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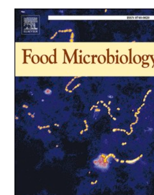

Reduction of ethanol content in wine with an improved combination of yeast strains and process conditions

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ABSTRACT

One interesting strategy to address the increasing alcohol content of wines, associated with climate change, is to reduce the ethanol yield during fermentation. Within this strategy, the approach that would allow the clearest reduction in alcohol content is the respiration of part of the grape sugars by yeasts. Non-*Saccharomyces* species can be used for this purpose but suffer from a limited ability to dominate the process and complete fermentation. In turn, *Saccharomyces cerevisiae* shows a high production of acetic acid under the growth conditions required for respiration. Previously proposed procedures used combinations of non-*Saccharomyces* and *S. cerevisiae* starters, or a strain of *S. cerevisiae* (PR1018), with unique metabolic properties. In both cases, precise management of oxygen availability was required to overcome the acetic acid problem. In this work, we have developed a laboratory scale process to take advantage of the properties of PR1018 and a strain of *Metschnikowia pulcherrima*. This process is more robust than the previous ones and does not rely on strict control of oxygenation or even the use of this particular strain of *S. cerevisiae*. Aeration can be interrupted instantly without impairing the volatile acidity. Under the selected conditions, an ethanol reduction of around 3% (v/v) was obtained compared to the standard fermentation control.

1. Introduction

According to OIV (International Organisation of Vine and Wine) wine is primarily the result of the fermentation of grape sugars by yeasts (OIV, 2022). In spontaneously fermented wines there is a dominance of *Saccharomyces cerevisiae* during the main stages of alcoholic fermentation (Gonzalez and Morales, 2022), despite its low abundance among fungal communities in grape berries (Tronchoni et al., 2022b), and regardless of the involvement, intended or unintended, of other microorganisms. Ethanol and CO_2 are the main products of sugar fermentation by yeasts, and *S. cerevisiae* shows low biological diversity regarding ethanol yield on sugar (Camarasa et al., 2011). Therefore, alcohol formation is inherent to winemaking.

Wine is a common component of the Mediterranean Diet (Trichopoulou et al., 2014). The scientific information on the impact of wine consumption on human health was recently revised by Minzer et al. (2020). They conclude that light to moderate wine intake seems to have beneficial effects to some extent in several diseases and conditions, but no definitive recommendations can be made on a specific intake level that can benefit most diseases. Instead, there is a medical consensus, backed by the WHO (2019), on the direct risks of excessive alcohol consumption in relation to various diseases, as well as indirect risks like violent behaviour, self-harm, or road safety consequences.

In this context, all stakeholders in the wine industry have been trying to develop processes that allow wines of lower alcoholic strength to be produced, without detriment to their quality (Gonzalez et al., 2021; Varela et al., 2015). The demand for such improvements is pressing because, due to various factors, including global warming, wines on the market have experienced a significant increase in alcohol content over the last decades (Jones et al., 2005; Mira de Orduña, 2010; Martinez de Toda and Balda, 2015; Van Leeuwen and Darriet, 2016).

Some years ago, our group proposed the use of mixed cultures of *S. cerevisiae* with non-*Saccharomyces* species to reduce the overall alcohol yield from yeast metabolism during fermentation (Gonzalez et al., 2013). *Metschnikowia pulcherrima* was selected to develop a process relying on respiration under controlled aeration conditions

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(Morales et al., 2015; Tronchoni et al., 2018). The same species, *M. pulcherrima*, has been explored by several authors to reduce ethanol yield during fermentation, both under aerated and non-aerated conditions; while the aeration strategy has also been explored by several authors with different non-conventional species (Contreras et al., 2015; Röcker et al., 2016; Alonso-del-Real et al., 2017; Canonico et al., 2019a; Canonico et al., 2019b; Aplin and Edwards, 2021; Edwards and Aplin, 2022). The success of this process depended on the use of controlled oxygenation conditions, which are difficult to implement in the winery. One of the main difficulties was the excessive production of acetic acid by *S. cerevisiae* in the presence of oxygen (Quirós et al., 2014; Curiel et al., 2016). More recently, we described a strain of *S. cerevisiae* that did not present this limitation. It could be used as a single starter for alcohol level reduction under aerated fermentation conditions. However, a gradual reduction of the available oxygen, using automated control of dissolved oxygen, was required (Tronchoni et al., 2022a). In the current work, we have developed a process that combines the advantages of the two previous ones, while overcoming some of the hurdles, using strains of *M. pulcherrima* and *S. cerevisiae*.

2. Materials and methods

2.1. Yeast strains and media

Two strains of *S. cerevisiae*, PR1018 (Tronchoni et al., 2022a), and Lalvin EC1118 (Lallemand Inc, Montreal, Canada); and fifteen *M. pulcherrima* strains from the institute collection were used in this work (Table S1). All of them are wild isolates from La Rioja (Spain). Yeasts were maintained at 4 °C on YPD agar plates (2% glucose, 2% peptone, 1% yeast extract, and 2% agar), or as glycerol stocks at −80 °C. Inocula were grown on YPD for 48 h at 25 °C, washed and suspended in water.

Natural white must from the 2019 harvest (a blend of Malvasia and Viura varieties) was kept frozen. It contained 220 g/L of sugar and 186 mg/L yeast assimilable nitrogen, pH 3.45. Enough volume for each experiment was thawed and pasteurized. Pasteurization consisted of heating to 105 °C for less than 1 min and then allowing it to cool down inside the closed autoclave. Where indicated, cultures were supplemented with 0.3 g/L of a filter-sterilised commercial yeast autolysate (Helper 100% origin, Oenofrance, France).

