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Technological affinity index for interaction between lactic acid bacteria and *Saccharomyces cerevisiae* strains to modulate the fruity and floreal aroma of Catarratto wines

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ABSTRACT

Microbial interactions during the fermentation process influence the sensory characteristics of wines. Alongside alcoholic fermentation, malolactic fermentation also plays a crucial role in determining the aromatic traits of wines. The time (t), rate (m) and volatile organic compounds (VOCs) of malolactic fermentation are linked to the interaction between yeasts and lactic acid bacteria. The study investigated the interactions between *Lactiplantibacillus plantarum* or *Oenococcus oeni* with *Saccharomyces cerevisiae* by using the Technological Affinity Index (TAIndex). The co-inoculation of *L. plantarum/S. cerevisiae* resulted in a higher TAIndex than the co-inoculation of *O. oeni/S. cerevisiae* conditions. A low TAIndex led to increased aromaticity of the wines. The time and rate of malolactic fermentation have a strong impact on the synthesis of VOCs with a high olfactory impact. Therefore, knowledge of the TAIndex could play a decisive role in improving winemaking planning to produce wines with higher fruit and floral perceptions.

1. Introduction

The aroma of wine is the most important factor that influences consumer acceptance (Morata, 2018).

The different aroma components in wine have different origins, including cultivars (Yang et al., 2021), agronomic techniques (Alem, Rigou, Schneider, Ojeda, & Torregrosa, 2019; Coletta et al., 2021), and geographic area.

However, the microbial components of the must plays role in the synthesis of volatile organic compounds, which are responsible for the aroma of wine (Liu et al., 2023).

Lactic acid bacteria (LAB) and yeasts produce a large number of secondary metabolites that create a sensory buffer in wine regulating the antagonisms and synergies of odor perceptions (Ferreira et al., 2016). LAB are microbial entities that most influence the organoleptic and technological framework of wine. For instance, the main technological function of LAB is the conversion of L-malic acid to L-lactic acid, which has deep implications on the gustatory, olfactory and microbial levels (Morata, 2021). The loss of a carboxylic group by L-malic acid leads to a biological deacidification of the wine (Lasik, 2013), which is associated with an improvement in taste. Thus, many of these sensory effects are the direct result of increasing pH and decreasing total acidity. In red

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wine, this is the case with the decrease in the reactivity of phenolic hydroxyls to salivary proteins. As a result of malolactic fermentation, wines became less tannic and have greater smoothness characters (Costello, Siebert, Solomon, & Bartowsky, 2013).

Malolactic fermentation has been reported to improve aromatic profiles and olfactory complexity of wines (Cappello, Zapparoli, Logrieco, & Bartowsky, 2017). However, the information in the literature is quite discordant. While some authors (Avedovech, Mcdaniel, Watson, & Sandine, 1992; Sauvageot & Vivier, 1997) reported a decrease in the olfactory intensity and fruity characters coming from the cultivar as a result of malolactic fermentation, others like Knoll et al. (2012) and Malherbe, Tredoux, Nieuwoudt, and du Toit (2012) reported an increase in the fruity components of wines due to the production of ethyl esters and acetates. The conflicting information in the bibliography poses uncertainty for technicians who want to adopt malolactic fermentation as a biotechnological means of maintaining or improving the fruit and floral perceptions of wines while ensuring microbial stability.

The main interfering agent in the fruity and floral perceptions of wines is 2,3-butanedione, which is a by-product of malolactic fermentation. Bartowsky and Henschke (2004) pointed out several factors that lead to the production of 2,3-butanedione, including sulphur dioxide, dissolved oxygen, and temperature of malolactic fermentation performance. Furthermore, Olguín, Bordons, and Reguant (2009) reported that gene expression of the citric acid pathway, as well as 2,3-butanedione production, is stimulated by ethanol in the medium.

Therefore, the different strategies of LAB inoculation, sequential (at the end of alcoholic fermentation, in the presence of ethanol) or co-inoculation (24 h after yeast starter inoculation, in the absence of ethanol), may play a crucial role in the productive suppression of 2,3-butanedione. In fact, many authors (Lasik-Kurdyś, Majcher, & Nowak, 2018; Plavša, Jagatić Korenika, Lukić, Bubola, & Jeromel, 2021; Tristezza et al., 2016) reported that the technique of simultaneous inoculation of LAB at 24 h from the yeast strain has positive effects on fruity perception by shielding them from missed buttery hints resulting from 2,3-butanedione.

The effects of LAB-yeast co-inoculation on the aroma profiles of wines are well documented. During the fermentation phase, the relationships established between LAB and *S. cerevisiae* strains allows the 2,3-butanedione produced by the LAB to be reduced to 2,3-butanediol and acetoin by reductive metabolism of the yeast strains.

Although the interactive metabolic aspect between LAB and *S. cerevisiae* is sufficient to explain the validity of co-inoculation in the technological objective of the intensity of the fruity and floral perceptions of wines, limited information is available in the literature on the metabolic effects of microbial consociation between LAB and *S. cerevisiae* strains in the kinetics of different aromatic classes produced through the co-fermentation of LAB and *S. cerevisiae* during winemaking.

An additional cognitive requirement is represented by the effects of LAB-*S. cerevisiae* consociation on the balance between volatile organic compounds (VOCs) concentration and olfactory threshold, thus determining odor perception in wines.

The study introduces the technological affinity index (TAIndex), which evaluates interactions between LAB and *S. cerevisiae* during wine fermentation. Specifically, TAIndex considers factors like malic acid degradation rates, malolactic fermentation time, and VOCs. Thus, winemakers can easily collect relevant data in the cellar using untrained staff and TAIndex allows the wine industry to quickly adjust wine flavor profiles based on consumer preferences.

In particular, in the present research, three commercial LAB strains (two *Oenococcus oeni* and one *Lactiplantibacillus plantarum* strains) were used in different co-inoculation with two *S. cerevisiae* strains. The research activity aimed to evaluate the effects of the LAB-*S. cerevisiae* consociation on: (*i*) kinetics of malolactic fermentation in terms of duration and yield; (*ii*), improvement of high olfactory impact VOC concentration; (iii) sensory characterization of aroma of Catarratto after malolactic fermentation; and (iv) TAIndex calculation.

2. Material and methods

2.1. Experimental design and sampling

The experimentation set, as shown in Fig. 1, consisted of co-inoculation of different LAB and *S. cerevisiae* strain during winemaking of Catarratto white grape. The first experimental set comprised the CO1, CO3 and CO5 trials, which were inoculated with the *S. cerevisiae* NF213 strain. After 24 h, the LAB strains were added: MLB6 (*O. oeni*) in the CO1 trial; MLA4 (*O. oeni*) in the CO3 trial; MLPK45H (*L. plantarum*) in the CO5 trial. The control CONT A1 trial was inoculated only with *S. cerevisiae* NF213 strain.

The second experimental set comprised the CO6, CO8 and CO10 trials, which were inoculated with the *S. cerevisiae* QA23™ strain. After 24 h, the LAB strains were added: MLB6 (*O. oeni*) in the CO6 trial; MLA4 (*O. oeni*) in the CO8 trial; MLPK45H (*L. plantarum*) in the CO10 trial. The control CONT A2 was inoculated only with *S. cerevisiae* QA23™ strain.

The QA23™, MLB6™, MLA4™ and MLPK45H™ strains were used as described by the manufacturer (Lallemand Inc. Italia, Castel D'Azzano, Verona, Italy). The strain NF213, which belongs to the strain collection of the Department of Agriculture, University of Palermo, was used at a dose of 20 g/hL. Before yeast inoculation, total nitrogen levels were adjusted as reported by Kemsawasd, Viana, Ardö, and Arneborg (2015) using an organic nutrient. Additionally, in the control trials CONT A1 and CONT A2, 10 g/hL of lysozyme (Esseco s.r.l. San Martino, Novara, Italy) were added before inoculation of *S. cerevisiae* to prevent the development of indigenous LAB.

Samples were taken for analysis from the clarified must before, after *S. cerevisiae* yeast inoculation, after LAB inoculation and at the end of alcoholic fermentation (days 1, 2, 3 and 11).

The samples were collected in triplicate and transported under refrigerated conditions, placed in a climate chamber at 4 °C, and analytically processed within 24 h. Samples for VOCs analysis were collected at the end of sugar or malic acid depletion.

For brevity of the manuscript and ease of reference of the data by the reader, only data collected at significant sampling points have been reported in the manuscript.

2.2. Winemaking process

The grapes were destemmed and crushed, and 4 g/q of metabisulfite (Laffort, France) was added. Two g/hL of pectolytic enzyme LALLZYME HC™ (Lallemand Inc. Italy, Castel D'Azzano, Verona, Italy) was added to the must during the static settling stage. In addition, a temperature of 10 °C was maintained for 24 h to facilitate the catalytic action of the pectins. Then, the must was aliquoted into 24 (1 hL each) steel tanks to constitute eight different trials, each of which was conducted in triplicates. The trials were inoculated as described in the experimental plan when they reached a temperature of 15 °C. Fermentation took place inside a climatic cell in such a way as to allow the constant and uniform maintenance of 20 °C for the fermentation activities to take place. The addition of potassium metabisulfite (Esseco s.r.l., San Martino, Novara, Italy) was carried out one week after the complete degradation of malic acid. Therefore, microbial inactivity was ensured by 0.8 mg/L molecular SO_2 (Tomasset, 1978).

2.3. Microbial counts and identification of yeasts and LAB strains

During alcoholic fermentation, plate counts were performed to estimate the levels of total yeasts (Pallmann et al., 2001), which were differentiated into *Saccharomyces* and non-*Saccharomyces* colonies as described by Varela (2016). LAB population was monitored according to the procedure described by Tristezza et al. (2016). To ensure the most accurate CFU/mL data during both fermentation and post-fermentation

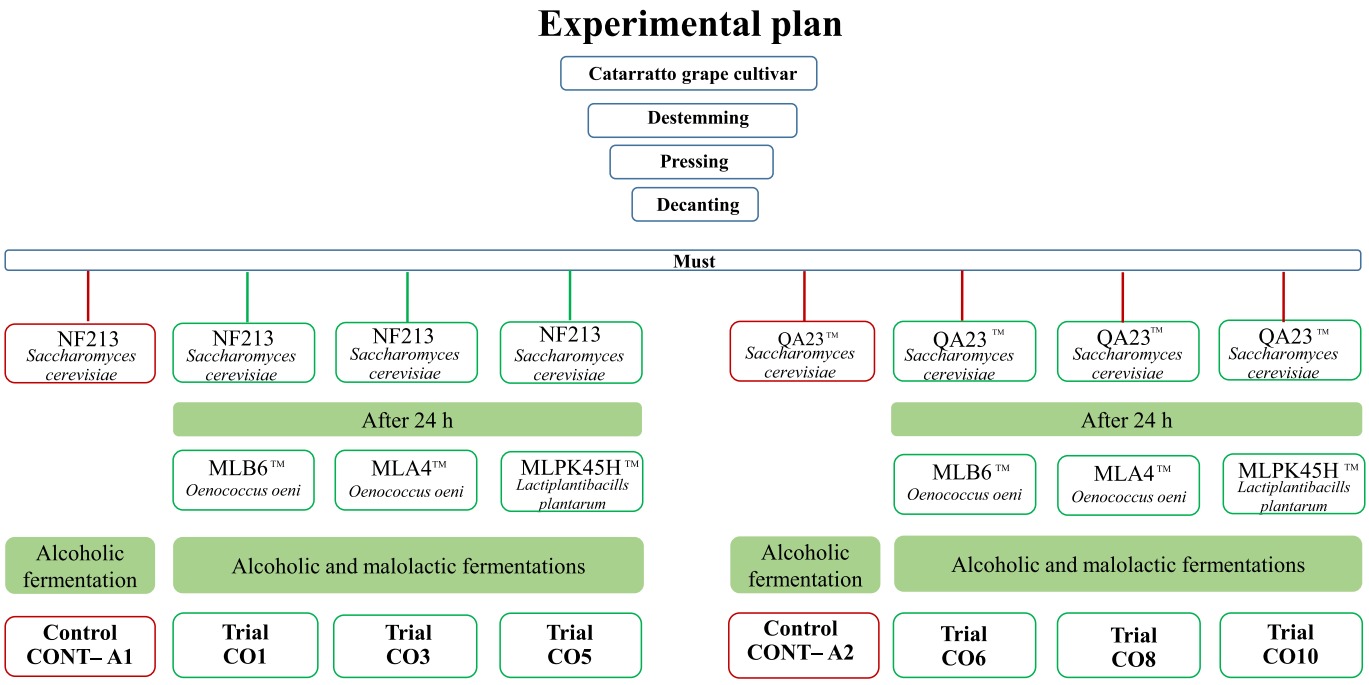


Fig. 1. Experimental plan.

phases, the mass was homogenized under sterile conditions.

Yeast isolates were purified and phenotypically grouped as reported by Alfonzo et al. (2020). The selection and molecular identification of yeast isolates at species level (Francesca et al., 2024) and genetic strain characterization (Alfonzo et al., 2021) were also performed. The isolation and genetic characterization of LAB strains were conducted as reported by Solieri, Genova, De Paola, & Giudici, 2010. By using DNA fingerprinting, the study allowed us to assess the prevalence of all the introduced strains in the microbial populations.

