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Volatile profile of reduced alcohol wines fermented with selected non-*Saccharomyces* yeasts under different aeration conditions

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ABSTRACT

Over the last decades there has been an increase in ethanol concentration in wine. High ethanol concentration may impact negatively wine flavor and can be associated with harmful effects on human health. In this study, we investigated a microbiological approach to reduce wine ethanol concentration, using three non-*Saccharomyces* yeast strains (*Metschnikowia pulcherrima*, *Torulaspora delbrueckii* and *Zygosaccharomyces bailii*) in sequential fermentations with *S. cerevisiae* under different aeration conditions. At the same time, we evaluated the volatile profile of the resulting reduced alcohol Chardonnay wines. Results showed that the non-*Saccharomyces* yeasts tested were able to reduce wine ethanol concentration when oxygen was provided. Compared to *S. cerevisiae* wines, ethanol reduction was 1.6% v/v, 0.9% v/v and 1.0% v/v for *M. pulcherrima*, *T. delbrueckii* and *Z. bailii* sequential fermentations, respectively. Under the conditions evaluated here, aeration did not affect acetic acid production for any of the non-*Saccharomyces* strains tested. Although aeration affected wine volatile profiles, this was depended on yeast strain. Thus, wines produced with *M. pulcherrima* under aeration of 0.05 volume of air per volume of culture per minute (VVM) showed excessive ethyl acetate content, while *Z. bailli* wines produced with 0.05 VVM aeration had increased concentrations of higher alcohols and volatile acids. Increased concentrations of these compounds over their sensory thresholds, are likely to impact negatively on wine sensory profile. Contrarily, all three non-*Saccharomyces* strains under 0.025 VVM aeration conditions produced wines with reduced ethanol concentration and acceptable chemical volatile profiles.

1. Introduction

In the last two decades, ethanol concentration in wine has increased about 2% v/v in most winemaking regions. This increase has been mainly due to climate change and consumer's preferences towards well-structured wines with optimal phenolic maturity (Alston et al., 2011; Godden and Muhlack, 2010; Jones et al., 2005; Mozell and Thach, 2014; Robinson et al., 2012). High ethanol content can alter the sensory profile of wine increasing the perception of bitterness, astringency and hotness, and masking some volatile compounds (Fischer and Noble, 1994; Wilkinson and Jiranek, 2013). Additionally, wines with high alcohol concentration can have negative effects on human health (Gronbaek, 2009) and are subjected to higher taxes in many countries (Gil et al., 2013). Different strategies have been proposed for the production of wine with reduced alcohol concentration, including vineyard management, grape must pre-fermentation practices, microbiological approaches during fermentation and post-fermentation processing technologies (Ciani et al., 2016; Garcia-Martin et al., 2010; Gil et al., 2013; Gonzalez et al., 2013; Longo et al., 2017; Poni, 2014; Schmidtke et al., 2012; Varela et al., 2015). Particularly, a wide range of different biological approaches have been suggested to reduce ethanol concentration in wine, including the use of non-*Saccharomyces* yeasts (Kutyna et al., 2010; Longo et al., 2017).

Non-*Saccharomyces* yeasts have been widely used to improve wine aroma and flavor profile (Ciani and Comitini, 2015; Jolly et al., 2014; Maturano et al., 2012, 2015) and to control the growth of undesired microflora in wine (Oro et al., 2014, 2016). In addition, several non-*Saccharomyces* yeast species, in combination with *Saccharomyces cerevisiae*, have been shown to produce wines with reduced ethanol concentration (Canonico et al., 2016; Ciani et al., 2016; Contreras et al., 2014, 2015; Gobbi et al., 2014; Loira et al., 2014; Quiros et al., 2014). Unlike *S. cerevisiae*, which favours fermentative metabolism over aerobic respiration when sugar concentration exceeds 10 g/L (due to the Crabtree effect), many non-*Saccharomyces* yeasts are able to use

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oxygen for growth regardless of sugar concentration (Alexander and Jeffries, 1990; de Deken, 1966) and thus, divert carbon into other metabolites decreasing ethanol formation. Several reports have explored the oxidative metabolism observed in some non-*Saccharomyces* species with the aim of reducing wine ethanol concentration (Contreras et al., 2015; Morales et al., 2015; Rocker et al., 2016; Tronchoni et al., 2018). While aeration can enhance the growth and the persistence of non-*Saccharomyces* yeasts during wine fermentation (Englezos et al., 2018; Shekhawat et al., 2017), it can also negatively impact wine sensory profile (Rocker et al., 2016; Tronchoni et al., 2018). Thus, although effective to reduce ethanol concentration, the main limiting factor of this strategy is the over-production of undesirable volatile compounds such as acetic acid and ethyl acetate.

In the present study, we describe the volatile profile of reduced alcohol Chardonnay wines produced with three non-*Saccharomyces* yeast strains in sequential fermentations with *S. cerevisiae*, under different aeration conditions. Growth kinetics for *Metschnikowia pulcherrima*, *Torulaspora delbrueckii* and *Zygosaccharomyces bailii* strains, and their interactions with *S. cerevisiae* were also evaluated.

2. Materials and methods

2.1. Yeast strains

M. pulcherrima DiSVA269 was obtained from the Yeast Collection of the Department of Life and Environmental Sciences of the Polytechnic University of Marche (Italy), while *S. cerevisiae* AWRI 838, *T. delbrueckii* AWRI1152 and *Z. bailii* AWRI1578 were obtained from the Australian Wine Research Institute (AWRI) Wine Microorganism Culture Collection (WMCC). The non-*Saccharomyces* strains described above were previously selected for their ability to reduce wine ethanol concentration in sequential fermentation trials at laboratory scale (Canonico et al., 2016; Contreras et al., 2015). Strains were maintained at −80 °C for long-term storage in cryovials supplemented with 80% (w/v) glycerol as cryoprotective agent. Subsequently, the strains were cultured on Yeast Peptone Dextrose (YPD) agar medium at 25 °C for 48–72 h, and stored at 4 °C.

