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Torulaspora delbrueckii promotes malolactic fermentation in high polyphenolic red wines

Aitor Balmaseda [a], Nicolas Rozès [b], Albert Bordons [a], Cristina Reguant [a,*]

[a] Grup de Biotecnologia Enològica, Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Facultat d'Enologia, Spain
[b] Grup de Biotecnologia Microbiana dels Aliments, Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Facultat d'Enologia, C/ Marcel·lí Domingo s/n, 43007 Tarragona, Catalonia, Spain

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ABSTRACT

Using *Torulaspora delbrueckii* as starter culture for alcoholic fermentation (AF) is a current trend for enhancing the quality of red wines. As red winemaking usually requires subsequent malolactic fermentation (MLF), the compatibility of this yeast and *Oenococcus oeni* is a key factor for a successful fermentative process. In this work we study the interactions of *T. delbrueckii* and *O. oeni* in wines from grapes with different degrees of maturity. The results showed higher total polyphenolic index (TPI) values in *T. delbrueckii* wines. Moreover, the aromatic characteristics were improved in these wines, compared to the wines inoculated only with *Saccharomyces cerevisiae*. There was also a reduction in some inhibitor compounds for *O. oeni*, for instance medium chain fatty acids, as a result of the fermentation with this non-*Saccharomyces*. Overall, the use of *T. delbrueckii* resulted in better MLF performances.

1. Introduction

Yeasts and lactic acid bacteria (LAB) are the most important microorganisms for determining the quality of wine through their metabolism and interactions (Petruzzi et al., 2017). *Saccharomyces cerevisiae* is the predominant yeast species in the final stages of alcoholic fermentation (AF). High yeast diversity is found in the first stages of AF, including species of *Hanseniaspora, Torulaspora* and *Metschnikowia*. These non-*Saccharomyces* lose their viability when the ethanol concentration begins to increase (Vilela, 2019). LAB, mainly *Oenococcus oeni,* play an important role in the winemaking process through malolactic fermentation (MLF), by decarboxylating L-malic acid into L-lactic acid, improving wine quality and microbial stability (Davis, Wibowo, Eschenbruch, Lee, & Fleet, 1985; Lonvaud-Funel, 1999).

Some non-*Saccharomyces*, such as *T. delbrueckii,* are a current winemaking trend. This yeast can be found in late AF due to its high metabolic activity under winemaking conditions and also due to its resistance to ethanol and SO_2 (Benito, 2018). Moreover, *T. delbrueckii* and *S. cerevisiae* are genetically close (Masneuf-Pomarede, Bely, Marullo, & Albertin, 2016).

The microbial community and the specific inoculated strains play an important role in the organoleptic profile of wine. Indeed, some strains can help in the extraction of aromas and polyphenols from grape skins, which improves both aroma complexity and colour. This is currently of great interest because climate change means that grapes must be harvested with a low secondary metabolite concentration to meet a compromise with the sugar concentrations (Ubeda, Hornedo-Ortega, Cerezo, García-Pattilla, & Troncoso, 2020). Typical red wines have a concentration of around 500 mg/L of anthocyanins (mainly monomeric anthocyanins), which leads to larger pigments with higher stability during AF, MLF and maturation (Watrelot & Norton, 2020). The standard concentration of tannins in red wines of *Vitis vinifera* cultivars is 1–4 g/L (Asenstorfer, Hayasaka, & Jones, 2001). In addition, high polyphenolic wines can cause difficulties for *O. oeni* to perform MLF (Reguant, Bordons, Arola, & Rozès, 2000).

T. delbrueckii is proposed as a tool for modulating the wine aromatic profile (Belda et al., 2015). Its metabolic activity helps to release terpene aromas such as α-terpineol and linalool (Čuš & Jenko, 2013). Its use is related to an enhancement in the fruity character of wines (Morata et al., 2020). Moreover, it can help to enhance red wine colour (Escribano-Viana et al., 2019), reduce the ethanol content (Belda, Zarraonaindia, Perisin, Palacios, & Acedo, 2017; Contreras et al., 2014), decrease the fatty acid concentration (Benito, 2018), and increase mannoprotein and glycerol contents (Belda et al., 2015; González-Royo

* Corresponding author.
E-mail address: cristina.reguant@urv.cat (C. Reguant).

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et al., 2015) in mixed fermentation together with *S. cerevisiae*. Altogether, *T. delbrueckii* is reported as a stimulating yeast for MLF (Balmaseda, Bordons, Reguant, & Bautista-Gallego, 2018; Martín-García, Balmaseda, Bordons, & Reguant, 2020). In this final point, the compatibility and microbial interaction mediated consequences of *T. delbrueckii* and *O. oeni* has been studied recently in white and red winemaking (Balmaseda, Rozès, Leal, Bordons, & Reguant, 2021).

To better understand the suitability of *T. delbrueckii* for red winemaking and its effect on MLF, we studied these microbial interactions in Merlot winemaking with two grape maturity levels using two strains of this yeast species in sequential inoculation with *S. cerevisiae*. We compared our results with a wine fermented only with *S. cerevisiae*. We monitored MLF in inoculated fermentations, evaluating two *O. oeni* strains, and also in spontaneous MLF. This work offers novel data regarding the impact of the use of *T. delbrueckii* on wine polyphenolic content along the red winemaking process. The use of different *T. delbrueckii* and *O. oeni* strains provided information about the metabolic traits affecting wine composition that may be species or strain dependent.

2. Materials and methods

2.1. Microorganisms

AF was carried out using three yeast strains: *T. delbrueckii* Biodiva (TdB) (Lallemand Inc., Montréal, Canada), *T. delbrueckii* Viniferm (TdV) (Agrovin, Alcázar de San Juan, Spain) and *S. cerevisiae* Lalvin-QA23 (Sc) (Lallemand Inc.). Yeasts were stored as active dry yeasts at 4 °C. Two strains of *O. oeni* were used: PSU-1 (ATCC BAA-331) and Viniflora-CH11 (Chr. Hansen AS, Hoersholm, Denmark), which were kept on MRSmf plates (Margalef-Català et al., 2017) and stored at 4 °C.

2.2. Fermentation trials

Fermentations were carried out with red Merlot grapes from a vineyard in Vilafranca del Penedès (Catalonia, Spain). The vineyard was harvested before the optimal maturity level (Merlot 1) and at the optimal maturity level 10 days after (Merlot 2) during the 2019 vintage. The maturity of Merlot 2 resulted in a less acid must and increased YAN (yeast assimilable nitrogen) concentration, while the other parameters were similar to Merlot 1 (Suppl. Table 1). Grapes and the resulting musts were processed as in Balmaseda et al. (2021). Briefly, about 100 kg of red grapes were manually harvested each time and then processed in the experimental cellar of *Rovira i Virgili* University.

Alcoholic fermentations were carried out with each *T. delbrueckii* strain and *S. cerevisiae* was inoculated after 48h. Fermenters were supplemented with nutrients (0.4 g/L Nutrient Vit NatureTM, Lallemand Inc.) together with *S. cerevisiae* inoculation. Each yeast was inoculated for a population of 2.5×10^6 cells/mL with active dry yeast after rehydration with water following the manufacturer's instructions. There was also a control fermentation with *S. cerevisiae* as a sole starter (Sc). All fermentations were performed in triplicate. Samples of 6 mL were taken every 48h to monitor the density decrease and yeast population evolution. YPD agar medium (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, 17 g/L agar, Panreac Química SLU, Castellar del Vallès, Spain) was used to calculate the total number of yeast cells, and lysine agar medium (Oxoid LTD., England) for quantification of non-*Saccharomyces* yeasts (Wang, Mas, & Esteve-Zarzoso, 2016), after incubation at 28 °C for 48h. AF was considered finished when the sugar concentration was below 2 g/L. Fermentations were carried out at 27 °C. Fermenting must was manually punched down every 48 h during AF. Grape skins were always submerged thanks to a flat strainer used as a stopper in the fermenter.

After AF, wines were pressed and transferred to another container, cooled for 5 days and decanted. Then, wine samples were centrifugated and stored at −20 °C. Later, equal volumes of each triplicate (0.5 L) were mixed and sulphited (10 mg/L $K_2S_2O_5$) in two 0.75 L bottles, which were stored at 4 °C until wine tasting. The residual volume of the mixed wines was supplemented with L-malic acid for a concentration of 2 g/L. Then, the pH was corrected to the value before L-malic acid addition. Adjusted wines were inoculated with two *O. oeni* strains, each in 1 L flasks at 20 °C for a population of 2×10^7 cells/mL. In addition, a spontaneous MLF was followed. These fermentations were also carried out in triplicate. Samples were taken every 24h to monitor L-malic acid and the bacterial population. Samples were plated on MRSmf supplemented with nystatin (100 mg/L), sodium azide (25 mg/L) and tomato juice (100 mL/L, Aliada, Madrid, Spain), and incubated at 27 °C in a 10% CO_2 atmosphere for 7–15 days. MLF was considered finished when the L-malic acid was below 0.05 g/L. After AF and MLF, wines for tasting were sulphited (25 mg/L $K_2S_2O_5$).