2.4. Physico-chemical analysis

The samples were centrifuged at 9000 rpm at 4 °C and then filtered through a polyethersulfone membrane with a pore size of 0.20 μm (VWR®). CO_2 stripping was performed using a vacuum pump to minimize errors during instrumental reading. The pH, total acidity, and ethanol were measured using a FOSS-WineScanTM Flex system (FOSS, Hillerød, Denmark), according to the procedure described in OIV Res. Oeno 390/10 All.2. The values of L-malic, L-lactic, acetic acids, together with reducing sugars, glucose and fructose, and glycerol were measured by means of an iCubio iMagic M9 enzymatic analyser (Shenzhen iCubio Biomedical Technology Co. Ltd. Shenzhen, China), as reported by Matraxia et al. (2021).

2.5. Analysis of VOCs in wine samples

2.5.1. Standard solutions

Standards for each compound were purchased individually from Sigma-Aldrich (82,024 Taufkirchen, Germany). 2,3-butanediol was used as standard for the alcohol fraction, acetoin as standard for the carboxyl-function fraction and ethyl lactate as standard for the ester fraction. In addition, n-alkane standards (C8 to C40) were purchased from Aldrich Chemical Co. (St. Louis, Mo., USA). Standard solutions of each compound were prepared at five different concentrations: 2,3-butanediol, 53.25 mg/L, 112.50 mg/L, 225.00 mg/L, 262.00 mg/L, 450. 00 mg/L; acetoin: 24.70 mg/L, 45.70 mg/L, 64.70 mg/L, 115.60 mg/L, 173.30 mg/L, 289.80 mg/L; ethyl lactate, 79.00 mg/L, 134.00 mg/L, 224.00 mg/L, 326.00 mg/L, 477.00 mg/L.

2.5.2. Extraction, identification and quantification of VOCs by GC–MS

To determine the volatile compound composition, wine samples (10 mL) from all trials were mixed with MS SupraSolv® dichloromethane (5 mL) in a 50-mL conical flask. The mixture was stirred at room temperature for 30 min and then centrifuged at 4000 rpm for 10 min using a Low Speed Centrifuge (ScanSpeed 416) with Swing Rotor (LaboGene ApS Industrivej 6–8, Vassingerød, DK- 3540 Lynge, Denmark). The aqueous phase was removed, and anhydrous sodium sulphate (1 g) was added before centrifugation at 4000 rpm for 5 min. The dichloromethane layer was removed and dried under N_2 gas to 0.3 mL.

Gas chromatographic analyses were performed with Agilent 7000C GC system, fitted with a fused silica Agilent DB-5MS capillary column (30 m × 0.25 mm i.d.; 0.25 μm film thickness), coupled to an Agilent triple quadrupole Mass Selective Detector MSD 5973; ionization voltage 70 eV; electron multiplier energy 2000 V; transfer line temperature, 295 °C. Solvent Delay: 3.5 min. Helium was the carrier gas (1 mL/min).

The temperature was initially maintained at 40 °C for 1 min. Then it was gradually increased to 250 °C at a rate of 3 °C/min for 30 min and finally maintained at 250 °C at 10 °C/min. One μL of sample was injected at 250 °C automatically and in the splitless mode; transfer line temperature, 295 °C. The individual peaks were analysed using the GC MS Solution package, Version 2.72. Identification of compounds was carried out using Adams, NIST 11, Wiley 9 and FFNSC 2 mass spectral database. These identifications were also confirmed by other published mass spectra. Quantification was carried out using the three calibration lines. For compounds belonging to other classes than the standards, similarity was used for quantification. A dilution factor was used for the reported data.

To determine which VOCs were actively contributing to the wine's aroma profile, the detected concentrations were transformed into odor activity units (OAV) using the method described by Butkhup et al. (2011). The method reported by Butkhup et al. (2011) was also used to calculate the aroma series per individual wine (fruity, floral, fatty, solvent and sulfurous). The sum of the individual odourant active values (OAV) determined for each volatile organic compound per trial defined the olfactory intensity of the test wine. The VOCs with OAV > 0.1 were organized into tables for convenient reference and analysis (Peng, Wen, Tao, & Lan, 2013).

2.6. Sensory analysis

The sensory profiles of the wines were evaluated by submitting the different theses to a trained panel of judges, as described by Jackson (2022). The sensory evaluation was conducted by a 15-member panel of judges, consisting of eight women and seven men, with ages ranging from 27 to 45 years. The panel was preliminarily submitted for organoleptic performance evaluation. The organoleptic profiles of the wines were elaborated in triplicate by three different wine tasting committees for both test batteries. Quantification of the different descriptors detected was performed through a 9-point intensity scale, as described by Alfonzo et al. (2020). Sensory analysis was performed as reported by Naselli et al. (2023).

2.7. Determination of technological affinity index

The technological affinity index (TAIndex) of LAB-*S. cerevisiae* has been proposed by this study and estimated using linear regression with the following formula:

$$\text{TAIndex} = \{[\text{Vi}/(-\text{m})]/\text{t}_c\}, \text{expressed in: } \text{t}^{-1};$$

where:

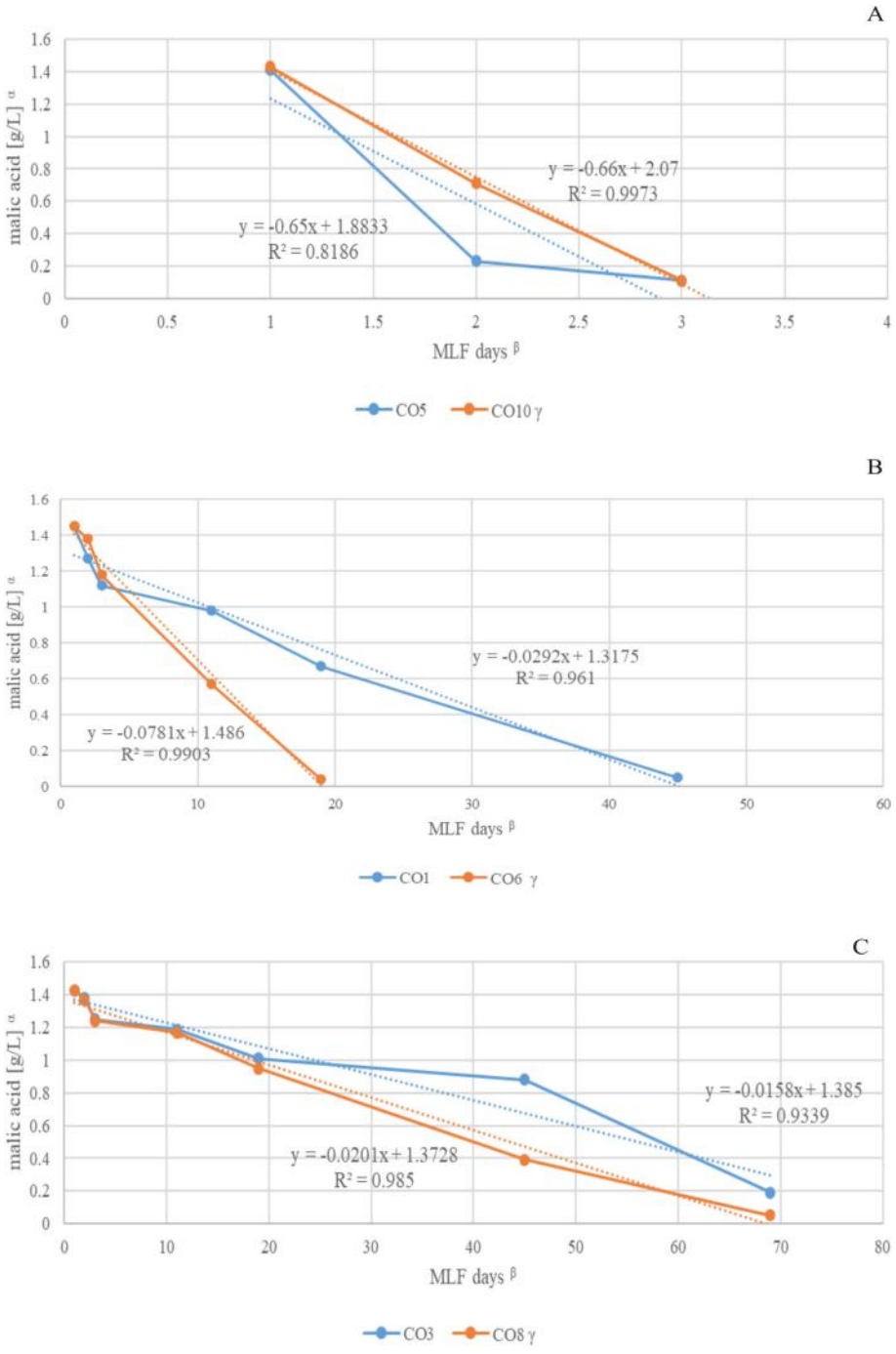


Fig. 2. Trend to degradation of malic acid as a function of time under constant temperature conditions: (A) *Lactiplantibacillus plantarum* MLPK45H with NF213 and QA23 strains *S. cerevisiae*; (B) *Oenococcus oeni* MLB6 with NF213 and QA23 strains *S. cerevisiae*; (C) *O. oeni* MLA4 with NF213 and QA23 strains *S. cerevisiae*. Simbols: $^{\alpha}$ malic acid [g/L]; $^{\beta}$ malolactic fermentation days; $^{\gamma}$ Trials.

– "Vi": is the instantaneous speed of the reaction for the conversion of malic acid to lactic acid (malo-lactic fermentation), expressed in: [malic acid] g/L × day $^{-1}_{MLF}$;
– "m": is the degradation rate of malic acid in the unit of time, expressed in: [malic acid] g/L × day $^{-1}_{MLF}$;
– "t_c" is the time of the reaction for the conversion of malic acid to lactic acid (malo-lactic fermentation) corrected by the graphical method modified of Di Stefano and Cravero (1989).

The parameter V_i was calculated using the following formula:

$$Vi = \frac{\Delta[malic\ acid](g/L)}{\Delta[tc]}$$

$$Vi = \frac{[\text{malic acid}]\text{inoculum LAB}(g/L) - [\text{malic acid}]\text{end malo\_lactic fermentation}(g/L)}{\text{"tc" end malo\_lactic fermentation LAB inoculum} - \text{"t" inoculum LAB}}$$

The parameter "m" represents the angular coefficient of the straight line obtained from the linear regression equation of the malic acid trend of malolactic fermentation versus time (Fig. 2a, b, c).

The parameter "t_c" is calculated graphically by measuring the distance between the two points on the malic acid concentration trend line as a function of time using the following formula:

$$tc = d\,(AB) = \sqrt{(X_2 - X_1)^2 + (Y_2 - Y_1)^2}$$

where:

– X_1, coordinate point referring to the day malolactic fermentation started (LAB inoculum).
– X_2, coordinate point referring to the day of the end of malolactic fermentation.
– Y_1, coordinate point referring to the concentration of malic acid detected at the end of malolactic fermentation.
– Y_2, coordinate point referring to the malic acid concentration detected at the start of malolactic fermentation (LAB inoculum).

2.8. Statistical analysis

The ANOVA test was applied to determine the significance of the differences between the technological, microbial, VOC, and sensory values of the different tests. In addition, the Tukey's test was used to compare the different data, and values of $P < 0.05$ determined significance. The relationships between VOCs, sensory attributes and biotechnological associations were determined by means of agglomerative hierarchical clustering (AHC) and principal component analysis (PCA) (Naselli et al., 2023).

3. Results and discussion

3.1. Microbial growth dynamics and strain monitoring

The trends of yeast monitoring during the course of fermentation are shown in Fig. S1a, b, c, d. At the beginning of the trial, the detection of *S. cerevisiae* and non-*Saccharomyces* populations in Catarratto must were 4.11 (Fig. S1a, b) and 3.67 Log CFU/mL (Fig. S1c, d), respectively. After yeast inoculation, the cell density of the first experimental set using *S. cerevisiae* NF213 strain ranged from 7.20 and 7.47 Log CFU/mL between trials. The levels of *S. cerevisiae* detected after adding QA23 strain in the second test set were comparable, with a degree of variability between trials of 7.21 and 7.51 Log CFU/mL. The population of non-*Saccharomyces* decreased correspondingly with the increase in *S. cerevisiae*. These decreases were at values of <2.0 Log CFU/mL on the second day of fermentation. The monitoring of non-*Saccharomyces* was consistent with the findings of Wang, Mas, and Esteve-Zarzoso (2016).