2.2. Sequential fermentations in chardonnay grape juice

Three different aeration conditions were performed to evaluate *M. pulcherrima*, *T. delbrueckii* and *Z. bailii* in sequential fermentations with *S. cerevisiae* in sterilized Chardonnay. These conditions were i) no air addition, ii) 5 mL/min aeration (0.025 volume of air per volume of culture per minute - VVM) for 72 h, and iii) 10 mL/min aeration (0.05 VVM) for 72 h. Chardonnay must was prepared from grapes obtained from Rowland Flat (South Australia) during the 2017 vintage. Chardonnay juice was filter sterilised (0.2 µm Millipore, USA) and contained 220 g/L of sugar (equal amounts of glucose and fructose), 349 mg N/L of yeast assimilable nitrogen (YAN), pH 3.3 and 9 mg/L free sulfur dioxide.

Yeast strains were pre-cultured in 10 ml YPD overnight at 28 °C in an orbital shaker. This culture was then used to inoculate 100 mL of sterile grape must, diluted 1:1 with water, in 250 mL Erlenmeyer flasks. Flasks were incubated for 3 day at 22 °C with shaking (120 rpm) under aerobic conditions and then used to inoculate grape must. For the treatment with no air addition, sequential fermentations were performed in 250 mL fermentation flasks equipped with fermentation locks and containing 200 mL of sterilized grape must. Ferments were incubated at 22 °C (120 rpm) under initial semi-aerobic conditions; flasks fitted with air-locks ensure anaerobic conditions after all oxygen in the headspace is consumed. Treatments with air addition were performed in small-scale (250 mL) bioreactors (Medicel Oy, Finland) containing 200 mL of sterilized Chardonnay juice. Sterile filtered air (0.22 µm, Millipore) was continuously sparged (72 h) into the medium with a stainless-steel diffuser. Aeration rate for each bioreactor was controlled by using a flow meter (Medicel Oy, Finland). Dissolved oxygen in each bioreactor was monitored with a fiber-optic FireSting O_2 system (PyroScience, Germany). All ferments were performed in triplicate, inoculated at an optical density of 0.1 (OD600 nm) and incubated at 22 °C, under shaking or agitation at 200 rpm. After 72 h *S. cerevisiae* AWRI 838 was sequentially inoculated (OD_{600} equivalent to 0.1) to ensure completion of fermentation. Samples were taken daily for analysis until the end of fermentation (< 2 g/L of residual sugars). Anaerobic controls with *S. cerevisiae* AWRI 838 were performed introducing nitrogen at the same rates as the air addition treatments.

Growth kinetics were determined by plate counts on both WL Nutrient Agar (Oxoid, Hampshire, UK) and Lysine Agar (Oxoid, Hampshire, UK) which does not support the growth of *S. cerevisiae* (Lin, 1975). Thus, both cultures provide differentiation between non-*Saccharomyces* and *S. cerevisiae*.

2.3. Analytical techniques

Ethanol, glucose, fructose, glycerol and organic acids were quantified by high-performance liquid chromatography (HPLC) using a BioRad HPX87H column as described previously (Varela et al., 2004). Analysis of higher alcohols, acetate-, and ethyl esters was performed using gas chromatography–mass spectrometry (GCMS) using a stable isotope dilution analysis (SIDA) as previously described (Bizaj et al., 2012). Quantification of acetic acid, propanoic acid, 2-methylpropanoic acid, butanoic acid, 2-/3-methylbutanoic acid, hexanoic acid, octanoic acid and decanoic acid in was performed using headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME/GCMS), with polydeuterated internal standards for stable isotope dilution analysis as described previously (Varela et al., 2017).

2.4. Statistical analysis

Analysis of variance (ANOVA) was applied to the experimental data for the main enological characteristics of the wines. Means were analyzed using the STATISTICA7 software, differences were determined using Duncan tests and were considered significant if the associated *P*-values were < 0.05. Principal component analysis (PCA) was applied to discriminate among wines using their volatile profile. PCA was carried out using the statistical software package JMP 11®. The mean data were normalized, to neutralize any influence of hidden factors. The PCA provides a graphical representation of the overall differences not only within the same species with different oxygen condition, but also between all species tested.

3. Results

Three non-*Saccharomyces* yeast strains, *M. pulcherrima, T. delbrueckii* and *Z. bailii* were evaluated in sequential inoculation trials with *S. cerevisiae* for their ability to produce reduced-alcohol wines under different aeration conditions. Three different conditions were tested, anaerobic conditions throughout fermentation, 0.025 VVM aeration for 72 h followed by anaerobic conditions, and 0.05 VVM aeration for 72 h followed by anaerobic conditions. Since aeration can increase the production of undesirable flavor compounds, the volatile profile of the resulting Chardonnay wines was also evaluated.

3.1. Aeration increased sugar utilization kinetics

Fermentation kinetics for pure *S. cerevisiae* and non-*Saccharomyces*/*S. cerevisiae* sequential fermentations are shown in Fig. 1. For *S. cerevisiae*, aeration increased fermentation kinetics shortening fermentation length proportionally to aeration level. Fermentations performed with *S. cerevisiae* and 0.05 VVM aeration exhibited the fastest fermentation kinetics of all cultures (Fig. 1A). *M. pulcherrima* ferments under anaerobic conditions showed slower fermentation kinetics than *S. cerevisiae*

