ± 0.13 ^{b}	1.23 ± 0.11 ^{c}	0.16 ± 0.01 ^{a}	1.25 ± 0.05 ^{c}	n.d	1.25 ± 0.04 ^{c}	0.03 ± 0.02 ^{a}
L-lactic acid (g/L)	n.d	0.34 ± 0.30 ^{ab}	n.d	0.86 ± 0.44 ^{b}	n.d	1.51 ± 0.02 ^{c}	n.d	0.81 ± 0.11 ^{b}
D-lactic acid (g/L)	0.13 ± 0.01 ^{a}	0.13 ± 0.01 ^{a}	0.13 ± 0.05 ^{a}	0.32 ± 0.01 ^{b}	0.07 ± 0.01 ^{a}	0.49 ± 0.01 ^{c}	0.15 ± 0.04 ^{a}	0.09 ± 0.01 ^{a}
Glycerol (g/L)	5.89 ± 0.80 ^{abc}	7.25 ± 0.41 ^{bc}	5.56 ± 0.67 ^{ab}	7.55 ± 0.17 ^{c}	4.93 ± 0.97 ^{a}	5.97 ± 0.86 ^{abc}	4.86 ± 0.46 ^{a}	5.67 ± 0.41 ^{ab}
Ethanol (% v/v)	9.11 ± 0.37 ^{a}	9.43 ± 0.03 ^{ab}	9.71 ± 0.24 ^{abc}	9.77 ± 0.13 ^{bcd}	10.02 ± 0.22 ^{bcd}	9.70 ± 0.09 ^{abc}	10.34 ± 0.33 ^{d}	10.09 ± 0.13 ^{cd}
pH	3.37 ± 0.02 ^{bc}	3.33 ± 0.03 ^{b}	3.19 ± 0.01 ^{a}	3.42 ± 0.01 ^{c}	3.33 ± 0.01 ^{b}	3.40 ± 0.04 ^{c}	3.24 ± 0.04 ^{a}	3.40 ± 0.01 ^{c}
Eq-mannose (mg/L)	143.5 ± 18.2 ^{ab}	164.9 ± 19.4 ^{bc}	115.7 ± 7.6 ^{ab}	210.6 ± 16.5 ^{c}	113.7 ± 40.5 ^{ab}	117.1 ± 34.1 ^{ab}	93.9 ± 14.4 ^{a}	109.6 ± 7.5 ^{b}

Table 3
Composition of wine in volatile compounds (mg/L). FAs correspond to Fatty Acids, SCFA correspond to Short-Chain Fatty Acids, MCFA correspond to Medium-Chain Fatty Acids and LCFA correspond to Long-Chain Fatty acids. Wines: H corresponds to high temperature of fermentation, L corresponds to low temperature of fermentation, S correspond to wines fermented in presence of grape skins (Skin-fermented wines), W correspond to White wines with racking (White wines), Sc corresponds to fermentation solely with *S. cerevisiae* and Td corresponds to sequential fermentation with *T. delbrueckii* and *S. cerevisiae*. Different lowercase letters indicate the existence of significant difference between samples at the end of alcoholic fermentation (p-value <0.05). All data is expressed as the arithmetic average of three biological replicates ± standard deviation (n = 3).

	H-S-Sc	H-S-Td	L-S-Sc	L-S-Td	H-W-Sc	H-W-Td	L-W-Sc	L-W-Td
Fusel alcohol acetates	0.83 ± 0.19 ^{ab}	1.37 ± 0.16 ^{abc}	1.08 ± 0.62 ^{ab}	0.49 ± 0.08 ^{a}	2.71 ± 0.19 ^{d}	1.80 ± 0.17 ^{bcd}	2.31 ± 0.20 ^{cd}	1.81 ± 0.67 ^{bcd}
Ethyl esters of FAs	2.42 ± 0.41 ^{ab}	1.56 ± 0.06 ^{a}	1.57 ± 0.11 ^{a}	3.25 ± 0.75 ^{bc}	4.36 ± 0.65 ^{c}	2.99 ± 0.15 ^{abc}	4.37 ± 0.23 ^{c}	3.31 ± 1.32 ^{bc}
Fusel alcohols	328 ± 16 ^{abc}	407 ± 13 ^{c}	354 ± 75 ^{bc}	298 ± 12 ^{ab}	264 ± 25 ^{ab}	264 ± 10 ^{ab}	269 ± 14 ^{ab}	254 ± 32 ^{a}
SCFA	37.49 ± 1.41 ^{e}	26.38 ± 2.42 ^{d}	17.60 ± 2.52 ^{bc}	19.37 ± 1.77 ^{c}	6.87 ± 0.65 ^{a}	23.07 ± 2.59 ^{cd}	5.84 ± 0.58 ^{a}	10.55 ± 2.84 ^{b}
MCFA	1.12 ± 0.21 ^{a}	0.83 ± 0.07 ^{a}	2.22 ± 0 0.33 ^{bc}	2.22 ± 0.12 ^{bc}	2.76 ± 0.27 ^{c}	1.48 ± 0.06 ^{ab}	1.03 ± 0.17 ^{a}	1.46 ± 0.88 ^{ab}
LCFA	9.66 ± 1.86 ^{bc}	7.07 ± 0.63 ^{ab}	11.36 ± 1.38 ^{c}	5.79 ± 0.09 ^{a}	8.30 ± 1.52 ^{abc}	6.64 ± 0.79 ^{ab}	7.16 ± 0.19 ^{ab}	5.54 ± 0.90 ^{a}

et al., 1990). Other researchers examined the effects of pre-fermentative maceration on white wines. For example, an investigation of the Muscat grape variety revealed that a 23-h maceration at 18 °C led to higher levels of free and glycosylated-bound aroma compounds (Sánchez Palomo et al., 2006). However, grape and yeast glycoside activity may not be sufficient under fermentative conditions. As a potential solution, the addition of glycosidic and pectolytic enzymes enhanced the release of varietal volatile compounds in skin-fermented white wines (Cabaroglu et al., 2003).

Significant differences were observed in SCFA and LCFA concentrations, with higher levels detected in skin-fermented wines. These results are consistent with the known presence of fatty acids in grape skins, which, as previously discussed, may influence the progression and efficiency of AF. This phenomenon could be related to the presence of FAs in the skins of the grapes, which, as discussed above, contribute to the improved performance of AF observed in these wines. Similarly, Herraiz et al. (1990) also noted an increase in SCFA with the inclusion of grape skins during AF. Temperature effects became evident for SCFA and MCFA. Notably, SCFA concentrations increased at higher temperatures in skin-fermented and white wines. The MCFA decreased in white wines fermented at lower temperatures with *S. cerevisiae* (L-W-Sc). In contrast, MCFA levels increased at lower temperatures in skin-fermented wines.

Vinification type and AF temperature influenced the behavior of *T. delbrueckii*. Its presence increased SCFA levels in white wines (H-W-Td and L-W-Td), while in skin-fermented wines (H-S-Td), SCFA concentrations decreased at higher temperatures but remained stable at lower ones (L-S-Td). At low temperatures, L-S-Td wines showed elevated ester levels, as *T. delbrueckii* has been characterized by the production of this family of compound by Zhang et al. (2024) and reduced LCFA compared to controls. High temperatures led to significantly lower MCFA concentrations in *T. delbrueckii* inoculated wines, especially in H-W-Td, with a similar trend in H-S-Td. In contrast, no MCFA reduction was observed at low temperatures (L-W-Td and L-S-Td). Sequential fermentations with *T. delbrueckii* reduce the concentration of MCFA in the final wine (Balmaseda, Rozès, Leal, et al., 2021; B. Q. Zhang et al., 2018), and this reduction depends on the strain and AF conditions (Balmaseda et al., 2023). This reduction was confirmed by Cheng et al. (2025), who reported significant MCFA decreases in co-fermentations with *T. delbrueckii*.