Before inoculation of the *S. cerevisiae* strains, LAB values of 3.1 Log CFU/mL were detected (Fig. S1e, f). After inoculation of the LAB, which occurred 24 h after the addition of the yeast strain, the bacterial populations in the CO1 and CO3 and CO6 and CO8 trials (inoculated with *O. oeni* strains) were between 5.41 and 5.51 Log CFU/mL. These values were similar to those reported by Celik, Cabaroglu, and Krieger-Weber (2019). In the trials involving the addition of the 24-h MLPK45H strain of *S. cerevisiae*, a cell density of 7.71 Log CFU/mL was found. The highest levels of LAB were 5.88–5.93 Log CFU/mL for *O. oeni* and 8.0 Log CFU/mL for L. *plantarum*, respectively. At the end of alcoholic fermentation, which occurred for both experimental sets on the 11th fermentation day, the yeasts were at a cell density of 7.39 Log CFU/mL. At the 19th fermentative day, the CO5 and CO10 trials resulted in a lower concentration in LAB than the CO1, CO3 and CO6, and CO8 trials (Fig. S1e, f). This phenomenon is imputable to the depletion of malic acid and to the addition of potassium metabisulphite. At the 19th and 45th fermentation days, corresponding to the end of malolactic fermentation for CO1 and CO6 trials, the levels of LAB were 5.21 and 5.00 Log CFU/mL, respectively. On the 69th day fermentation, the CO3 and CO8 trials completed malic acid degradation and LAB reached densities of 4.43 and 3.76 Log CFU/mL, respectively.

3.2. Kinetics of the main oenological parameters

Table S1 shows the technological parameters of the starting must.

The CO5 and CO10 trials exhibited the most rapid depletion of malic acid, which occurred in two days (Table S2). The consociations with the two different strains of *S. cerevisiae* (QA23 and NF213) did not affect the degradation of malic acid, except for timing; Fig. 2a).

The higher speed of malic acid depletion could be attributed to the TAIndex found when using the LAB strain MLPK45H with the two *S. cerevisiae* strains NF213 and QA23 (Table 1) respectively.

TAIndex values ranging from 0.348 to 0.351 (Table 1) microbiologically stabilized the must before the end of alcoholic fermentation

Table 1
Technological affinity index between LAB and *S. cerevisiae* and validity of the method.

Trials [α]	Microbic consociations [β]	TAIndex [γ]	m [δ]	R^2 [ε]	MLF days [ζ]
CO1	MLB6/NF213	0.009	−0.0292	0.9610	44
CO3	MLA4/NF213	0.013	−0.0201	0.9903	68
CO5	MLPK45H/NF213	0.351	−0.6500	0.9339	2
CO6	MLB6/QA23	0.148	−0.0781	0.9850	18
CO8	MLA4/QA23	0.019	−0.0158	0.9339	68
CO10	MLPK45H/QA23	0.348	−0.6600	0.8186	2

[α]Trials.
[β]Microbic consociations: LAB strains–*S. cerevisiae* strains.
[γ]TAIndex. Technological Affinity Index LAB–*S. cerevisiae*.
[δ]m, degradation rate of malic acid [g/L] in the unit of time (day) (angular coefficient, derived from the equation of the straight lines shown in Fig. 2.
[ε]R^2, coefficient of determination (derived from the equation of the straight lines shown in Fig. 2).
[ζ]MLF days, days of malolactic fermentation.

that occurred in 11 days (Table S3).

The kinetics of malic acid degradation were influenced by the association of LAB strain MLB6 with the two different yeasts, QA23 and NF213. In fact, the CO6 trial, (MLB6-QA23) terminated malic acid degradation within day 19 of winemaking (Table S4), compared to the CO1 trial, (MLB6-NF213), that terminated at day 45 (Fig. 2b; Table S5). The difference in the timing of malolactic fermentation of the two different consociations, MLB6-QA23 and MLB6-NF213, leads to the hypothesis of a different requirement of the two yeasts to assimilate metal cofactors, such as Mn^{2+} or Mg^{2+} elements, which are essential to trigger the endogenous process of enzymatic decarboxylation in *O. oeni* (Lonvaud-Funel, 2022). On the other hand, *S. cerevisiae* may respond differently to counteract the co-presence of LAB in the medium through the production of volatile catabolites (Alexandre, Costello, Remize, Guzzo, & Guilloux-Benatier, 2004). In contrast, CO3 and CO8 trials (MLA4-NF213; MLA4-QA23) required 69 days to complete malolactic fermentation. (Fig. 2c; Table S6).

Table S6 shows the best technological performances were recorded by the trials involving the use of LAB strain MLPK45H, specifically the CO5 and CO10 trials. In spite of the consumption of malic acid, the total acidity values were the highest in each of the trial batteries compared to the trials involving the use of *O. oeni*; 4.98 g/L H_2T versus 4.61 and 4.64 g/L H_2T (for the CO5 trials versus CO1 and CO3, respectively); 4.95 g/L H_2T versus 4.59 and 4.58 g/L H_2T (for the CO10 trials versus CO6 and CO8, respectively). The higher lactic acid yield of *L. plantarum* strains and their low production of acetic acid contributed to these values.

This behaviour is the result of homofermentative metabolism of the MLPK45H strain of *L. plantarum*, as described by Krieger-Weber, Heras, and Suarez (2020).

However, the consumption of citric acid by the heterofermentative *O. oeni* strains, MLB6 and MLA4 (trials CO3, CO5 and CO6, CO8) occurred significantly in comparison to both the control trials, CONT A1 and CONT A2, and the trials involving the inoculation of the *L. plantarum* strain, CO5 and CO10. The acetic acid values, although different between the trials, remained below the technological levels compatible with high quality wines.

3.3. Volatile organic compound composition

3.3.1. Higher alcohols

Alcohols were identified as the most abundant aromatic fraction in the wines object of investigation (Table 2). The trials that predicted LAB-*S. cerevisiae* microbial consociations showed lower amounts of higher alcohols than the control trials (CONT A1 and CONT A2), with the exception of the CO3 trial (Table 2). These results confirm the findings of Knoll et al. (2012). The CO3 trial stood out by registering a higher total amount of alcohols than the CONT A1 control (Table 2). The production of three different enantiomers of 2,3-butanediol in comparison to the CONT A1 control trial contributed to this result (Table 2). This peculiarity was exclusively recorded in the MLA4-NF213 consociation (CO3 trial), indicating that this microbial combination has a higher conversion rate of 2,3-butanediol than the corresponding CO8 trial (MLA4-QA23) and all other microbial interaction trials. Furthermore, the microbial consociation MLB6-NF213 (CO1 trial) exhibited a peculiarity in its favor, recording a significant production of 3-ethoxy-1-propanol in comparison to the CONT A1 trial (Table 2). This peculiarity was attributed to the *S. cerevisiae* yeast strain used in the microbial combination, as it was not produced in the corresponding CO6 trial (MLB6-QA23; Table 2).

The differences in higher alcohols detected between the trials could be due to trophic competitions that occurred between LAB and yeasts during fermentation (Maarman, 2014). The antagonisms between the different microorganisms are presumed to have occurred for the amino acid compounds, in particular, leucine, phenylalanine, tyrosine and methionine (Maarman, 2014). The significant differences between trials in the formation of 3-methyl-1-butanol, hydroxyethylbenzene, 4-(2-hydroxyethyl)-phenol, and 3-methylsulfanyl-1-propanol through the Ehrlich pathway in yeast could support this inference (Ribereau-Gayon, 2018). Therefore, the varying concentrations of these volatile organic compounds could be a result of a specific physiological nutritional requirement by the LAB strains (Ribereau-Gayon, 2018).

3.3.2. Esters

3.3.2.1. Acetate esters. Trial showed significant differences in terms of acetate ester amount and composition (Table S7, S8). The total acetate ester values were higher in CO8 trials (3.77 mg/L) than the CONT A2 control (3.34 mg/L). The lowest values were found in the CO6 and CO10 trials (2.68 and 2.19 mg/L, respectively). 3-methyl-1-butyl acetate, phenylethyl acetate, and 2-methyl-1-butyl acetate were the VOCs that contributed the most to the increase in acetate ester content in the CO8 trial compared to the CONT A2 control (Table 2). These increases observed under experimental pH conditions contrast with the findings of Costello et al., 2013. This phenomenon suggests that wine limiting conditions (Costello et al., 2013) or those created by the coexistence of LAB and yeasts during fermentation are crucial in activating some biosynthetic processes (Liu et al., 2017). The hypothesis is supported by the varying recorded production of phenylethyl acetate in the CO8 trials and 2-methyl-1-butylacetate in the CO6, CO8 and CO10 trials compared to the control, CONT A2. In such cases, the esterification process due to LAB activity can decrease hydroxybutylbenzene concentration, thereby reducing the potential toxicity against bacterial cells (Table 1, Corre, Lucchini, Mercier, & Cremieux, 1990; Romano, Ciani, & Cocolin, 2022). Indeed, Romano et al. (2022) have reported that hydroxybutylbenzene produced by yeasts is well known to inhibit the transport of sugars and amino acids within the bacterial cell.

MLPK45H strain co-inoculated with different strains of *S. cerevisiae* (CO5 and CO10 trials) resulted in the lowest concentration of acetate esters compared to the other consociated trials and the related CONT A1 and CONT A2 controls (Table S7 and S8).

3.3.2.2. Ethyl esters. Fig. 3a shows that the olfactively active esters depend on the rate of malolactic fermentation “m” as well as the rate of malic acid degradation in the unit of time. Especially ethyl octanoate and ethyl decanoate, seem to be favored by a range of the malic acid degradation rate, “m”, between −0.0201 and − 0.0292 (Table 1).

The timing of malolactic fermentation significantly impacts the aromatic profile of wine. Although oenological conditions are standardized, different strains or species of LAB inoculated into the same grape must can lead to varying malolactic fermentation durations. To assess the effect of the malolactic process on volatile organic compound composition, it is crucial to sample wines at the end of this fermentation. Considering the time factor, specifically the interval between the end of malolactic fermentation in two experimental trials, helps identify its impact on volatile organic compounds. Ethyl esters from organic acids do not directly interact with LAB-*S. cerevisiae*, but they result from chemical esterification occurring during different time periods (Ancín-Azpilicueta, González-Marco, & Jiménez-Moreno, 2009).

The primary influence of the biotechnological component lies in the synthesis of specific organic acids, including succinic acid, which contributes to the production of methyl succinate and diethyl succinate. These esters, along with diethyl malate, significantly elevate the total ester content of wine due to chemical esterification processes (Ancín-Azpilicueta et al., 2009; Shinohara, Shimizu, & Shimazu, 1979) catalyzed by factors such as ethanol availability, acid pKa, hydrogenionic activity, and, especially, temperature (Tomasset, 1978). However, in this study, the relatively high odor thresholds of diethyl succinate and diethyl malate esters (200 mg/L and 760 mg/L, respectively) (García-Carpintero, Sánchez-Palomo, Gallego, & González-Viñas, 2011) indicated that they had only a minor influence on the wine’s aroma.

Table 2
Volatile organic compounds detected in Catarratto experimental wines (all values in mg/L).