2.3. Yeast and bacterial identification

2.3.1. Yeast identification

Twenty-five colonies were randomly selected for yeast identification isolated from must before the first inoculation, must before inoculating *S. cerevisiae* (48h) and wine at the end of AF (density below 995 g/L and residual sugars below 2 g/L). Isolate species were identified based on the amplicon size of the ITS-5.8S rDNA region (Esteve-Zarzoso, Belloch, Uruburu, & Querol, 1999).

2.3.2. LAB identification and strain typing of Oenococcus oeni

Colonies (10 from inoculated MLF to confirm the imposition of the inoculated strain and 20 from spontaneous MLF to evaluate the strain diversity) were randomly selected for LAB identification from MRSmf plates at the end of MLF. The identification of LAB species and strain typing of *O. oeni* were performed as described in Balmaseda et al. (2021). DNA was extracted with a High Pure PCR Template Preparation Kit (Roche, Barcelona, Spain). Briefly, LAB isolates with a cocci morphology were confirmed to be *O. oeni* by the species-specific PCR (Zapparoli, Torriani, Pesente, & Dellaglio, 1998). Non-*Oenococcus* isolates were identified with the 16S-ARDRA method and *Mse*I digestion according to Rodas, Ferrer, and Pardo (2003). Isolates identified as *O. oeni* were typed by the multilocus variable number tandem repeat (VNTR) (Claisse & Lonvaud-Funel, 2014).

2.4. General oenological analytical parameters

Concentrations of sugars (glucose and fructose), L-malic acid, acetic acid, glycerol, D- and L-lactic, NH_4, primary ammonium nitrogen (NOPA), total and free SO_2 and citric acid were determined with a Miura One Multianalyzer (TDI, Barcelona, Spain) using enzymatic kits from TDI and Biosystems S.A. (Barcelona, Spain). Acetaldehyde and succinic acid were determined using the corresponding assay kits K-ACHYD and K-SUCC (Megazyme, Wicklow, Ireland), respectively. pH was determined using a Crison micro pH 2002 pH-meter (Barcelona, Spain) and alcoholic degree was determined by ebulliometry (Electronic ebulliometer uEBU6576, GabSystem) following the methods of the Compendium of International Methods of Analysis of Musts and Wines (OIV, 2009).

2.5. Analyses of volatile compounds

Wine samples (10 mL) were taken after AF and MLF. The volatile compounds were liquid/liquid extracted with 0.4 mL dichloromethane and 2.5 g $(NH_4)_2SO_4$ using 4-methyl-2-pentanol (0.8 g/L) and heptanoic acid (0.7 g/L) as internal standards following Ortega, López, Cacho, and Ferreira (2001). After 90 min agitation at room temperature and centrifugation (5080 g, 5 min), 2 μL of the organic phase was injected in split mode (10:1, 30 mL/min) into a gas chromatography HP-FFAP (30 m × 0.25 mm × 0.25 μm, Agilent Technologies, Böbligen, Germany). The temperature of the program was as follows: 35 °C during 5 min,

increased 3 °C/min to 200 °C, then 8 °C/min to 220 °C. The temperature of the injector and detector were 180 °C and 280 °C respectively. The gas carrier was He at 3 mL/min. Aromatic volatile compounds were identified and quantified by comparison with standards.

2.6. Colour parameters and phenolic characterization

The colour of wine samples was analysed directly in a quartz cuvette with a 1 mm optical length based on the method of Glories (1984). CIELab coordinates: lightness (L), chroma (C), hue (h), red-greenness (a) and yellow-blueness (b) were determined according to Ayala, Echávarri, and Negueruela (1997) and data processing was performed with the MSCV software.

The phenolic composition was analysed in terms of the total polyphenol index (TPI), tannin concentration and anthocyanin concentration. TPI was analysed by measuring the 280 nm absorbance of a 1:100 dilution of wines with a spectrophotometer, using a 10 mm quartz cuvette and multiplying the absorbance value by 100 as described by Ribéreau-Gayon, Dubordieu, Donèche, & Lonvaud-Funel (2006). Tannin concentration was determined based on the Bate-Smith method (Ribéreau-Gayon & Stonestreet, 1966) with some modifications (Vignault et al., 2018).

2.7. Wine tasting

Sensory analyses were performed after AF and MLF. Triplicates were blended for simplifying the analysis. Wines were evaluated by at least 12 tasters, considered as experts, from the Oenology Faculty of *Rovira i Virgili* University. The experts were given 20 mL of wine in dark glasses to avoid subjectivity due to the colour of the samples.

Samples were randomly numbered with 3-digit codes. Wines were served anonymously according to a Latin square of Williams design to avoid the range and carry-over effect.

Triangle tests were performed to evaluate differences between the produced wines. In addition, tasters were asked to write down their preference in each sequence. In addition to triangular tests, a classification test was performed. We selected some wines with distinct classifications and correctly assigned on triangle tests. Tasters were asked to classify the wines in terms of increasing intensity for three attributes: red fruit, lactic character and astringency.

2.8. Statistical analysis

The statistical software XLSTAT version 2020.1.1.64570 (Addinsoft, Paris, France) was used. The data obtained were submitted to one-way ANOVA with a subsequent analysis using the Tukey test, with a confidence interval of 95%. Results were considered significant when p-value ≤ 0.05. Principal Component Analysis (PCA) was also performed with the same statistical software to determine differences between the wines. The level of significance of sensory triangle tests was determined following Jackson's method (Jackson, 2002). The sensorial classification test was analysed based on the Friedman test with a significance level of p-value ≤ 0.05 (Olkin, Lou, Stokes, & Cao, 2015).

3. Results and discussion

3.1. Fermentation performance

Wine AF was very quick in both Merlot grape musts at two different maturity levels. In both cases *S. cerevisiae* fermented wines finished AF in 8 days (Table 1). Little delay was observed in the wines fermented sequentially with *T. delbrueckii* and *S. cerevisiae*: AF finished in 10 days in Merlot 1 and 12 days in Merlot 2 (Table 1). The observed AF extension in sequential inoculations is a common behaviour due to competition events between the inoculated fermenting yeasts (Balmaseda et al., 2021; Belda et al., 2015). Basically, the AF took longer because the yeasts needed more time to dry the wines (glucose + fructose < 2 g/L). The difference in the sugar consumption rate was not very remarkable (Table 1) as only a significant difference was observed in Sc wines regarding TdB and TdV wines with Merlot 2 must. The consumption rates of the AF (Table 1) were higher in Merlot 2 wines, probably due to higher nitrogen available (Suppl. Table 1) in this more mature grape must (Ali et al., 2011).

Larger differences were observed in MLF duration (Table 1, Fig. 1). Generally, MLF took longer in Merlot 2 wines than in Merlot 1 wines. All MLFs finished with the exception of the spontaneous MLF of Sc wines in Merlot 2. The duration of the MLF inoculated with *O. oeni* PSU-1 in Merlot 1 was significantly lower in TdB and TdV compared to Sc wines. The same tendency was observed in Merlot 2 wines for the two *O. oeni* strains: TdB and TdV had a shorter MLF duration than Sc wines. This was not the case for *O. oeni* CH11 in Merlot 1, where the MLF took less time in Sc wine (8 days) than in TdB (13 days) or TdV (17 days) wines. This could be due to the strain specific interactions between these yeasts and LAB (Balmaseda et al., 2018).

The scenario found in Merlot 2 wines can be summarised as longer MLF with more difficulties involved in its development (Fig. 1, Table 1). Wines from Merlot 1 and Merlot 2 resulted in similar ethanol concentration, pH and organic acid concentration (Table 2). Altogether, large differences were observed in the polyphenolic composition (Fig. 2). Merlot 2 wines had a significantly higher concentration of anthocyanins, tannins and TPI, which have been previously related to harsh conditions for the development of MLF (Reguant et al., 2000). Their effect on LAB may be positive or negative depending on the nature and concentration of the compounds and on the bacterial strains (Breniaux et al., 2018; García-Ruiz, Moreno-Arribas, Martín-Álvarez, & Bartolomé, 2011). Recently, phenolic compounds have been described as stress compounds (Bech-Terkilsen, Westman, Swiegers, & Siegumfeldt, 2020). Indeed some of them, such as stilbenes, are related to an inhibition in malic acid degradation and CFU decline during MLF in *O. oeni* (Zimdars, Caspers-Weiffenbach, Wegmann-Herr, & Weber, 2020). The inhibition of polyphenols is dependent on their structure (Devi & Anu-Appaiah, 2018; García-Ruiz et al., 2011) and on the *O. oeni* strain (Zimdars et al., 2020).

