

# Food Chemistry

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

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<b>Abstract:</b>	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 (VOC) of malolactic fermentation are linked to the interaction between yeast and lactic acid bacteria. The study investigated the interactions between <i>Lactiplantibacillus plantarum</i> or <i>Oenococcus oeni</i> with <i>Saccharomyces cerevisiae</i> by using the Technological Affinity Index (TAIndex). The co-inoculation of <i>L. plantarum</i> / <i>S. cerevisiae</i> resulted in a higher TAIndex than the co-inoculation of <i>O. oeni</i> / <i>S. cerevisiae</i> 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.
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DIPARTIMENTO SCIENZE AGRARIE,  
ALIMENTARI e FORESTALI

**SAAF**  
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SCIENZE  
AGRARIE  
ALIMENTARI  
FORESTALI

Palermo, 14/03/2023

Dear Editor,

We are pleased to submit our paper titled “Technological affinity index for interaction between lactic acid bacteria and *Saccharomyces cerevisiae* strains to modulate the fruity and floral aroma of Catarratto wines” for your consideration. Thank you for your time and consideration.

The aim of this study was to investigate the effects of interactions between lactic acid bacteria and *Saccharomyces cerevisiae* on volatile organic compounds with high olfactory impact. The Technological Affinity Index (TAIndex) was formulated to explain the effects of lactic acid bacteria-*S. cerevisiae* co-inoculation by relating the time and rate of malic acid degradation, duration of malolactic fermentation with the composition of VOCs. The microbiological, physicochemical, and sensory parameters, as well as the composition of volatile organic compounds, were analysed for the different wines. The results show that the interaction between lactic acid bacteria and *S. cerevisiae* is a strategy that can improve the sensory characteristics of wines. The rate and duration of malolactic fermentation influence the composition of volatile organic compounds and, consequently, the olfactory profiles of wine. Furthermore, the TAIndex can be used to plan the vinification of wines by enhancing their fruity and floral notes.

Currently, there is a lack of scientific research on these topics.  
Our research article may be of interest for Food Chemistry.  
The manuscript has been prepared following FC authors’ guidelines.

I hope the paper could be revised by FC reviewers.

With my best personal regards,  
Nicola Francesca

## Highlights

- Technological Affinity Index as a criterion for managing wine aromaticity
- The time and rate of malolactic fermentation are crucial to the wine aromas
- The degradation of malic acid depends on the yeast strains used in co-inoculation
- LAB-*Saccharomyces cerevisiae* co-inoculation influenced fruity and floral perceptions
- mid-chain fatty acids and 3-methyl-1-butanol influence floral/fruity perceptions

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.

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

Trial <sup>α</sup>	Microbic consociations <sup>β</sup>	TAIndex <sup>γ</sup>	m <sup>δ</sup>	R <sup>2</sup> <sup>ε</sup>	MLF days <sup>ζ</sup>
CO1	MLB6/NF213	0.009	- 0.0781	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	- 0.0292	0.9850	18
CO8	MLA4/QA23	0.019	- 0.0158	0.9339	68
CO10	MLPK45H/QA23	0.348	- 0.6600	0.8186	2

<sup>α</sup> Trials;<sup>β</sup> Microbic consociations: LAB strains – *S. cerevisiae* strains.<sup>γ</sup> TAIndex. Technological Affinity Index LAB – *S. cerevisiae*.<sup>δ</sup> 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).<sup>ε</sup> R<sup>2</sup>, coefficient of determination (derived from the equation of the straight lines shown in Fig. 2).<sup>ζ</sup> MLF days, days of malolactic fermentation.