KI [α]	KI [β]	Compounds [γ]	CONT A1 [δ]	CO 1 [δ]	CO 3 [δ]	CO 5 [δ]	S.S. [ε]
		∑ Alcohols	**121.74 ± 4.53 [a]**	**111.16 ± 4.14 [b]**	**128.09 ± 4.76 [a]**	**98.2 ± 3.65 [c]**	***
758	759	3-methyl-1-butanol	55.56 ± 1.30 [a]	44.37 ± 1.04 [c]	50.28 ± 1.18 [b]	42.55 ± 1.00 [c]	***
765	765	1,2,-propanediol	1.24 ± 0.04 [a]	1.10 ± 0.03 [b]	1.07 ± 0.03 [b]	0.78 ± 0.02 [c]	***
809	809	2.3-butanediol [ζ]	20.22 ± 0.63 [c]	22.90 ± 0.71 [b]	27.66 ± 0.86 [a]	16.68 ± 0.52 [d]	***
816	816	2.3-butanediol [η]	5.34 ± 0.08 [b]	5.61 ± 0.08 [b]	7.80 ± 0.11[a]	4.08 ± 0.06[c]	***
824	824	2.3-butanediol [θ]	3.44 ± 0.09 [c]	9.86 ± 0.25 [b]	10.42 ± 0.26 [b]	11.00 ± 0.28 [a]	***
848	848	3-ethoxy-1-propanol	0.28 ± 0.01[c]	0.65 ± 0.01[a]	0.29 ± 0.01[b]	0.29 ± 0.01[b]	***
878	878	1-hexanol	0.52 ± 0.01 [b]	0.49 ± 0.01 [c]	0.56 ± 0.01 [a]	0.39 ± 0.01 [d]	***
1038	1039	Phenyl methanol	0.20 ± 0.01[a]	0.18 ± 0.01[b]	0.19 ± 0.01 [ab]	0.00 ± 0.00 [c]	***
1089	1088	1,2,3-propanetriol	1.43 ± 0.06 [a]	0.00 ± 0.00 [c]	0.96 ± 0.04 [b]	1.44 ± 0.06 [a]	***
1116	1117	Hydroxyethylbenzene	27.04 ± 0.90 [a]	21.79 ± 0.72 [b]	26.87 ± 0.89 [a]	20.59 ± 0.68 [b]	***
1428	1428	4-(2-hydroxyethyl)-phenol	1.79 ± 0.06 [a]	0.85 ± 0.03 [c]	1.59 ± 0.05 [b]	0.00 ± 0.00 [d]	***
1502	1503	2,4-di-tert-butylphenol	4.68 ± 0.10 [a]	3.36 ± 0.07 [b]	0.4 ± 0.01 [c]	0.40 ± 0.01 [c]	***
		∑ Aldehydes	**2.03 ± 0.07 [a]**	**0.93 ± 0.03 [c]**	**0.26 ± 0.01 [d]**	**1.59 ± 0.04 [b]**	***
1105	1105	Nonanal	1.01 ± 0.03 [a]	0.60 ± 0.02 [b]	0.08 ± 0.01[c]	0.00 ± 0.00 [d]	***
1211	1211	3.4-dimethylbenzaldehyde	0.00 ± 0.00 [b]	0.00 ± 0.00 [b]	0.00 ± 0.00 [b]	0.49 ± 0.0 [a]	***
1271	–	4-propyl benzaldehyde	1.02 ± 0.04 [a]	0.33 ± 0.0 [b]	0.18 ± 0.0 [c]	0.00 ± 0.00 [d]	***
1811	1812	Hexadecanal	0.00 ± 0.00 [b]	0.00 ± 0.00 [b]	0.00 ± 0.00 [b]	0.33 ± 0.01[a]	***
–	2020	Octadecanal	0.00 ± 0.00 [b]	0.00 ± 0.00 [b]	0.00 ± 0.00 [b]	0.77 ± 0.02 [a]	***
		∑ Carboxylic acids	**8.05 ± 0.27 [bc]**	**7.92 ± 0.24 [c]**	**8.89 ± 0.24 [b]**	**11.08 ± 0.41 [a]**	***
914	916	4-hydroxybutanoic acid	0.24 ± 0.01[c]	0.38 ± 0.01[b]	0.66 ± 0.02 [a]	0.71 ± 0.03 [a]	***
928	932	Lactic acid	0.00 ± 0.00 [b]	1.47 ± 0.04 [a]	0.00 ± 0.00 [b]	0.00 ± 0.00 [b]	***
1013	1016	Hexanoic acid	2.78 ± 0.12 [a]	2.15 ± 0.09 [b]	1.54 ± 0.06 [c]	2.05 ± 0.09 [b]	***
1189	1188	Octanoic acid	1.93 ± 0.08 [b]	0.86 ± 0.04 [c]	1.82 ± 0.07 [b]	5.83 ± 0.24 [a]	***
1377	1377	Decanoic acid	3.10 ± 0.06 [b]	3.06 ± 0.06 [b]	4.87 ± 0.09 [a]	2.49 ± 0.05 [c]	***
		∑ Esters	**14.84 ± 0.46 [a]**	**11.73 ± 0.37 [b]**	**13.87 ± 0.43 [a]**	**9.31 ± 0.57 [c]**	***
889	884	3-methyl-1-butyl acetate	2.78 ± 0.09 [a]	2.16 ± 0.07 [b]	2.18 ± 0.07 [b]	0.92 ± 0.03 [c]	***
885	886	2-methyl-1-butyl acetate	0.14 ± 0.01[a]	0.13 ± 0.01[b]	0.08 ± 0.01[c]	0.00 ± 0.00 [d]	***
941	941	Ethyl 3-hydroxybutanoate	0.12 ± 0.03 [a]	0.18 ± 0.05 [a]	0.14 ± 0.04 [a]	0.13 ± 0.04 [a]	**
1000	1001	Ethyl hexanoate	1.62 ± 0.04 [a]	0.91 ± 0.02 [d]	1.20 ± 0.03 [c]	1.32 ± 0.03 [b]	***
1181	1181	Diethyl succinate	0.41 ± 0.01[b]	0.30 ± 0.01[c]	0.48 ± 0.01 [a]	0.50 ± 0.02 [a]	***
1195	1196	Ethyl octanoate	3.16 ± 0.09 [a]	3.22 ± 0.09 [a]	2.25 ± 0.07 [b]	2.05 ± 0.06 [c]	***
1206	1205	Monoethyl succinate	4.92 ± 0.19 [a]	2.78 ± 0.11 [b]	5.46 ± 0.21 [a]	2.50 ± 0.10 [b]	***
1253	1253	Phenylethl acetate	0.62 ± 0.02 [a]	0.53 ± 0.02 [b]	0.60 ± 0.02 [a]	0.54 ± 0.02 [b]	***
1264	1264	Diethyl malate	0.23 ± 0.01[b]	0.28 ± 0.01[a]	0.14 ± 0.01[c]	0.07 ± 0.01[d]	***
1390	1392	Ethyl decanoate	0.84 ± 0.04 [b]	1.24 ± 0.05 [a]	0.70 ± 0.03 [c]	0.71 ± 0.03 [c]	***
1590	1590	Ethyl dodecanoate	0.00 ± 0.00 [c]	0.00 ± 0.00 [c]	0.64 ± 0.03 [a]	0.57 ± 0.23 [b]	***
		∑ Ketones	**1.08 ± 0.03 [b]**	**1.08 ± 0.03 [b]**	**1.99 ± 0.05 [a]**	**0.76 ± 0.02 [c]**	***
723	722	3-hydroxy-2-butanone	0.13 ± 0.01 [c]	0.19 ± 0.01 [b]	0.96 ± 0.03 [a]	0.09 ± 0.01 [c]	***
963	954	4-hydroxy-2-butanone	0.95 ± 0.02 [b]	0.89 ± 0.02 [c]	1.03 ± 0.02 [a]	0.67 ± 0.01 [d]	***
		∑ Anhydrides	**0.79 ± 0.02 [b]**	**0.63 ± 0.01 [d]**	**1.03 ± 0.02 [a]**	**0.69 ± 0.02 [c]**	***
993	994	Glutaconic anhydride	0.79 ± 0.02 [b]	0.63 ± 0.01 [d]	1.03 ± 0.02 [a]	0.69 ± 0.02 [c]	***
		∑ Others	**3.05 ± 0.10 [a]**	**2.91 ± 0.09 [a]**	**0.00 ± 0.00 [b]**	**0.00 ± 0.00 [b]**	***
1246	1245	1,3-di-tert-butylbenzene	3.05 ± 0.10 [a]	2.91 ± 0.09 [a]	0.00 ± 0.00 [b]	0.00 ± 0.00 [b]	***

KI [α]	KI [β]	Compounds [γ]	CONT A2 [δ]	CO 6 [δ]	CO 8 [δ]	CO 10 [δ]	S.S. [ε]
		∑ Alcohols	**147.31 ± 3.45 [a]**	**100.14 ± 2.31 [c]**	**110.79 ± 2.44 [b]**	**86.82 ± 1.95 [d]**	***
758	759	3-methyl-1-butanol	61.54 ± 1.62 [a]	39.4 ± 1.04 [c]	51.8 ± 1.36 [b]	33.40 ± 0.88 [d]	***
765	765	1.2.-propanediol	1.72 ± 0.06 [a]	1.19 ± 0.04 [b]	0.00 ± 0.00 [c]	0.00 ± 0.00 [c]	***
809	809	2.3-butanediol [ζ]	38.01 ± 0.84 [a]	22.44 ± 0.50 [b]	13.94 ± 0.31 [d]	19.74 ± 0.44 [c]	***
816	816	2.3-butanediol [η]	8.83 ± 0.32 [a]	5.98 ± 0.22 [b]	3.06 ± 0.11 [d]	4.54 ± 0.16 [c]	***
824	824	2.3-butanediol [θ]	3.37 ± 0.05 [d]	11.24 ± 0.16 [b]	14.01 ± 0.20 [a]	9.17 ± 0.13 [c]	***
848	848	3-ethoxy-1-propanol	1.15 ± 0.04 [a]	0.41 ± 0.02 [c]	0.64 ± 0.02 [b]	0.35 ± 0.01 [c]	***
878	878	1-hexanol	0.76 ± 0.02 [a]	0.37 ± 0.01 [c]	0.53 ± 0.01 [b]	0.36 ± 0.01 [c]	***
983	985	3-methylsulfanyl-1-propanol	1.56 ± 0.04 [a]	0.76 ± 0.02 [c]	0.95 ± 0.03 [b]	0.68 ± 0.02 [c]	***
1089	1088	Phenyl methanol	0.00 ± 0.00 [d]	0.07 ± 0.01 [c]	0.37 ± 0.01 [a]	0.18 ± 0.01 [b]	***
1116	1117	Hydroxyethylbenzene	26.42 ± 0.38 [a]	15.98 ± 0.23 [c]	22.54 ± 0.32 [b]	15.58 ± 0.22 [c]	***
1305	1315	2-methoxy-4-vinyl phenol	1.86 ± 0.05 [a]	0.82 ± 0.02 [c]	1.16 ± 0.03 [b]	1.83 ± 0.05 [a]	***
1428	1428	4-(2-hydroxyethyl)-phenol	2.09 ± 0.05 [a]	1.15 ± 0.03 [c]	1.27 ± 0.03 [b]	0.63 ± 0.01 [d]	***
1502	1503	2,4-di-tert- butylphenol	0.00 ± 0.00 [d]	0.33 ± 0.01 [c]	0.52 ± 0.01 [a]	0.36 ± 0.01 [b]	***
		∑ Aldehydes	**1.80 ± 0.06 [b]**	**1.49 ± 0.05 [c]**	**3.11 ± 0.10 [a]**	**1.87 ± 0.06 [b]**	***
1083	1079	4-methylbenzalddehyde	0.00 ± 0.00 [c]	0.00 ± 0.00 [c]	0.49 ± 0.02 [a]	0.36 ± 0.01 [b]	***
1211	1211	3,4-dimethylbenzaldehyde	0.00 ± 0.00 [d]	0.45 ± 0.01 [b]	0.53 ± 0.02 [a]	0.40 ± 0.01 [c]	***
1271	–	4-propyl benzaldehyde	1.80 ± 0.06 [a]	0.00 ± 0.00 [b]	0.00 ± 0.00 [b]	0.00 ± 0.00 [b]	***
1811	1812	Hexadecanal	0.00 ± 0.00 [c]	0.34 ± 0.01 [b]	0.66 ± 0.01 [a]	0.35 ± 0.01 [b]	***
–	2020	Octadecanal	0.00 ± 0.00 [d]	0.70 ± 0.02 [c]	1.43 ± 0.03 [a]	0.76 ± 0.02 [b]	***
		∑ Carboxylic acids	**16.11 ± 0.20 [a]**	**9.49 ± 0.12 [a]**	**11.91 ± 0.15 [b]**	**9.53 ± 0.12 [b]**	***
914	916	4-hydroxybutanoic acid	0.40 ± 0.01 [b]	0.45 ± 0.01 [a]	0.40 ± 0.01 [b]	0.32 ± 0.01 [c]	***
928	932	Lactic acid	0.00 ± 0.00 [c]	0.93 ± 0.04 [b]	0.00 ± 0.00 [c]	1.09 ± 0.04 [a]	***
1013	1016	Hexanoic acid	5.26 ± 0.19 [a]	2.73 ± 0.10 [d]	4.63 ± 0.17 [b]	3.33 ± 0.12 [c]	***
1189	1188	Octanoic acid	3.09 ± 0.09 [a]	2.59 ± 0.08 [b]	2.97 ± 0.09 [a]	2.29 ± 0.07 [c]	***
1377	1377	Decanoic acid	7.36 ± 0.31 [a]	2.79 ± 0.12 [c]	3.91 ± 0.17 [b]	2.50 ± 0.11 [c]	***
		∑ Esters	**18. 38 ± 0.48 [a]**	**8.42 ± 0.20 [c]**	**13.91 ± 0.38 [b]**	**7.57 ± 0.20 [c]**	***
883	884	3-methyl-1-butyl acetate	2.76 ± 0.05 [b]	2.12 ± 0.04 [c]	3.08 ± 0.06 [a]	1.72 ± 0.03 [d]	***

(*continued on next page*)

Table 2 (*continued*)