2.2. Fermentation experiments

Shake flasks (100 mL nominal volume) were filled with 20 mL of reconstituted grape must (from industrial concentrate), inoculated at 0.2 final OD_{600}, covered with a loosened GL25 screw cap (to allow gas exchange), and incubated at 25 °C, 180 rpm. After 4 days, samples were withdrawn, centrifuged, and supernatants were kept frozen for HPLC analysis.

For bioreactor assays, batch cultures were performed using DASGIP bioreactors (400 mL nominal volume) or Applikon MiniBio bioreactors (250 mL nominal volume), equipped with refrigerated gas condensers. Applikon bioreactors were filled with 150 mL of pasteurized natural white grape must, and 200 μL of antifoam 204 (Sigma-Aldrich, Spain). Temperature was set to 25 °C and stirring to 1000 rpm. The cultures were sparged with compressed air at 10, 5, or 2.5 VVH (volumes of gas/volume culture/hour). Anaerobic controls were flushed with nitrogen at 10 VVH for the same period. DASGIP bioreactors were filled with 200 mL of reconstituted grape must, and 200 μL of antifoam 204 (Sigma-Aldrich, Spain). Temperature was set to 25 °C and stirring to 250 rpm. Gas flow was controlled with MFC17 mass flow controllers (Aalborg Instruments and Controls, Inc., Orangeburg, NY), whose calibration was regularly verified with an electronic flowmeter (Agilent Technologies, Santa Clara, CA). Bioreactors were inoculated to 0.2 final OD_{600}, with independent inocula for each replicate. During the experiment, 1 mL samples were collected at the times indicated in the figures for HPLC analysis. A final 10 mL sample was preserved for the analysis of volatile compounds. Samples were centrifuged and supernatants were kept frozen for chromatographic analyses. Dissolved oxygen was monitored by Lumisens sensors (Applikon).

2.3. Determination of metabolite concentration

The concentration of glucose, fructose, glycerol, ethanol, and acetic acid was determined using a Surveyor Plus liquid chromatograph (Thermo Fisher Scientific, Waltham, MA) equipped with a refraction index and a photodiode array detector (Surveyor RI Plus and Surveyor PDA Plus, respectively) on a 300 × 7.7 mm PL Hi-Plex H^+ (8 μm particle size) column (Agilent Technologies, Santa Clara, CA) and 4 × 3 mm ID Carbo-H guard (Phenomenex, Torrance, CA). The column was maintained at 50 °C and 1.5 mM H_2SO_4 was used as the mobile phase at a flow rate of 0.6 mL/min. Prior to injection in duplicate, the samples were filtered through 0.22 μm pore size nylon filters (Micron Analitica).

2.4. Monitoring of population dynamics

Viable cells for each of the inoculated strains were quantified by daily plating three independent appropriate dilutions in YPD plates and incubating for 48–72 h at 25 °C. Colonies of *M. pulcherrima* in mixed cultures were distinguished from those of *S. cerevisiae* by the development of a pink coloration. This colony colour is due to the production of pulcherrimin, common among strains of *M. pulcherrima* (Kurtzman et al., 2011).

2.5. Analysis of volatile compounds

Samples for gas chromatography-mass spectrometry (GC-MS) analysis contained 2 mL of the original sample, and ethanol was added to get the same amount of ethanol in all samples (up to 14% ethanol), 1 g NaCl, and 20 μL internal standard, in 20 mL flasks. Internal standard contained 1000 ppm each of 4-methyl 2-pentanol and heptanoic acid, and 100 ppm each of ethyl nonanoate and 1-nonanol, in water, prepared from 10000 ppm individual solutions in ethanol. Sample was preincubated for 10 min at 45 °C with 10" agitation intervals, followed by 30 min of extraction with 50/30 μm DBV/CAR/PDMS SPME fiber (Stableflex, SUPELCO, Bellefonte, PA), in the same conditions. Fiber was desorbed for 5 min at 250 °C.

GC-MS was carried out in a Thermo TRACE GC Ultra device coupled to a Thermo ISQ mass detector, equipped with a Thermo TriPlus autosampler. Gas chromatography was carried in a Thermo Scientific fused-silica capillary column TG-WAXMS A (30 m long; 0.25 mm OD; 0.25 μm film thickness). Chromatographic conditions were as follows: 5 min at 40 °C, 3 °C/min up to 200 °C, 15 °C/min up to 240 °C, 10 min at 240 °C. Helium was used as carrier gas at a flow rate of 1 mL/min, operating in split mode (ratio 30). Total analysis time was 71 min. Detection was performed with the mass spectrometer operating in the Full Scan mode (dwell time 500 ms), with 70 eV ionization energy, and source and quadrupole temperatures of 250 °C. Detection was stopped during the time interval for ethanol elution. Peak identification was made by comparison of ion spectra with NIST mass spectral library. For each compound, including internal standards, the sum of the areas of the peaks of selected characteristic ions was obtained.

2.6. Colour analysis

CIELab colour parameters, L*, a*, and b*, were determined according to OIV (2021), using an Agilent Cary60 UV–Vis spectrophotometer, connected to a computer running CaryWinUV software version 5.0.0.999.

2.7. Statistical analysis

The yield on substrate and concentration levels of the main fermentation metabolites, volatile compounds, and CIELab parameters were compared by one-way analysis of variance. Comparison of means was carried by Tukey test. All statistical analyses, including PCA, were performed using SPSS Statistics v. 25 program (IBM, Armonk, NY, United States).