**Table 2**

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

KI <sup>α</sup>	KI <sup>β</sup>	Compounds <sup>γ</sup>	CONT A1 <sup>δ</sup>	CO 1 <sup>δ</sup>	CO 3 <sup>δ</sup>	CO 5 <sup>δ</sup>	S.S. <sup>ε</sup>	→ continued
		<b>∑ Alcohols</b>	<b>121.74±4.53 <sup>a</sup></b>	<b>111.16±4.14 <sup>b</sup></b>	<b>128.09±4.76 <sup>a</sup></b>	<b>98.2±3.65 <sup>c</sup></b>	***	
758	759	3-methyl-1-butanol	55.56±1.30 <sup>a</sup>	44.37±1.04 <sup>c</sup>	50.28±1.18 <sup>b</sup>	42.55±1.00 <sup>c</sup>	***	
765	765	1,2,-propanediol	1.24±0.04 <sup>a</sup>	1.10±0.03 <sup>b</sup>	1.07±0.03 <sup>b</sup>	0.78±0.02 <sup>c</sup>	***	
809	809	2,3-butanediol <sup>ζ</sup>	20.22±0.63 <sup>c</sup>	22.9±0.71 <sup>b</sup>	27.66±0.86 <sup>a</sup>	16.68±0.52 <sup>d</sup>	***	
816	816	2,3-butanediol <sup>η</sup>	5.34±0.08 <sup>b</sup>	5.61±0.08 <sup>b</sup>	7.80±0.11 <sup>a</sup>	4.08±0.06 <sup>c</sup>	***	
824	824	2,3-butanediol <sup>θ</sup>	3.44±0.09 <sup>c</sup>	9.86±0.25 <sup>b</sup>	10.42±0.26 <sup>b</sup>	11.00±0.28 <sup>a</sup>	***	
848	848	3-ethoxy-1-propanol	0.28±0.01 <sup>c</sup>	0.65±0.01 <sup>a</sup>	0.29±0.01 <sup>b</sup>	0.29±0.01 <sup>b</sup>	***	
878	878	1-hexanol	0.52±0.01 <sup>b</sup>	0.49±0.01 <sup>c</sup>	0.56±0.01 <sup>a</sup>	0.39±0.01 <sup>d</sup>	***	
1038	1039	Phenyl methanol	0.20±0.01 <sup>a</sup>	0.18±0.01 <sup>b</sup>	0.19±0.01 <sup>ab</sup>	0.00±0.00 <sup>c</sup>	***	
1089	1088	1,2,3-propanetriol	1.43±0.06 <sup>a</sup>	0.00±0.00 <sup>c</sup>	0.96±0.04 <sup>b</sup>	1.44±0.06 <sup>a</sup>	***	
1116	1117	Hydroxyethylbenzene	27.04±0.90 <sup>a</sup>	21.79±0.72 <sup>b</sup>	26.87±0.89 <sup>a</sup>	20.59±0.68 <sup>b</sup>	***	
1428	1428	4-(2-hydroxyethyl)-phenol	1.79±0.06 <sup>a</sup>	0.85±0.03 <sup>c</sup>	1.59±0.05 <sup>b</sup>	0.00±0.00 <sup>d</sup>	***	
1502	1503	2,4-di-tert-butylphenol	4.68±0.10 <sup>a</sup>	3.36±0.07 <sup>b</sup>	0.4±0.01 <sup>c</sup>	0.40±0.01 <sup>c</sup>	***	
		<b>∑ Aldehydes</b>	<b>2.03±0.07 <sup>a</sup></b>	<b>0.93±0.03 <sup>c</sup></b>	<b>0.26±0.01 <sup>d</sup></b>	<b>1.59±0.04 <sup>b</sup></b>	***	
1105	1105	Nonanal	1.01±0.03 <sup>a</sup>	0.60±0.02 <sup>b</sup>	0.08±0.01 <sup>c</sup>	0.00±0.00 <sup>d</sup>	***	
1211	1211	3,4-dimethylbenzaldehyde	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.49±0.0 <sup>a</sup>	***	
1271	-	4-propyl benzaldehyde	1.02 ±0.04 <sup>a</sup>	0.33±0.0 <sup>b</sup>	0.18±0.0 <sup>c</sup>	0.00±0.00 <sup>d</sup>	***	
1811	1812	Hexadecanal	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.33±0.01 <sup>a</sup>	***	
-	2020	Octadecanal	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.77±0.02 <sup>a</sup>	***	
		<b>∑ Carboxylic acids</b>	<b>8.05±0.27 <sup>bc</sup></b>	<b>7.92±0.24 <sup>c</sup></b>	<b>8.89±0.24 <sup>b</sup></b>	<b>11.08±0.41 <sup>a</sup></b>	***	
914	916	4-hydroxybutanoic acid	0.24±0.01 <sup>c</sup>	0.38±0.01 <sup>b</sup>	0.66±0.02 <sup>a</sup>	0.71±0.03 <sup>a</sup>	***	
928	932	Lactic acid	0.00±0.00 <sup>b</sup>	1.47±0.04 <sup>a</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	***	
1013	1016	Hexanoic acid	2.78±0.12 <sup>a</sup>	2.15±0.09 <sup>b</sup>	1.54±0.06 <sup>c</sup>	2.05±0.09 <sup>b</sup>	***	
1189	1188	Octanoic acid	1.93±0.08 <sup>b</sup>	0.86±0.04 <sup>c</sup>	1.82±0.07 <sup>b</sup>	5.83±0.24 <sup>a</sup>	***	
1377	1377	Decanoic acid	3.10±0.06 <sup>b</sup>	3.06±0.06 <sup>b</sup>	4.87±0.09 <sup>a</sup>	2.49±0.05 <sup>c</sup>	***	
		<b>∑ Esters</b>	<b>14.84±0.46 <sup>a</sup></b>	<b>11.73±0.37 <sup>b</sup></b>	<b>13.87±0.43 <sup>a</sup></b>	<b>9.31±0.57 <sup>c</sup></b>	***	
889	884	3-methyl-1-butyl acetate	2.78±0.09 <sup>a</sup>	2.16±0.07 <sup>b</sup>	2.18±0.07 <sup>b</sup>	0.92±0.03 <sup>c</sup>	***	
885	886	2-methyl-1-butyl acetate	0.14±0.01 <sup>a</sup>	0.13±0.01 <sup>b</sup>	0.08±0.01 <sup>c</sup>	0.00±0.00 <sup>d</sup>	***	
941	941	Ethyl 3-hydroxybutanoate	0.12±0.03 <sup>a</sup>	0.18±0.05 <sup>a</sup>	0.14±0.04 <sup>a</sup>	0.13±0.04 <sup>a</sup>	**	
1000	1001	Ethyl hexanoate	1.62±0.04 <sup>a</sup>	0.91±0.02 <sup>d</sup>	1.20±0.03 <sup>c</sup>	1.32±0.03 <sup>b</sup>	***	
1181	1181	Diethyl succinate	0.41±0.01 <sup>b</sup>	0.30±0.01 <sup>c</sup>	0.48±0.01 <sup>a</sup>	0.50±0.02 <sup>a</sup>	***	
1195	1196	Ethyl octanoate	3.16±0.09 <sup>a</sup>	3.22±0.09 <sup>a</sup>	2.25±0.07 <sup>b</sup>	2.05±0.06 <sup>c</sup>	***	
1206	1205	Monoethyl succinate	4.92±0.19 <sup>a</sup>	2.78±0.11 <sup>b</sup>	5.46±0.21 <sup>a</sup>	2.50±0.10 <sup>b</sup>	***	
1253	1253	Phenylethyl acetate	0.62±0.02 <sup>a</sup>	0.53±0.02 <sup>b</sup>	0.60±0.02 <sup>a</sup>	0.54±0.02 <sup>b</sup>	***	
1264	1264	Diethyl malate	0.23±0.01 <sup>b</sup>	0.28±0.01 <sup>a</sup>	0.14±0.01 <sup>c</sup>	0.07±0.01 <sup>d</sup>	***	
1390	1392	Ethyl decanoate	0.84±0.04 <sup>b</sup>	1.24±0.05 <sup>a</sup>	0.70±0.03 <sup>c</sup>	0.71±0.03 <sup>c</sup>	***	
1590	1590	Ethyl dodecanoate	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>c</sup>	0.64±0.03 <sup>a</sup>	0.57±0.23 <sup>b</sup>	***	
		<b>∑ Ketones</b>	<b>1.08±0.03 <sup>b</sup></b>	<b>1.08±0.03 <sup>b</sup></b>	<b>1.99±0.05 <sup>a</sup></b>	<b>0.76±0.02 <sup>c</sup></b>	***	
723	722	3-hydroxy-2-butanone	0.13±0.01 <sup>c</sup>	0.19±0.01 <sup>b</sup>	0.96±0.03 <sup>a</sup>	0.09±0.01 <sup>c</sup>	***	
963	954	4-hydroxy-2-butanone	0.95±0.02 <sup>b</sup>	0.89±0.02 <sup>c</sup>	1.03±0.02 <sup>a</sup>	0.67±0.01 <sup>d</sup>	***	
		<b>∑ Anhydrides</b>	<b>0.79±0.02 <sup>b</sup></b>	<b>0.63±0.01 <sup>d</sup></b>	<b>1.03±0.02 <sup>a</sup></b>	<b>0.69±0.02 <sup>c</sup></b>	***	
993	994	Glutaconic anhydride	0.79±0.02 <sup>b</sup>	0.63±0.01 <sup>d</sup>	1.03±0.02 <sup>a</sup>	0.69±0.02 <sup>c</sup>	***	
		<b>∑ Others</b>	<b>3.05±0.10 <sup>a</sup></b>	<b>2.91±0.09 <sup>a</sup></b>	<b>0.00±0.00 <sup>b</sup></b>	<b>0.00±0.00 <sup>b</sup></b>	***	
1246	1245	1,3-di-tert-butylbenzene	3.05±0.10 <sup>a</sup>	2.91±0.09 <sup>a</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	***	