KI [α]	KI [β]	Compounds [γ]	CONT A2 [δ]	CO 6 [δ]	CO 8 [δ]	CO 10 [δ]	S.S. [ε]
885	886	2-methyl-1-butyl acetate	0.00 ± 0.00 [d]	0.12 ± 0.01 [a]	0.10 ± 0.01 [b]	0.06 ± 0.01 [c]	***
941	941	Ethyl 3-hydroxybutanoate	0.14 ± 0.01 [a]	0.15 ± 0.01 [a]	0.15 ± 0.01 [a]	0.15 ± 0.01 [a]	***
1000	1001	Ethyl hexanoate	1.84 ± 0.03 [a]	1.13 ± 0.02 [c]	1.58 ± 0.03 [b]	1.18 ± 0.02 [c]	***
1181	1181	Diethyl succinate	0.59 ± 0.02 [a]	0.40 ± 0.01 [b]	0.38 ± 0.01 [b]	0.41 ± 0.01 [b]	***
1195	1196	Ethyl octanoate	3.69 ± 0.09 [a]	1.91 ± 0.04 [b]	2.01 ± 0.05 [b]	1.69 ± 0.04 [c]	***
1206	1205	Monoethyl succinate	6.04 ± 0.19 [a]	1.60 ± 0.05 [c]	4.91 ± 0.15 [b]	1.38 ± 0.04 [c]	***
1253	1253	Phenylethyl acetate	0.58 ± 0.01 [a]	0.44 ± 0.01 [b]	0.59 ± 0.01 [a]	0.41 ± 0.01 [c]	***
1264	1264	Diethyl malate	0.42 ± 0.02 [a]	0.00 ± 0.00 [c]	0.12 ± 0.01 [b]	0.00 ± 0.00 [c]	***
1294	–	Ethyl decanoate	1.30 ± 0.03 [a]	0.55 ± 0.01 [c]	0.73 ± 0.01 [b]	0.45 ± 0.01 [d]	***
1390	1392	Ethyl dodecanoate	0.00 ± 0.00 [c]	0.00 ± 0.00 [c]	0.15 ± 0.02 [a]	0.07 ± 0.01 [b]	***
1590	1590	Ethyl 5-oxo-2-pyrrolidine-carboxylate	1.02 ± 0.03 [a]	0.00 ± 0.00 [c]	0.15 ± 0.01 [b]	0.05 ± 0.01 [c]	***
		∑ Ketones	**1.62 ± 0.04 [a]**	**0.76 ± 0.03 [d]**	**1.23 ± 0.03 [b]**	**0.91 ± 0.03 [c]**	***
723	722	3-hydroxy-2-butanone	0.27 ± 0.01 [b]	0.07 ± 0.01 [d]	0.33 ± 0.01 [a]	0.21 ± 0.01 [c]	***
963	954	4-hydroxy-2-butanone	1.35 ± 0.03 [a]	0.69 ± 0.02b [c]	0.90 ± 0.02 [b]	0.70 ± 0.02 [c]	***
		∑ Others	**2.26 ± 0.07 [a]**	**0.00 ± 0.00 [b]**	**0.00 ± 0.00 [b]**	**0.00 ± 0.00 [b]**	***
1246	1245	1.3-di-tert-butylbenzene	2.26 ± 0.07 [a]	0.00 ± 0.00 [b]	0.00 ± 0.00 [b]	0.00 ± 0.00 [b]	***

[α] Kovats index obtained through the modulated chromatogram reported for DB-5 MS apolar column;
[β] Kovats index based on literature (https://webbook.nist.gov/);
[γ] Compounds are classified in order of Kovats index;
[δ] Relative amounts expressed as mg/L with respect to calibration curves of ethyl lactate, 3-hydroxy-2-butanone, 2.3-butanediol;
[ε] statistical significance. Data in the same line followed by the same letter are not significantly different according to Tukey's test. P value: * $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s., not significant.
[ζ]Unidentified stereoisomer.
[η]Unidentified stereoisomer.
[θ] Unidentified stereoisomer.

3.3.3. Carboxylic acids

Different trends in fatty acid production were registered depending on the LAB-*S. cerevisiae* consociation (Table 2). In particular, malolactic fermentations conducted at the same time as alcoholic fermentation resulted in a significant increase in decanoic and octanoic fatty acids in the CO3 and CO5 trials (MLA4-NF213 and MLPK45H-NF213) compared to the CONT A2 control. The concentration of decanoic acid was 4.87 mg/L in CO3 trial, which was higher than the 3.10 mg/L in the CONT A1 trial. Similarly, the concentration of octanoic acid was 5.83 mg/L in CO5 trial, which was higher than the 1.93 mg/L in the CONTA1 trial. These values are consistent with those reported by Knoll et al. (2012) and Sun, Chen, and Jin (2018).

The significant production of decanoic and octanoic acids in the CO3 and CO5 trials involving the combination of LAB MLA4 and MLPK45H with the *S. cerevisiae* strain NF213 could be attributed to an antagonistic response exerted by the *S. cerevisiae* strain NF213 to alter and inhibit the metabolic physiology of the co-fermentative LAB (Rossouw, Du Toit, & Bauer, 2012). Therefore, the lower concentrations of decanoic and octanoic acids found in the CO1, CO6, CO8 and CO10 trials (Table 1) could be explained as an increased response of LAB to matrix detoxification by esterification. However, the different response recorded in the complementary tests of the two different sets (CO3 vs CO8 for decanoic acid; and CO5 vs CO10 for octanoic acid; Table 1) indicates that this is a strongly *S. cerevisiae* strain-dependent effect. The data suggested that only certain consociations of LAB-*S. cerevisiae* strains have a higher technological affinity, as shown in Table 1. Therefore, having noted the inverse correlation between octanoic and decanoic fatty acids with their corresponding ethyl esters (Table S2), the production of fatty acids by the *S. cerevisae* strain as an antagonistic-inhibitory effect (Alexandre et al., 2004; Rossouw et al., 2012) toward LAB represents a synthesis of aromatic precursors.

3.3.4. Aldehydes

The microbial strains used in this study showed a different response in the synthesis of aldehydes. The LAB microbial consociations with the yeast strain NF213 resulted in significantly reduced aldehyde concentrations compared to the CONT A1 control. These results are consistent with those of Liu (2002). Therefore, the decrease in aldehydes would allow the decrease in herbaceous hints in favor of fruity sensorial intensity. On the contrary, microbial consociations with the yeast strain QA23 resulted in an increase of aldehydes, with the exception of the CO6 trial (Table 2). The concentration of aldehydes in the CO8 trial was 3.11 mg/L, while it was 1.87 mg/L in the CO10 trial (non-significant difference compared to the CONT A2 control, (1.80 mg/L). The concentration of aldehydes in the CO6 trial was 1.49 mg/L, which was lower than the concentration in the CONT A2 trial (1.80 mg/L).

According to ours study, the VOCs that contributed most to the increase in total aldehyde values in the CO8 and CO10 trials were 4-methylbenzaldehyde and 3,4-dimethylbenzaldehyde (Table 2). These compounds result from the methylation of carbons 3 and 4 of the aromatic ring of benzylaldehyde. The formation of the benzylaldehyde during fermentation is due to the degradation of phenylalanine by LAB (Nierop Groot & de Bont, 1998). Thus, the different production of these aldehydes in the various trials could be explained by a trophic competition for phenylalanine between the co-fermenting microorganisms.

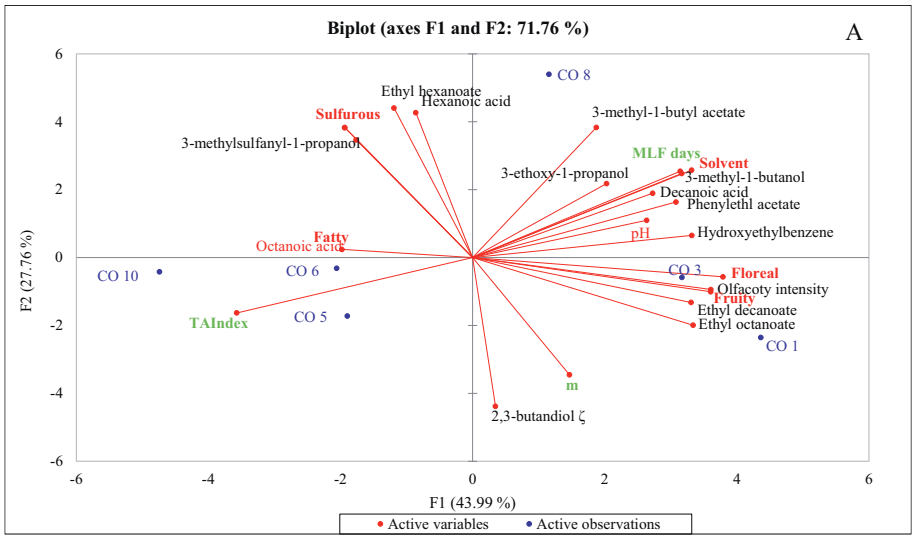
3.3.5. The 2,3-butanedione, 2,3-butanediol and 3-hydroxy-2-butanone compounds

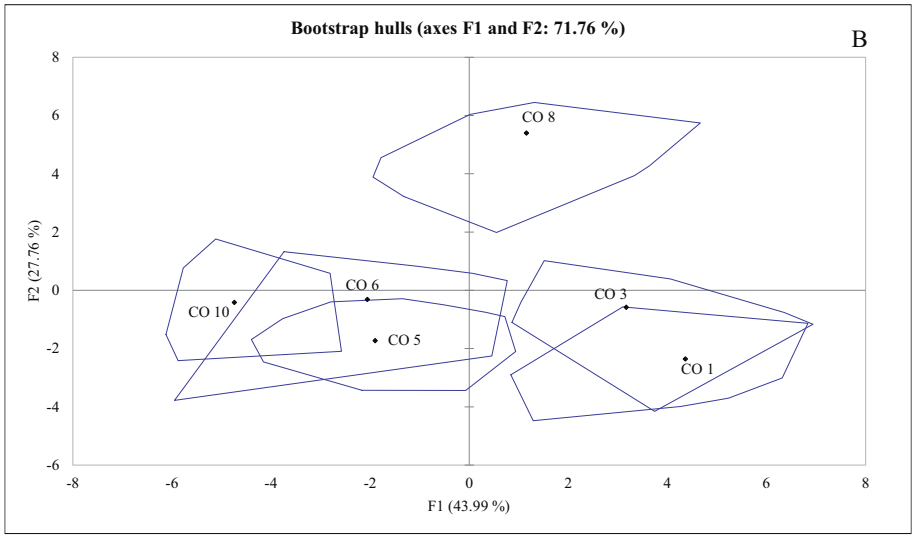
2,3-butanedione, also known as diacetyl, is a diketone whose production has always been attended to by technicians because of its olfactory perceptions. In fact, aromas resulting from the production of this compound can also result in off-flavors depending on the concentration produced. In wine, LAB play a key role in the production of diacetyl, which is synthesized due to the degradation of citric acid.

According to Bartowsky and Henschke (2004), using citrate-negative LAB to carry out malolactic fermentation in wines may not always be a valid strategy to prevent diacetyl formation; in fact, its synthesis can also be triggered from pyruvate formed by glycolysis (Ochando, Mouret, Humbert-Goffard, Sablayrolles, & Farines, 2018). The joint creation of a reducing environment by the two *S. cerevisiae* strains and the reductive metabolisms of the different LAB tested favored the total reduction of 2,3-butanedione to 3-hydroxy-2-butanone and, subsequently, to 2,3-butanediol (Table 2) (Bartowsky & Henschke, 2004) This behaviour was also observed in trials with a significant decrease in citric acid (Table S6) in contrast to that reported by Bartowsky and Henschke (2004).

3.4. Active volatile compound analysis

The aroma profiles of the wines were characterized by VOCs from the





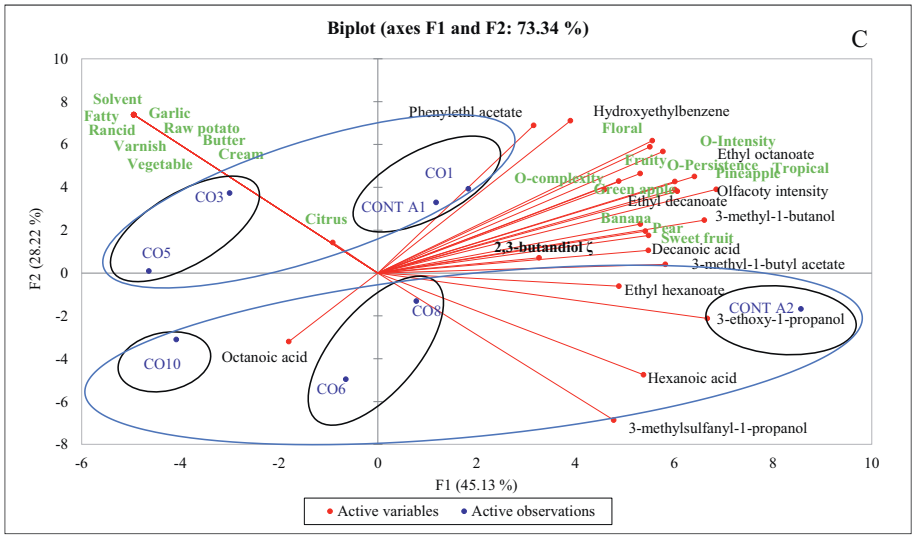


Fig. 3. Principal component analysis (PCA) biplot: (A) OAV > 0.1 and TAIndex; m, malolactic fermentation days and pH; (B) Bootstrap hull's for OAV > 0.1; (C) OAV > 0.1 and aroma attributes.

Table 3
Odor activity value of volatile organic compounds detected above the perception threshold in Catarratto experimental wines.