In these wines, the use of *T. delbrueckii* clearly resulted in shorter MLF in the inoculated wines and in successful spontaneous MLF, although high polyphenolic concentration was detected (Fig. 2). The wine fermented just with *S. cerevisiae* in Merlot 2 could not undergo spontaneous MLF since the LAB viable population did not reach 10^2 CFU/mL in more than three months (data not shown).

In general, calculated consumption rates were higher in *T. delbrueckii* wines (Table 1). In addition, high consumption rates were observed in spontaneous MLF compared to the inoculated wines because of a quick L-malic acid consumption in the final stages of MLF (Fig. 2).

3.2. Analysis of the microbial population

The two grape musts had similar yeast concentrations: 9.6×10^4 CFU/mL (total yeasts) and 1.7×10^4 CFU/mL (non-*Saccharomyces* yeasts) for Merlot 1 and 3.5×10^5 CFU/mL (total yeasts) and 6.1×10^4 CFU/mL (non-*Saccharomyces*) for Merlot 2. The inoculation of the selected yeasts was successful in both fermenting musts: the imposition of *T. delbrueckii* at 48h before *S. cerevisiae* inoculation, and the imposition of *S. cerevisiae* at the end of AF were in all cases 85% or higher (data not shown). In fact, by the end of AF, non-*Saccharomyces* were not detected. On the second day of fermentation, all wines, including those initially inoculated with *T. delbrueckii*, had a yeast population of around 10^8 CFU/mL (data not shown). After three days of fermentation, the viable population of non-*Saccharomyces* was lost (less than 10^2 CFU/mL) in all fermentations (data not shown), due to the high imposition ability of the inoculated *S. cerevisiae* strain, also observed in the previous vintage (Balmaseda et al., 2021).

No significant indigenous LAB population (<10 CFU/mL) was

Table 1
Alcoholic (AF) and malolactic (MLF) fermentation duration and consumption rate of sugar and L-malic acid. Values shown are the mean of triplicates ± SD. Sc (*S. cerevisiae*), TdB (*T. delbrueckii* Biodiva and *S. cerevisiae*), TdV (*T. delbrueckii* Viniferm and *S. cerevisiae*) fermented wines. PSU, CH11 and Spontaneous refer to the MLF strategy where *O. oeni* PSU-1, *O. oeni* CH11 or non-*O. oeni* were inoculated.

		Duration[a] (days)				Consumption rate[b] (g/L·day)			
		AF	PSU	CH11	Spontaneous	AF	PSU	CH11	Spontaneous
Merlot 1	Sc	8	6 (14)	8 (16)	36 (44)	15.5 ± 1^{a}	0.60 ± 0.04^{aB}	0.34 ± 0.05^{aA}	0.18 ± 0.01^{aA}
	TdB	10	2 (12)	13 (23)	34 (44)	15.5 ± 0.5^{a}	1.77 ± 0.08^{aB}	0.22 ± 0.01^{aA}	0.18 ± 0.01^{aA}
	TdV	10	2 (12)	17 (23)	34 (44)	15.0 ± 2.1^{a}	1.86 ± 0.06^{aB}	0.14 ± 0.03^{aA}	0.14 ± 0.01^{aA}
Merlot 2	Sc	8	23 (31)	30 (38)	–	19.2 ± 0.6^{b}	0.03 ± 0.01^{aA}	0.39 ± 0.08^{aB}	–
	TdB	12	14 (26)	17 (29)	44 (56)	16.4 ± 0.9^{a}	0.16 ± 0.01^{bA}	0.48 ± 0.01^{bB}	0.40 ± 0.03^{bAB}
	TdV	12	17 (29)	21 (33)	40 (52)	17.1 ± 0.5^{a}	0.08 ± 0.01^{aA}	0.1 ± 0.01^{aB}	0.27 ± 0.04^{aAB}

[a–b]. Lowercase indicate significant differences at $p \leq 0.05$ according to a Tukey post-hoc comparison test regarding to the yeast used. [A–B]. Uppercase indicate significant differences at $p \leq 0.05$ regarding to the MLF strategy used.

[a] Durations in brackets in MLF represent the length of the complete fermentative process (AF + MLF).

[b] Calculation based on consumption rate of sugar as density (AF) and L-malic acid (MLF) considering the period of exponential decrease of these values. Statistics are calculated regarding to the values inside each square corresponding to each fermentation (AF or MLF) in the two Merlot grape musts.

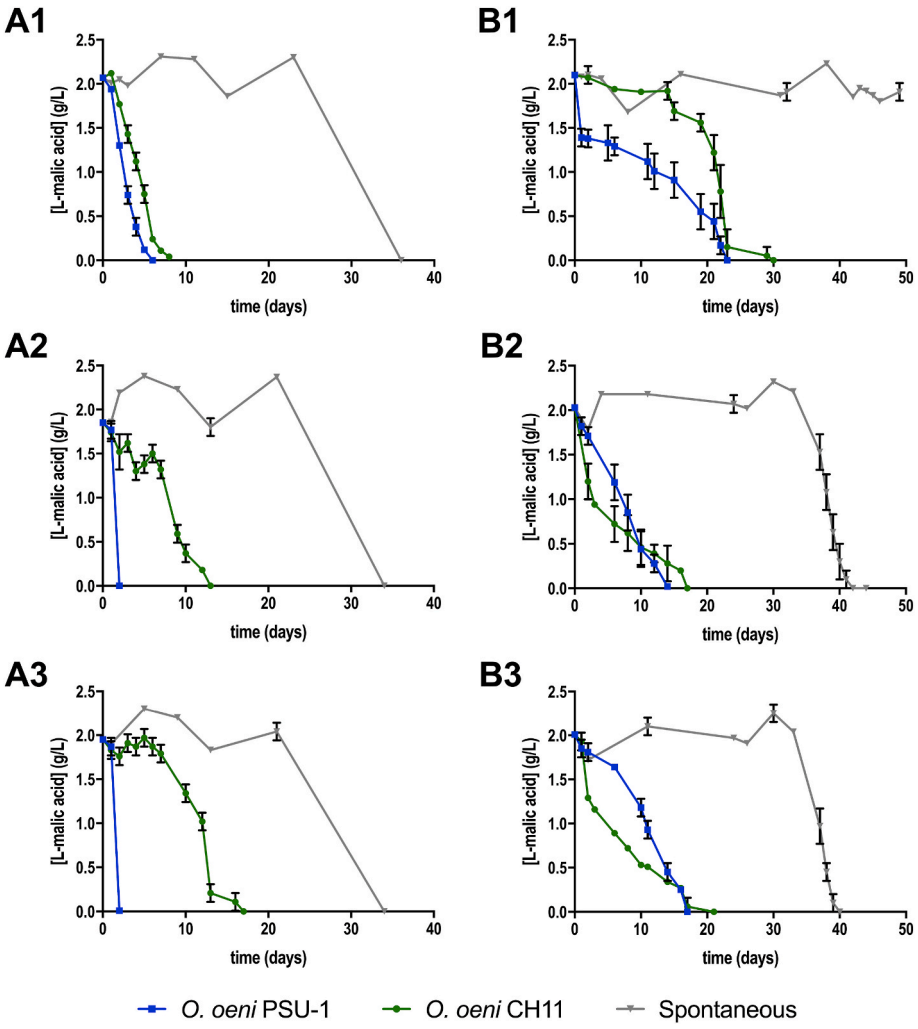


Fig. 1. Evolution of malolactic fermentation after AF by monitoring the L-malic acid consumption. Left: Merlot 1 wines fermented with *S. cerevisiae* (A1), *T. delbrueckii* Biodiva-*S. cerevisiae* (A2), *T. delbrueckii* Viniferm-*S. cerevisiae* (A3). Right Merlot 2 wines fermented with *S. cerevisiae* (B1), *T. delbrueckii* Biodiva-*S. cerevisiae* (B2), *T. delbrueckii* Viniferm-*S. cerevisiae* (B3).

detected during the entire AF process (data not shown). This correlates with the previous data about spontaneous MLF where the LAB population needed more than a month to undergo the fermentation (Table 1). Generally, during the MLF the inoculated wines maintained a population of 1-4 x 10^{7} CFU/mL until L-malic acid was completely consumed. In contrast, Sc wines of Merlot 2 lost 2 logarithmic units of viable *O. oeni* cells at the end of the MLF process (data not shown).