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

P &lt; 0.05; \*\*, P &lt; 0.01; \*\*\*, P &lt; 0.001; n.s., not significant.

<sup>ζ</sup> unidentified stereoisomer<sup>η</sup> unidentified stereoisomer<sup>θ</sup> unidentified stereoisomer

→ continued	KI <sup>α</sup>	KI <sup>β</sup>	Compounds <sup>γ</sup>	CONT A2 <sup>δ</sup>	CO 6 <sup>δ</sup>	CO 8 <sup>δ</sup>	CO 10 <sup>δ</sup>	S.S. <sup>ε</sup>
			<b>∑ Alcohols</b>	<b>147.31±3.45 <sup>a</sup></b>	<b>100.14±2.31 <sup>c</sup></b>	<b>110.79±2.44 <sup>b</sup></b>	<b>86.82±1.95 <sup>d</sup></b>	<b>***</b>
	758	759	3-methyl-1-butanol	61.54±1.62 <sup>a</sup>	39.4±1.04 <sup>c</sup>	51.8±1.36 <sup>b</sup>	33.40±0.88 <sup>d</sup>	<b>***</b>
	765	765	1,2.-propanediol	1.72±0.06 <sup>a</sup>	1.19±0.04 <sup>b</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>c</sup>	<b>***</b>
	809	809	2,3-butanediol <sup>ζ</sup>	38.01±0.84 <sup>a</sup>	22.44±0.50 <sup>b</sup>	13.94±0.31 <sup>d</sup>	19.74±0.44 <sup>c</sup>	<b>***</b>
	816	816	2,3-butanediol <sup>η</sup>	8.83±0.32 <sup>a</sup>	5.98±0.22 <sup>b</sup>	3.06±0.11 <sup>d</sup>	4.54±0.16 <sup>c</sup>	<b>***</b>
	824	824	2,3-butanediol <sup>θ</sup>	3.37±0.05 <sup>d</sup>	11.24±0.16 <sup>b</sup>	14.01±0.20 <sup>a</sup>	9.17±0.13 <sup>c</sup>	<b>***</b>
	848	848	3-ethoxy-1-propanol	1.15±0.04 <sup>a</sup>	0.41±0.02 <sup>c</sup>	0.64±0.02 <sup>b</sup>	0.35±0.01 <sup>c</sup>	<b>***</b>
	878	878	1-hexanol	0.76±0.02 <sup>a</sup>	0.37±0.01 <sup>c</sup>	0.53±0.01 <sup>b</sup>	0.36±0.01 <sup>c</sup>	<b>***</b>
	983	985	3-methylsulfanyl-1-propanol	1.56±0.04 <sup>a</sup>	0.76±0.02 <sup>c</sup>	0.95±0.03 <sup>b</sup>	0.68±0.02 <sup>c</sup>	<b>***</b>
	1089	1088	Phenyl methanol	0.00±0.00 <sup>d</sup>	0.07±0.01 <sup>c</sup>	0.37±0.01 <sup>a</sup>	0.18±0.01 <sup>b</sup>	<b>***</b>
	1116	1117	Hydroxyethylbenzene	26.42±0.38 <sup>a</sup>	15.98±0.23 <sup>c</sup>	22.54±0.32 <sup>b</sup>	15.58±0.22 <sup>c</sup>	<b>***</b>
	1305	1315	2-methoxy-4-vinyl phenol	1.86±0.05 <sup>a</sup>	0.82±0.02 <sup>c</sup>	1.16±0.03 <sup>b</sup>	1.83±0.05 <sup>a</sup>	<b>***</b>
	1428	1428	4-(2-hydroxyethyl)-phenol	2.09±0.05 <sup>a</sup>	1.15±0.03 <sup>c</sup>	1.27±0.03 <sup>b</sup>	0.63±0.01 <sup>d</sup>	<b>***</b>
	1502	1503	2,4-di-tert- butylphenol	0.00±0.00 <sup>d</sup>	0.33±0.01 <sup>c</sup>	0.52±0.01 <sup>a</sup>	0.36±0.01 <sup>b</sup>	<b>***</b>
			<b>∑ Aldehydes</b>	<b>1.80±0.06 <sup>b</sup></b>	<b>1.49±0.05 <sup>c</sup></b>	<b>3.11±0.10 <sup>a</sup></b>	<b>1.87±0.06 <sup>b</sup></b>	<b>***</b>
	1083	1079	4-methylbenzaldehyde	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>c</sup>	0.49±0.02 <sup>a</sup>	0.36±0.01 <sup>b</sup>	<b>***</b>
	1211	1211	3,4-dimethylbenzaldehyde	0.00±0.00 <sup>d</sup>	0.45±0.01 <sup>b</sup>	0.53±0.02 <sup>a</sup>	0.40±0.01 <sup>c</sup>	<b>***</b>
	1271	-	4-propyl benzaldehyde	1.80±0.06 <sup>a</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	<b>***</b>
	1811	1812	Hexadecanal	0.00±0.00 <sup>c</sup>	0.34±0.01 <sup>b</sup>	0.66±0.01 <sup>a</sup>	0.35±0.01 <sup>b</sup>	<b>***</b>
	-	2020	Octadecanal	0.00±0.00 <sup>d</sup>	0.70±0.02 <sup>c</sup>	1.43±0.03 <sup>a</sup>	0.76±0.02 <sup>b</sup>	<b>***</b>