3. Results

3.1. Selection of a suitable *M. pulcherrima* strain

The first step of this work consisted in the selection of a suitable *M. pulcherrima* strain. This screening involved fifteen *M. pulcherrima* natural isolates. Analyses of metabolites after four days of aerobic growth are summarized in Table S1. Most of the assayed strains consumed above 200 g/L of reducing sugars during the four days of aerobic culture. Ethanol yield on sugar was below 0.36 g/g. This agreed with previous observations for aerobic cultures of *M. pulcherrima* (Quirós et al., 2014; Morales et al., 2015; Rodrigues et al., 2016). Acetic acid yield ranged from 0.79 to 2.95 mg/g (above 1.4 mg/g for most strains), while glycerol yield ranged from 14 to 38 mg/g. Two strains were selected for further characterization. Strain Mp704 was selected because it showed one of the lowest ethanol yield values (0.306 g/g), with a good sugar consumption level (212 g/L) (Table S1). In turn Mp734 showed one of the lowest acetic acid yield values (1.37 mg/g), with good sugar consumption (208 g/L) (Table S1). Although Mp402 stood out for its low ethanol yield (0.262 g/g) and acetic acid yield (0.79 mg/g), it was discarded due to low sugar consumption (166 g/L) (Table S1).

The two selected strains were tested for aerobic fermentation in DASGIP bioreactors for seven days. Under these conditions, sugar consumption stalled by day 4 of the experiment for both strains, leaving around 50 g/L of residual sugar for Mp734 and above 80 g/L for Mp704 (data not shown). Ethanol yield was very low, 0.12 mg/g for Mp704 and 0.19 mg/g for Mp734; and acetic acid yield was lower for Mp734 (0.65 mg/g) than for Mp704 (1.89 mg/g) (data not shown). Considering the lower acetic acid yield and the higher sugar consumption, Mp734 was retained for the development of an aerobic, co-inoculated fermentation with *S. cerevisiae* PR1018.

3.2. Adjusting the proportion of yeast species

Several *S. cerevisiae* inoculation rates (0.02, 0.05, 0.1 OD_{600}) were tested in Applikon bioreactors by triplicate, with *M. pulcherrima* inoculation fixed at 0.20 OD_{600}. Aeration was stablished at 10 VVH with compressed air. It was interrupted, along with stirring, after 72 h. The aim was to allow a reduction in ethanol yield, while lowering the risk of acetic acid build-up. Results are shown in Fig. 1 along with results from anaerobic fermentation. All mixed-inoculum fermentations showed slower sugar consumption and higher acetic acid yield. Ethanol yield was low, resulting in wines with lower ethanol content by 2.5–3% (v/v) (Table 1). After 10 days of fermentation, residual sugar in mixed cultures was high (16–24 g/L), as compared to anaerobic cultures (2 g/L) (Table 1).

3.3. Adjusting the gas flow

Several glass flow values were tested to minimize the level of dissolved oxygen during aerobic fermentation and reduce the risk of oxidation. In the previous experiments, no negative impact on ethanol or acetic acid yield was observed with increasing levels of *S. cerevisiae* inoculation. Therefore, the highest level (0.1 OD_{600}) was used for these