Due to the low bacterial population detected in must and wines after AF, LAB isolates were only analysed from wines after MLF. Firstly, 240 isolates were identified by species-specific PCR as *O. oeni*. Then, the 41 isolates that resulted in no amplification by species-specific PCR were analysed by 16S rRNA ARDRA and all of them were identified as *O. oeni*.

Table 2
Oenological parameters of wines after alcoholic and malolactic fermentations. Values shown are the means of triplicates ± SD. Statistics were calculated independently for each grape variety. Sc, TdB and TdV correspond to *S. cerevisiae*, *T. delbrueckii* Biodiva- *S. cerevisiae* and *T. delbrueckii* Viniferm-*S. cerevisiae* fermented wines, respectively. P, C and S refers to the MLF strategy were *O. oeni* PSU-1, *O. oeni* CH11 or non-*O. oeni* was inoculated. n.d.: not detected.

		L-lactic acid (g/L)	D-lactic acid (g/L)	Acetic acid (g/L)	Citric acid (g/L)	Glycerol (g/L)	pH	Acetaldehyde (mg/L)	Succinic acid (g/L)	SO_2 T (mg/L)	Ethanol (% vol/vol)
Merlot 1	Sc	n.d.^{d}	0.17 ± 0.01^{efg}	0.19 ± 0.01^{e}	0.13 ± 0.01^{ab}	6.1 ± 0^{c}	3.3 ± 0^{cd}	45.7 ± 1.53^{a}	0.24 ± 0.02^{a}	17 ± 1^{a}	13.1 ± 0.2^{a}
	Sc–P	1.50 ± 0.01^{ab}	0.18 ± 0.01^{defg}	0.29 ± 0.02^{ab}	0.03 ± 0.01^{c}	5.8 ± 0.2^{c}	3.4 ± 0^{b}				
	Sc–C	1.50 ± 0.02^{bc}	0.18 ± 0.01^{defg}	0.25 ± 0.01^{cd}	0.12 ± 0.01^{ab}	6 ± 0.3^{c}	3.4 ± 0^{bc}				
	Sc–S	1.50 ± 0.6^{bc}	0.19 ± 0.02^{cdef}	0.31 ± 0.02^{a}	n.d.^{cd}	6.2 ± 0.1^{c}	3.2 ± 0.1^{d}				
	TdB	n.d.^{d}	0.15 ± 0.01^{g}	0.19 ± 0.03^{e}	0.15 ± 0.02^{a}	7.4 ± 0.2^{ab}	3.4 ± 0^{bc}	42.8 ± 0.71^{a}	0.19 ± 0.01^{b}	11.7 ± 1.5^{b}	13 ± 0.2^{a}
	TdB-P	1.55 ± 0.04^{ab}	0.21 ± 0.01^{cd}	0.29 ± 0.02^{ab}	n.d.^{d}	7 ± 0.2^{ab}	3.4 ± 0.1^{ab}				
	TdB-C	1.48 ± 0.05^{c}	0.16 ± 0.02^{fg}	0.22 ± 0.01^{cde}	0.12 ± 0.02^{ab}	6.9 ± 0.1^{b}	3.4 ± 0^{ab}				
	TdB-S	1.46 ± 0.01^{bc}	0.26 ± 0.01^{a}	0.31 ± 0.02^{ab}	n.d.^{d}	7.1 ± 0.1^{ab}	3.4 ± 0^{ab}				
	TdV	n.d.^{d}	0.20 ± 0.01^{cde}	0.19 ± 0.02^{e}	0.13 ± 0.01^{ab}	7.3 ± 0.2^{ab}	3.4 ± 0^{bc}	41.5 ± 1.6^{a}	0.12 ± 0.02^{c}	13.3 ± 2.1^{ab}	13.1 ± 0.2^{a}
	TdV-P	1.53 ± 0.02^{a}	0.25 ± 0.01^{ab}	0.27 ± 0^{bc}	n.d.^{d}	7.6 ± 0.3^{a}	3.5 ± 0^{a}				
	TdV-C	1.40 ± 0.05^{c}	0.22 ± 0.02^{bc}	0.22 ± 0.01^{de}	0.11 ± 0.01^{b}	7.5 ± 0.3^{a}	3.5 ± 0^{a}				
	TdV-S	1.48 ± 0.04^{bc}	0.27 ± 0.01^{a}	0.29 ± 0.01^{ab}	n.d.^{d}	7.3 ± 0.2^{ab}	3.2 ± 0^{d}				
Merlot 2	Sc	n.d.^{d}	0.16 ± 0.02^{c}	0.22 ± 0.02^{ef}	0.16 ± 0.01^{ab}	6.5 ± 0.2^{bc}	3.3 ± 0.1^{a}	52.2 ± 4.9^{a}	0.27 ± 0.01^{a}	18 ± 2^{a}	13.3 ± 0.2^{a}
	Sc–P	1.40 ± 0.01^{c}	0.16 ± 0.01^{bc}	0.22 ± 0.01^{cd}	0.12 ± 0.02^{bc}	6.9 ± 0.1^{c}	3.4 ± 0^{a}				
	Sc–C	1.19 ± 0.05^{bc}	0.19 ± 0.01^{bc}	0.28 ± 0.01^{de}	0.13 ± 0.01^{ab}	6.3 ± 0.1^{bc}	3.4 ± 0^{a}				
	Sc–S	0.10 ± 0.05^{d}	0.17 ± 0.02^{bc}	0.22 ± 0.01^{ef}	0.16 ± 0.01^{ab}	6.3 ± 0.4^{c}	3.2 ± 0^{ab}				
	TdB	n.d.^{d}	0.15 ± 0.02^{c}	0.19 ± 0.01^{f}	0.14 ± 0.01^{abc}	7.4 ± 0.3^{a}	3.3 ± 0^{a}	42.8 ± 0.7^{b}	0.27 ± 0.04^{a}	13.5 ± 0.5^{b}	13 ± 0.7^{a}
	TdB-P	1.51 ± 0.04^{ab}	0.17 ± 0.02^{bc}	0.27 ± 0.01^{cd}	0.13 ± 0.02^{bc}	7.2 ± 0.1^{a}	3.4 ± 0.1^{a}				
	TdB-C	1.40 ± 0.12^{ab}	0.17 ± 0.02^{bc}	0.29 ± 0.02^{bcd}	0.14 ± 0.02^{ab}	7.6 ± 0.2^{a}	3.4 ± 0.1^{a}				
	TdB-S	1.55 ± 0.03^{a}	0.27 ± 0.01^{a}	0.29 ± 0.01^{bcd}	0.07 ± 0^{d}	7.1 ± 0.2^{ab}	3.3 ± 0^{a}				
	TdV	n.d.^{d}	0.18 ± 0.03^{bc}	0.27 ± 0.01^{cd}	0.15 ± 0.01^{ab}	7.5 ± 0.4^{a}	3 ± 0.2^{b}	43.1 ± 3.12^{b}	0.29 ± 0.02^{a}	14.3 ± 1.2^{b}	13.4 ± 0^{a}
	TdV-P	1.38 ± 0.12^{ab}	0.22 ± 0.05^{ab}	0.31 ± 0.03^{bc}	0.07 ± 0.01^{d}	7.7 ± 0.2^{a}	3.4 ± 0^{a}				
	TdV-C	1.29 ± 0.04^{ab}	0.22 ± 0.01^{ab}	0.36 ± 0.01^{a}	0.17 ± 0.02^{a}	7.4 ± 0.2^{a}	3.3 ± 0.2				
	TdV-S	1.55 ± 0.03^{ab}	0.27 ± 0.01^{a}	0.32 ± 0.01^{ab}	0.10 ± 0.03^{cd}	7.2 ± 0^{a}	3.4 ± 0^{a}				

$^{a–g}$. Values are significantly different at $p \leq 0.05$ according to a Tukey HSD post-hoc comparison test.

From them 16 VNTR profiles were obtained, two of which (IN1 and IN2) corresponded to the inoculated strains PSU-1 and CH11. These VNTR profiles can be regarded as bacterial strains (Claisse & Lonvaud-Funel, 2014).