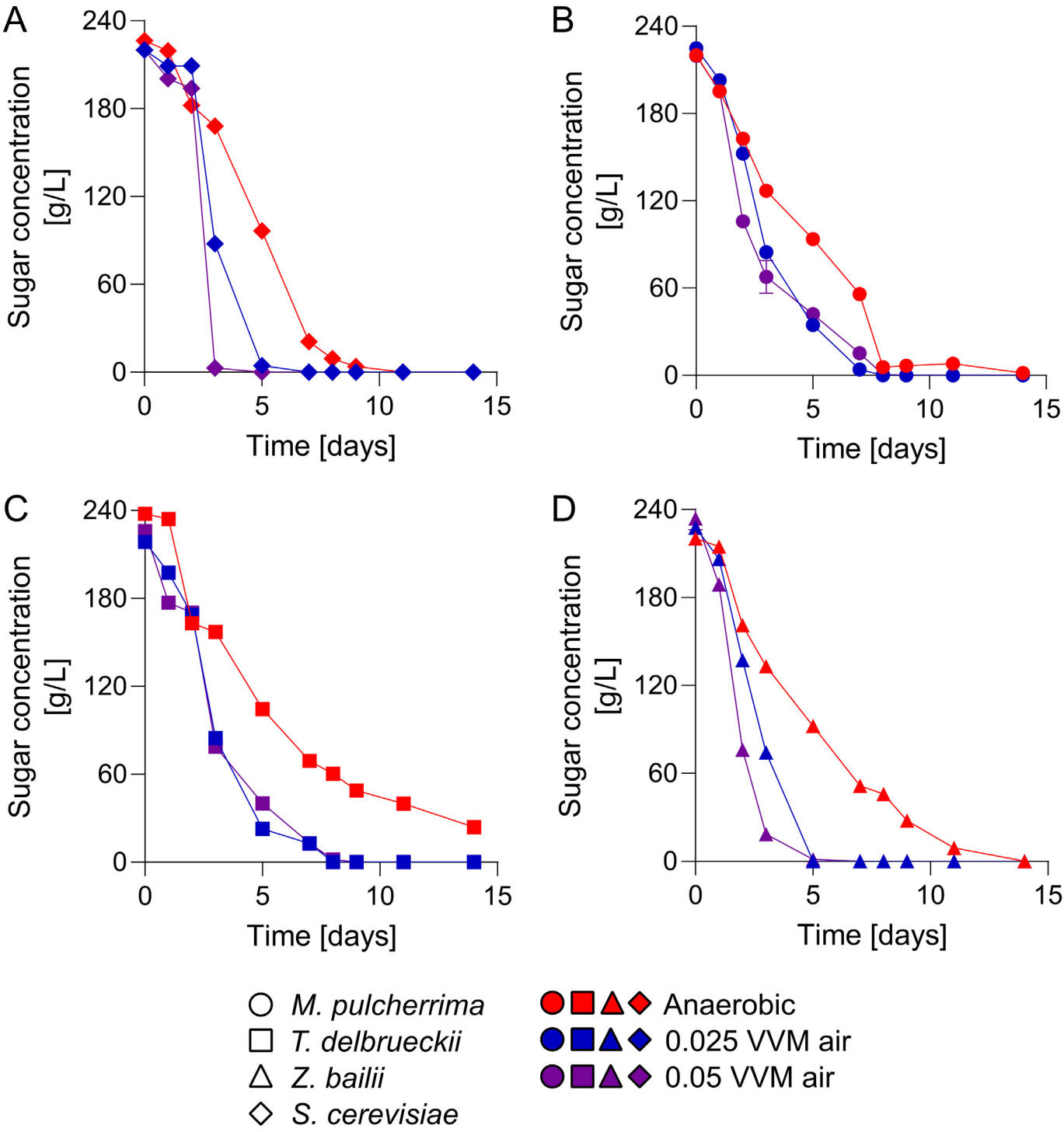

Fig. 1. Sugar consumption kinetics for *S. cerevisiae* ferments (A) and sequentially inoculated trials with *M. pulcherrima* (B), *T. delbrueckii* (C) and *Z. bailii* (D) under different aeration conditions. Anaerobic conditions (red), aeration 0.025 VVM (blue), aeration 0.05 VVM (purple). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

pure culture without aeration, while both aeration treatments (0.025 and 0.05 VVM) showed faster fermentation kinetics (Fig. 1B). *T. delbrueckii* ferments under anaerobic conditions were unable to finish fermentation in 14 days leaving 26 g/L of residual sugar. In contrast, *T. delbrueckii* ferments with both aeration treatments finished fermentation and showed similar kinetics than *S. cerevisiae* pure culture without aeration (Fig. 1C). *Z. bailii* ferments under anaerobic conditions showed sluggish fermentation kinetics completing sugar consumption after 11 days (Fig. 1D). Aeration however, strongly influenced fermentation kinetics with both aeration treatments of *Z. bailii* showing faster kinetics than *S. cerevisiae* ferments without aeration. Additionally, *Z. bailii* ferments with 0.05 VVM aeration showed fermentation kinetics comparable to that exhibited by *S. cerevisiae* with 0.05 VVM air addition.

For each aeration condition, all three non-*Saccharomyces* strains showed similar growth kinetics with only minor differences (Fig. 2). Thus, under anaerobic conditions, non-*Saccharomyces* strains attained maximum cell numbers 2–3 days after inoculation. Cell numbers decreased 5–7 days after inoculation to disappear completely at the end of