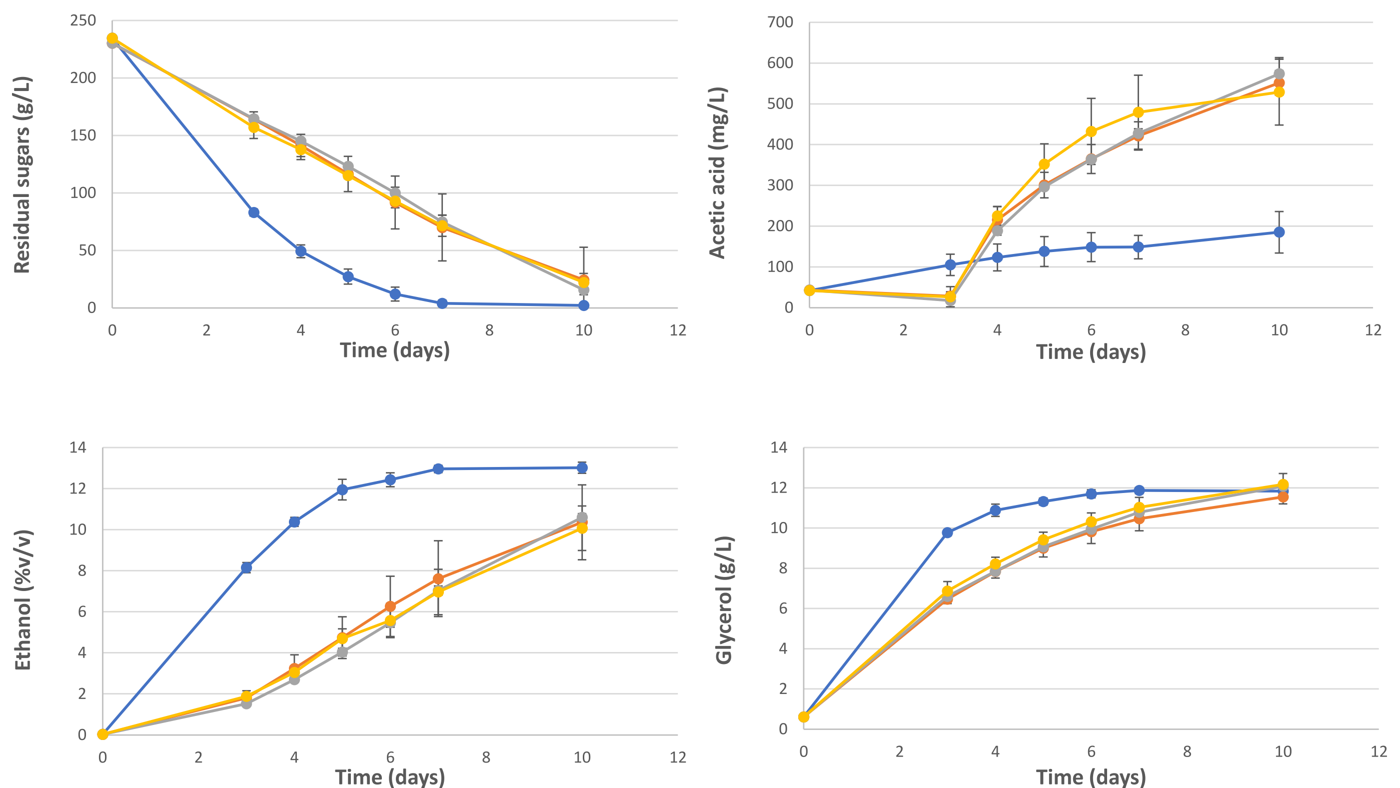

Fig. 1. Evolution of main fermentation parameters with different proportions of *S. cerevisiae* in the starter culture. Aeration and stirring were stopped three days after inoculation. *M. pulcherrima* inoculated at 0.2 OD_{600} in all aerated conditions. *S. cerevisiae* 0.10 OD_{600}, orange; 0.05 OD_{600}, grey; 0.02 OD_{600}, yellow lines. Control fermentation (*S. cerevisiae* 0.20 OD_{600}, anaerobic), blue lines. Data are the average of three biological replicates ±SD. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Main fermentation parameters by the end of fermentation of aerated and control fermentations under the indicated conditions of *S. cerevisiae* inoculation level, aeration regime, or nitrogen nutrient (YAN) supplementation.

S. cerevisiae OD_{600}	VVH	YAN	Acetic acid (mg/L)	Ethanol % (v/v)	Glycerol (g/L)	Ethanol yield (g/g)	Acetic acid yield (mg/g)	Residual sugar (g/L)
0.20	**10 (N_2)**	-	185 ± 51 A	13.0 ± 0.3 B	11.8 ± 0.1 B	0.44 ± 0.01 C	0.80 ± 0.22 A	2.1 ± 0.2
0.02	**10**	-	529 ± 81 C	10.1 ± 1.1 A	12.2 ± 0.6 B	0.37 ± 0.03 AB	2.49 ± 0.40 C	22.0 ± 7.9
0.05	**10**	-	574 ± 40 C	10.6 ± 0.2 A	12.1 ± 0.2 B	0.39 ± 0.01 B	2.67 ± 0.20 C	15.7 ± 4.2
0.10	**10**	-	551 ± 30 C	10.4 ± 1.8 A	11.5 ± 0.4 B	0.40 ± 0.02 B	2.71 ± 0.39 C	24.2 ± 28.6
0.10	**5**	+	388 ± 68 B	10.9 ± 0.2 AB	10.5 ± 0.6 B	0.38 ± 0.01 AB	1.70 ± 0.30 B	2.3 ± 0.3
0.10	**5**	-	346 ± 19 B	9.9 ± 0.6 A	10.5 ± 0.9 B	0.35 ± 0.02 A	1.54 ± 0.09 B	3.0 ± 1.3
0.10	**2.5**	-	315 ± 52 B	10.2 ± 0.0 A	8.4 ± 0.7 A	0.37 ± 0.00 AB	1.43 ± 0.25 AB	3.2 ± 1.9

Shown values correspond to average ± SD from three biological replicates (six for 5VVH without added nitrogen).
Different capital letters in the same column indicate statistically significant differences.

fermentations. They were carried out with aeration rates of 2.5 VVH and 5 VVH under the same conditions as used for the previous tests at 10 VVH. Several features became improved by reducing gas flow, including faster fermentation kinetics, lower acetic acid yield, and reduced residual sugar content (Fig. 2; Table 1). In addition, ethanol yield of cultures at 2.5 or 5 VVH was similar to that at 10 VVH, and hence, clearly reduced compared to anaerobic conditions (Table 1).

3.4. Nutrient supplementation

During the aerobic phase, biomass production by both yeast strains is expected to consume all the available nitrogen nutrients. This would contribute to the slow fermentation rates observed in the final stages of the process in the co-inoculated fermentations. To ensure sufficient population of active *S. cerevisiae* cells in the final fermentation stages, the effect of nitrogen supplementation was tested. As the aim was to enhance the activity of *S. cerevisiae*, nitrogen was supplied after the aeration was stopped, to reduce the chances of uptake by *M. pulcherrima*.

A subtle improvement in fermentation kinetics was observed but it was not statistically significant (data not shown). However, there were differences in the profile of volatile compounds (see below) and *M. pulcherrima* was substituted by *S. cerevisiae* quicker in the supplemented fermentations (Fig. 3). This subtle effect on yeast strain replacement suggests that, in the way it was done, nitrogen supplementation preferentially favoured *S. cerevisiae*, as expected. In addition, viable cell counts indicate that *M. pulcherrima* was still dominating the yeast population at the time of aeration stop (60–80% of the culturable cells).

3.5. Profile of volatile compounds in different fermentation setups

Relative concentration data were recovered for 54 volatile compounds on final fermentation samples. Principal component analysis showed samples from the standard fermentation conditions clustering