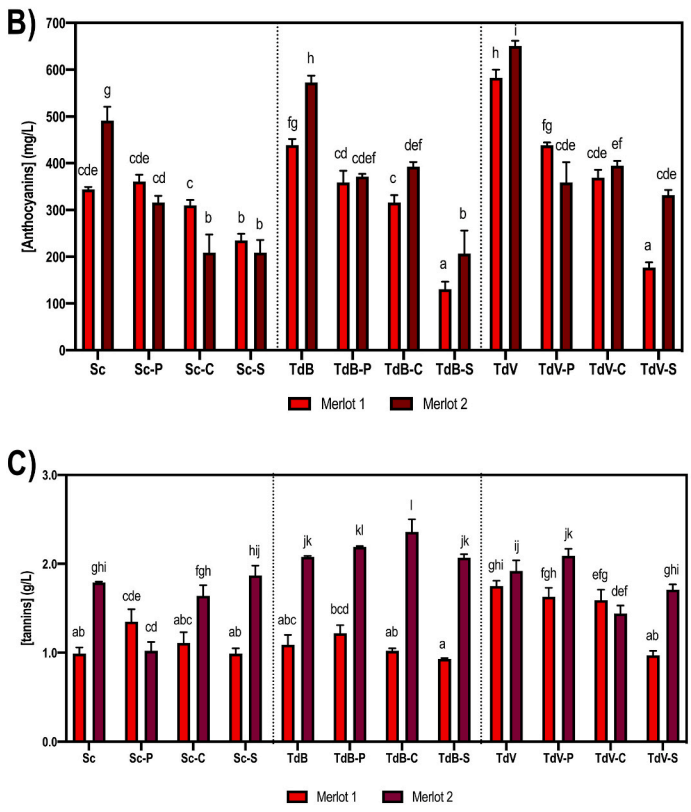
The imposition of the inoculated strain was successful and complete in most of the cases (Fig. 3). *O. oeni* PSU-1 reached the total imposition (100%) in all wines. However, *O. oeni* CH11 was not completely imposed in TdB wines, especially in Merlot 1 where it represented just 20% of the analysed population. Indeed, wines inoculated with CH11 had the slowest MLFs (Table 1), even if it was imposed at the end of MLF. It is interesting to observe that these two *O. oeni* strains behaved differently, highlighting the strain-specific interaction between yeasts and *O. oeni* (Balmaseda et al., 2018). This could be related to a higher concentration of polyphenols in Merlot 2, which allowed better adaptation of the oenological commercial strain CH11 upon the autochthonous microbiota.

Spontaneous MLFs were characterised by a strain diversity dependent on the grape maturity with the exception of the strain AiB9 that was detected in Merlot 1 TdV wine and Merlot 2 TdB wine (Fig. 3). Different strain compositions characterised the obtained wines after spontaneous MLF (Fig. 3). Nevertheless, some of them appeared in more than one wine (AiB5, AiB8, AiB9 and AiB13). Altogether, the previous fermenting yeasts affected the observed strain diversity at the end of MLF. Moreover, using *T. delbrueckii* somehow promoted the MLF performance since the spontaneous MLF of these wines was quicker than that of *S. cerevisiae* (Table 1).

The strain diversity observed in Sc and TdB wines was similar, but not their imposition percentages. In addition, TdB and TdV wines share these dominant strains. As a result, we observed a more different microbiota in TdV compared to Sc. Moreover, the suitability of *O. oeni* CH11 in TdB was low but more efficient in Merlot 2. Altogether, there is probably a higher concentration of inhibiting compounds, which could explain the non-successful spontaneous MLF in Sc wine and the low diversity observed at the end of MLF in TdB wine, which could be related

A)

TPI	Sc	Sc-P	Sc-C	Sc-S	TdB	TdB-P	TdB-C	TdB-S	TdV	TdV-P	TdV-C	TdV-S
M1	30.2±4^{c}	33.1±0.8^{bc}	33.4±0.2^{abc}	31.9±0.2^{bc}	31.8±0.8^{bc}	33.7±0.3^{abc}	32.7±1.5^{bc}	32.6±0.3^{bc}	37.4±2^{a}	35.9±0.3^{ab}	35.4±0.1^{ab}	34.9±0.8^{ab}
M2	43.4±0.8^{bc}	41.6±0.9^{c}	41.6±0.5^{c}	41.7±0.5^{c}	46.7±1.8^{a}	45.4±1.3^{ab}	45.8±0.7^{ab}	45±0.4^{ab}	45±1.4^{ab}	44.1±0.8^{abc}	44±0.3^{abc}	43.3±0.4^{bc}



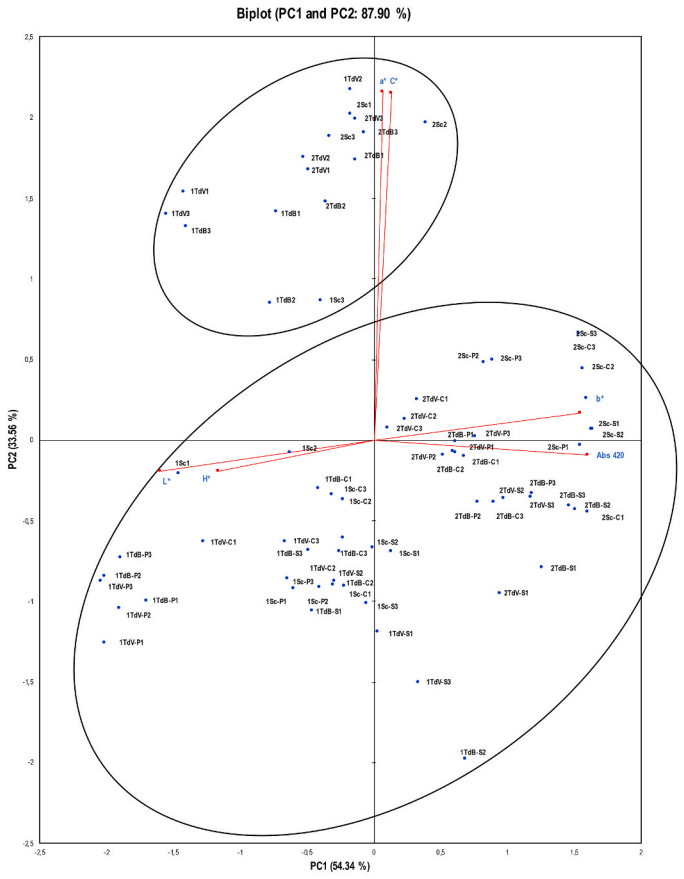


Fig. 2. Polyphenolic and colour parameters of wines after alcoholic and malolactic fermentation. M1 and M2 correspond to Merlot 1 and Merlot 2 grape musts. Sc, TdB and TdV correspond to the wines fermented with *S. cerevisiae*, *T. delbrueckii* Biodiva-*S. cerevisiae* and *T. delbrueckii* Viniferm-*S. cerevisiae* respectively. P, C and S correspond malolactic fermentation strategy were *O. oeni* PSU-1, *O. oeni CH11* or non-*O. oeni* was inoculated. A) Total polyphenolic index (TPI). B) Anthocyanin concentration of wines. C) Tannin concentration of wines. All the values shown are the mean of triplicates ±SD. Statistics were calculated independently for each grape must. Values are significantly different at $p \leq 0.05$ according to a Tukey HSD post-hoc comparison test. D) Principal component analysis (PCA) biplots of varimax rotated PCA for wine volatile composition in which observations (the three replicates of each wine) and variables are plotted. The last number of each observation indicates the replica number (1–3). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

to a higher concentration of some polyphenols (Fig. 2).

3.3. General oenological parameters of wines

They were particularly homogenous (Table 2) in both wines Merlot 1 and 2. All wines after AF presented an L-malic acid concentration of around 1 g/L (data not shown). This little reduction from the must concentration (Suppl. Table 1) could be related to the yeast metabolism (Belda et al., 2015; du Plessis et al., 2017) and not to LAB activity since no L-lactic acid was detected in these wines (Table 2). D-lactic acid generally increased a little during the MLF performance, and it was only noticeable in the spontaneous MLFs with the exception of Sc Merlot 2 wine, which did not undergo MLF.

O. oeni can assimilate citric acid as a source of energy in wines as a response to stress (Bartowsky & Henschke, 2004; Lonvaud-Funel, 1999), but its consumption is dependent on the *O. oeni* strain (Bartowsky & Henschke, 2004). Here, citric acid was consumed by *O. oeni* PSU-1 and the spontaneous MLF, but not by *O. oeni* CH11 in Merlot 1 wines (Table 2), and in general, its consumption in Merlot 2 wines was lower than in Merlot 1. As a result, *O. oeni* released acetic acid, increasing the volatile acidity, but as citric acid content was low, no remarkable changes in acetic acid concentration were observed.