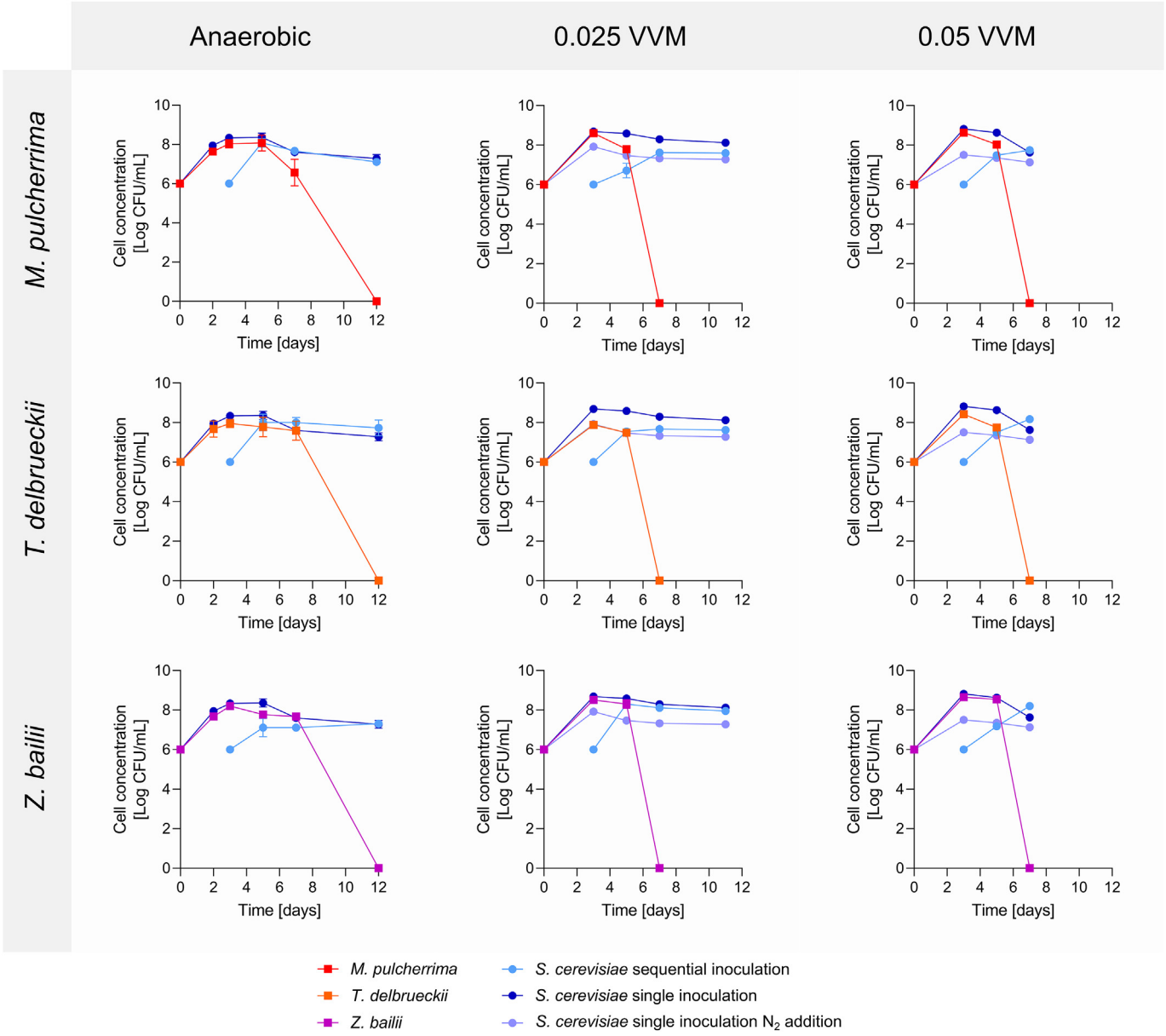

Fig. 2. Yeast growth kinetics for *S. cerevisiae* in pure (dark blue) and mixed culture (light blue), and non-*Saccharomyces* strains *M. pulcherrima* (red), *T. delbrueckii* (orange) and *Z. bailii* (purple) in sequentially inoculated fermentations under different aeration conditions. *S. cerevisiae* controls sparged with nitrogen are indicated in grey. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

fermentation. Conversely, sequentially inoculated *S. cerevisiae* remained viable until the end of fermentation showing growth kinetics similar to the *S. cerevisiae* pure culture. For treatments with 0.025 VVM aeration, *M. pulcherrima* and *Z. bailii* attained higher cell numbers than under anaerobic conditions, while *T. delbrueckii* showed similar cell concentration. All non-*Saccharomyces* strains disappeared 7 days after inoculation, whereas *S. cerevisiae* remained viable until the end of fermentation. Similar growth kinetics were observed for 0.05 VVM air addition, with all three non-*Saccharomyces* strains exhibiting higher cell numbers than under anaerobic conditions.

3.2. Oxygen utilization kinetics differed depending on yeast strain

In treatments with 0.025 VVM aeration, *M. pulcherrima* and *Z. bailii* were able to consume all dissolved oxygen in 20 h, faster than *S. cerevisiae* (Fig. 3A). *T. delbrueckii,* however consumed all oxygen in approximately 45 h, well before sequential inoculation with *S. cerevisiae*. In treatments with 0.05 VVM air addition, *M. pulcherrima* was again the fastest strain to consume all oxygen (< 20 h), whereas *Z. bailii* and *S. cerevisiae* needed 25 h. Once more, *T. delbrueckii* showed the slowest oxygen consumption rate exhibiting a long lag phase before utilizing all oxygen in 60 h.

3.3. Aeration shaped wine chemical composition

The chemical composition of Chardonnay wines produced with non-*Saccharomyces* strains in sequential fermentation with *S. cerevisiae* is summarized in Table 1. Aeration affected the production of ethanol, glycerol and organic acids. Wines produced with *M. pulcherrima* under aeration conditions exhibited a significant ethanol reduction compared to anaerobic conditions. In particular, 0.05 VVM air addition enabled an ethanol reduction of 1.4% v/v. Glycerol concentration decreased only when air was provided at 0.05 VVM, whereas succinic acid content increased significantly at both aeration levels. Wines produced with *T.*