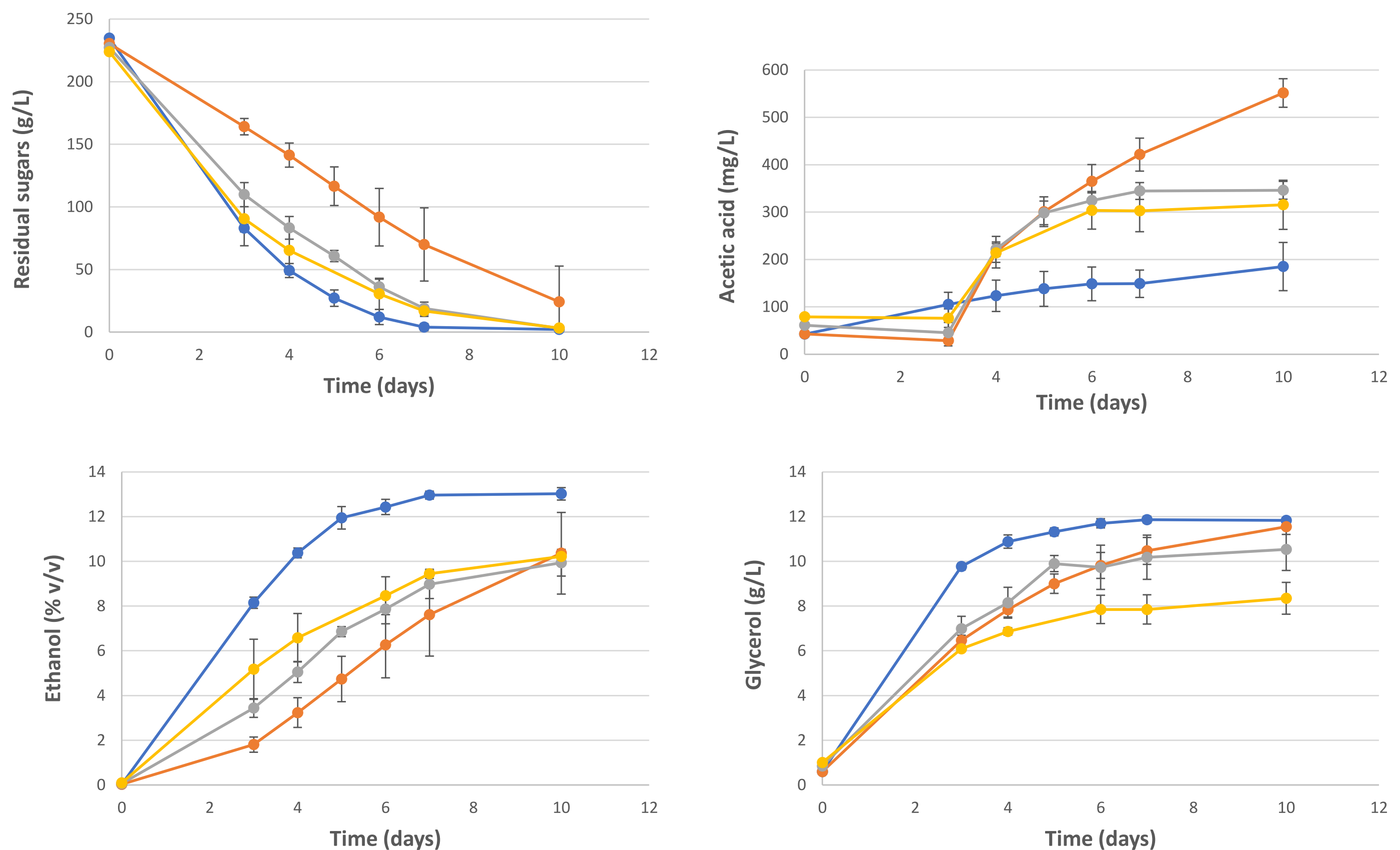

Fig. 2. Evolution of main fermentation parameters with different gas flow rates. Aeration and stirring were stopped three days after inoculation. 10 VVH, orange; 5 VVH, grey; 2.5 VVH, yellow. Control fermentation (*S. cerevisiae* 0.20 OD_{600}, anaerobic), blue lines. Data are the average of three biological replicates ±SD. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

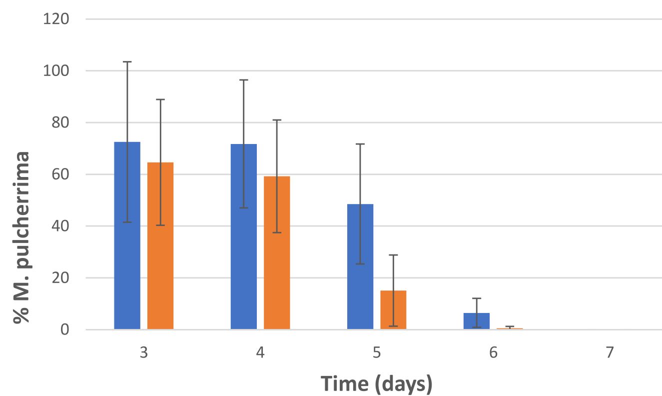

Fig. 3. Evolution of the percentage of *M. pulcherrima* cfu counts (as percentage of global cfu) depending on nitrogen nutrient supplementation. Supplemented fermentation, orange bars; non supplemented, blue bars. Data are average of three biological replicates ±SD. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

far apart from all the mixed culture samples in the PCA1 axis (Fig. 4). The main drivers for this differentiation were the content of different ethyl, isoamyl, and other esters, as well as some fatty acids; and that of some terpene compounds (HO-trienol, α-terpineol, β-citronellol) and low molecular mass esters like ethyl acetate and isoamyl acetate (Fig. 4; Fig. 5). The standard fermentation samples showed the highest values for esters and the lowest ones for terpene compounds.

Samples from mixed inoculum fermentation were distributed along the PCA2 axis, showing a good clustering for biological replicates. Volatiles from experiments aerated at 10 VVH do not seem to be affected by the different inoculation levels of *S. cerevisiae*, since they group together also for this second axis. Samples from experiments aerated at 5 VVH or 2.5 VVH fall slightly separated from those at 10 VVH (Fig. 4); mainly due to their higher content in acetaldehyde and some derivatives (Fig. 5). PCA did not indicate major differences in volatile compounds between samples supplemented or not with nitrogen (Fig. 4). However, some volatile compounds, like acetaldehyde and metabolically related molecules (but not Erlich pathway derived compounds), seem to be specifically enriched in supplemented samples (Fig. 5). This suggests that the main impact of added nitrogen sources comes through a regulatory impact on central carbon metabolism, rather than as direct precursors of volatile compounds.