An increase in the concentration of glycerol was detected in *T. delbrueckii* wines (Table 2). It can be explained because some non-*Saccharomyces* have a more active glyceropyruvic pathway than *S. cerevisiae* (Belda et al., 2015). The role of glycerol in *O. oeni* metabolism is still unclear (Balmaseda et al., 2018). In these vinifications little variation in glycerol concentration was observed after MLF compared to after AF. (Table 2).

Generally, wines fermented with *T. delbrueckii* resulted in higher pH levels (Table 2), which is usually associated with an improvement in MLF performance since it can attenuate the inhibitory effect of ethanol and medium chain fatty acids (MCFA). The variation in the pH, higher or lower, is very dependent on the medium where the yeast is fermenting, as seen in previous studies (Balmaseda et al., 2021; Martín-García et al., 2020). Only a small increase in pH was observed in wines after MLF (Table 2).

Mixed fermentations with *T. delbrueckii* are reported to decrease acetaldehyde content in wine (Benito, 2018). In the present work, the use of *T. delbrueckii* showed a tendency to decrease its concentration,

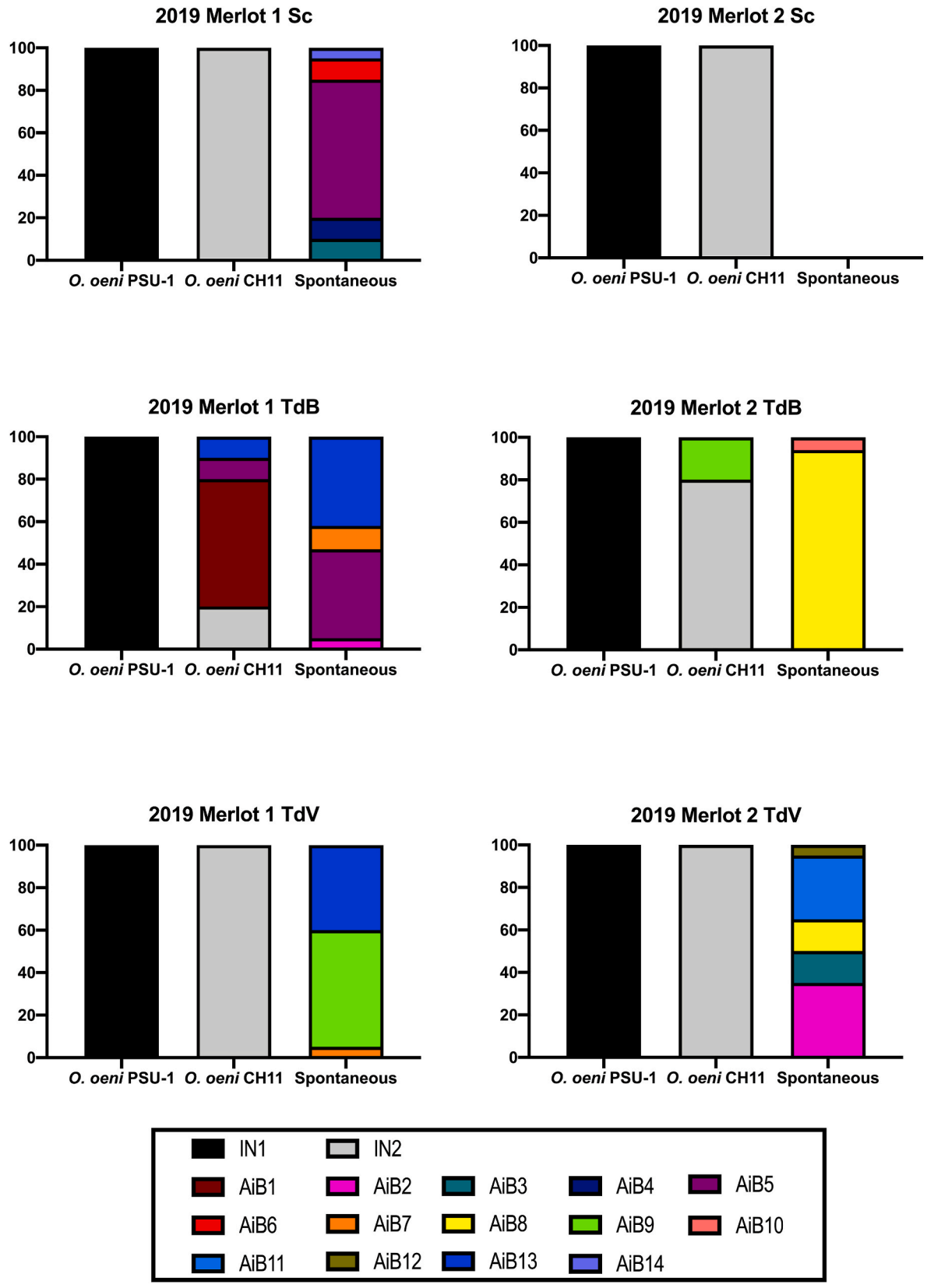


Fig. 3. Percentage of imposition of the different VNTR profiles of *O. oeni* after malolactic fermentation. IN1 and IN2 refers to *O. oeni* PSU-1 and *O. oeni* CH11, respectively. AB named VNTR profiles correspond to naturally appeared profiles. Sc, TdB and TdV correspond to the wines fermented with *S. cerevisiae*, *T. delbrueckii* Biodiva-*S. cerevisiae* and *T. delbrueckii* Viniferm-*S. cerevisiae* respectively. *O. oeni* PSU-1 and *O. oeni* CH11 correspond to the wines where one of each *O. oeni* strains was inoculated. Spontaneous refers to the wines where no *O. oeni* was inoculated.

which was significantly different in Merlot 2 wines (Table 2).

Succinic acid content in wine after AF was reduced in *T. delbrueckii* wines of Merlot 1 must (Table 2), being its concentration half in 1TdV wine compared to the control 1Sc wine. This reduction is interesting for the subsequent MLF since succinic acid acts as a competitive inhibitor of the malolactic enzyme, delaying or inhibiting the MLF (Balmaseda et al., 2018). In Merlot 2 wines no reduction was observed.

Sulphur dioxide was significantly reduced in *T. delbrueckii* wines (Table 2) as observed in the previous vintage (Balmaseda et al., 2021). Nevertheless, its concentrations detected were lower than the threshold of 35 mg/L, which is considered as inhibitory for some *O. oeni* strains (Lerm, Engelbrecht, & du Toit, 2010).

Although some non-*Saccharomyces* can reduce the ethanol content in wine (Balmaseda et al., 2018) and particularly *T. delbrueckii* (Benito, 2018), in our work no reduction in ethanol content was detected in either of the Merlot wines. Still, this ability is very dependent on the medium (Balmaseda et al., 2021).

Additionally, it should be considered that there may be other compounds, not determined in this work, that could explain the enhanced MLF performance in *T. delbrueckii* wines.

3.4. Analyses of volatile compounds

The wines produced had differences regarding the volatile composition (Fig. 4A, Table 3). In general, wines fermented with *T. delbrueckii* and those with Merlot 2 grapes had a higher concentration of volatile compounds (Table 3).

As a result of PCA (Fig. 4A), wines were separated through the PC2 into those fermented only with *S. cerevisiae* and those sequentially fermented with both yeasts, with the exception of Sc–S wines. Almost all the volatile components of the PCA point to TdB and TdV (Suppl. Figure 1), which were the wines with the highest concentration of volatile compounds (Table 3, Suppl. Table 3). In contrast 1Sc wines are plotted on the opposite side, where there are mainly ethyl esters and