3.2.3. Metabolomic analysis

To better understand the effect of these three factors on the wines obtained, a metabolomic analysis was performed. First, a PCA was performed to determine which metabolites contributed to the overall variation observed in the wines under these conditions (type of vinification and inoculation and fermentation temperature) (Fig. 1).

The first principal component (PC1) explained 58.62 % of the variance, and the second (PC2) explained 18.23 %. The loading variables presented in Fig. 1B indicate the contributions provided by the two components related to their length and direction. The loadings on PC1 show that the variables with the highest contributions, in decreasing order, are organic Krebs acids, malic acid 4-ethyl ester and malic acid 1-ethyl ester. Additionally, the sum of phenolic acids, tyrosol, pyruvate and 2-isopropylmalate also contribute positively (toward the right side in Fig. 1B).

Fig. 1A shows that all of the skin-fermented wines (S) were primarily

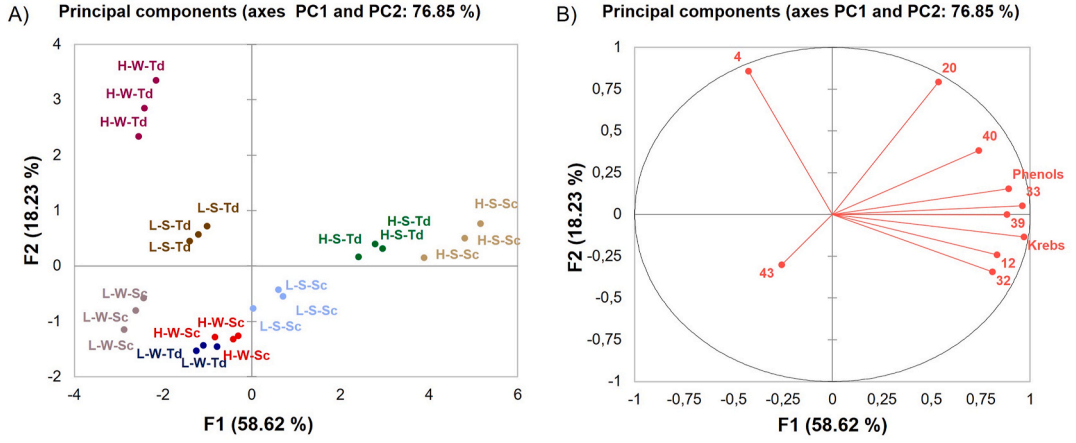

Fig. 1. Principal component analysis of the metabolites analysed. The two-dimensional principal subspace for wine data (A) and the variables used (B). Variables: 4, Lactic acid; 12, Pyruvic acid; 20, Succinic acid mono ethyl ester; 32, Malic acid 1-ethyl ester; 33, Malic acid 4-ethyl ester; 39, Tyrosol; 40, 2-Isopropylmalic acid; 43, Tartaric acid ethyl ester; Krebs, sum of organic acids in the Krebs cycle; Phenols, sum of phenolic acids. Wines: H corresponds to high temperature of fermentation, L corresponds to low temperature of fermentation, S correspond to wines fermented in presence of grape skins (Skin-fermented wines), W correspond to White wines with racking (White wines), Sc corresponds to fermentation solely with *S. cerevisiae* and Td corresponds to sequential fermentation with *T. delbrueckii* and *S. cerevisiae*.

related to a high content of organic Krebs acids and phenolic acids, and the wines obtained by grape must racking (W) are shown in the left part of Fig. 2A, which indicates the presence of phenolic acids and the lowest content of organic Krebs acids. However, there is an exception for L-S-Td. This result was expected because the phenolic acids were primarily located in the grape skins. The skin-fermented wines formed four clusters based on inoculation type and fermentation temperature. Higher temperature (25 °C) increased phenolic acid levels, with single inoculations showing higher concentrations than sequential ones at the same temperature. This characteristic was also linked to a higher content of ethyl malate esters, organic Krebs acids, pyruvate and 2-isopropylmalate. The loadings on PC2 explained the lactate and succinate mono-ethyl ester contents. Therefore, the sequential fermentations were separated from the rest of the conditions according to PC2 because of the spontaneous MLF in H-W-Td wines.

The most interesting compounds were individually analysed. Fig. 2 shows the variation between organic acids in the Krebs cycle, phenolic acids and 2-isopropylmalic acid. The amount of organic acids from the Krebs cycle (Fig. 2A) significantly increased in skin-fermented wines at high temperature, especially wines fermented with only *S. cerevisiae* (H-S-Sc). However, in *S. cerevisiae* wines, organic acids dropped at both temperatures. In contrast, organic acids decreased significantly only at high temperature in the presence of *T. delbrueckii* (H-W-Td).

The phenolic acid content (Fig. 2B) was significantly lower in white wines. This result was expected because the phenolic compounds present in the grape skin were released with skin maceration during AF (Beara et al., 2024; Bene & Kállay, 2019; Maante-Kuljus et al., 2024)). A notable reduction in hydroxycinnamic acids was found in the presence of *T. delbrueckii* compared to *S. cerevisiae*, especially p-coumaric acid, specially at lower temperatures. Ngqumba et al. (2017) reported a reduction in certain phenolic acids (ferulic and gallic acid) in wines produced via monoculture and the co-inoculation of *T. delbrueckii* and *S. cerevisiae*.

These findings are consistent with the hydroxycinnamate decarboxylase (HCDC) activity reported in *T. delbrueckii* strains (Božič et al., 2020), which is also present in other non-*Saccharomyces* species (Benito et al., 2011; Božič et al., 2020). Although the intensity of this activity is lower than that typically observed in *S. cerevisiae* (Božič et al., 2020), suggesting potential complementary roles when both yeasts are present. This observation suggests that *T. delbrueckii* metabolises hydroxycinnamic acids into vinylphenols. This effect is particularly relevant in red wines, where these compounds can react with anthocyanins to form stable vinylphenolic pyranoanthocyanins (Schwarz et al., 2003; Benito et al., 2011; Ruiz-de-Villa et al., 2023a). In white wines, however, the reduction of hydroxycinnamic acids contribute to lower astringency (Ferrer-Gallego et al., 2014), which could be advantageous in skin-fermented styles.

Regarding *S. cerevisiae*, a decrease in phenolic acid content was observed at lower temperatures (Fig. 2B), consistent with strain-dependent HCDC activity that is less active at higher fermentation temperatures (Božič et al., 2020).

Finally, although hydroxycinnamic acid degradation could potentially involve LAB, all strains isolated during AF and MLF were identified as *O. oeni*, which lacks the ability to decarboxylate hydroxycinnamic acids (De Las Rivas et al., 2009).