			<b>∑ Carboxylic acids</b>	<b>16.11±0.20 <sup>a</sup></b>	<b>9.49±0.12 <sup>a</sup></b>	<b>11.91±0.15 <sup>b</sup></b>	<b>9.53±0.12 <sup>b</sup></b>	<b>***</b>
	914	916	4-hydroxybutanoic acid	0.40±0.01 <sup>b</sup>	0.45±0.01 <sup>a</sup>	0.40±0.01 <sup>b</sup>	0.32±0.01 <sup>c</sup>	<b>***</b>
	928	932	Lactic acid	0.00±0.00 <sup>c</sup>	0.93±0.04 <sup>b</sup>	0.00±0.00 <sup>c</sup>	1.09±0.04 <sup>a</sup>	<b>***</b>
	1013	1016	Hexanoic acid	5.26±0.19 <sup>a</sup>	2.73±0.10 <sup>d</sup>	4.63±0.17 <sup>b</sup>	3.33±0.12 <sup>c</sup>	<b>***</b>
	1189	1188	Octanoic acid	3.09±0.09 <sup>a</sup>	2.59±0.08 <sup>b</sup>	2.97±0.09 <sup>a</sup>	2.29±0.07 <sup>c</sup>	<b>***</b>
	1377	1377	Decanoic acid	7.36±0.31 <sup>a</sup>	2.79±0.12 <sup>c</sup>	3.91±0.17 <sup>b</sup>	2.50±0.11 <sup>c</sup>	<b>***</b>
			<b>∑ Esters</b>	<b>18.38±0.48 <sup>a</sup></b>	<b>8.42±0.20 <sup>c</sup></b>	<b>13.91±0.38 <sup>b</sup></b>	<b>7.57±0.20 <sup>c</sup></b>	<b>***</b>
	883	884	3-methyl-1-butyl acetate	2.76±0.05 <sup>b</sup>	2.12±0.04 <sup>c</sup>	3.08±0.06 <sup>a</sup>	1.72±0.03 <sup>d</sup>	<b>***</b>
	885	886	2-methyl-1-butyl acetate	0.00±0.00 <sup>d</sup>	0.12±0.01 <sup>a</sup>	0.10±0.01 <sup>b</sup>	0.06±0.01 <sup>c</sup>	<b>***</b>
	941	941	Ethyl 3-hydroxybutanoate	0.14±0.01 <sup>a</sup>	0.15±0.01 <sup>a</sup>	0.15±0.01 <sup>a</sup>	0.15±0.01 <sup>a</sup>	<b>***</b>
	1000	1001	Ethyl hexanoate	1.84±0.03 <sup>a</sup>	1.13±0.02 <sup>c</sup>	1.58±0.03 <sup>b</sup>	1.18±0.02 <sup>c</sup>	<b>***</b>
	1181	1181	Diethyl succinate	0.59±0.02 <sup>a</sup>	0.40±0.01 <sup>b</sup>	0.38±0.01 <sup>b</sup>	0.41±0.01 <sup>b</sup>	<b>***</b>
	1195	1196	Ethyl octanoate	3.69±0.09 <sup>a</sup>	1.91±0.04 <sup>b</sup>	2.01±0.05 <sup>b</sup>	1.69±0.04 <sup>c</sup>	<b>***</b>
	1206	1205	Monoethyl succinate	6.04±0.19 <sup>a</sup>	1.60±0.05 <sup>c</sup>	4.91±0.15 <sup>b</sup>	1.38±0.04 <sup>c</sup>	<b>***</b>
	1253	1253	Phenylethyl acetate	0.58±0.01 <sup>a</sup>	0.44±0.01 <sup>b</sup>	0.59±0.01 <sup>a</sup>	0.41±0.01 <sup>c</sup>	<b>***</b>
	1264	1264	Diethyl malate	0.42±0.02 <sup>a</sup>	0.00±0.00 <sup>c</sup>	0.12±0.01 <sup>b</sup>	0.00±0.00 <sup>c</sup>	<b>***</b>
	1294	-	Ethyl decanoate	1.30±0.03 <sup>a</sup>	0.55±0.01 <sup>c</sup>	0.73±0.01 <sup>b</sup>	0.45±0.01 <sup>d</sup>	<b>***</b>
	1390	1392	Ethyl dodecanoate	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>c</sup>	0.15±0.02 <sup>a</sup>	0.07±0.01 <sup>b</sup>	<b>***</b>
	1590	1590	Ethyl 5-oxo-2-pyrrolidine-carboxylate	1.02±0.03 <sup>a</sup>	0.00±0.00 <sup>c</sup>	0.15±0.01 <sup>b</sup>	0.05±0.01 <sup>c</sup>	<b>***</b>
			<b>∑ Ketones</b>	<b>1.62±0.04 <sup>a</sup></b>	<b>0.76±0.03 <sup>d</sup></b>	<b>1.23±0.03 <sup>b</sup></b>	<b>0.91±0.03 <sup>c</sup></b>	<b>***</b>
	723	722	3-hydroxy-2-butanone	0.27±0.01 <sup>b</sup>	0.07±0.01 <sup>d</sup>	0.33±0.01 <sup>a</sup>	0.21±0.01 <sup>c</sup>	<b>***</b>
	963	954	4-hydroxy-2-butanone	1.35±0.03 <sup>a</sup>	0.69±0.02 <sup>b</sup>	0.90±0.02 <sup>b</sup>	0.70±0.02 <sup>c</sup>	<b>***</b>
			<b>∑ Others</b>	<b>2.26±0.07 <sup>a</sup></b>	<b>0.00±0.00 <sup>b</sup></b>	<b>0.00±0.00 <sup>b</sup></b>	<b>0.00±0.00 <sup>b</sup></b>	<b>***</b>
	1246	1245	1,3-di-tert-butylbenzene	2.26±0.07 <sup>a</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	<b>***</b>