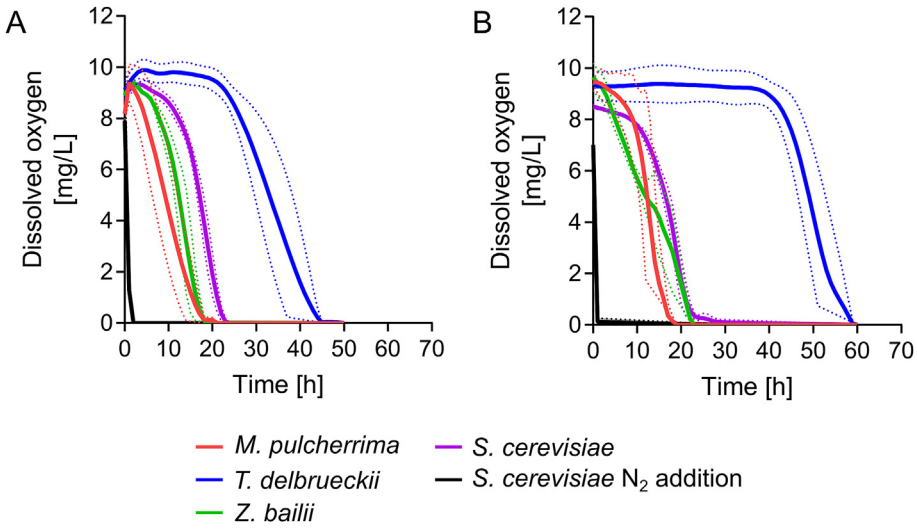

Fig. 3. Dissolved oxygen concentration for *S. cerevisiae* ferments (purple) and sequentially inoculated trials with *M. pulcherrima* (red), *T. delbrueckii* (blue) and *Z. bailii* (green) 0.025 VVM aeration (A) and 0.05 VVM aeration (B). *S. cerevisiae* controls sparged with nitrogen are indicated in black. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

delbrueckii were able to reach dryness (< 4 g/L sugar) only when air was provided. Aeration increased the production of succinic acid, whereas the concentration of glycerol did not show clear trends. Wines produced with *Z. bailii* showed a similar trend to *M. pulcherrima* wines in response to air addition. *S. cerevisiae* wines showed similar ethanol concentration regardless of aeration level, whereas glycerol and succinic acids increased with aeration. Moreover, the aeration condition 0.025VVM significantly increase the malic acid content in *M. pulcherrima*, *T. delbrueckii* and *S. cerevisiae*, while *Z. bailii* showed a significant increase in anaerobic condition.

Compared to *S. cerevisiae* wines produced under anaerobic conditions, wines produced with non-*Saccharomyces* strains and aeration showed a reduction in ethanol concentration between 0.9 and 1.6% v/v (Table 1). Thus, wines produced with *M. pulcherrima* had 1.6% v/v less alcohol than control wines, while *T. delbrueckii* wines and wines fermented with *Z. bailii* had 0.9% v/v and 1.0% v/v lower ethanol concentration, respectively.

3.4. Wine volatile composition was affected by yeast strain and aeration level

The concentration of esters, higher alcohols and volatile acids found in the Chardonnay wines are reported in Fig. 4 and Table S1. Ethyl acetate concentration was the lowest for *T. delbrueckii* regardless of aeration level, while *M. pulcherrima* produced the highest concentration at 0.05 VVM aeration. The concentration of total esters decreased with aeration for most strains except *Z. bailii*, which showed slightly increased ester production at 0.05 VVM aeration. This increase was due to higher concentrations of ethyl propanoate and 2-phenylethyl acetate in these wines. *M. pulcherrima* wines showed increased concentration of 2-methylpropyl acetate, 3-methylbutyl acetate, 2-phenylethyl acetate and ethyl octanoate, particularly under anaerobic conditions. Wines produced with *S. cerevisiae* under anaerobic conditions or with nitrogen sparging, had the highest concentrations of ethyl hexanoate, ethyl octanoate and ethyl decanoate (Fig. 4).

All non-*Saccharomyces* species showed a significant increase in the total concentration of higher alcohols compared to *S. cerevisiae*. This increase was the result of high concentrations of 2-methylpropanol, 2-methylbutanol, 3-methylbutanol and 2-phenyl ethanol (Fig. 4). Air addition increased the total concentration of higher alcohols in all wines. Thus, *Z. bailii* wines produced with 0.05 VVM aeration showed the highest total concentration of higher alcohols (Table S1).

Acetic acid concentration was the highest in *S. cerevisiae* wines produced anaerobically, whereas wines produced with *M. pulcherrima* or *T. delbrueckii* showed the lowest concentrations regardless of aeration level (Fig. 4). Ferments inoculated with *Z. bailii* had the highest concentrations of propanoic, 2-methyl propanoic and butanoic acids, while *T. delbrueckii* and *S. cerevisiae* wines produced with aeration contained the highest concentrations of 2- and 3-methyl butanoic acids. The concentration of the medium-chain fatty acids (MCFAs), hexanoic,

Table 1
Chemical composition of Chardonnay wines produced with *M. pulcherrima, T. delbrueckii* and *Z. bailii* in sequential fermentation with *S. cerevisiae* under different aeration conditions.