The identification of 2-isopropylmalic acid (2-IPMA) (Fig. 2C) is particularly noteworthy due to the limited knowledge of this compound in the wine environment. Along with 3-isopropylmalic acid (3-IPMA), 2-IPMA is an intermediate in the leucine biosynthesis pathway in yeast (Dumlao et al., 2008; Marobbio et al., 2008). These intermediates can be secreted into the medium, particularly under amino acid starvation conditions In *S. cerevisiae*, it is formed in the mitochondria from α-ketoisovalerate and acetyl-CoA by the enzyme isopropylmalate synthase and then exported to the cytosol for further conversion. While typically intracellular, 2-IPMA can be secreted into the medium, especially under amino acid limitation or metal stress (Dumlao et al., 2008). Its recent detection in wines via untargeted metabolomics revealed concentrations up to ~30 mg/L, particularly in red wines, and suggests yeast metabolic overflow during nitrogen-stressed fermentation conditions (Ricciutelli et al., 2020). Although 2-IPMA is non-volatile and does not contribute directly to aroma or taste, its presence serves as a metabolic marker of anabolic yeast activity. It may correlate indirectly with sensory outcomes, given that leucine biosynthesis intersects with the Ehrlich pathway leading to higher alcohols such as isoamyl alcohol (Lu et al., 2023).

At elevated temperatures, significantly higher 2-IPMA

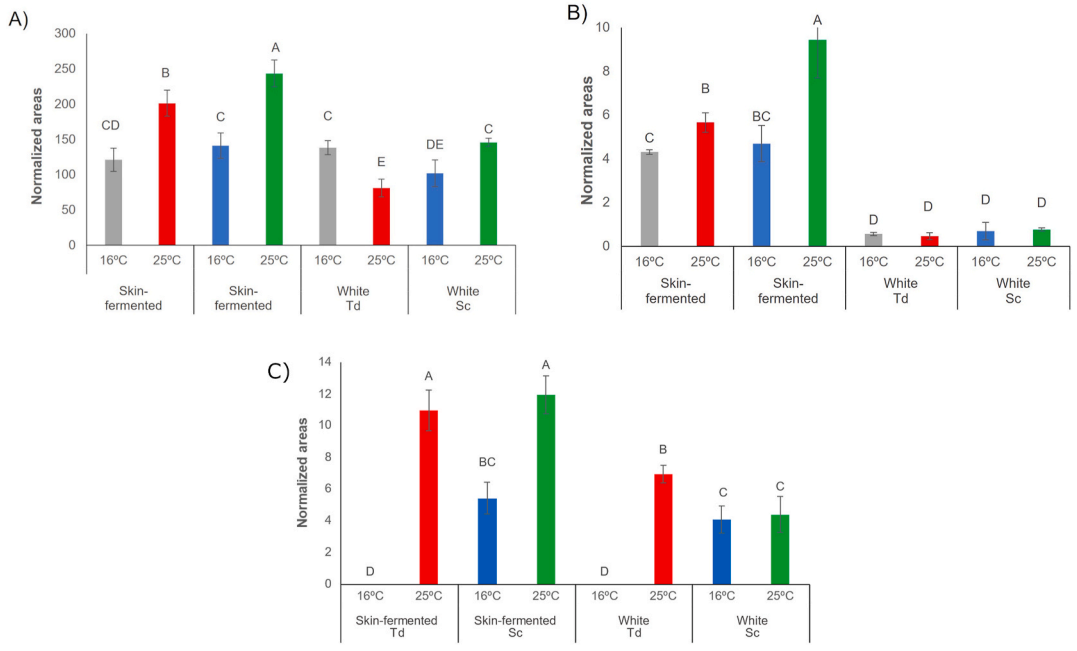

Fig. 2. Normalized areas of more highlighted compounds: A) Organic acids from the Krebs Cycle, B) Phenolic compounds and C) 2-isopropylmalic acid. Wine codes described in Table 1. Letters indicate significant changes between conditions.

concentrations were observed in skin-fermented wines compared to white wines, suggesting a potential contribution of grape skins. This aligns with previous reports showing higher 2-IPMA levels in red wines (Ricciutelli et al., 2019, 2020) and with its presence in apple skins (Sugimoto et al., 2021).

Fermentations with *T. delbrueckii* showed significant differences in 2-IPMA levels, indicating a possible species-specific influence on its metabolism. Additionally, lower concentrations of this metabolite were found in low temperatures wines.

Beyond its metabolic role, 2-IPMA exhibits antimicrobial activity against several pathogenic bacteria (Ricciutelli et al., 2020). Further research is needed to elucidate its potential effects on wine-relevant microorganisms such as LAB and acetic acid bacteria.

3.3. Assessment of variability caused by oenological practices

An ASCA model was used to study the variability in metabolome and volatile data associated with the different factors considered: type of vinification and inoculation and fermentation temperature, individualizing their effects and interactions, complementing univariate analyses. ASCA results are expressed as a percentage effect, which indicates the contribution of each factor to matrix variability. Thus, Table 4 summarizes the results for the 62 and 23 molecules identified in the metabolomic and volatile analyses, respectively.

As shown by the ASCA results (Table 4), all of the considered factors significantly impacted the metabolome and volatile composition of the obtained wines. The "type of winemaking" factor made a greater contribution to the overall variability of the wines (28.16 %), which highlights the great contribution of grape skins to the characteristics of the wine. However, "Fermentation temperature" and "Yeast" are also significant factors, which was suspected from the recent literature on the use of *T. delbrueckii* (Azzolini et al., 2015; Puertas et al., 2017) and the impact of temperature on organoleptic wine characteristics (Beltran et al., 2008). The binary combinations of the factors was also significant, which means that different factors have different effects depending on the other factors. This result would mean that temperature does not affect wines fermented with *S. cerevisiae* the same as compared to sequential inoculation with *T. delbrueckii*. The remaining unexplained variability (24.30 %) may be due to the inherent variability of biological replicates (Schorn-García et al., 2023). These results indicate that winemakers will be able to obtain different wines by modulating the three factors.

The ASCA individualises the variability of the data for each of the considered factors. Therefore, it is possible to attribute the greater variability to a specific factor. For the "type of winemaking", the factor had the greatest influence on the phenolic composition of wines macerated by skins and other compounds, such as tyrosol and succinate, fumarate and oxaloacetate (Krebs cycle metabolites) in terms of the metabolome, and fusel alcohols (especially 2-phenylethanol) and SCFA for volatile composition. The vinification of white wine is more related to higher acetate and ester concentrations. For the factor "Fermentation temperature", the high temperature was related to an increase in the 2-IPMA concentration, as previously observed, and a low temperature was related to an increase in the MCFA and LCFA concentrations. The "yeast" factor was related to *T. delbrueckii* inoculation, which was associated with higher concentrations of acids from the Krebs cycle and valeric acid.

Table 4
ASCA results for the metabolomic and volatile analysis showing the percentage of variance (Effect (%)) for each factor and the *p*-value obtained from the permutation test. * Corresponds to a *p*-value <0.05, which means that the factor is significant.