<sup>α</sup> Kovats index obtained through the modulated chromatogram reported for DB-5 MS apolar column;

<sup>β</sup> Kovats index based on literature (<https://webbook.nist.gov/>);

<sup>γ</sup> compounds are classified in order of Kovats index;

<sup>δ</sup> Relative amounts expressed as mg/L with respect to calibration curves of ethyl lactate, 3-hydroxy-2-butanone, 2,3-butanediol;

<sup>ε</sup> 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.

<sup>ζ</sup> unidentified stereoisomer

<sup>η</sup> unidentified stereoisomer

<sup>θ</sup> unidentified stereoisomer

**Table 3**

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

Compounds <sup>α</sup>	Aroma description <sup>β</sup>	Aromatic Series <sup>γ</sup>	Reference Aromatic Series <sup>δ</sup>	Odor threshold <sup>ε</sup>	Reference Odor threshold <sup>ζ</sup>	CONT A1 <sup>η</sup>	CO 1 <sup>η</sup>	CO 3 <sup>η</sup>	CO 5 <sup>η</sup>	S.S. <sup>θ</sup>	CONT A2 <sup>η</sup>	CO 6 <sup>η</sup>	CO 8 <sup>η</sup>	CO 10 <sup>η</sup>	S.S. <sup>ι</sup>
3-methyl-1-butanol	Fusel	4	[1]	40	[2]	1.39±0.03 <sup>a</sup>	1.11±0.03 <sup>c</sup>	1.26±0.03 <sup>b</sup>	1.06±0.03 <sup>c</sup>	***	1.54±0.04 <sup>a</sup>	0.99±0.03 <sup>c</sup>	1.30±0.03 <sup>b</sup>	0.84±0.02 <sup>d</sup>	***
3-ethoxy-1-propanol	Fruity	1	[1]	0.1	[3]	2.80±0.10 <sup>b</sup>	6.50±0.10 <sup>a</sup>	2.90±0.10 <sup>b</sup>	2.90±0.10 <sup>b</sup>	***	11.50±0.40 <sup>a</sup>	4.10±0.20 <sup>c</sup>	6.40±0.20 <sup>b</sup>	3.50±0.10 <sup>c</sup>	***
3-methylsulfanyl-1-propanol	Raw potato, Garlic	5	[4]	0.5	[5,6]	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	***	3.12±0.08 <sup>a</sup>	1.52±0.04 <sup>b</sup>	1.90±0.06 <sup>c</sup>	1.36±0.04 <sup>c</sup>	***
Hydroxyethylbenzene	Rose	2	[7,8]	10	[7,8]	2.70±0.09 <sup>a</sup>	2.18±0.07 <sup>b</sup>	2.69±0.09 <sup>a</sup>	2.06±0.07 <sup>b</sup>	***	2.64±0.04 <sup>a</sup>	1.60±0.02 <sup>c</sup>	2.25±0.03 <sup>b</sup>	1.56±0.02 <sup>c</sup>	***
Hexanoic acid	Cheese Fatty	3	[9]	0.4	[3,10]	6.62±0.29 <sup>a</sup>	5.12±0.21 <sup>b</sup>	3.67±0.14 <sup>b</sup>	4.88±0.21 <sup>c</sup>	***	12.52±0.45 <sup>a</sup>	6.50±0.24 <sup>d</sup>	11.02±0.40 <sup>b</sup>	7.92±0.29 <sup>c</sup>	***
Octanoic acid	Rancid, Cheese, Fatty	3	[9]	0.5	[7,8]	3.86±0.16 <sup>c</sup>	1.72±0.08 <sup>b</sup>	3.64±0.14 <sup>b</sup>	11.66±0.48 <sup>a</sup>	***	6.18±0.18 <sup>a</sup>	5.18±0.16 <sup>b</sup>	5.94±0.18 <sup>a</sup>	4.58±0.14 <sup>c</sup>	***
Decanoic acid	Fatty Rancid	3	[9]	1	[11]	3.10±0.06 <sup>b</sup>	3.06±0.06 <sup>b</sup>	4.87±0.09 <sup>a</sup>	2.49±0.05 <sup>c</sup>	***	7.36±0.31 <sup>a</sup>	2.79±0.12 <sup>c</sup>	3.91±0.17 <sup>b</sup>	2.50±0.11 <sup>c</sup>	***
3-methyl-1-butyl acetate	Banana	1	[12]	0.03	[7,8]	92.67±3.00 <sup>a</sup>	72.00±2.33 <sup>b</sup>	72.67±2.33 <sup>b</sup>	30.66±1.00 <sup>c</sup>	***	92.00±1.67 <sup>b</sup>	70.67±1.33 <sup>c</sup>	102.67±2.00 <sup>a</sup>	57.33±1.00 <sup>d</sup>	***
Ethyl hexanoate	Apple, Banana	1	[12]	0.005	[7,8]	324.00±8.00 <sup>a</sup>	182.00±4.00 <sup>d</sup>	240.00±6.00 <sup>c</sup>	264.00±6.00 <sup>b</sup>	***	368.00±6.00 <sup>a</sup>	226.00±4.00 <sup>c</sup>	316.00±6.00 <sup>b</sup>	236.00±4.00 <sup>c</sup>	***
Ethyl octanoate	Pineapple, Pear	1	[12]	0.002	[7,8]	1580.00±45.00 <sup>a</sup>	1610.00±45.00 <sup>a</sup>	1125.00±35.00 <sup>b</sup>	1025.00±30.00 <sup>b</sup>	***	1845.00±45.00 <sup>a</sup>	955.00±20.00 <sup>b</sup>	1005.00±25.00 <sup>b</sup>	845.00±20.00 <sup>c</sup>	***
Phenylethyl acetate	Rosa, Floreal	2	[8]	0.25	[8]	2.48±0.08 <sup>a</sup>	2.12±0.08 <sup>b</sup>	2.40±0.08 <sup>a</sup>	2.16±0.08 <sup>b</sup>	***	2.32±0.04 <sup>a</sup>	1.76±0.04 <sup>b</sup>	2.36±0.04 <sup>a</sup>	1.64±0.04 <sup>c</sup>	***
Ethyl decanoate	Floreal	2	[3]	0.20	[3]	4.20±0.20 <sup>b</sup>	6.20±0.25 <sup>a</sup>	3.50±0.15 <sup>c</sup>	3.55±0.15 <sup>c</sup>	***	6.50±0.15 <sup>a</sup>	2.75±0.05 <sup>c</sup>	3.65±0.05 <sup>b</sup>	2.25±0.05 <sup>d</sup>	***
Olfactory intensity						2023.82±57.01 <sup>a</sup>	1892.01±52.22 <sup>a</sup>	1462.59 ±44.15 <sup>b</sup>	1350.43±38.17 <sup>b</sup>	***	2358.68±54.36 <sup>a</sup>	1278.85±26.23 <sup>c</sup>	1462.40±34.17 <sup>b</sup>	1164.48±25.81 <sup>d</sup>	***