Compounds [α]	Aroma description [β]	AromaticSeries [γ]	ReferenceAromaticSeries [δ]	Odor threshold [ε]	ReferenceOdor treshold [ζ]	CONT A1 [η]	CO 1 [η]	CO 3 [η]	CO 5 [η]	S. S.[θ]	CONT A2 [η]	CO 6 [η]	CO 8 [η]	CO 10 [η]	S.S. [ι]
3-methyl-1-butanol	Fusel	4	[1]	40	[2]	1.39 ± 0.03 a	1.11 ± 0.03 c	1.26 ± 0.03 b	1.06 ± 0.03 c	***	1.54 ± 0.04 a	0.99 ± 0.03 c	1.30 ± 0.03 b	0.84 ± 0.02 d	***
3-ethoxy-1-propanol	Fruity	1	[1]	0.1	[3]	2.80 ± 0.10 b	6.50 ± 0.10 a	2.90 ± 0.10 b	2.90 ± 0.10 b	***	11.50 ± 0.40 a	4.10 ± 0.20 c	6.40 ± 0.20 b	3.50 ± 0.10 c	***
3-methylsulfanyl-1-propanol	Raw potato, Garlic	5	[4]	0.5	[5,6]	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	***	3.12 ± 0.08 a	1.52 ± 0.04 b	1.90 ± 0.06 c	1.36 ± 0.04 c	***
Hydroxyethylbenzene	Rose	2	[7,8]	10	[7,8]	2.70 ± 0.09 a	2.18 ± 0.07 b	2.69 ± 0.09 a	2.06 ± 0.07 b	***	2.64 ± 0.04 a	1.60 ± 0.02 c	2.25 ± 0.03 b	1.56 ± 0.02 c	***
Hexanoic acid	Cheese Fatty	3	[9]	0.4	[3,10]	6.62 ± 0.29 a	5.12 ± 0.21 b	3.67 ± 0.14 b	4.88 ± 0.21 c	***	12.52 ± 0.45 a	6.50 ± 0.24 d	11.02 ± 0.40 b	7.92 ± 0.29 c	***
Octanoic acid	Rancid, Cheese, Fatty	3	[9]	0.5	[7,8]	3.86 ± 0.16 c	1.72 ± 0.08 b	3.64 ± 0.14 b	11.66 ± 0.48 a	***	6.18 ± 0.18 a	5.18 ± 0.16 b	5.94 ± 0.18 a	4.58 ± 0.14 c	***
Decanoic acid	Fatty Rancid	3	[9]	1	[11]	3.10 ± 0.06 b	3.06 ± 0.06 b	4.87 ± 0.09 a	2.49 ± 0.05 c	***	7.36 ± 0.31 a	2.79 ± 0.12 c	3.91 ± 0.17 b	2.50 ± 0.11 c	***
3-methyl-1-butyl acetate	Banana	1	[12]	0.03	[7,8]	92.67 ± 3.00 a	72.00 ± 2.33 b	72.67 ± 2.33 b	30.66 ± 1.00 c	***	92.00 ± 1.67 b	70.67 ± 1.33 c	102.67 ± 2.00a	57.33 ± 1.00 d	***
Ethyl hexanoate	Apple, Banana	1	[12]	0.005	[7,8]	324.00 ± 8.00 a	182.00 ± 4.00 d	240.00 ± 6.00 c	264.00 ± 6.00 b	***	368.00 ± 6.00 a	226.00 ± 4.00 c	316.00 ± 6.00 b	236.00 ± 4.00 c	***
Ethyl octanoate	Pineapple. Pear	1	[12]	0.002	[7,8]	1580.00 ± 45.00a	1610.00 ± 45.00a	1125.00 ± 35.00 b	1025.00 ± 30.00 b	***	1845.00 ± 45.00 a	955.00 ± 20.00 b	1005.00 ± 25.00 b	845.00 ± 20.00 c	***
Phenylethyl acetate	Rosa. Floreal	2	[8]	0.25	[8]	2.48 ± 0.08 a	2.12 ± 0.08 b	2.40 ± 0.08 a	2.16 ± 0.08 b	***	2.32 ± 0.04 a	1.76 ± 0.04 b	2.36 ± 0.04 a	1.64 ± 0.04 c	***
Ethyl decanoate	Floreal	2	[3]	0.20	[3]	4.20 ± 0.20 b	6.20 ± 0.25 a	3.50 ± 0.15 c	3.55 ± 0.15 c	***	6.50 ± 0.15 a	2.75 ± 0.05 c	3.65 ± 0.05 b	2.25 ± 0.05 d	***
2,3-butandiol ζ	Fruity	1	[13]	150	[12]	0.13 ± 0.00 c	0.15 ± 0.00 b	0.18 ± 0.00 a	0.11 ± 0.00 d	***	0.25 ± 0.00 a	0.15 ± 0.00 b	–	0.13 ± 0.00 c	***
Olfactory intensity						2023.95 ± 57.01a	1892.16 ± 52.22a	1462.17 ± 44.15b	1350.54 ± 38.17b	***	2358.93 ± 54.36 a	1279.00 ± 26.23 c	1462.40 ± 34.17 b	1164.61 ± 25.81 d	***

[α] Compounds with OAV > 0.1.
[β] Aroma description.
[γ] Aromatic series, 1: fruity; 2: floral; 3: fatty; 4: solvent; 5: sulfurous.
[δ] Reference Aromatic Series: [1] Butkhup et al., 2011; [3] Kelebek & Selli, 2011; [4] Celik et al., 2019; [7] Selli et al., 2004; [8] Cañas et al., 2008;[9] Cai et al., 2014; [12] Bayram & Kayalar, 2018; [13] García-Carpintero et al., 2011.
[ε] Odor threshold (mg/L).
[ζ] Reference Odor threshold: [2] Herrero, Cuesta, Garcia, & Diaz, 1999; [3] Kelebek & Selli, 2011; [5] Krieger-Weber, Silvano, & Loubser, 2015; [6] Davis, Wibowo, Eschenbruch, Lee, & Fleet, 1985; [7] Selli et al., 2004; [8] Cañas et al., 2008; [10] Moio et al., 1995; [11] Delequis et al., 2000; [12] García-Carpintero et al., 2011.
[η] Relative amounts expressed in OAV (odor activity value).
[θ] Statistical significance among CONT A1, CO1, CO3, CO5 trials; Data in the same line followed by the same letter are not significantly different according to Tukey's test. P value: ***, P < 0.001; n.s., not significant.
[ι] Statistical significance among CONT A2, CO6, CO8, CO10 trials; Data in the same line followed by the same letter are not significantly different according to Tukey's test. P value: ***, P < 0.001; n.s., not significant.

metabolic processes of the biotechnology used. The study found that the active volatile component, represented by VOCs above the perception threshold (OAV > 0.1) (Peng et al., 2013), was composed of 12 compounds for the trials involving the combination of the LAB MLB6, MLA4, MLPK45H strains with the *S. cerevisiae* NF213 (CONT A1, CO1, CO3 and CO5 trials) (Table 3). For the test set in which the same LAB were combined with *S. cerevisiae* QA23 strain for the initiation of fermentation (trials CONT A2, CO6, CO8 and CO10) the active volatile component was composed of 12 compounds (Table 3). According to Ferreira et al. (2016), the sensorial buffer of wine is composed of a total of three higher alcohols (methyl-1-butanol, 3-ethoxy-1-propanol, hydroxyethylbenzene), three medium chain fatty acids (hexanoic acid, octanoic acid and decanoic acid), two acetate esters and three ethyl esters (3-methyl-1-butyl acetate, phenylethyl acetate, ethyl hexanoate, ethyl octanoate and ethyl decanoate). 3-methylsulfanyl-1-propanol was the VOC that significantly differentiated trials in addition to the amount of OAVs detected (Tables 2 and 3).

This study found that the active volatile component is a function of TAIndex; the negative correlation between the two would be explained by 45.96% of the total variance in the PCA analysis (Fig. 3a). Data suggest that a higher degree of esterification by LAB to neutralize the deleterious effect of medium-chain fatty acids in the cell (Costello et al., 2013) could result in the decrease in TAIndex and increase in olfactory intensity, producing much more aromatic wines. The negative correlation found between octanoic acid and ethyl octanoate (45.96% of the total variance), and decanoic acid with ethyl decanoate (25.93% of the total variance; Fig. 3a) could further confirm this hypothesis. These mechanisms were found to be positively correlated with MLB6-NF213 consociation in the CO1 trial, which had a higher endowment of the fruit and floral component among the trials (Table 4).

The increase in TAIndex found among the trials associated with the MLPK45H, CO5 and CO10 trials (Table 1) resulted in a significant decrease in active volatile components (Table 3) and, thus, in olfactory intensity. This is probably due to the rapid degradation of malic acid that occurred when the culture medium had low limiting factors, low concentrations of ethanolic substrate (Table S2) and, presumably, low concentrations of medium-chain fatty acids by the metabolism of *S. cerevisiae*.

The limited concentrations of these two compounds in solution during the first three days of vinification would not have led LAB to catalyse the octanoic and decanoic acid esterification reactions. Thus, the failure of LAB to contribute to esterification resulted in a decrease in octane intensity. This hypothesis formulation would also explain the positive correlation of octanoic acid with the CO5 and CO10 trials (Fig. 3a).

The delayed times of potential esterification by LAB and octanoic acid synthesis by the *S. cerevisiae* strain favored the accumulation of octanoic acid in the medium. These hypotheses are confirmed by the negative correlations recorded between: the days of malolactic fermentation and ethyl octanoate and olfactory intensity (25.97% of the total variance); TAIndex and olfactory intensity (45.96% % of the total variance); and TAIndex and days of malolactic fermentation (45.96% % of the total variance) (Fig. 3a).

Furthermore, the dynamics described above made it possible to discriminate three groups of wines according to their TAIndex values (Fig. 3b). Values between 0.009 and 0.013 (CO1 and CO3 trials) (Table 1) marked a single grouping with partially overlapping olfactory profiles (Fig. 3b). This similarity is explained by 45.96% of the total variance from the production of ethyl octanoate and ethyl decanoate (Fig. 3a, b) for the MLB6-NF213 and MLA4-NF213 consociations.

Single clustering resulted for the CO8 trial. The TAIndex value of 0.019 in this thesis allowed the olfactory active VOCs to be distinguished from the other theses (Fig. 3b). In contrast, TAIndex values above 0.148 outlined partially overlapping olfactory profiles. These peculiarities were found among the CO5, CO6 and CO10 trials, which formed the third group (Fig. 3b).

This group was found to be closely related to octanoic acid. This result suggests that the production of ethyl octanoate, in a microbial consociation, is enabled by a malic acid degradation rate of less than -0.0292 g/L × day^{-1} and a malolactic fermentation of >18 days.

The technological affinity between LAB and *S. cerevisiae* is calculated by using data from the relationship between malic acid degradation as a function of time. The resulting trend reveals the extent to which the decarboxylative activity of LAB is affected by the activity of the *S. cerevisiae* strain during the co-fermentation phase (Alexandre et al., 2004).

Assuming that the secondary metabolisms and thus esterifications of LAB are activated by ATP hydrolysis and that the synthesis of adenosine triphosphate is enabled by the decarboxylative activity of malic acid to recover protons from the acid function of the malate anion (Versari, Parpinello, & Cattaneo, 1999), any interference of the f *S. cerevisiae* strain on the slowing of decarboxylative capacity toward LAB would interfere in the timing of malolactic fermentation. This interference would consequently affect the ability of LAB to detoxify the culture medium from *S. cerevisiae* catabolites (medium-chain fatty acids and higher alcohols). Thus, the timing of malolactic fermentation and the ability of LAB to detoxify the culture medium from *S. cerevisiae* catabolites are closely related.

Therefore, by identifying the malic acid trend over time with its graphical representation, it was possible to obtain an equation of the type y = mx + q from the linear regression (Bevilacqua, Speranza, Petruzzi, Sinigaglia, & Corbo, 2023; Caponigro et al., 2010; Hsiao & Siebert, 1999). The corresponding coefficient of determination R^2 indicates the link between the variability of the data and the correctness of the statistical model.

3.5. The sensory analysis

Table 5 presents the data from the sensory measurements. The microbial consociation trials in the two experimental sets resulted in significantly different sensory profiles. The appearance of the wine was influenced by the variability of colour attributes. The yellow colour

Table 4
Aroma profiles of Catarratto experimental wines derived from odor activity values (all values in OAV).

Aroma series [α]	CONT A1 [β]	CO 1 [β]	CO 3 [β]	CO 5 [β]	S.S. [γ]	CONT A2 [β]	CO 6 [β]	CO 8 [β]	CO 10 [β]	S. S.[δ]
Fruity	1999.59 ± 56.10 [a]	1870.65 ± 51.43 [a]	1440.74 ± 43.43 [b]	1322.67 ± 37.10 [b]	***	2316.75 ± 53.07 [a]	1255.92 ± 25.53 [c]	1430.07 ± 33.20 [b]	1141.96 ± 25.10 [d]	***
Floreal	9.38 ± 0.10 [b]	10.49 ± 0.40 [a]	8.58 ± 0.32 [c]	7.77 ± 0.30 [d]	***	11.46 ± 0.23 [a]	6.11 ± 0.11 [c]	8.26 ± 0.12 [b]	5.45 ± 0.11 [d]	***
Fatty	13.58 ± 0.51 [b]	9.90 ± 0.35 [c]	12.18 ± 0.37 [b]	19.03 ± 0.74 [a]	***	26.06 ± 0.94 [a]	14.47 ± 0.52 [c]	20.87 ± 0.75 [b]	15.01 ± 0.53 [c]	***
Solvent	1.39 ± 0.03 [a]	1.11 ± 0.03 [c]	1.26 ± 0.03 [b]	1.06 ± 0.03 [c]	***	1.54 ± 0.04 [a]	0.99 ± 0.03 [c]	1.30 ± 0.03 [b]	0.84 ± 0.02 [d]	***
Sulfurous	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	***	3.12 ± 0.08 [a]	1.52 ± 0.04 [c]	1.90 ± 0.06 [b]	1.36 ± 0.04 [c]	***

[α] Aroma series.
[β] Aroma profile calculated by summing and of the odorous activity values (OAV) of the aromatic series from Table 3.
[γ] Statistical significance among CONT A1, CO1, CO3, CO5 trials.
[δ] Statistical significance among CONT A2, CO6, CO8, CO10 trials.