Strain	Aeration conditions	Residual sugar [g/L]	Glycerol [g/L]	Succinic acid [g/L]	Malic acid [g/L]	Ethanol [% v/v]	Ethanol yield [g/g]
M. pulcherrima	Anaerobic	0.00 ± 0.00^{a}	7.8 ± 0.1^{a}	0.4 ± 0.0^{b}	3.8 ± 0.3^{b}	11.6 ± 0.1^{a}	38.1 ± 0.4^{a}
	0.025 VVM	0.00 ± 0.00^{a}	7.4 ± 0.5^{a}	0.8 ± 0.2^{a}	4.4 ± 0.0^{a}	11.1 ± 0.1^{b}	36.6 ± 0.4^{a}
	0.05 VVM	0.00 ± 0.00^{a}	5.5 ± 0.1^{b}	0.9 ± 0.1^{a}	3.6 ± 0.2^{b}	10.2 ± 0.4^{c}	34.4 ± 1.8^{b}
T. delbrueckii	Anaerobic	24.12 ± 0.89^{a}	7.9 ± 0.0^{b}	0.6 ± 0.1^{c}	2.3 ± 0.3^{b}	9.8 ± 0.1^{b}	36.4 ± 0.5^{a}
	0.025 VVM	0.00 ± 0.00^{b}	6.5 ± 0.1^{c}	1.6 ± 0.2^{b}	3.0 ± 0.3^{a}	10.8 ± 0.1^{a}	36.5 ± 1.5^{a}
	0.05 VVM	0.00 ± 0.00^{b}	11.5 ± 0.2^{a}	2.2 ± 0.1^{a}	2.2 ± 0.1^{b}	10.9 ± 0.2^{a}	35.9 ± 0.5^{a}
Z. bailii	Anaerobic	0.00 ± 0.00^{a}	7.3 ± 0.3^{a}	0.7 ± 0.1^{b}	3.2 ± 0.2^{a}	11.4 ± 0.1^{a}	36.2 ± 2.6^{a}
	0.025 VVM	0.00 ± 0.00^{a}	7.8 ± 0.0^{a}	1.7 ± 0.0^{a}	1.7 ± 0.1^{c}	10.8 ± 0.1^{b}	37.3 ± 0.4^{a}
	0.05 VVM	0.00 ± 0.00^{a}	5.6 ± 0.3^{b}	1.5 ± 0.1^{a}	2.7 ± 0.1^{b}	10.6 ± 0.1^{b}	34.0 ± 2.6^{a}
S. cerevisiae	Anaerobic	0.00 ± 0.00^{c}	4.9 ± 0.2^{c}	0.3 ± 0.0^{b}	3.2 ± 0.1^{c}	11.8 ± 0.0^{a}	39.2 ± 0.8^{a}
	0.025 VVM	2.40 ± 0.06^{a}	6.5 ± 0.2^{a}	1.6 ± 0.1^{a}	4.0 ± 0.1^{a}	11.6 ± 0.1^{a}	39.2 ± 0.8^{a}
	0.05 VVM	1.00 ± 0.03^{b}	5.8 ± 0.2^{b}	1.7 ± 0.1^{a}	3.6 ± 0.1^{b}	11.7 ± 0.1^{a}	38.8 ± 2.5^{a}
	0.025 VVM N_2	2.34 ± 0.62^{a}	5.0 ± 0.1^{c}	0.3 ± 0.0^{b}	3.1 ± 0.1^{c}	11.7 ± 0.1^{a}	39.9 ± 1.5^{a}
	0.05 VVM N_2	1.00 ± 0.30^{b}	4.7 ± 0.2^{c}	0.3 ± 0.0^{b}	3.5 ± 0.2^{b}	11.7 ± 0.1^{a}	38.8 ± 2.5a

Data are means ± standard deviation from three independent replicates. Data with different superscript letters (^{a,b,c}) within each column and for each species are statistically different according to Duncan tests (0.05%).

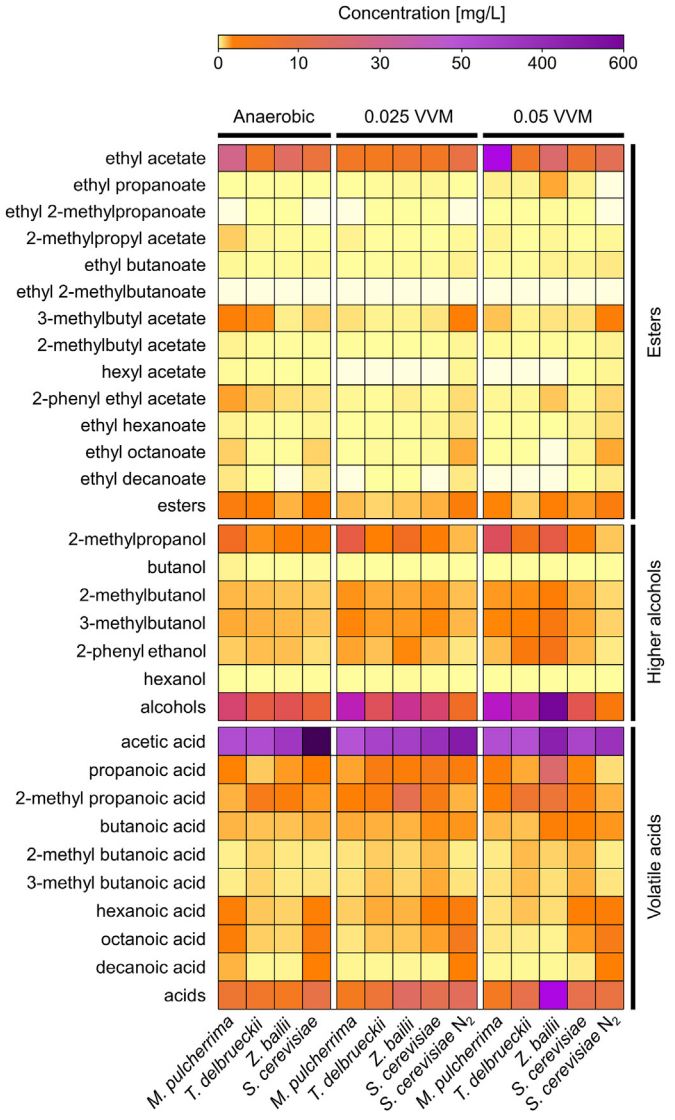

Fig. 4. Heatmap indicating the concentration of esters, higher alcohols and volatile acids for *S. cerevisiae* ferments and sequentially inoculated trials with *M. pulcherrima*, *T. delbrueckii* and *Z. bailii* under different aeration conditions. Total ester concentration excludes ethyl acetate, total concentration of volatile acids excludes acetic acid.

octanoic and decanoic acids, was the highest in wines produced with *M. pulcherrima* or *S. cerevisiae* under anaerobic conditions and in *S. cerevisiae* wines with nitrogen sparging (Fig. 4).