<sup>α</sup> Compounds with OAV > 1<sup>β</sup> Aroma description<sup>γ</sup> Aromatic series, 1: fruity; 2: floral; 3: fatty; 4: solvent; 5: sulfurous.<sup>δ</sup> Reference Aromatic Series: <sup>[1]</sup> Butkhup et al., 2011; <sup>[3]</sup> Kelebek et al., 2011; <sup>[4]</sup> Celik et al., 2019; <sup>[7]</sup> Selli et al., 2004; <sup>[8]</sup> Izquierdo-Cañas et al., 2008; <sup>[9]</sup> Cai et al., 2014; <sup>[12]</sup> Bayram et al., 2018;<sup>ε</sup> Odor threshold (mg/L)<sup>ζ</sup> Reference Odor threshold: <sup>[2]</sup> Herrero et al., 1999; <sup>[3]</sup> Kelebek et al., 2011; <sup>[5]</sup> Krieger-Weber et al., 2015; <sup>[6]</sup> Davis et al., 1985; <sup>[7]</sup> Selli et al., 2004; <sup>[8]</sup> Izquierdo-Cañas et al., 2008; <sup>[10]</sup> Moio et al., 1995;<sup>[11]</sup> Delequis et al., 2000.<sup>η</sup> Relative amounts expressed in OAV (odor activity value)<sup>θ</sup> 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.<sup>ι</sup> 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.

**Table 4**

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

Aroma series <sup>α</sup>	CONT A1 <sup>β</sup>	CO 1 <sup>β</sup>	CO 3 <sup>β</sup>	CO 5 <sup>β</sup>	S.S. <sup>γ</sup>	CONT A2 <sup>β</sup>	CO 6 <sup>β</sup>	CO 8 <sup>β</sup>	CO 10 <sup>β</sup>	S.S. <sup>δ</sup>
Fruity	1999.46 ± 56.10 <sup>a</sup>	1870.50 ± 51.43 <sup>a</sup>	1440.56 ± 43.43 <sup>b</sup>	1322.56 ± 37.10 <sup>b</sup>	***	2316.50 ± 53.07 <sup>a</sup>	1255.77 ± 25.53 <sup>c</sup>	1430.07 ± 33.20 <sup>b</sup>	1141.83 ± 25.10 <sup>d</sup>	***
Floreal	9.38 ± 0.10 <sup>b</sup>	10.49 ± 0.40 <sup>a</sup>	8.58 ± 0.32 <sup>c</sup>	7.77 ± 0.30 <sup>d</sup>	***	11.46 ± 0.23 <sup>a</sup>	6.11 ± 0.11 <sup>c</sup>	8.26 ± 0.12 <sup>b</sup>	5.45 ± 0.11 <sup>d</sup>	***
Fatty	13.58 ± 0.51 <sup>b</sup>	9.90 ± 0.35 <sup>c</sup>	12.18 ± 0.37 <sup>b</sup>	19.03 ± 0.74 <sup>a</sup>	***	26.06 ± 0.94 <sup>a</sup>	14.47 ± 0.52 <sup>c</sup>	20.87 ± 0.75 <sup>b</sup>	15.01 ± 0.53 <sup>c</sup>	***
Solvent	1.39 ± 0.03 <sup>a</sup>	1.11 ± 0.03 <sup>c</sup>	1.26 ± 0.03 <sup>b</sup>	1.06 ± 0.03 <sup>c</sup>	***	1.54 ± 0.04 <sup>a</sup>	0.99 ± 0.03 <sup>c</sup>	1.30 ± 0.03 <sup>b</sup>	0.84 ± 0.02 <sup>d</sup>	***
Sulfurous	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	***	3.12 ± 0.08 <sup>a</sup>	1.52 ± 0.04 <sup>c</sup>	1.90 ± 0.06 <sup>b</sup>	1.36 ± 0.04 <sup>c</sup>	***