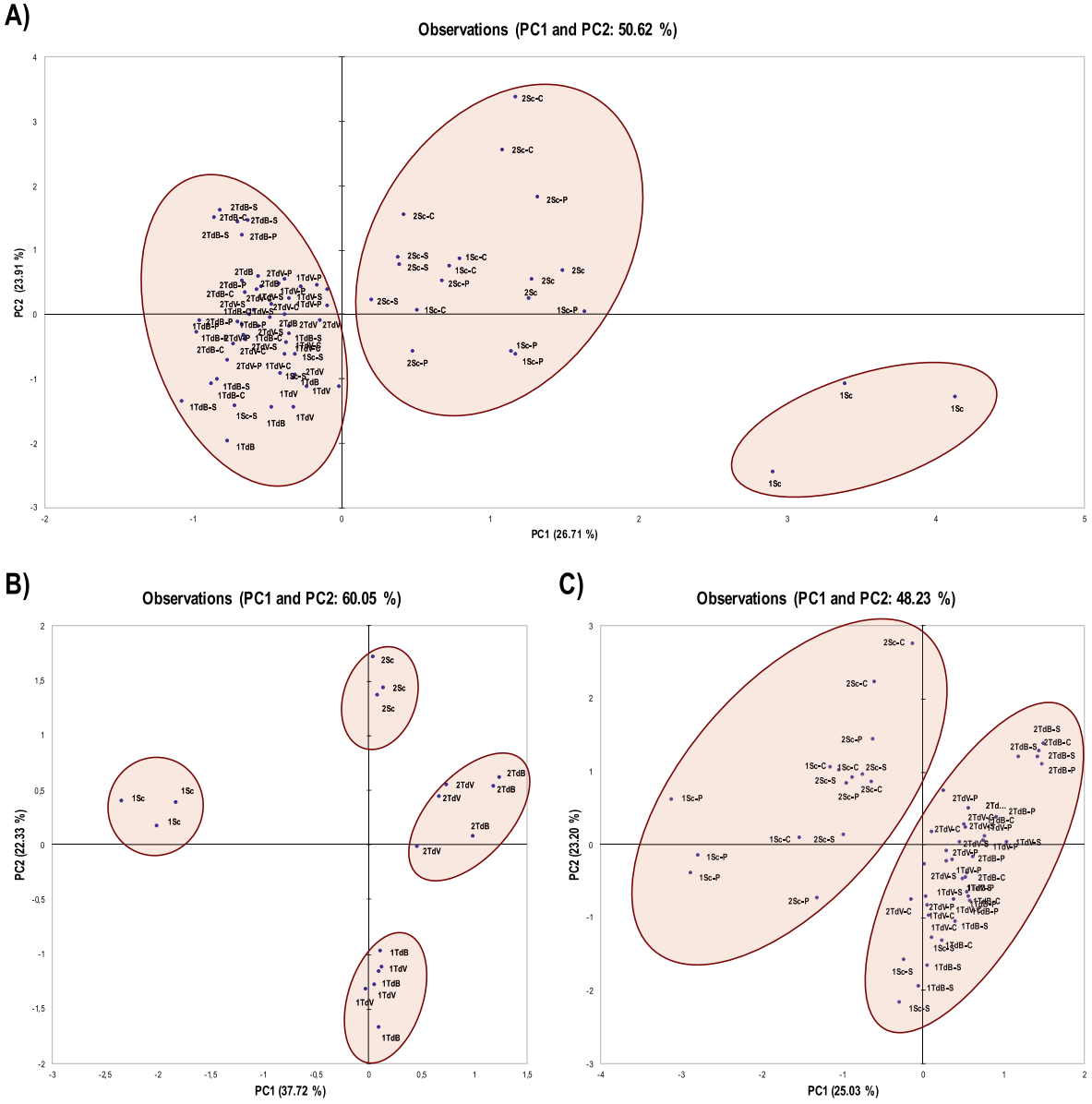


Fig. 4. Principal component analysis (PCA) biplots of varimax rotated PCA for wine volatile composition in which observations are plotted. A) Produced wines with all strain combinations in the two grape musts. B) Wines after AF in the two grape musts. C) Wines after MLF in the two grape musts. 1 and 2 represent the two grape musts: Merlot1 and Merlot2 respectively. Sc, TdB and TdV correspond to the wines fermented with *S. cerevisiae*, *T. delbrueckii* Biodiva-*S. cerevisiae* and *T. delbrueckii* Viniferm-*S. cerevisiae* respectively. P, C and S correspond malolactic fermentation strategy were *O. oeni* PSU-1, *O. oeni* CH11 or non-*O. oeni* was inoculated.

Table 3
Concentrations of wine volatile compounds (mg/L) after AF grouped as family compounds. SCFA (propionic, isobutyric, butyric 3-methylbutanoic and valeric acids), MCFA (hexanoic, octanoic and decanoic acids), Ethyl esters of FA (ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate and ethyl dodecanoate), Fusel alcohols (2-methyl-propanol, 1-butanol, 2- methyl-1-butanol, benzyl alcohol, 2-phenylethanol, 1-propanol, 1-pentanol, 1-hexanol and *cis*-3-hexen-1-ol), Fusel alcohol acetates (2-phenylethanol acetate, isobutyl acetate and isoamyl acetate). Sc, TdB and TdBV correspond to *S. cerevisiae*, *T. delbrueckii* Biodiva- *S. cerevisiae* and *T. delbrueckii* Viniferm-*S. cerevisiae* fermented wines, respectively. Values shown are the mean of triplicates ± SD. Statistics were calculated independently for each grape must.

		ΣSCFA (mg/L)	ΣMCFA (mg/L)	ΣEthyl esters of FA (mg/L)	Ethyl lactate (mg/L)	ΣFusel alcohols (mg/L)	ΣFusel alcohol acetates (mg/L)
Merlot 1	Sc	6.7 ± 1.1^{a}	3.6 ± 0.4^{b}	6.3 ± 0.3^{c}	7.7 ± 0.5^{a}	245.9 ± 28.8^{a}	5.7 ± 0.3^{b}
	TdB	9.1 ± 1.2^{ab}	0.5 ± 0.1^{a}	4.0 ± 0.5^{b}	6.2 ± 0.2^{a}	220.0 ± 24.8^{a}	3.1 ± 0.2^{a}
	TdV	9.5 ± 0.5^{b}	0.6 ± 0.1^{a}	2.9 ± 0.1^{a}	6.0 ± 0.6^{a}	221.2 ± 4.9^{a}	3.3 ± 0.2^{a}
Merlot 2	Sc	17.0 ± 1.1^{b}	0.8 ± 0.0^{b}	6.1 ± 0.4^{a}	n.d.	228.0 ± 5.9^{a}	5.5 ± 0.1^{b}
	TdB	13.6 ± 2.0^{ab}	0.5 ± 0.1^{a}	6.0 ± 0.7^{a}	7.4 ± 0.1^{b}	249.8 ± 7.6^{a}	5.3 ± 0.1^{ab}
	TdV	11.5 ± 2.4^{a}	0.5 ± 0.0^{a}	6.6 ± 0.2^{a}	8.6 ± 0.3^{c}	235.3 ± 12.6^{a}	5.1 ± 0.1^{a}

* $^{a–c}$. values are significantly different at $p \leq 0.05$ according to a Tukey HSD post-hoc comparison test. n.d.: not detected

MCFA. In this case, *S. cerevisiae* wines (1Sc and 2Sc) showed a poor volatile composition related to their less aromatic enzymatic activities (Romano, Fiore, Paraggio, Caruso, & Capece, 2003) and lower maturation level of the grapes (Ferreira & López, 2019). However, the maturity level did not have a significant impact when *T. delbrueckii* fermented. TdB and TdV wines from Merlot 1 and Merlot 2 were clustered in the most aromatic group (Fig. 4A).

When we analysed wines after AF (Fig. 4B), we observed large differences. Samples were clustered in four groups: *T. delbrueckii* wines from Merlot 1, the same from Merlot 2 and two clusters for *S. cerevisiae* wines. It is remarkable that although TdB and TdV showed high homogeneity in the two grape musts, Sc wines had much more variability. 1Sc and 2Sc wines had significantly higher concentrations of MCFA (Table 3), more remarkable in 1Sc. Previous studies showed that the use of non-*Saccharomyces* can reduce the concentration of MCFA (Balmaseda et al., 2018), which can be toxic to *O. oeni* (Capucho & San Romao, 1994), by destabilizing its cell membrane (Carreté, Vidal, Bordons, & Constantí, 2002). Nevertheless, the concentration of this compound family was low, then it should not have a large impact on *O. oeni.* The contribution to SCFA was mainly due to high isobutyric and propionic acid concentrations (Suppl. Table 2). In this sense, higher concentrations of isobutyric acid have been recently associated with *T. delbrueckii* (Sereni, Phan, Osborne, & Tomasino, 2020). Sc wines had the highest ethyl ester of FA concentration in Merlot 1 (Table 2). Low concentrations of ethyl lactate were quantified in wines after AF (Table 2), which increased at the end of the MLF due to the LAB metabolism (Suppl. Table 2), as previously known (Liu, 2002).

Regarding volatile composition after MLF (Fig. 4C), two clusters of wines can be observed. PC1 separates Merlot 1 and Merlot 2 wines and PC2 separates Sc wines from TdB and TdV wines, with some exceptions. The most aromatic wines are grouped in the positive PC1 and PC2 quadrant: 2TdB and 2TdV wines. As described, the use of different non-*Saccharomyces* enhances the wine volatile composition (Englezos et al., 2016; Liu, Lu, Duan, & Yan, 2016; Tofalo et al., 2016; Tufariello et al., 2020). Of course, this was clearer in Merlot 2 wines, which came from more mature grapes.