Factor	Effect (%)
Yeast	11.51*
Type of winemaking	28.16*
Fermentation temperature	13.23*
Yeast x Type of winemaking	6.08*
Yeast x Fermentation temperature	7.50*
Type of winemaking x Fermentation temperature	9.22*

3.4. Sensory analysis

The organoleptic analysis focused on the sensory characteristics resulting from the use of *T. delbrueckii*. Sensory analysis was important to verify that the differences in chemical composition observed in the wines were also perceived organoleptically. For this purpose, the wines after AF were subjected to a triangular analysis. This analysis compared wines fermented solely with *S. cerevisiae* to wines produced using sequential fermentation with *T. delbrueckii* (H-S-Sc vs. H-S-Td, L-S-Sc vs. L-S-Td, H-W-Sc vs. H-S-Td, and L-W-Sc vs. L-S-Td) according to different vinification conditions. The results showed significant differences in all combinations, which indicated that the use of *T. delbrueckii* in sequential fermentation had a distinct impact on the organoleptic properties of the wines regardless of temperature or the presence of grape skins during AF (data not shown). These differences were attributed to spontaneous MLF, but the tasters differentiated the wine fermented with *T. delbrueckii* even in conditions with incomplete MLF.

A descriptive analysis of the wines was subsequently performed and focused on tropical, pear, terpenic and grass aromas, acidity, bitterness, and overall harmony. These attributes were selected based on the grape cultivar and wine type. As shown by the PCA results in Fig. 5A, the skin-fermented wines subjected to sequential fermentation were separated from wines fermented solely with *S. cerevisiae* based on PC1. The H-S-Td wines exhibited positive correlations with pear, tropical, and terpenic aromas and global harmony, and the L-S-Td wines showed stronger associations with terpenic and grass aromas. Conversely, L-S-Sc and H-S-Sc negatively correlated with these variables. Notably, L-S-Td had an inverse correlation with acidity due to its spontaneous MLF. For the white wines (Fig. 5B), PC2 distinguished the wines fermented with *T. delbrueckii* from wines fermented solely with *S. cerevisiae*. L-W-Td and H-W-Td positively correlated with terpenic aromas and overall harmony, particularly L-W-Td. They also exhibited negative correlations with acidity due to the occurrence of spontaneous MLF. PC1 separated L-S-Td and L-S-Sc from H-W-Td and H-W-Sc, because white wines fermented at lower temperatures showed stronger associations with all analysed aromas. Fermentation temperature, particularly for white wines, significantly influences the aromatic composition of wines (Beltran et al., 2008).

There was an influence of the factors considered and the interaction between them, which justified the absence of significant differences in many cases. Although only a limited number of significant variations were found in the analysis of volatile compounds between sequential wines with *T. delbrueckii* and wines solely fermented with *S. cerevisiae*, a more intricate difference in aromatic profiles became evident in the sensory analysis. This phenomenon is attributed to aromatic synergy, wherein the interplay of various compounds contributes to overall sensory perception.

3.5. Malolactic fermentation

The goal was to initiate MLF after AF, but musts had unexpectedly high indigenous LAB levels (4×10^4 CFU/mL). In H-S-Td, L-S-Td, H-W-Td, and L-W-Td wines, LAB populations remained high until the end of AF (1×10^5 CFU/mL), leading to spontaneous MLF, except in H-S-Td. In Sc wines, LAB levels were lower (2×10^3 CFU/mL), preventing spontaneous MLF.

These results align with previous studies showing that *T. delbrueckii* can promote spontaneous MLF due to its synergy with *O. oeni*, accelerating L-malic acid degradation (Balmaseda, Rozès, Bordons, & Reguant, 2021; Balmaseda et al., 2023; Ruiz-de-Villa et al., 2023b; Ruiz-de-Villa

et al., 2023c). This effect has been observed in red wines produced using traditional (Balmaseda, Rozès, Leal, et al., 2021) and carbonic maceration (Ruiz-de-Villa et al., 2023b) methods.

For wines without spontaneous MLF (H-O-Td, H-O-Sc, L-O-Sc, H-W-Sc, L-W-Sc), MLF was induced using two *O. oeni* strains. As shown in Fig. 3, L-malic acid was consumed fastest in H-O-Td by both strains. Among spontaneous fermentations, L-O-Sc completed MLF most quickly. Notably, L-malic acid consumption in H-O-Td occurred 11 days earlier than in its control (H-O-Sc), completing in 3 days versus 17.

The beneficial impact of *T. delbrueckii* has been documented across diverse matrices, including white wines (Balmaseda, Rozès, Leal, et al., 2021). This effect is the result of a combination of factors, where *T. delbrueckii* positively influences the composition of the wine after AF by enhancing the activity of *O. oeni*. This process begins with the reduction of certain compounds toxic to *O. oeni*, such as MCFA (Edwards & Beelman, 1987). Notably, the reduction of these compounds depends on the strain of *T. delbrueckii* and the specific winemaking conditions. A significant reduction in MCFA was only observed in the H-W-Td replicates compared to *S. cerevisiae* (Table 3).

Conversely, the increased release of mannoproteins into media by *T. delbrueckii* has the potential to improve the performance of MLF. These mannoproteins can be metabolised by *O. oeni* (Balmaseda, Rozès, Bordons, & Reguant, 2021; Jamal et al., 2013) and contribute to the detoxification of media from inhibitory compounds (Lafon-Lafourcade et al., 1984), which supports MLF progression. This study revealed a mannoproteins concentration increase in sequential fermentations with *T. delbrueckii,* especially at low fermentation temperatures (Table 1). Hydroxycinnamic acids, especially p-coumaric acid, have been shown to inhibit *O. oeni* (Reguant et al., 2000). Their reduction in skin-fermented wines with sequential AF using *T. delbrueckii* may be linked to the acceleration of MLF.

3.5.1. *Lactic acid bacteria identification and strain typing*

Considering the large population of LAB detected, species identification and strain typing were performed. All isolates were identified as *O. oeni* using species-specific PCR. Nineteen different VNTR profiles were identified (Fig. 4), which may be associated with different *O. oeni* strains (Claisse & Lonvaud-Funel, 2014). In L-S-Td, H-W-Td and L-W-Td wines, inoculated strain identification was not possible due to spontaneous MLF during AF.

LAB profile variability differed across conditions. Two profiles (M1 and M2) were detected in must, with M2 persisting through AF and spontaneous MLF, especially in H-W-Sc and H-W-Td wines, and showing 20–50 % prevalence in other spontaneous cases. In H-S-Sc wine, M2 even outcompeted the inoculated strain OoVP41, suggesting potential for future use. Another recurrent profile, C5, was present at the end of MLF in all wines inoculated with OoVP41, indicating good adaptation and competitiveness.

OoVP41 showed weak dominance, likely due to indigenous LAB competition or slower MLF kinetics, though it appeared more often after MLF in low-AF-temperature wines (e.g., L-W-Sc). A matching profile (VP41*) was also found in non-inoculated wines, suggesting it had colonized the winery. In contrast, OoPSU-1 showed strong dominance (>70 %) in all inoculated wines, in line with previous findings of up to 100 % imposition (Balmaseda, Rozès, Bordons, & Reguant, 2021, Balmaseda, Rozès, Leal, et al., 2021).

4. Conclusion

This study combined a factorial design, including vinification style, sequential *T. delbrueckii* inoculation, and fermentation temperature. Furthermore, by integrating classical oenological parameters with metabolomic, volatile compound, microbiological, and sensory analyses, the work provided a broad perspective on how these factors jointly influenced alcoholic fermentation performance, malolactic fermentation dynamics, and the resulting composition and perception of skin-fermented white wines.