<sup>α</sup> Aroma series

<sup>β</sup> Aroma profile calculated by summing and of the odorous activity values (OAV) of the aromatic series from Table 3

<sup>γ</sup> Statistical significance among CONT A1, CO1, CO3, CO5 trials

<sup>δ</sup> Statistical significance among CONT A2, CO6, CO8, CO10 trials

**Table 5**

Sensory score for experimental Catarratto wines.

Attributes <sup>α</sup>		Trials								Statistical <sup>γ</sup> Significance		
		CONT A1 <sup>β</sup>	CO1 <sup>β</sup>	CO3 <sup>β</sup>	CO5 <sup>β</sup>	CONT A2 <sup>β</sup>	CO6 <sup>β</sup>	CO8 <sup>β</sup>	CO10 <sup>β</sup>	Judge	Wine	
Apparence	Yellow colour	7.26 ± 0.02 <sup>bc</sup>	7.21 ± 0.02 <sup>cd</sup>	7.41 ± 0.02 <sup>a</sup>	7.37 ± 0.02 <sup>a</sup>	7.12 ± 0.02 <sup>e</sup>	7.21 ± 0.02 <sup>cd</sup>	7.18 ± 0.02 <sup>d</sup>	7.28 ± 0.02 <sup>b</sup>	***	***	
	Green reflexes	6.14 ± 0.11 <sup>b</sup>	6.71 ± 0.12 <sup>a</sup>	6.31 ± 0.11 <sup>b</sup>	6.24 ± 0.11 <sup>b</sup>	6.27 ± 0.11 <sup>b</sup>	6.23 ± 0.11 <sup>b</sup>	6.16 ± 0.11 <sup>b</sup>	6.48 ± 0.12 <sup>ab</sup>	***	***	
	Green apple	7.70 ± 0.22 <sup>a</sup>	6.27 ± 0.18 <sup>cd</sup>	6.80 ± 0.19 <sup>bc</sup>	6.87 ± 0.19 <sup>b</sup>	7.83 ± 0.23 <sup>a</sup>	6.11 ± 0.17 <sup>d</sup>	6.18 ± 0.17 <sup>d</sup>	6.03 ± 0.17 <sup>d</sup>	***	***	
	Banana	6.61 ± 0.14 <sup>ab</sup>	6.10 ± 0.13 <sup>c</sup>	6.18 ± 0.13 <sup>bc</sup>	4.81 ± 0.10 <sup>d</sup>	6.62 ± 0.14 <sup>a</sup>	6.12 ± 0.13 <sup>c</sup>	6.97 ± 0.15 <sup>a</sup>	5.10 ± 0.11 <sup>d</sup>	***	***	
	Citrus	3.27 ± 0.01 <sup>b</sup>	3.12 ± 0.01 <sup>e</sup>	3.23 ± 0.01 <sup>c</sup>	3.71 ± 0.01 <sup>a</sup>	3.30 ± 0.01 <sup>b</sup>	3.18 ± 0.01 <sup>d</sup>	3.21 ± 0.01 <sup>c</sup>	3.00 ± 0.01 <sup>f</sup>	***	***	
	Fatty	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	***	*
	Floral	6.80 ± 0.08 <sup>b</sup>	7.12 ± 0.09 <sup>a</sup>	6.76 ± 0.08 <sup>b</sup>	6.46 ± 0.08 <sup>bc</sup>	7.31 ± 0.09 <sup>a</sup>	6.01 ± 0.07 <sup>d</sup>	6.61 ± 0.08 <sup>bc</sup>	5.83 ± 0.07 <sup>d</sup>	***	***	
	Fruity	8.12 ± 0.15 <sup>b</sup>	8.61 ± 0.15 <sup>a</sup>	7.30 ± 0.13 <sup>c</sup>	6.84 ± 0.12 <sup>d</sup>	8.18 ± 0.15 <sup>b</sup>	7.18 ± 0.18 <sup>cd</sup>	7.13 ± 0.13 <sup>cd</sup>	7.21 ± 0.13 <sup>cd</sup>	***	***	
	Tropical	8.25 ± 0.12 <sup>a</sup>	8.31 ± 0.12 <sup>a</sup>	7.10 ± 0.10 <sup>b</sup>	6.35 ± 0.09 <sup>c</sup>	8.36 ± 0.12 <sup>a</sup>	6.12 ± 0.09 <sup>cd</sup>	6.38 ± 0.09 <sup>c</sup>	6.01 ± 0.09 <sup>d</sup>	***	***	
	O-Intensity	8.34 ± 0.22 <sup>a</sup>	8.69 ± 0.22 <sup>a</sup>	7.00 ± 0.18 <sup>b</sup>	6.68 ± 0.17 <sup>bc</sup>	8.42 ± 0.23 <sup>a</sup>	6.51 ± 0.17 <sup>bc</sup>	7.00 ± 0.18 <sup>b</sup>	6.23 ± 0.15 <sup>c</sup>	***	***	
Odour	Pear	6.58 ± 0.17 <sup>ab</sup>	6.08 ± 0.15 <sup>b</sup>	6.12 ± 0.16 <sup>b</sup>	5.21 ± 0.13 <sup>c</sup>	6.42 ± 0.16 <sup>ab</sup>	6.11 ± 0.16 <sup>b</sup>	6.74 ± 0.17 <sup>a</sup>	5.20 ± 0.13 <sup>c</sup>	***	***	