3.5. Colour parameters and phenolic characterization

The different maturity levels of Merlot 1 and Merlot 2 grapes resulted in higher TPI, anthocyanin and tannin concentrations in Merlot 2 wines (Fig. 2).

TPI varied from 30 to 37 in Merlot 1 and from 32 to 47 in Merlot 2 wines (Fig. 2A). The use of *T. delbrueckii* could be noted in wines after AF, and this tendency was clearer in Merlot 2 for which the two *T. delbrueckii* wines had a significantly higher index than the *S. cerevisiae* wine. The higher levels of polyphenols in non-*Saccharomyces* wines have been previously described (Escribano-Viana et al., 2019).

The anthocyanin concentration was also greater after AF as a consequence of the use of *T. delbrueckii* (Fig. 2B), and particularly in the case of the *T. delbrueckii* Viniferm strain in Merlot 2 wine. This behaviour seems to be strain specific (Carew, Smith, Close, Curtin, & Dambergs, 2013; Chen et al., 2018); therefore, the selection of strains in terms of anthocyanin adsorption is crucial for red winemaking (Benito, 2018). After MLF, we found that its decreased in all wines (Costello, Francsi, & Bartowsky, 2012; Davis et al., 1985), and a dramatic drop in concentration was observed in spontaneous MLF, probably because it took a long time to finish (Fig. 2, Table 1).

Tannin concentration varied from 1 to 2.4 g/L in all wines (Fig. 2C). It was also significantly higher in *T. delbrueckii* wines (Fig. 2C), especially in Merlot 2. Indeed, wines coming from Merlot 2 grapes can be considered as high tannic wines as they have more tannins than the average for this variety (Harbertson et al., 2008). *T. delbrueckii* is described as a non-*Saccharomyces* with low adsorption of polyphenols (Benito, 2018).

According to the colour parameters, wines were clustered in two groups in the PCA (Fig. 2D). One of them, characterised by higher a* and Chroma values, grouped AF wines except for some replicates of Sc wines. Wines after MLF were grouped in another cluster in the opposite direction, with higher heterogeneity. In addition, wines in the two clusters tended to be closer to other wines produced with the same Merlot maturity level.

In summary, the use of *T. delbrueckii* increased the TPI due to the accumulation of both anthocyanin and tannins, mainly after AF. After MLF there was a tendency for the polyphenolic composition to decrease, probably due to oxidation or precipitation or even attachment to yeast cell walls (Escribano-Viana et al., 2019), or also due to the interaction with *O. oeni* cell envelopes (Campos, Couto, & Hogg, 2003; Campos et al., 2009). A dramatic fall was observed in anthocyanin concentrations after spontaneous MLF, presumably as a consequence of a long fermenting period.

It is important to highlight that a higher concentration of phenolic compounds is usually related to more stressful conditions for MLF (Bech-Terkilsen et al., 2020). Even so, wines fermented with *T. delbrueckii*, with a higher concentration of these compounds (Fig. 2), were also the ones where MLF performance was enhanced, especially with strain Biodiva (Table 1, Fig. 2). Therefore, the use of *T. delbrueckii* seems to promote changes in composition that favour *O. oeni* adaptation to wine stressful conditions, as seen in Balmaseda et al. (2021).

3.6. Wine tasting

The results of the triangle tests, including those after AF and MLF, are shown in Table 4. Regarding wines after AF tasters preferred, in general, wines only fermented with *S. cerevisiae*, although they were not able to distinguish 1Sc and 1TdB. Interestingly, wines produced with the two strains of *T. delbrueckii* resulted in significantly different wines, and the TdV wine was the preferred one. The results were robust for the wines after MLF. Tasters clearly preferred the inoculated ones with *O. oeni* from those spontaneously fermented, and no differences were observed

Table 4
Results of the sensorial analysis from both, triangular and classification tests. 1 and 2 refers to Merlot 1 or Merlot 2 grapes. Sc, TdB and TdV correspond to *S. cerevisiae*, *T. delbrueckii* Biodiva- *S. cerevisiae* and *T. delbrueckii* Viniferm-*S. cerevisiae* fermented wines, respectively. P, C and S refers to the MLF strategy were *O. oeni* PSU-1, *O. oeni* CH11 or non-*O. oeni* was inoculated.

Triangular tests			
		Preference[b]	
Compared wines	Positive identifications	A	B
1Sc vs 1TdB	9/25	7 ^{NS}	2
1TdB vs 1TdV	15/25	3	12 $^{\alpha}$
1Sc vs 1TdV	15/25	11 $^{\alpha}$	4
1Sc–P vs 1Sc–C	4/17	3 ^{NS}	1
1Sc–C vs 1Sc–S	10/17	10 ^{ß}	0
1Sc–P vs 1Sc–S	12/17	9	3 $^{\alpha}$
2ScC vs 2TdB-C	8/13	1	7 $^{\alpha}$
2Sc–C vs 2TdV-C	8/13	0	8 ^{ß}
2TdB-C vs 2TdV-C	5/13	5 $^{\alpha}$	0
2Sc–P vs 2TdB-P	8/13	2	6 ^{NS}
2Sc–P vs 2TdV-P	8/13	3	5 ^{NS}
2TdB-P vs 2TdV-P	9/13	4	5 ^{NS}
Classification test			
Attribute	Wines classification as increasing intensity of the attribute[a]		
Red fruit	$1Sc^{1,2,3} > 2Sc^{4} >$ 1Sc–S > 2Sc–P > 2TdB-S > 2TdB-$P^{1} >$ 1Sc-$P^{2} >$ 1TdB-$P^{3,4}$		
Lactic character	$2Sc^{1} > 1Sc^{2} >$ 2TdB-S > 1Sc–P > 2TdB-P > 2Sc–P > 1Sc–S > 1TdB-$P^{1,2}$		
Astringency	1Sc-$S^{1,2,3} >$ 1Sc-$P^{4,5} >$ 1Sc > 2TdB-S > 1TdB-P > 2Sc-$P^{2} >$ 2TdB-$P^{3,4} > 2Sc^{1,5}$		

^{NS}, No significant.
$^{\alpha}$, Significant at $p < 0.05$.
^{ß}, Significant at $p < 0.001$.
[a] Wines sharing the same superscript number represent statistical significance ($p \leq 0.05$).
[b] Only the preference of positive identifications is shown. A and B correspond to the first and second wine for each comparison.

comparing strains PSU-1 and CH11. In addition, tasters preferred *T. delbrueckii* wines after MLF and differentiated them from *S. cerevisiae* ones.

Considering the information obtained from the triangle tests, eight representatives wines were selected to perform a classification test according to three attributes (Table 4, below). Wines fermented only with *S. cerevisiae* were the ones with less intensity of the red fruit aroma attribute. However, after MLF these wines showed an increased red fruit aroma. This is also related to the volatile composition of wines, and *S. cerevisiae* wines were the least aromatic of the produced wines (Fig. 4). The lactic character typical of wines after MLF was correctly assessed because wines after MLF showed an increased intensity in this attribute. Nevertheless, not many significant differences were found. Considering astringency, wines from Merlot 2 grapes, in general, had higher astringency than Merlot 1 wines. This correlates with the higher TPI and tannin concentrations observed in Merlot 2 wines (Fig. 2).

4. Conclusion

The use of *T. delbrueckii* in more mature grape wines reduced the duration of the fermentative process and enabled spontaneous MLF. In this way, the diversity of *O. oeni* strains was dependent on the maturity level and the fermenting yeast combination. Volatile complexity and polyphenolic composition was enhanced due to the use of *T. delbrueckii*, mainly in wines made from more mature grapes. These effects were more remarkable for some strains, such as Biodiva for promoting MLF or Viniferm for the polyphenolic concentration. Therefore, careful attention should be given to strain selection and yeast-*O. oeni* strain compatibility to benefit from their oenological advantages in red winemaking.

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CRediT authorship contribution statement

Aitor Balmaseda: Conceptualization, Methodology, Investigation, Writing – original draft, preparation. **Nicolas Rozès:** Conceptualization, Writing – review & editing, Funding acquisition. **Albert Bordons:** Conceptualization, Supervision, Writing – review & editing. **Cristina Reguant:** Conceptualization, Supervision, Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

That there is no conflict of interest related to the funding received for the work under consideration for publication.

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Appendix A. Supplementary data

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