3.6. *Impact of fermentation conditions on colour parameters*

The analysis of the CIELab parameters L*, a*, and b* for all the fermentation experiments is shown in Figure S1. L* values varied between 97 and 99, and only one of the aerated wines showed statistically significant difference against the wine fermented under standard conditions. No relevant differences between wines were observed for parameter a*, relative to the green–red axis, with values ranging from −0.44 to −0.05. Finally, parameter b* shows a slight trend towards more positive (yellowness) values for aerated fermentation, as compared to anaerobic fermentation (Figure S1). The control wine showed a value of 4.62, while the aerated wines fluctuated from 5.90 to 7.52. No relationship was observed between different levels of aeration or different proportions of yeast inoculum with the measured colour parameters.

3.7. *Suitability of other S. cerevisiae wine strains*

The results shown above suggest that the high affinity of *M. pulcherrima* for oxygen might by critical in preventing the increase in acetic acid production that was previously identified for *S. cerevisiae* in the shift from aerobic to anaerobic conditions. If this was correct, oxygen sequestration by *M. pulcherrima* might also help avoiding acetic acid

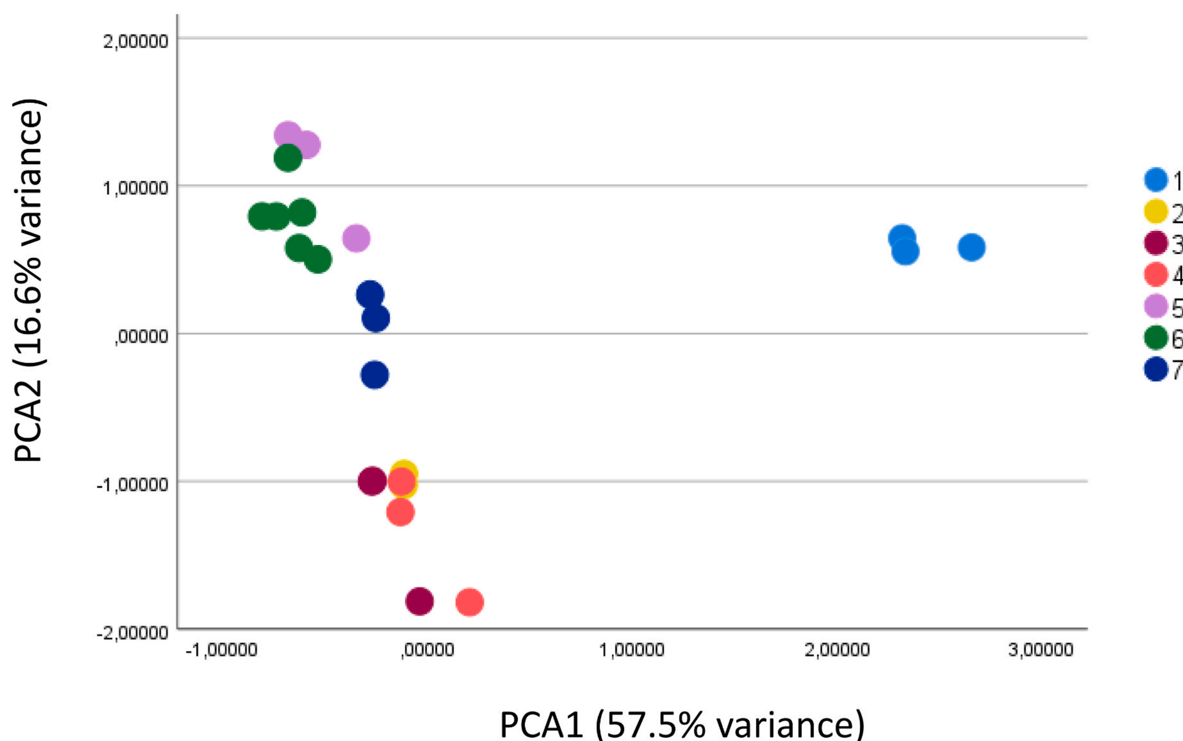

Fig. 4. PCA analysis of 54 volatile compounds identified at the end of the different fermentations. *M. pulcherrima* inoculated at 0.2 OD_{600} in all aerated conditions. 1, control fermentation (*S. cerevisiae* 0.20 OD_{600}, anaerobic). 2, *S. cerevisiae* 0.02 OD_{600}; air 10 VVH. 3, *S. cerevisiae* 0.05 OD_{600}; air 10 VVH. 4, *S. cerevisiae* 0.10 OD_{600}; air 10 VVH. 5, *S. cerevisiae* 0.10 OD_{600}; air 5 VVH (supplemented with nitrogen nutrients as described in the text). 6, (not supplemented). 7, *S. cerevisiae* 0.10 OD_{600}; air 2.5 VVH.