To assess the overall effect of aeration on the volatile profile of the Chardonnay wines a Principal Component Analysis (PCA) was performed (Fig. 5). The first principal component (PC1) enabled discrimination according to aeration conditions, while a combination of both PC1 and PC2 allowed separation by yeast strain particularly for anaerobic conditions. *S. cerevisiae* wines produced with nitrogen sparging and under anaerobic conditions clustered relatively close and were associated with MCFAs, ethyl butanoate, MCFA ethyl esters and acetic acid. Wines produced anaerobically with *M. pulcherrima* located separately to other ferments and associated with 2-phenyl ethyl acetate, 2-methyl propyl acetate and butanol, while wines produced anaerobically with *T. delbrueckii* and *Z. bailii* grouped together in the middle of the plot (Fig. 5). While aeration level did not separate wines produced with *S. cerevisiae*, it enabled separation of wines produced with non-*Saccharomyces* strains. Wines fermented with *Z. bailii* under aeration conditions were particularly affected by aeration level, with wines produced with 0.025 VVM aeration located in the upper right quadrant, whereas wines made with 0.05 VVM aeration placed in the bottom right quadrant.

4. Discussion

One of the most pressing concerns in winemaking is the progressive increase of ethanol concentration in wine. Although several approaches can be used to decrease alcohol concentration in wine, the use yeast strains with reduced alcohol production has emerged as an attractive strategy (Varela and Varela, 2018). In particular, the use of non-*Saccharomyces* yeasts in co-culture or sequential inoculation with *S. cerevisiae* has been shown to lower wine ethanol concentration under anaerobic conditions (Canonico et al., 2016; Ciani et al., 2016; Englezos et al., 2018; Furlani et al., 2017; Rolle et al., 2018; Varela, 2016) and under controlled aeration during fermentation (Alonso-del-Real et al., 2017; Contreras et al., 2015; Quiros et al., 2014; Rocker et al., 2016). Here we evaluated the effect of aeration on the volatile composition and yeast population dynamics of reduced alcohol Chardonnay wines produced with *M. pulcherrima*, *T. delbrueckii* and *Z. bailli*, sequentially inoculated with *S. cerevisiae*.

The early addition of oxygen stimulates wine fermentation, favoring yeast growth, viability and fermentation activity by promoting sterol and unsaturated fatty acids (UFAs) biosynthesis (Blateyron and Sablayrolles, 2001; Fornairon-Bonnefond et al., 2003; Larue et al., 1980; Mauricio et al., 1997). Conversely, lack of oxygen and lipids in anaerobic growth media causes stress for yeast cells and inhibits cell growth (Bardi et al., 1998; Deytieux et al., 2005; Fornairon-Bonnefond et al., 2002). In this study, for all the yeast species evaluated aeration enhanced biomass formation and increased sugar utilization kinetics which resulted in shorter fermentation times. Interestingly, *T. delbrueckii* was able to complete Chardonnay fermentation only when air was provided, suggesting a higher requirement for oxygen and/or lipids for this species. In addition, the slow oxygen consumption kinetics observed for *T. delbrueckii* may indicate a different mechanism for oxygen utilization in this species compared to the other yeasts evaluated in this study.

In addition to improving fermentation performance, oxygen has been shown to affect yeast physiology and metabolism (Aceituno et al., 2012; Shekhawat et al., 2018; Tronchoni et al., 2018). Unlike *S. cerevisiae*, for which respiration is repressed by high concentrations of hexoses even in the presence of oxygen, several non-*Saccharomyces* species are able to aerobically respire sugar (Alexander and Jeffries, 1990; de Deken, 1966). This oxidative metabolism results in altered formation of the main metabolites produced during fermentation, including ethanol, glycerol and organic acids (Contreras et al., 2015; Morales et al., 2015; Quiros et al., 2014). Compared to *S. cerevisiae* wines, the ethanol reduction achieved with aeration was 1.6% v/v, 0.9% v/v and 1.0% v/v for *M. pulcherrima*, *T. delbrueckii* and *Z. bailii* sequential fermentations, respectively. These results are comparable with previous reports performed under similar fermentation conditions (Contreras et al., 2015; Quiros et al., 2014).

Although ethanol concentration in wine can be reduced by providing non-*Saccharomyces* yeasts with oxygen, this sometimes has undesirable side-effects. Several non-*Saccharomyces* species can produce increased concentrations of acetic acid (Contreras et al., 2015; Rocker et al., 2016; Shekhawat et al., 2017). Acetic acid has a sour and bitter taste and concentrations higher than 0.9 g/L are considered detrimental for wine quality (Ribéreau-Gayon et al., 2006). Indeed, wines produced with non-*Saccharomyces* strains under aerobic conditions have shown increased scores for the 'vinegar' sensory descriptor (Rocker et al., 2016). None of the strains evaluated in this study, however showed increased acetic acid production in response to aeration, which indicates that the composition of the media/grape juice can modulate its formation. Similar results have been obtained with other white grape varieties (Tronchoni et al., 2018).