Table 5
Sensory score for experimental Catarratto wines.

Attributes [α]		Trials									Statistical [γ] Significance
		CONT A1 [β]	CO1 [β]	CO3 [β]	CO5 [β]	CONT A2 [β]	CO6 [β]	CO8 [β]	CO10 [β]	Judge	Wine
Apparence	Yellow colour	7.26 ± 0.02 [bc]	7.21 ± 0.02 [cd]	7.41 ± 0.02 [a]	7.37 ± 0.02 [a]	7.12 ± 0.02 [e]	7.21 ± 0.02 [cd]	7.18 ± 0.02 [d]	7.28 ± 0.02 [b]	***	***
	Green reflexes	6.14 ± 0.11 [b]	6.71 ± 0.12 [a]	6.31 ± 0.11 [b]	6.24 ± 0.11 [b]	6.27 ± 0.11 [b]	6.23 ± 0.11 [b]	6.16 ± 0.11 [b]	6.48 ± 0.12 [ab]	***	***
Odor	Green apple	7.70 ± 0.22 [a]	6.27 ± 0.18 [cd]	6.80 ± 0.19 [bc]	6.87 ± 0.19 [b]	7.83 ± 0.23 [a]	6.11 ± 0.17 [d]	6.18 ± 0.17 [d]	6.03 ± 0.17 [d]	***	***
	Banana	6.61 ± 0.14 [ab]	6.10 ± 0.13 [c]	6.18 ± 0.13 [bc]	4.81 ± 0.10 [d]	6.62 ± 0.14 [a]	6.12 ± 0.13 [c]	6.97 ± 0.15 [a]	5.10 ± 0.11 [d]	***	***
	Citrus	3.27 ± 0.01 [b]	3.12 ± 0.01 [e]	3.23 ± 0.01 [c]	3.71 ± 0.01 [a]	3.30 ± 0.01 [b]	3.18 ± 0.01 [d]	3.21 ± 0.01 [c]	3.00 ± 0.01 [f]	***	***
	Fatty	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	***	*
	Floral	6.80 ± 0.08 [b]	7.12 ± 0.09 [a]	6.76 ± 0.08 [b]	6.46 ± 0.08 [bc]	7.31 ± 0.09 [a]	6.01 ± 0.07 [d]	6.61 ± 0.08 [bc]	5.83 ± 0.07 [d]	***	***
	Fruity	8.12 ± 0.15 [b]	8.61 ± 0.15 [a]	7.30 ± 0.13 [c]	6.84 ± 0.12 [d]	8.18 ± 0.15 [b]	7.18 ± 0.18 [cd]	7.13 ± 0.13 [cd]	7.21 ± 0.13 [cd]	***	***
	Tropical	8.25 ± 0.12 [a]	8.31 ± 0.12 [a]	7.10 ± 0.10 [b]	6.35 ± 0.09 [c]	8.36 ± 0.12 [a]	6.12 ± 0.09 [cd]	6.38 ± 0.09 [c]	6.01 ± 0.09 [d]	***	***
	O-Intensity	8.34 ± 0.22 [a]	8.69 ± 0.22 [a]	7.00 ± 0.18 [b]	6.68 ± 0.17 [bc]	8.42 ± 0.23 [a]	6.51 ± 0.17 [bc]	7.00 ± 0.18 [b]	6.23 ± 0.15 [c]	***	***
	Pear	6.58 ± 0.17[ab]	6.08 ± 0.15 [b]	6.12 ± 0.16 [b]	5.21 ± 0.13 [c]	6.42 ± 0.16 [ab]	6.11 ± 0.16 [b]	6.74 ± 0.17 [a]	5.20 ± 0.13 [c]	***	***
	O-Persistence	7.49 ± 0.15 [a]	7.61 ± 0.15 [a]	6.54 ± 0.13 [b]	6.36 ± 0.12 [b]	7.64 ± 0.15 [a]	6.54 ± 0.12 [b]	6.38 ± 0.12 [b]	6.37 ± 0.12 [b]	***	***
	Pineapple	8.20 ± 0.02 [c]	8.40 ± 0.02 [b]	6.20 ± 0.02 [h]	6.32 ± 0.02 [g]	8.60 ± 0.02 [a]	6.62 ± 0.02 [f]	7.48 ± 0.02 [d]	6.68 ± 0.02 [e]	***	***
	Sweet fruit	6.59 ± 0.12 [ab]	6.09 ± 0.11 [b]	6.15 ± 0.11 [c]	5.01 ± 0.09 [d]	6.52 ± 0.12 [b]	6.12 ± 0.11 [c]	6.86 ± 0.12 [a]	5.15 ± 0.09 [d]	***	*
	Solvent	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	***	*
	Garlic	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	***	*
	Raw potato	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	***	*
	Cream	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	***	*
	Butter	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	***	*
	Rancid	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	***	*
	Varnish	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	***	*
	Vegetable	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	***	*
Taste	O-complexity	7.21 ± 0.18 [b]	7.66 ± 0.19 [a]	6.23 ± 0.16 [c]	6.41 ± 0.18 [c]	7.18 ± 0.16 [b]	6.43 ± 0.16 [c]	6.27 ± 0.16 [c]	6.31 ± 0.16 [c]	***	***
	Sweet	5.36 ± 0.11 [d]	6.12 ± 0.12 [a]	6.18 ± 0.12 [a]	5.63 ± 0.11 [cd]	5.42 ± 0.11 [d]	6.01 ± 0.12 [ab]	6.06 ± 0.12 [ab]	5.78 ± 0.11 [bc]	***	***
	Sour	6.70 ± 0.02 [b]	4.81 ± 0.01 [g]	4.92 ± 0.01 [e]	5.21 ± 0.01 [c]	6.80 ± 0.02 [a]	4.91 ± 0.01 [e]	4.86 ± 0.01 [f]	5.16 ± 0.01 [d]	***	***
	Salty	6.56 ± 0.12 [a]	5.60 ± 0.10 [bc]	5.78 ± 0.10 [b]	5.61 ± 0.10 [bc]	6.71 ± 0.12 [a]	5.21 ± 0.09 [d]	5.46 ± 0.10 [cd]	5.57 ± 0.10 [bc]	***	***
	Bitter	4.62 ± 0.14 [abc]	4.65 ± 0.14 [ab]	4.71 ± 0.14 [ab]	4.68 ± 0.14 [ab]	4.35 ± 0.13 [bc]	4.84 ± 0.14 [a]	4.24 ± 0.12 [c]	4.35 ± 0.13 [bc]	***	***
	Body	6.12 ± 0.13 [a]	6.21 ± 0.13 [a]	6.36 ± 0.14 [a]	6.28 ± 0.14 [a]	6.25 ± 0.14 [a]	6.23 ± 0.13 [a]	6.18 ± 0.13 [a]	6.23 ± 0.13 [a]	***	**
	Balance	7.10 ± 0.02 [a]	6.21 ± 0.02 [f]	6.27 ± 0.02 [e]	6.70 ± 0.02 [b]	7.15 ± 0.02 [a]	6.36 ± 0.02 [d]	6.21 ± 0.02 [f]	6.5 ± 0.02 [c]	***	***
Flavor	Banana-like	7.69 ± 0.01 [b]	7.6 ± 0.01 [c]	6.49 ± 0.01 [e]	5.83 ± 0.01 [h]	7.86 ± 0.01 [a]	6.29 ± 0.01 [f]	6.94 ± 0.01 [d]	5.93 ± 0.01 [g]	***	***
	F-Citrus	3.32 ± 0.04 [b]	3.12 ± 0.04 [de]	3.23 ± 0.04 [bcd]	3.48 ± 0.04 [a]	3.25 ± 0.04 [bc]	3.14 ± 0.04 [cde]	3.16 ± 0.04 [cde]	3.06 ± 0.04 [e]	***	***
	F-Fruity	7.45 ± 0.20 [a]	7.04 ± 0.19 [ab]	6.55 ± 0.18 [bc]	5.97 ± 0.16 [cd]	7.54 ± 0.20 [a]	6.29 ± 0.17 [d]	6.82 ± 0.18 [bc]	5.81 ± 0.16 [d]	***	***
	F-Intensity	8.61 ± 0.13 [a]	8.57 ± 0.13 [a]	6.76 ± 0.10 [b]	6.47 ± 0.10 [bc]	8.64 ± 0.13 [a]	6.41 ± 0.09 [c]	6.74 ± 0.10 [b]	6.23 ± 0.09 [c]	***	***
	F-Persistence	7.12 ± 0.18 [a]	7.32 ± 0.19 [a]	6.21 ± 0.16 [b]	6.03 ± 0.15 [b]	7.18 ± 0.18 [a]	5.92 ± 0.15 [b]	5.98 ± 0.15 [b]	5.83 ± 0.15 [b]	***	***
	F-Complexity	7.31 ± 1.85 [a]	7.76 ± 1.97 [b]	6.23 ± 1.58 [a]	6.36 ± 1.61 [b]	7.43 ± 1.88 [a]	6.42 ± 1.63 [b]	5.58 ± 1.42 [c]	6.14 ± 1.56 [b]	***	***
	F-Pineapple	7.80 ± 0.15 [a]	8.10 ± 0.16 [a]	5.90 ± 0.12 [c]	6.03 ± 0.12 [c]	7.90 ± 0.16 [a]	5.70 ± 0.11 [c]	7.20 ± 0.14 [b]	5.80 ± 0.11 [c]	***	***

(*continued on next page*)

Table 5 (*continued*)

Attributes [α]		Trials									Statistical [γ] Significance
		CONT A1 [β]	CO1 [β]	CO3 [β]	CO5 [β]	CONT A2 [β]	CO6 [β]	CO8 [β]	CO10 [β]	Judge	Wine
Finish	F-Sweet fruit	6.38 ± 0.02 [a]	6.16 ± 0.02 [c]	6.00 ± 0.02 [d]	5.80 ± 0.02 [d]	6.31 ± 0.02 [b]	5.96 ± 0.02 [d]	6.29 ± 0.02 [b]	5.30 ± 0.01 [f]	***	***
	Overall quality	8.69 ± 0.02 [b]	8.91 ± 0.02 [a]	7.53 ± 0.02 [h]	6.91 ± 0.02 [g]	8.59 ± 0.02 [c]	7.62 ± 0.02 [e]	7.78 ± 0.02 [d]	7.46 ± 0.02 [f]	***	***
	Odor	8.81 ± 0.16 [a]	8.76 ± 0.16 [a]	6.25 ± 0.11 [bc]	6.16 ± 0.11 [bc]	8.86 ± 0.16 [a]	6.26 ± 0.11 [bc]	6.54 ± 0.12 [b]	6.12 ± 0.11 [c]	***	***
	Taste	7.64 ± 0.22 [a]	7.36 ± 0.22 [a]	7.21 ± 0.21 [a]	7.18 ± 0.21 [a]	7.56 ± 0.22 [a]	7.18 ± 0.21 [a]	7.46 ± 0.22 [a]	7.36 ± 0.22 [a]	***	***
	Mouth-feel	7.46 ± 0.16 [a]	7.26 ± 0.16 [a]	7.18 ± 0.16 [a]	7.16 ± 0.15 [a]	7.36 ± 0.16 [a]	7.18 ± 0.16 [a]	7.34 ± 0.16 [a]	7.12 ± 0.15 [a]	***	***
	Flavor	7.48 ± 0.02 [ab]	7.43 ± 0.02 [b]	6.22 ± 0.02 [d]	6.00 ± 0.02 [e]	7.51 ± 0.02 [a]	5.94 ± 0.02 [f]	6.28 ± 0.02 [c]	5.8 ± 0.02 [g]	***	***
	After-smell	8.46 ± 0.01 [b]	8.56 ± 0.01 [a]	7.26 ± 0.01 [d]	7.12 ± 0.01 [f]	8.48 ± 0.01 [b]	7.21 ± 0.01 [e]	7.45 ± 0.01 [c]	7.11 ± 0.01 [f]	***	***
	After-taste	8.23 ± 0.00 [a]	8.18 ± 0.10 [a]	7.18 ± 0.09 [b]	7.18 ± 0.09 [b]	8.36 ± 0.10 [a]	7.18 ± 0.09 [b]	7.35 ± 0.09 [b]	7.14 ± 0.09 [b]	***	***

Results indicate mean value of three replicate sessions.
[α] Sensorial attribute.
[β] Relative amounts expressed in on a numerical scale of 1 to 9.
[γ] Statistical significance. Data in the same line followed by the same letter are not significantly different according to Tukey's test. P value: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s., not significant.

showed a variability between the trials ranging from 7.12 and 7.47. This is consistent with the findings of Naselli et al. (2023).