The results showed that the performance of AF and the final wine composition varied depending on the experimental conditions: yeast (*T. delbrueckii* and *S. cerevisiae*), fermentation temperature (16 or 25 °C) and type of fermentation (presence or absence of grape skins).

For metabolic changes, the ASCA model revealed the collective influence of these factors on matrix variability. Remarkably, the type of vinification was the most influential factor contributing to the overall variance in the wines, followed by fermentation temperature and yeast species, all of which exhibited statistically significant affects. Sensory analysis observed that trained tasters differentiated the wines fermented in the presence of *T. delbrueckii* regardless of temperature or the presence of grape skins during AF.

Regarding the dynamics of MLF, this study highlights the influence of the presence of *T. delbrueckii* and its synergistic relationship with *O. oeni* in this type of vinification.

Further research expanding the experimental conditions, including broader microbial diversity, greater grape variety, and larger winemaking scales would enhance the generalizability of these findings. As sensory analysis was limited to the post-fermentation stage, the long-term impact after aging or bottling remains to be determined. In terms of applicability, this study offers valuable insights for winemakers interested in skin-fermented white wines and the use of *T. delbrueckii*. It is interesting, as research on orange wines is limited, despite their increasing consumption and production in certain regions. Moreover, understanding how *T. delbrueckii* affects MLF and overall wine composition in different wine matrices is important, as it can guide decisions on yeast inoculation and vinification practices.

CRediT authorship contribution statement

Candela Ruiz-de-Villa: Writing – original draft, Methodology, Investigation. **Violeta García-Viñola:** Investigation. **Daniel Schorn-García:** Data curation. **Montse Poblet:** Visualization, Supervision.

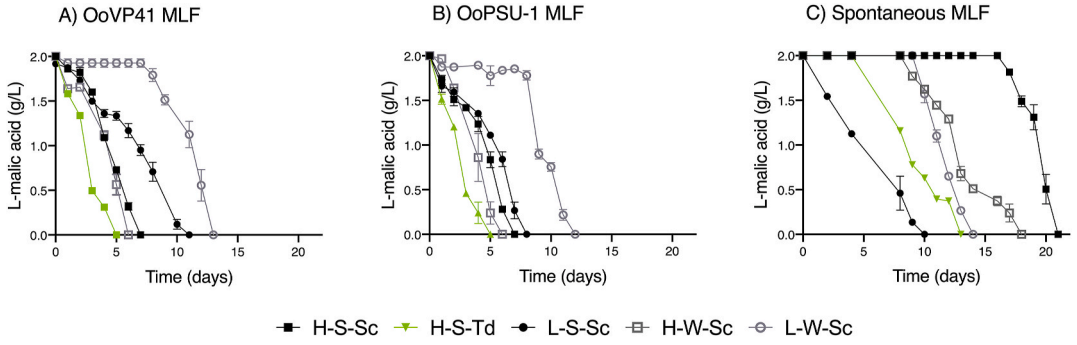

Fig. 3. Consumption of L-malic acid during malolactic fermentation. A) corresponds to OoVP41 MLF; B) corresponds to OoPSU-1 MLF, and C) corresponds to spontaneous MLF. Wine codes described in Table 1. All data is expressed as the arithmetic average of three biological replicates ± standard deviation (n = 3).

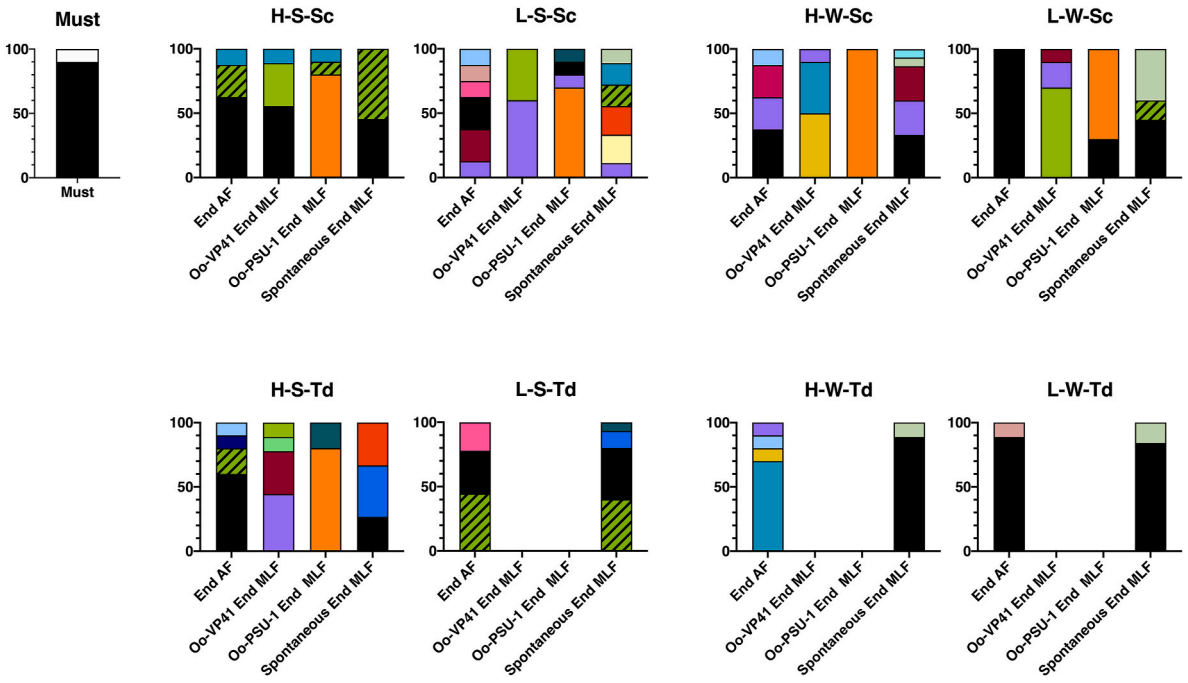

Fig. 4. Percentage of *O. oeni* strains during vinification, categorized by their VNTR profiles. Codes M1 and M2 refer to profiles of *O. oeni* identified in grape must at the start of fermentation. The code VP41* represents the OoVP41 strain isolated from the winery environment, while VP41 corresponds to the OoVP41 strain inoculated into the wine. PSU-1 corresponds to the OoPSU strain that was also inoculated. Profiles coded as C1 to C15 represent the distinct profiles of *O. oeni* strains identified throughout the alcoholic fermentation (AF) and malolactic fermentation (MLF) stages. Wine codes are detailed in Table 1.