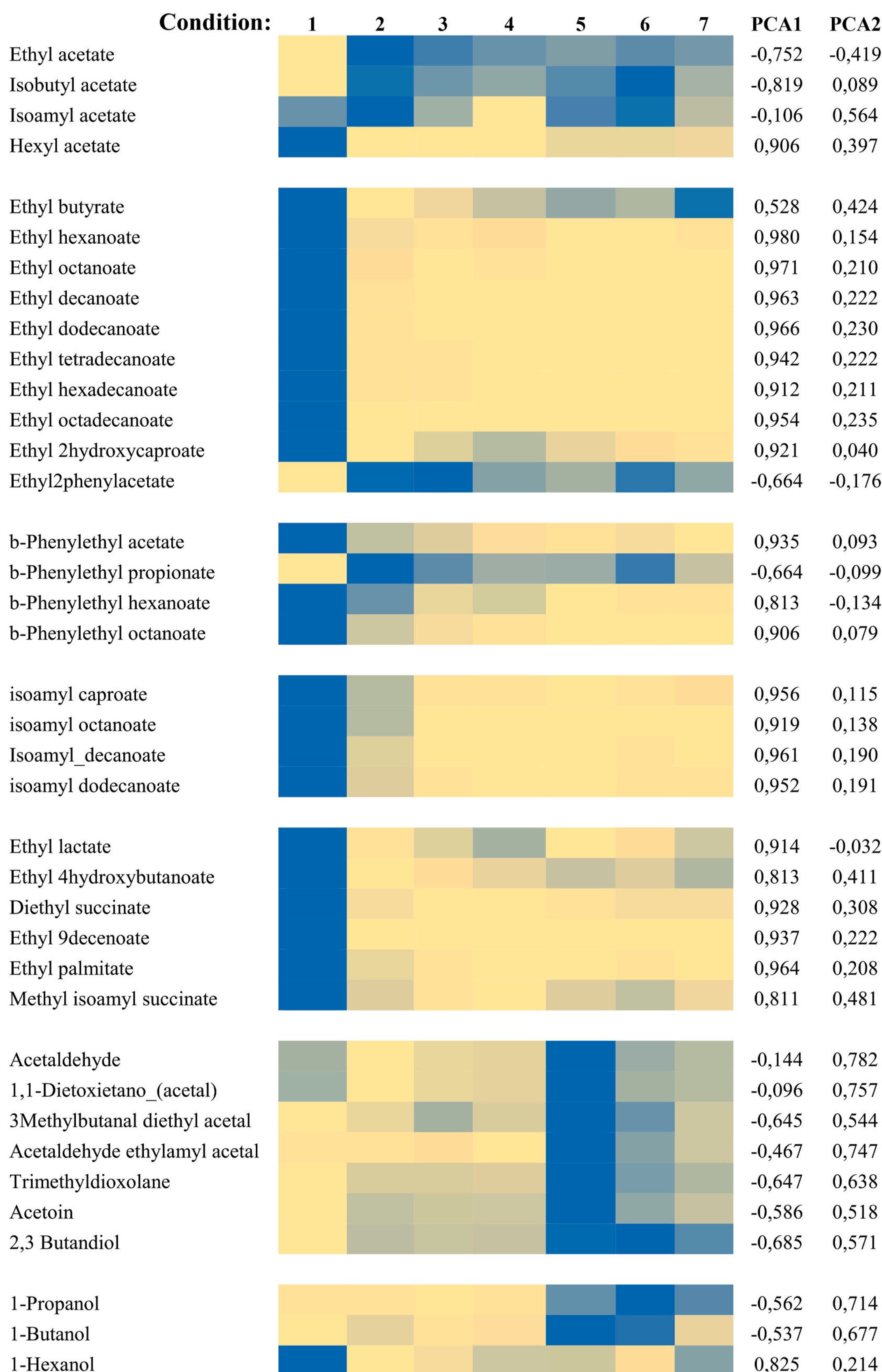

Fig. 5. Heatmap of 54 54 volatile compounds identified at the end of the different fermentations. Colours represent maximal and minimal values for each compound across the different fermentation conditions (as shown in the graphical legend). *M. pulcherrima* inoculated at 0.2 OD_{600} in all aerated conditions. 1, control fermentation (*S. cerevisiae* 0.20 OD_{600}, anaerobic). 2, *S. cerevisiae* 0.02 OD_{600}; air 10 VVH. 3, *S. cerevisiae* 0.05 OD_{600}; air 10 VVH. 4, *S. cerevisiae* 0.10 OD_{600}; air 10 VVH. 5, *S. cerevisiae* 0.10 OD_{600}; air 5 VVH (supplemented with nitrogen nutrients as described in the text). 6, *S. cerevisiae* 0.10 OD_{600}; air 5 VVH. 7, *S. cerevisiae* 0.10 OD_{600}; air 2.5 VVH. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