The green reflections in the trials were similar to each other, but had higher values than those described by Scacco et al. (2012). The CO1 trial stood out for having the highest value of green reflections compared to the control and compared to all trials in the two different experimental sets (Table 5). The wines were submitted to a panel, which outlined different olfactory profiles depending on the LAB-*S. cerevisiae* microbial consociation used. Among the microbial consociation trials, the CO1 trial (MLB6-NF213) stood out for its high values of intensity, persistence and tropical scents with values of 8.69, 7.61 and 8.31, respectively (Table 5). These olfactory attributes appeared not to differ from the CONT A2 control. However, the panel detected higher levels of the fruitiness and pineapple than the CONT A2 control.

The wines were subjected to a panel that outlined different olfactory profiles depending on the LAB–*S. cerevisiae* microbial association used. Among the microbial association tests, the CO1 trial (MLB6- NF213) stood out for its high intensity, persistence and tropical aroma values of 8.69, 7.61 and 8.31 respectively (Table 5). The study found that while olfactory attributes did not significantly differ from the CONT A1 control, the panel detected heightened fruitiness and pineapple aromas (Table 5). Surprisingly, the OAV values (Table 3) results contradicted sensory analysis for pineapple and fruity perceptions. This discrepancy might be due to the inhibitory effects of compounds like 3-methyl-1-butanol and medium-chain fatty acids on fruitiness and pineapple hint perception (Cameleyre, Lytra, Tempere, & Barbe, 2015). additionally, sulphur-related perceptions differed based on the detection analysis adopted. The OAV system, which isolates VOCs from interactive influences (Gómez-Míguez, Cacho, Ferreira, Vicario, & Heredia, 2007), revealed the effect of LAB-*S. cerevisiae* QA23 strain associations on these scents. The study observed statistically significant decreases in the CO6, CO8, and CO10 trials compared to the CONT A2 control (Table 3). These reductions may be due to suppression effects exerted by the LAB against the yeast. While the partial deactivation of the biosynthetic pathway responsible for 3-ethoxy-1-propanol production within yeast cells cannot be excluded (Irwin, 1992), sensory analysis, considering VOCs interactions (Gómez-Míguez et al., 2007), did not identify any sulphur-related perceptions associated with the olfactory attributes of garlic or raw potato (Table 5). These effects were consistent with rancid and solvent odours from fatty acids and 3-methyl-1-butanol. Interestingly, although undetected by human noses, 3-methyl-1-butanol and medium-chain fatty acids likely play a role in balancing olfactory perceptions trhough synergy or masking phenomena (Ferreira et al., 2016) within the sensory buffer (Ferreira, Escudero, Campo, & Cacho, 2008).

The interaction between LAB and *S. cerevisiae* significantly influences fruity wine flavor. In the CO1 trial, using MLB6 yeast led to a wine with distinct pineapple and pear aromas, attributed to ethyl octanoate (1610 olfactory units, Table 3; Fig. S4a). In contrast, the CO8 trial exhibited a more diverse fruit profile, including pineapple, pear, green apple, and banana, due to the synthesis of ethyl octanoate, ethyl hexanoate, and 3-methyl-1-butyl acetate (1005, 316 and 102.67 olfactory units, respectively (Table 3; Fig. 4b). Sensory analysis confirmed these fruity descriptors (banana, pear, and sweet fruit) in the CO8 (MLA4-QA23) trial. The CO1 trial exhibited the highest olfactory complexity value, differentiating itself from the CONT A1 control and other microbial association trials (Table 5). This distinction could be attributed to judges recording a stronger floral perception (Table 5) and an increase ethyl decanoate compared to the CONT A1 trial, as indicated by the OAV findings (Table 3). Additionally, the synergistic effect of the VOC mixture, although below the perception threshold, likely contributed to this outcome (Atanasova et al., 2005). In the context of olfactory intensity, the CO1 exhibited notable differences compared to the other LAB-*S. cerevisiae* consociation tests. Both OAV and sensory analysis assigned the highest scores: 1892 and 7.61, respectively. The data presented in Tables 3 and 5 indicated a robust correlation between this result and the specific *S. cerevisiae* strain, as well as its TAIndex value relative to LAB.

The taste fraction of the different trials was evaluated, and a further distinctive aspect emerged between the trials. Specifically, the CO1 and CO3 trials were characterized by a higher sweetness sensation than the CONT A1 control. Meanwhile, trials CO5 and CO10 were the most acidic of the trials that included microbial association. This sensory effect could result from the increased lactic acid synthesis and lack of citric acid degradation recorded when the *L. plantarum* MLPK45H strain was used (Table S6).

The judges perceived the bitter taste component differently in the different trials of the two experimental groups. The trials including microbial consortia were less bitter than the CONT A1 and CONT A2 controls. Specifically, the CO3 and CO8 trials reported the lowest bitterness value compared to the CONT A1 and CON A2 controls (4.28 and 4.24 vs 6.08 and 6.11) and the different microbial consociations CO1, CO5 and CO6, CO10 (4.28 and 4.24 vs 4.65–4.68, and 4.58–4.71, respectively). This phenomenon could be attributed to the protease enzymes synthesized by the LAB, which could promote yeast autolysis

from the fermentation phase (de Andrade Bulos et al., 2023).

The panel judges detected clear differences in the flavor of the wines between the trials. Among the trials inoculated with different microbial combinations, the CO1 trial showed the highest values of flavor intensity and persistence (Table 5). However, no difference was felt with the CONT A1 and CONT A2 controls. For taste sensations, the CO1 trial was shown to be different from the CONT A1 and CONT A2 controls in complexity and for the pineapple descriptor (Table 5). The CO1 trial also differed from the other trials in aftertaste (8.56). Although the aftertaste (8.18) was higher among the sister trials, it did not differ from the CONT A1 control. The panel judges concluded that the CO1 trial (MLB6-NF213) had the best overall quality with the distinction of 20 sensory descriptors. This result was higher than the CONT A1 control (15 sensory descriptors) and significantly different from the homologous CO6 trial (MLB6-QA23).

3.6. Sensory profiles associated with volatile organic compounds

To emphasize the connections between aromatic attributes and OAV values, the principal component analysis was employed. The F1 and F2 values explained 45.13% and 28.22% of the total variance, respectively (Fig. 3c). This analysis allowed us to categorize the tests into two distinct macro-groups based on the *S. cerevisiae* strain used. The inclusion of LAB strains in the microbial association facilitated further differentiation into subgroups. A variance of 45.13% revealed a positive correlation between the subgroups CONT A1, CO1 and CONT A2 (Fig. 3c) with respect to olfactory intensity and persistence. These attributes were closely tied to the odor perceptions associated with tropical fruits and pineapple. These olfactory attributes identified by the jury showed a correspondence with the OAVs (Table 3). Specifically, the tests and the subgroups CONT A2, CONT A1 and CO1 displayed positive correlations with the higher alcohol 3-ethoxy-1-propanol, as well as the ethyl esters hexanoate and ethyl octanoate. These findings are consistent with those reported by Ugliano and Moio (2005) and Vilanova and Martínez (2007). Furthermore, the subgroups CONT A1, CO1 and CONT A2 demonstrated further positive correlations between OAV and olfactory sensory attributes. Floral perception, in particular, showed positive correlations with phenylethylacetate, hydroxyethylbenzene, decanoic acid and 3-methyl-1-butanol.

These results are in agreement with those of previous studies conducted by Cañas, Romero, Alonso, and Herreros (2008), Selli et al. (2004) and Ferreira et al. (2016). However, the attribute of olfactory complexity was positively correlated with the CO1 trial in accordance with the sensory analysis (Table 5). PCA analysis attributed this result to the favorable relationship between floral and fruity perceptions. In particular, 2,3-butanediol played a significant role in enhancing fruity notes. The olfactory perceptions of sweet fruitiness, banana, and pear were positively correlated with it (García-Carpintero et al., 2011 and Etievant, 1991). Consistent with prior studies by Liang et al. (2023) and Escudero et al. (2004), our findings underscore the importance of VOCs with OAV values between 0.1 and 1. However, no direct olfactory correspondence was observed between 3-methyl-1-butanol, hexanoic acid, and decanoic acid and the corresponding scents of solvent, rancid, and fatty notes. Surprisingly, PCA analysis revealed an inverse correlation between sensory attributes and VOCs. Consequently, the judges were unable to discriminate between the correlated scents, even though they were detected above the perception threshold. Notably, a distinct subgroup emerged, represented by the CO3 and CO5 tests (specifically, MLA4 -NF213 and MLPK45H-NF213) (Fig. 3c). The perceived odours associated with these tests (such as rancid, grease, butter, paint, solvent, etc.) did not correspond to the VOCs detected above the perception threshold. It is probable that in a mixture characterized by low olfactory threshold VOCs, ethanol, hexanoic acid, octanoic acid, medium-chain fatty acids and higher alcohols like 3-methyl-1-butanol and 3-methylsulfanyl-1-propanol play a role in defining specific synergy and antagonism relationships (Ferreira, 2012; Ferreira et al., 2016) or incorporation (Naselli et al., 2023). These compounds may not be detectable by the human nose. Although cream and butter odours were positively correlated with the CO3 and CO5 tests, no identification of 2,3-butanedione was observed either by judges or by gas chromatographic analysis. Nevertheless, the statistical PCA analysis showed the potential production of 2,3-butanedione by the MLA4 strain, consistent with findings observed by Celik et al. (2019). The CO6 and CO8-CO10 strains formed two subgroups associated with the use of *S. cerevisiae* QA23 strain in various consociations with LAB strains. Interestingly, these strains exhibited negative correlations with fruity and floral perceptions. Specifically, the variance in the CO6 and CO10 trials was explained by 45.13%, while the CO8 trial accounted for 28.22% of the variance.

4. Conclusions

The TAIndex was calculated to determine the effects of interactions between LAB and *S. cerevisiae* strains. It allowed to understand how LAB strains behave when associated with different *S. cerevisiae* strains. The main technological effects associated with the variability of the TAIndex were observed in the kinetics of malolactic fermentation. Marginal deviations of the TAIndex in microbial consortia using the same LAB indicated a lower influence of the *S. cerevisiae* strain on LAB degradation of malic acid. Conversely, significant variability in TAIndex values revealed different effects on the timing and pace of malolactic fermentation. Specifically, when applying the LAB MLB6 strain (*O. oeni*) in different combinations with NF213 and QA23 strains (*S. cerevisiae*), TAIndex values ranging from 0.009 to 0.148 t^{-1} resulted in different malic acid degradation times in the wine, 44 and 18 d, respectively. However, notable impacts on the active volatile component of wines were observed due to these variations. When TAIndex values approached zero (0.009–0.013) t^{-1}, the wines exhibited higher production of the ethyl esters such as ethyl octanoate and ethyl decanoate, but TAIndex values close to 0.02 (specifically 0.019) t^{-1} were associated with wines characterized by olfactory VOCs primarily attributable to acetate esters and higher alcohols. Interestingly, TAIndex values exceeding 0.148 showed a negative correlation with the key olfactory VOCs, suggesting that rapid malolactic fermentations led to reduced VOCs in the wines.

In conclusion, the wines produced from the Catarratto grape variety following malolactic fermentation exhibit an olfactory profile characterized by fruity and floral notes, contributing to their aromatic complexity. Notably, the buttery hints associated with 2,3-butanedione production were absent in the final Catarratto wines. This underscores the effectiveness of microbial association techniques as a biotechnological strategy for shaping wine aromas and ensuring microbial stability. Additionally, it reinforces the significant role of the malolactic process in the formation of fruity and floral aromas. Further investigations will explore non-targeted metabolomics approaches to examine the effects on VOCs highlighted by the TAIndex. Additionally, there is potential for integrating the TAIndex into artificial intelligence calculation software, allowing the prediction and diversification of wine aroma profiles based on consumer preferences.

CRediT authorship contribution statement

Vincenzo Naselli: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Formal analysis, Data curation. **Antonino Pirrone:** Validation, Methodology, Investigation, Formal analysis, Data curation. **Enrico Viola:** Visualization, Formal analysis, Data curation. **Valentina Craparo:** Formal analysis, Data curation. **Antonella Maggio:** Software, Methodology, Formal analysis, Data curation. **Venera Seminerio:** Methodology, Formal analysis, Data curation. **Giuseppe Notarbartolo:** Methodology, Formal analysis. **Sibylle Krieger-Weber:** Writing – original draft, Visualization, Validation, Resources, Methodology, Data curation. **Paola Vagnoli:** Visualization, Validation, Resources, Conceptualization. **Stéphanie**

Weidmann: Writing – original draft, Visualization, Supervision. **Raffaele Guzzon:** Validation, Methodology, Conceptualization. **Luca Settanni:** Writing – review & editing, Writing – original draft, Visualization, Validation. **Giancarlo Moschetti:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Conceptualization. **Nicola Francesca:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition. **Antonio Alfonzo:** Writing – review & editing, Writing – original draft, Supervision, Software, Investigation, Data curation.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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