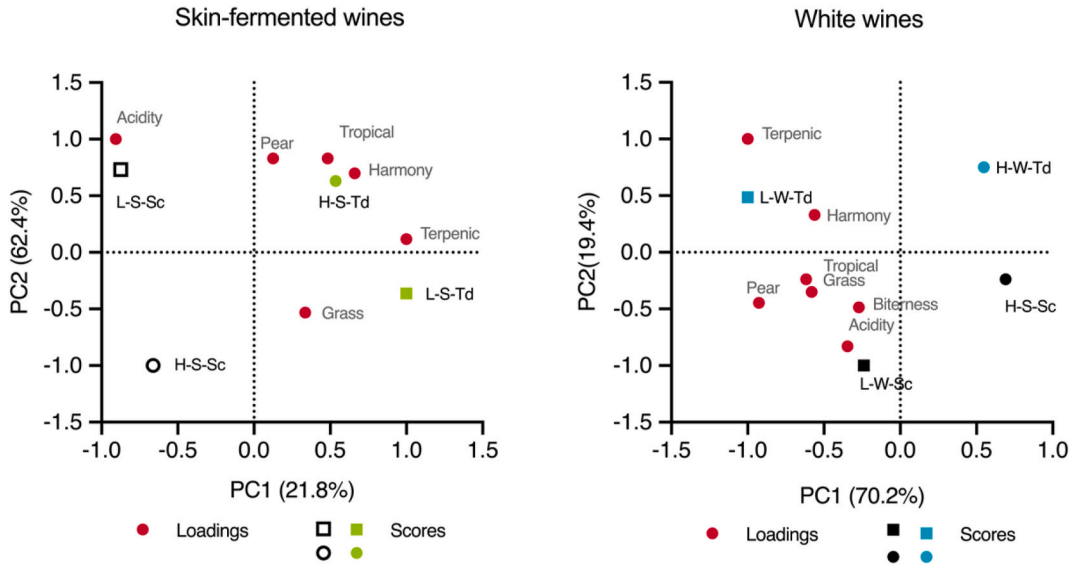

Fig. 5. Principal component analysis biplots built from the following variables: Pear, grass, terpenic aromas, bitterness, acidity and harmony. A) Correspond to skin-fermented wines and B) Corresponds to white wines. Wine codes described in Table 1.

Albert Bordons: Visualization, Supervision. **Cristina Reguant:** Writing – review & editing, Supervision, Funding acquisition. **Nicolas Rozès:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

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.

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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.2025.118608.

Data availability

Data will be made available on request.

References

Azzolini, M., Tosi, E., Lorenzini, M., Finato, F., & Zapparoli, G. (2015). Contribution to the aroma of white wines by controlled *Torulaspora delbrueckii* cultures in association with *Saccharomyces cerevisiae*. *World Journal of Microbiology and Biotechnology, 31*(2), 277–293. https://doi.org/10.1007/s11274-014-1774-1

Balmaseda, A., Rozès, N., Bordons, A., & Reguant, C. (2021). Simulated lees of different yeast species modify the performance of malolactic fermentation by *Oenococcus oeni* in wine-like medium. *Food Microbiology, 99*, Article 103839. https://doi.org/10.1016/j.fm.2021.103839

Balmaseda, A., Rozès, N., Bordons, A., & Reguant, C. (2023). The use of *Torulaspora delbrueckii* to improve malolactic fermentation. *Microbial Biotechnology, 7*(1), Article e14302. https://doi.org/10.1111/1751-7915.14302

Balmaseda, A., Rozès, N., Leal, M.Á., Bordons, A., & Reguant, C. (2021). Impact of changes in wine composition produced by non-*Saccharomyces* on malolactic fermentation. *International Journal of Food Microbiology, 337*, Article 108954. https://doi.org/10.1016/j.ijfoodmicro.2020.108954

Beara, I., Majkić, T., Milovanović, L., Svirčev, E., & Torović, L. (2024). Polyphenolic profile and in vitro biological activity of Serbian orange (skin fermented white) wines. *Food Chemistry, 447*. https://doi.org/10.1016/j.foodchem.2024.138933

Belda, I., Navascués, E., Marquina, D., Santos, A., Calderón, F., & Benito, S. (2016). Outlining the influence of non-conventional yeasts in wine ageing over lees. *Yeast, 33*(7), 329–338. https://doi.org/10.1002/yea.3165

Beltran, G., Novo, M., Guillamón, J. M., Mas, A., & Rozès, N. (2008). Effect of fermentation temperature and culture media on the yeast lipid composition and wine volatile compounds. *International Journal of Food Microbiology, 121*(2), 169–177. https://doi.org/10.1016/j.ijfoodmicro.2007.11.030

Bene, Z., & Kállay, M. (2019). Polyphenol contents of skin-contact fermented white wines. *Acta Alimentaria, 48*(4), 515–524. https://doi.org/10.1556/066.2019.48.4.13

Benito, S., Morata, A., Palomero, F., González, M. C., & Suárez-Lepe, J. A. (2011). Formation of vinylphenolic pyranoanthocyanins by *Saccharomyces cerevisiae* and *Pichia guillermondii* in red wines produced following different fermentation strategies. *Food Chemistry, 124*(1), 15–23. https://doi.org/10.1016/j.foodchem.2010.05.096

Bertinetto, C., Engel, J., & Jansen, J. (2020). ANOVA simultaneous component analysis: A tutorial review. *Analytica Chimica Acta X, 6*, Article 100061. https://doi.org/10.1016/J.ACAX.2020.100061

Bestulić, E., Rossi, S., Plavša, T., Horvat, I., Lukić, I., Bubola, M., Ilak Peršurić, A. S., Jeromel, A., & Radeka, S. (2022). Comparison of different maceration and non-maceration treatments for enhancement of phenolic composition, colour intensity, and taste attributes of Malvazija istarska (Vitis vinifera L.) white wines. *Journal of Food Composition and Analysis, 109*. https://doi.org/10.1016/j.jfca.2022.104472

Božič, J. T., Butinar, L., Albreht, A., Vovk, I., Korte, D., & Vodopivec, B. M. (2020). The impact of *saccharomyces* and non-*Saccharomyces* yeasts on wine colour: A laboratory study of vinylphenolic pyranoanthocyanin formation and anthocyanin cell wall adsorption. *Lebensmittel-Wissenschaft und -Technologie, 123*, Article 109072. https://doi.org/10.1016/j.lwt.2020.109072

Cabaroglu, T., Selli, S., Canbas, A., Lepoutre, J. P., & Günata, Z. (2003). Wine flavor enhancement through the use of exogenous fungal glycosidases. *Enzyme and Microbial Technology, 33*(5), 581–587. https://doi.org/10.1016/S0141-0229(03)00179-0

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 Microbiology, 84*, Article 103247. https://doi.org/10.1016/j.fm.2019.103247

Cheng, Y., Geng, S., Zhang, J., Zhao, X., Jiang, J., Liang, Y., Mu, H., Li, W., Qin, Y., Liu, Y., & Song, Y. (2025). A comprehensive study on fermentation and aroma contributions of Torulaspora delbrueckii in diverse wine varieties: Insights from pure and co-fermentation studies. *Food Research International, 199*, Article 115340. https://doi.org/10.1016/j.foodres.2024.115340

Claisse, O., & Lonvaud-Funel, A. (2014). Multiplex variable number of tandem repeats for *Oenococcus oeni* and applications. *Food Microbiology, 38*, 80–86. https://doi.org/10.1016/j.fm.2013.08.012

De Las Rivas, B., Rodríguez, H., Curiel, J. A., Landete, J. M., & Muñoz, R. (2009). Molecular screening of wine lactic acid bacteria degrading hydroxycinnamic acids. *Journal of Agricultural and Food Chemistry, 57*(2), 490–494. https://doi.org/10.1021/jf803016p

Dumlao, D. S., Hertz, N., & Clarke, S. (2008). Secreted 3-isopropylmalate methyl ester signals invasive growth during amino acid starvation in *Saccharomyces cerevisiae*. *Biochemistry, 47*(2), 698–709. https://doi.org/10.1021/bi7018157

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. *American Journal of Enology and Viticulture, 38*, 239–242. https://doi.org/10.5344/ajev.1987.38.3.239