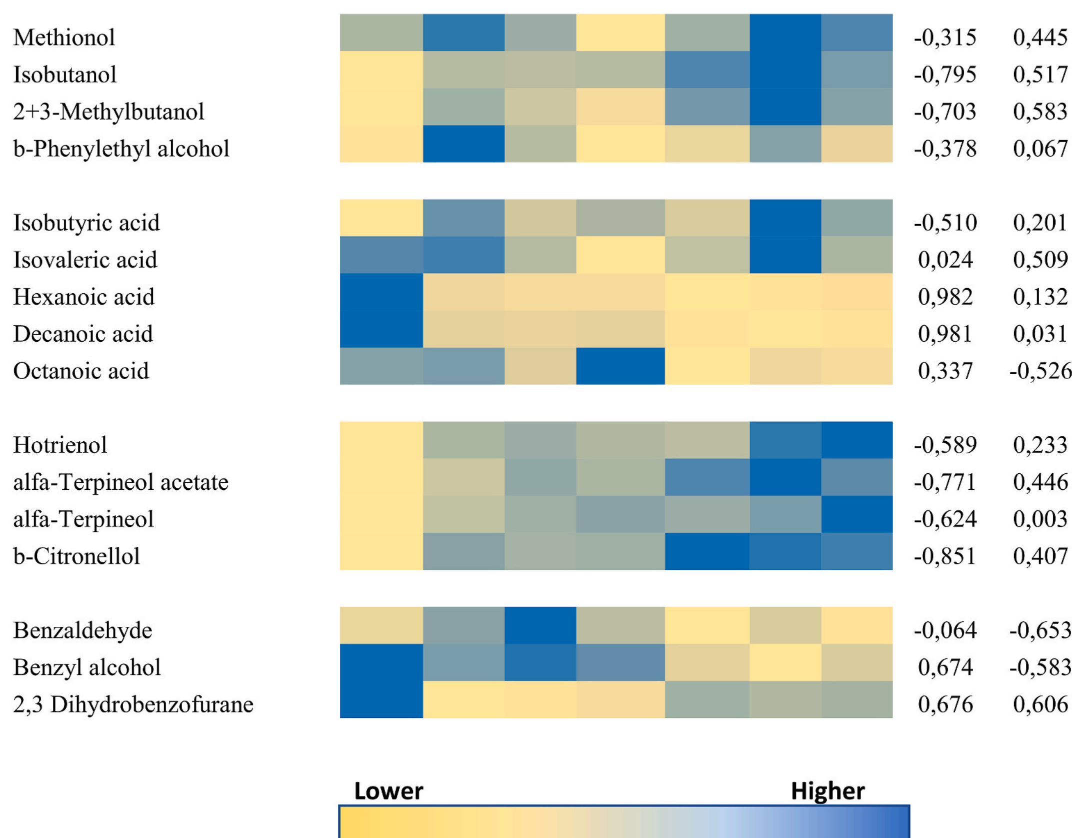

Fig. 5. (*continued*).

overproduction by standard *S. cerevisiae* wine yeast strains during the aerobic stage. To test this hypothesis, the commercial strain EC1118 was assayed in parallel with PR1018 under the final selected conditions. The results are shown in Figure S2. In both cases, the proportion of *M. pulcherrima* was around 80% of the viable cells in the bioreactor at the time of switching to anaerobic conditions. Sugar consumption kinetics, acetic acid yield and ethanol yield were almost indistinguishable using any of the two *S. cerevisiae* strains. These results confirm that the optimized conditions are also suitable for using other strains of *S. cerevisiae* for alcohol level reduction under aerobic conditions.

4. Discussion

This work builds on the idea of reducing alcohol yield during wine fermentation based on the respiration of sugars by yeasts (Gonzalez et al., 2013). The results presented in this manuscript allow to overcome the hurdle of automatic control of dissolved oxygen during wine fermentation (Tronchoni et al., 2022a). We combined one strain of *M. pulcherrima*, with a good respiration capacity; and the *S. cerevisiae* strain, PR1018, showing low acetic acid production under aeration conditions. On the other side, *M. pulcherrima* respiration capacity ensures low ethanol yield. The low impact of the sudden interruption of air supply on acetic acid production might be attributed to two factors: the lower population of *S. cerevisiae* in the moment of aeration arrest (around 20% of culturable cells), and the high oxygen affinity of *M. pulcherrima*, that might be buffering the impact of oxygen on *S. cerevisiae* metabolism. The system is robust, since it works with different strains of *S. cerevisiae*, air flow rates, or nitrogen supplementation.

The use of aeration and co-inoculation with *M. pulcherrima* starters resulted in a reduced production of several ethyl and acetate esters, and an increase in some terpene compounds, compared to fermentation under standard conditions. The increase in terpene compounds in fermentations inoculated with *M. pulcherrima* is in agreement with findings of other authors (Tufariello et al., 2021). The general reduction in ester content of the aerated samples might be related to changes in *S. cerevisiae* metabolism under aerobic conditions. Loss of some volatile compounds due to stripping during the aerobic stage does not seem to explain this result, as the controls were flushed with nitrogen at the maximum gas flow rate. Therefore, we found both potentially positive (terpens) and negative (loss of esters) contributions to the aroma profile of the lower alcohol wines. Nutrient addition, as tested in this work, showed a moderate global impact on the volatile profile, but there was a clear impact for acetaldehyde and metabolically related molecules. Sensory analysis of wines produced at a larger scale would be necessary to ascertain the acceptability and consumer preferences of these wines, and whether differences in volatile profiles between aerated fermentation conditions have any impact on this.

A weak trend was observed for aerated wines towards darker and more yellow colour, as compared to control wine. However, the colour parameters measured for these experimental wines fell globally in the range of commercial white wines and would be acceptable (Pérez-Gil et al., 2022; Gómez-Míguez et al., 2007). This is encouraging for obtaining quality wines by the proposed procedure.

One target during the adjustment process has been ensuring enough population of *S. cerevisiae* cells during the final steps of fermentation (anaerobic stage). To this end we resorted to increasing the inoculation levels of *S. cerevisiae* PR1018, and supplementing nutrients for them after aeration was stopped. Anyway, the impact of the later on fermentation kinetics was relatively low, and attention must be paid to the impact on the volatile profile, as described above. From the three stages of the adjusting strategy, the major improvement was attained by reducing air flow from 10 VVH to 5 or 2.5 VVH. This allowed the excess residual sugars observed at 10 VVH to be reduced to standard levels.

In summary, we have been able to propose a new fermentation procedure aimed at reducing ethanol yield during wine production. The

main advantages of this new procedure is that it do not depend on a single *S. cerevisiae* stain, and it avoids the need for specific devices for the automatic control of dissolved oxygen during fermentation. This would facilitate the transition from laboratory to pilot or industrial scale fermentation. It is also more robust than previous procedures, which also rely on the respiration of sugars by yeasts, in terms of the risk of uncontrolled production of acetic acid. Finally, it is worth noting that there are no indications of major oxidation problems associated with the aeration of the grape must during the early stages of alcoholic fermentation. Anyway, attention should be paid to the long-term impact of these practices on wine quality. This would require larger volume trials for tasting, as well as testing colour and aroma evolution during wine aging.

Declaration of competing interest

The authors of this manuscript 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.2023.104344.

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