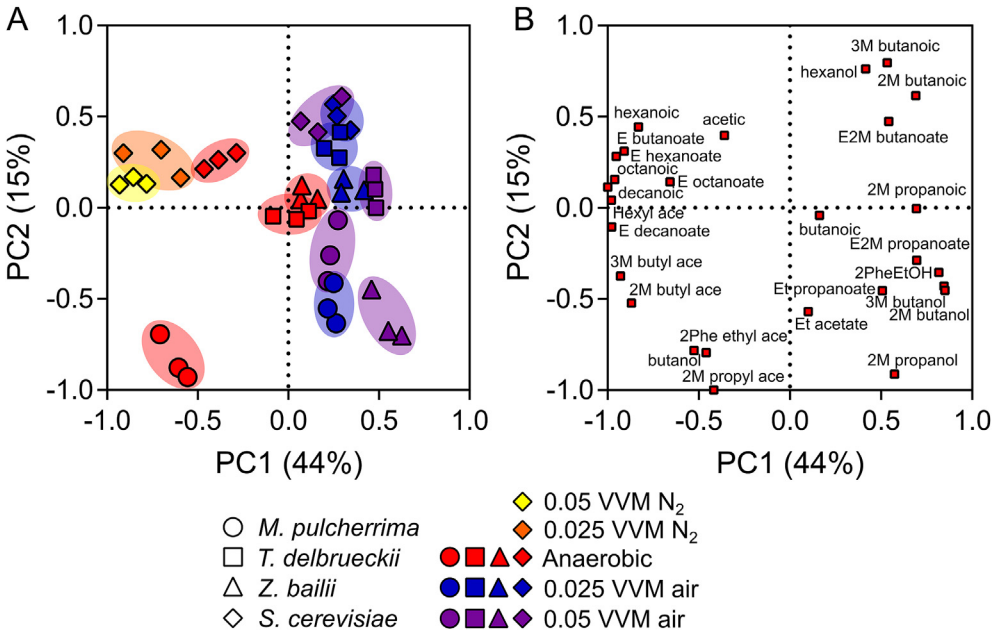

Fig. 5. Principal component analysis based on esters, higher alcohols and volatile acids for *S. cerevisiae* ferments (diamonds) and sequentially inoculated trials with *M. pulcherrima* (circles), *T. delbrueckii* (squares) and *Z. bailii* (triangles) under different aeration conditions. Scores (A), loadings (B). 2M butanoic, 2-methyl butanoic acid, 3M butanoic, 3-methyl butanoic acid, 2M butanol, 2-methyl butanol, 3M butanol, 3-methyl butanol, 2M butyl ace, 2-methyl butyl acetate, 3M butyl ace, 3-methyl butyl acetate, 2M propanoic, 2-methyl propanoic acid, 2M propanol, 2-methyl propanol, 2M propyl ace, 2-methyl propyl acetate, 2PheEtOH, 2-phenyl ethanol, 2Phe ethyl ace, 2-phenyl ethyl acetate, E2M butanoate, ethyl 2-methyl butanoate, E2M propanoate, ethyl-2 methyl propanoate, Et acetate, ethyl acetate, E butanoate, ethyl butanoate, E decanoate, ethyl decanoate, E hexanoate, ethyl hexanoate, E octanoate, ethyl octanoate, Et propanoate, ethyl propanoate, Hexyl ace, hexyl acetate.

Oxygen addition during fermentation also affects the formation of several volatile compounds found in wine. Oxygen supplementation of grape must has been described to increase the concentration of esters (Valero et al., 2002) and higher alcohols (Shekhawat et al., 2017; Valero et al., 2002; Varela et al., 2012). Conversely, oxygen addition has been reported to increase the concentration of ethyl esters and reduce the concentration of acetate esters (Bertrand and Torres-Alegre, 1984) and decrease the concentration of esters (Fariña et al., 2012) and higher alcohols (Bertrand and Torres-Alegre, 1984; Fariña et al., 2012). Oxygen has also been shown to alter the proportion of acetate to ethyl esters and the proportion of branch-chain acids to medium-chain fatty acids (Varela et al., 2012). These differences might, at least in part, be due to the use of different strains, media and fermentation conditions. Oxygen concentration can also influence the formation of the volatile thiols 4-methyl-mercaptopentan-2-one (4MMP), cis-3-mercaptohexan-1-ol (3 MH) and 3-mercaptohexyl acetate (3MHA) (Coetzee et al., 2013). In the current study, aeration did not affect the total ester concentration (excluding ethyl acetate) in *T. delbrueckii* and *Z. bailii* wines, but decreased ester concentration in *M. pulcherrima* and *S. cerevisiae* wines. Although air addition increased the concentration of higher alcohols, only one treatment, *Z. bailii* with 0.05 VVM aeration, showed concentrations over the limit (300 mg/L) considered by some authors as detrimental for wine quality (Rapp and Mandery, 1986).

Ethyl acetate is one of the main esters produced during wine fermentation, and while low concentrations impart a fruity aroma, concentrations above 150–160 mg/L are associated with undesirable 'nail polish remover' and 'solvent' sensory descriptors (Ribéreau-Gayon et al., 2006). Only one treatment, *M. pulcherrima* with 0.05 VVM aeration, produced wines with excessive ethyl acetate concentration (280 mg/L), most likely impacting negatively the flavor profile of these wines. Indeed, wines produced with this species under aerobic conditions have shown increased scores for the 'solvent' sensory descriptor (Rocker et al., 2016). Interestingly, increased ethyl acetate concentrations were not observed for the 0.025 VVM treatment, suggesting an oxygen threshold for ethyl acetate over-production in *M. pulcherrima*.

In summary, our results confirm the ability of non-*Saccharomyces* yeasts to produce wines with reduced ethanol content when supplied with small amounts of oxygen and indicate that the volatile composition of these wines can be modulated by the amount of oxygen provided during the early stages of fermentation. However, ethanol reduction and flavor profile must be balanced in order to provide wines with lower ethanol concentration and pleasant sensory profile. Given the high concentrations of ethyl acetate found in wines fermented with *M. pulcherrima* under 0.05 VVM aeration and the increased concentration of higher alcohols and volatile acids found in wines produced with *Z. bailii* with 0.05 VVM aeration, it is very likely that these wines have an unpleasant sensory profile. Nevertheless, wines fermented with *T. delbrueckii* under aerobic conditions, and wines produced with *M. pulcherrima* and *Z. bailii* under 0.025 VVM aeration showed a promising balance between ethanol reduction and volatile profile. Future work will focus on scaling up experiments to pilot scale using red and white grape musts and evaluating wine sensory profile.

Conflicts of interest

Submission of Manuscript FM_2019_19 entitled 'Volatile profile of reduced alcohol wines fermented with selected non-Saccharomyces yeasts under different aeration conditions'

The authors declare no conflict of interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2019.103247.

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