Esteve-Zarzoso, B., Belloch, C., Uruburu, F., & Querol, A. (1999). Identification of yeasts by RFLP analysis of the 5.8S rRNA gene and the two ribosomal internal transcribed spacers. *International Journal of Systematic Bacteriology, 49*(1), 329–337. https://doi.org/10.1099/00207713-49-1-329

Ferrer-Gallego, R., Hernández-Hierro, J. M., Rivas-Gonzalo, J. C., & Escribano-Bailón, M. T. (2014). Sensory evaluation of bitterness and astringency sub-qualities of wine phenolic compounds: Synergistic effect and modulation by aromas. *Food Research International, 62*, 1100–1107. https://doi.org/10.1016/J.FOODRES.2014.05.049

Garcia-Viñola, V., Ruiz-de-Villa, C., Gombau, J., Poblet, M., Bordons, A., Reguant, C., & Rozès, N. (2024). Simultaneous analysis of organic acids, glycerol and phenolic acids in wines using gas chromatography-mass spectrometry. *Foods, 13*(2), 186. https://doi.org/10.3390/foods13020186

Glonti, T. (2001). Le Bulletin de la OIV. Traditional technologies and history of Georgian wine. www.oiv.int.

Heard, G. M., & Fleet, G. H. (1988). The effects of temperature and pH on the growth of yeast species during the fermentation of grape juice the effects of temperature and pH on the growth of yeast species during the fermentation of grape juice. *Journal of Applied Bacteriology, 65*(1), 23–28. https://doi.org/10.1111/j.1365-2672.1988.tb04312.x

Herraiz, T., Martin-Alvarez, P. J., Reglero, G., Herraiz, M., & Cabezudo, M. D. (1990). Effects of the presence of skins during alcoholic fermentation on the composition of wine volatiles. *Vitis, 2*, 239–249.

Jamal, Z., Miot-Sertier, C., Thibau, F., Dutilh, L., Lonvaud-Funel, A., Ballestra, P., Le Marrec, C., & Dols-Lafargue, M. (2013). Distribution and functions of phosphotransferase system genes in the genome of the lactic acid bacterium *Oenococcus oeni*. *Applied and Environmental Microbiology, 79*(11), 3371–3379. https://doi.org/10.1128/AEM.00380-13

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

Kemp, B., Botezatu, A., Charnock, H., Inglis, D., Marchal, R., Pickering, G., Yang, F., & Willwerth, J. (2021). *White winemaking in cold climates. White Wine Technology*. Elsevier. https://doi.org/10.1016/B978-0-12-823497-6.00007-7

Lafon-Lafourcade, S., Geneix, C., & Ribéreau-Gayon, P. (1984). Inhibition of alcoholic fermentation of grape must by fatty acids produced by yeasts and their elimination by yeast ghosts. *Applied and Environmental Microbiology, 47*(6). https://journals.asm.org/journal/aem.

Le Fur, Y., Hory, C., Bard, M.-H., & Ülsson, A. (1994). Evolution of phytosterols in chardonnay grape berry skins during last stages of ripening. *Vitis, 33*.

Loira, I., Morata, A., González, C., & Suárez-Lepe, J. A. (2012). Selection of glycolytically inefficient yeasts for reducing the alcohol content of wines from hot regions. *Food and Bioprocess Technology, 5*(7), 2787–2796. https://doi.org/10.1007/s11947-011-0604-9

Lorteau, S. (2018). A comparative legal analysis of skin-contact wine definitions in Ontario and South Africa. *Journal of Wine Research, 29*(4), 265–277. https://doi.org/10.1080/09571264.2018.1532881

Lu, X., Yang, C., Yang, Y., & Peng, B. (2023). Analysis of the formation of characteristic aroma compounds by amino acid metabolic pathways during fermentation with *Saccharomyces cerevisiae*. *Molecules, 28*(7), 3100. https://doi.org/10.3390/molecules28073100

Luparia, V., Soubeyrand, V., Berges, T., Julien, A., & Salmon, J. M. (2004). Assimilation of grape phytosterols by *Saccharomyces cerevisiae* and their impact on enological fermentations. *Applied Microbiology and Biotechnology, 65*(1), 25–32. https://doi.org/10.1007/s00253-003-1549-3

Maante-Kuljus, M., Karp, K., Rätsep, R., Mainla, L., Koort, A., Põldma, P., Kaldmäe, H., & Moor, U. (2024). Polyphenol composition of skin-contact fermented 'Solaris' and 'Zilga' wines. *Beverages, 10*(3), 59. https://doi.org/10.3390/beverages10030059

Margalef-Català, M., Felis, G. E., Reguant, C., Stefanelli, E., Torriani, S., & Bordons, A. (2017). Identification of variable genomic regions related to stress response in *Oenococcus oeni*. *Food Research International, 102*, 625–638. https://doi.org/10.1016/j.foodres.2017.09.039

Marobbio, C. M. T., Giannuzzi, G., Paradies, E., Pierri, C. L., & Palmieri, F. (2008). α-Isopropylmalate, a leucine biosynthesis intermediate in yeast, is transported by the mitochondrial oxalacetate carrier. *Journal of Biological Chemistry, 283*(42), 28445–28453. https://doi.org/10.1074/jbc.M804637200

Mezey, J., Selnekovič, A., & Šuchma, J. (2025). Analytical and sensory comparison of white and orange wines. *Acta Horticulturae, 1*(1418), 417–422. https://doi.org/10.17660/ActaHortic.2025.1418.52

Ngqumba, Z., Ntushelo, N., Jolly, N. P., Ximba, B. J., & Minnaar, P. P. (2017). Effect of *Torulaspora delbrueckii* yeast treatment on flavanols and phenolic acids of chenin blanc wines. *South African Journal for Enology and Viticulture, 38*(2), 192–200. https://doi.org/10.21548/38-2-1391

Puertas, B., Jiménez, M. J., Cantos-Villar, E., Cantoral, J. M., & Rodríguez, M. E. (2017). Use of *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* in semi-industrial sequential inoculation to improve quality of palomino and chardonnay wines in warm climates. *Journal of Applied Microbiology, 122*(3), 733–746. https://doi.org/10.1111/jam.13375

Quirós, M., Rojas, V., Gonzalez, R., & Morales, P. (2014). Selection of non-*saccharomyces* yeast strains for reducing alcohol levels in wine by sugar respiration. *International Journal of Food Microbiology, 181*, 85–91. https://doi.org/10.1016/j.ijfoodmicro.2014.04.024

Reguant, C., Bordons, A., Arola, L., & Rozès, N. (2000). Influence of phenolic compounds on the physiology of *Oenococcus oeni* from wine. *Journal of Applied Microbiology, 88*(6), 1065–1071. https://doi.org/10.1046/j.1365-2672.2000.01075.x

Ricciutelli, M., Bartolucci, G., Campana, R., Salucci, S., Benedetti, S., Caprioli, G., Maggi, F., Sagratini, G., Vittori, S., & Lucarini, S. (2020). Quantification of 2- and 3-isopropylmalic acids in forty Italian wines by UHPLC-MS/MS triple quadrupole and evaluation of their antimicrobial, antioxidant activities and biocompatibility. *Food Chemistry, 321*, Article 126726. https://doi.org/10.1016/j.foodchem.2020.126726

Ricciutelli, M., Moretti, S., Galarini, R., Sagratini, G., Mari, M., Lucarini, S., Vittori, S., & Caprioli, G. (2019). Identification and quantification of new isomers of isopropylmalic acid in wine by LC-IT and LC-Q-Orbitrap. *Food Chemistry, 294*, 390–396. https://doi.org/10.1016/j.foodchem.2019.05.068

Roca-mesa, H., Sendra, S., Mas, A., Beltran, G., & Torija, M. J. (2020). Nitrogen preferences during alcoholic fermentation of different non-*Saccharomyces* yeasts of oenological interest. *Microorganisms, 8*(2). https://doi.org/10.3390/microorganisms8020157

Ruiz-de-Villa, C., Gombau, J., Poblet, M., Bordons, A., Canals, J., Zamora, F., Reguant, C., & Rozès, N. (2023a). *Torulaspora delbrueckii* improves organoleptic properties and promotes malolactic fermentation in carbonic maceration wines. *Fermentation, 9*(12), 1021. https://doi.org/10.3390/fermentation9121021

Ruiz-de-Villa, C., Gombau, J., Poblet, M., Bordons, A., Canals, J. M., Zamora, F., Reguant, C., & Rozès, N. (2023b). Sequential inoculation of *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* in rosé wines enhances malolactic fermentation and potentially improves colour stability. *Lebensmittel-Wissenschaft und -Technologie, 190*, Article 115540. https://doi.org/10.1016/j.lwt.2023.115540

Ruiz-de-Villa, C., Poblet, M., Bordons, A., Reguant, C., & Rozès, N. (2023c). Comparative study of inoculation strategies of *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* on the performance of alcoholic and malolactic fermentations in an optimized synthetic grape must. *International Journal of Food Microbiology, 404*, Article 110367. https://doi.org/10.1016/j.ijfoodmicro.2023.110367

Salvadó, Z., Arroyo-López, F. N., Barrio, E., Querol, A., & Guillamón, J. M. (2011). Quantifying the individual effects of ethanol and temperature on the fitness advantage of *Saccharomyces cerevisiae*. *Food Microbiology, 28*(6), 1155–1161. https://doi.org/10.1016/j.fm.2011.03.008

Sánchez Palomo, E., Pérez-Coello, M. S., Díaz-Maroto, M. C., González Viñas, M. A., & Cabezudo, M. D. (2006). Contribution of free and glycosidically-bound volatile compounds to the aroma of muscat "a petit grains" wines and effect of skin contact. *Food Chemistry, 95*(2), 279–289. https://doi.org/10.1016/j.foodchem.2005.01.012

Santos, L. P., Morais, D. R., Souza, N. E., Cottica, S. M., Boroski, M., & Visentainer, J. V. (2011). Phenolic compounds and fatty acids in different parts of Vitis labrusca and V. vinifera grapes. *Food Research International, 44*(5), 1414–1418. https://doi.org/10.1016/j.foodres.2011.02.022

Schorn-García, D., Giussani, B., García-Casas, M. J., Rico, D., Martin-Diana, A. B., Aceña, L., Busto, O., Boqué, R., & Mestres, M. (2023). Assessment of variability sources in grape ripening parameters by using FTIR and multivariate modelling. *Foods, 12*(5). https://doi.org/10.3390/foods12050962

Schwarz, M., Wabnitz, T. C., & Winterhalter, P. (2003). Pathway leading to the formation of anthocyanin-vinylphenol adducts and related pigments in red wines. *Journal of Agricultural and Food Chemistry, 51*(12), 3682–3687. https://doi.org/10.1021/jf0340963

Semon, M. J., Edwards, C. G., Forsyth, D., & Dinn, C. O. (2001). Inducing malolactic fermentation in chardonnay musts and wines using different strains of *Oenococcus oeni*. *Australian Journal of Grape and Wine Research, 7*(1), 52–59. https://doi.org/10.1111/j.1755-0238.2001.tb00194.x

Singleton, V. L., Sieberhagen, H. A., De Wet, P., & Van Wyk, C. J. (1975). Composition and sensory qualities of wines from white grapes by fermentation with and without grape olids. *J. Enol. Viticult, 26*(2).

Smilde, A. K., Jansen, J. J., Hoefsloot, H. C. J., Lamers, R. J. A. N., van der Greef, J., & Timmerman, M. E. (2005). ANOVA-simultaneous component analysis (ASCA): A new tool for analyzing designed metabolomics data. *Bioinformatics, 21*(13), 3043–3048. https://doi.org/10.1093/BIOINFORMATICS/BTI476

Sokolowsky, M., Rosenberger, A., & Fischer, U. (2013). Sensory impact of skin contact on white wines characterized by descriptive analysis, time-intensity analysis and temporal dominance of sensations analysis. *Food Quality and Preference, 39*, 285–297. https://doi.org/10.1016/j.foodqual.2014.07.002

Sugimoto, N., Engelgau, P., Jones, A. D., Song, J., Beaudry, R., & Klee, H. J. (2021). Citramalate synthase yields a biosynthetic pathway for isoleucine and straight-and branched-chain ester formation in ripening apple fruit. *Proceedings of the National Academy of Sciences, 118*(3). https://doi.org/10.1073/pnas.2009988118/-/DCSupplemental

Thurston, P. A., Taylor, R., & Ahvenainen, J. (1981). Effects of linoleic acid supplementation on the synthesis by yeast of lipids and acetate esters. *Journal of the Institute of Brewing, 87*(2), 92–95. https://doi.org/10.1002/j.2050-0416.1981.tb03995.x

Tumanov, S., Zubenko, Y., Greven, M., Greenwood, D. R., Shmanai, V., & Villas-Boas, S. G. (2015). Comprehensive lipidome profiling of sauvignon blanc grape juice. *Food Chemistry, 180*, 249–256. https://doi.org/10.1016/j.foodchem.2015.01.134

Velázquez, R., Zamora, E., álvarez, M. L., Hernández, L. M., & Ramírez, M. (2015). Effects of new *Torulaspora delbrueckii* killer yeasts on the must fermentation kinetics and aroma compounds of white table wine. *Frontiers in Microbiology, 6*(NOV). https://doi.org/10.3389/fmicb.2015.01222

Zapparoli, G., Torriani, S., Pesente, P., & Dellaglio, F. (1998). Design and evaluation of malolactic enzyme gene targeted primers for rapid identification and detection of *Oenococcus oeni* in wine. *Letters in Applied Microbiology, 27*(5), 243–246. https://doi.org/10.1046/j.1472-765X.1998.00448.x

Zhang, B. Q., Luan, Y., Duan, C. Q., & Yan, G. L. (2018). Use of *Torulaspora delbrueckii* Co-fermentation with two *Saccharomyces cerevisiae* strains with different aromatic characteristic to improve the diversity of red wine aroma profile. *Frontiers in Microbiology, 9*(APR). https://doi.org/10.3389/fmicb.2018.00606

Zhang, B., Yu, C., Wang, M., Zhao, X., Lin, L., Yan, G., & Zhang, C. (2024). Investigation of novel metabolites generated by *Torulaspora delbrueckii* to promote the aroma biosynthesis in *Saccharomyces cerevisiae* during wine fermentation. *LWT - Food Science and Technology, 211*, Article 116816. https://doi.org/10.1016/j.lwt.2024.116816