	O-Persistence	7.49 ± 0.15 <sup>a</sup>	7.61 ± 0.15 <sup>a</sup>	6.54 ± 0.13 <sup>b</sup>	6.36 ± 0.12 <sup>b</sup>	7.64 ± 0.15 <sup>a</sup>	6.54 ± 0.12 <sup>b</sup>	6.38 ± 0.12 <sup>b</sup>	6.37 ± 0.12 <sup>b</sup>	***	***	
	Pineapple	8.20 ± 0.02 <sup>c</sup>	8.40 ± 0.02 <sup>b</sup>	6.20 ± 0.02 <sup>h</sup>	6.32 ± 0.02 <sup>g</sup>	8.60 ± 0.02 <sup>a</sup>	6.62 ± 0.02 <sup>f</sup>	7.48 ± 0.02 <sup>d</sup>	6.68 ± 0.02 <sup>e</sup>	***	***	
	Sweet fruit	6.59 ± 0.12 <sup>ab</sup>	6.09 ± 0.11 <sup>b</sup>	6.15 ± 0.11 <sup>c</sup>	5.01 ± 0.09 <sup>d</sup>	6.52 ± 0.12 <sup>b</sup>	6.12 ± 0.11 <sup>c</sup>	6.86 ± 0.12 <sup>a</sup>	5.15 ± 0.09 <sup>d</sup>	***	*	
	Solvent	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	***	*	
	Garlic	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	***	*	
	Raw potato	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	***	*	
	Cream	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	***	*	
	Butter	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	***	*	
	Rancid	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	***	*	
Taste	Varnish	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	***	*	
	Vegetable	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	***	*	
	O-complexity	7.21 ± 0.18 <sup>b</sup>	7.66 ± 0.19 <sup>a</sup>	6.23 ± 0.16 <sup>c</sup>	6.41 ± 0.18 <sup>c</sup>	7.18 ± 0.16 <sup>b</sup>	6.43 ± 0.16 <sup>c</sup>	6.27 ± 0.16 <sup>c</sup>	6.31 ± 0.16 <sup>c</sup>	***	***	
	Sweet	5.36 ± 0.11 <sup>d</sup>	6.12 ± 0.12 <sup>a</sup>	6.18 ± 0.12 <sup>a</sup>	5.63 ± 0.11 <sup>cd</sup>	5.42 ± 0.11 <sup>d</sup>	6.01 ± 0.12 <sup>ab</sup>	6.06 ± 0.12 <sup>ab</sup>	5.78 ± 0.11 <sup>bc</sup>	***	***	
	Sour	6.70 ± 0.02 <sup>b</sup>	4.81 ± 0.01 <sup>g</sup>	4.92 ± 0.01 <sup>e</sup>	5.21 ± 0.01 <sup>c</sup>	6.80 ± 0.02 <sup>a</sup>	4.91 ± 0.01 <sup>e</sup>	4.86 ± 0.01 <sup>f</sup>	5.16 ± 0.01 <sup>d</sup>	***	***	
	Salty	6.56 ± 0.12 <sup>a</sup>	5.60 ± 0.10 <sup>bc</sup>	5.78 ± 0.10 <sup>b</sup>	5.61 ± 0.10 <sup>bc</sup>	6.71 ± 0.12 <sup>a</sup>	5.21 ± 0.09 <sup>d</sup>	5.46 ± 0.10 <sup>cd</sup>	5.57 ± 0.10 <sup>bc</sup>	***	***	
	Bitter	4.62 ± 0.14 <sup>abc</sup>	4.65 ± 0.14 <sup>ab</sup>	4.71 ± 0.14 <sup>ab</sup>	4.68 ± 0.14 <sup>ab</sup>	4.35 ± 0.13 <sup>bc</sup>	4.84 ± 0.14 <sup>a</sup>	4.24 ± 0.12 <sup>c</sup>	4.35 ± 0.13 <sup>bc</sup>	***	***	
	Body	6.12 ± 0.13 <sup>a</sup>	6.21 ± 0.13 <sup>a</sup>	6.36 ± 0.14 <sup>a</sup>	6.28 ± 0.14 <sup>a</sup>	6.25 ± 0.14 <sup>a</sup>	6.23 ± 0.13 <sup>a</sup>	6.18 ± 0.13 <sup>a</sup>	6.23 ± 0.13 <sup>a</sup>	***	**	
	Balance	7.10 ± 0.02 <sup>a</sup>	6.21 ± 0.02 <sup>f</sup>	6.27 ± 0.02 <sup>e</sup>	6.70 ± 0.02 <sup>b</sup>	7.15 ± 0.02 <sup>a</sup>	6.36 ± 0.02 <sup>d</sup>	6.21 ± 0.02 <sup>f</sup>	6.5 ± 0.02 <sup>c</sup>	***	***	
	Flavour	Banana-like	7.69 ± 0.01 <sup>b</sup>	7.6 ± 0.01 <sup>c</sup>	6.49 ± 0.01 <sup>e</sup>	5.83 ± 0.01 <sup>h</sup>	7.86 ± 0.01 <sup>a</sup>	6.29 ± 0.01 <sup>f</sup>	6.94 ± 0.01 <sup>d</sup>	5.93 ± 0.01 <sup>g</sup>	***	***

	F-Citrus	3.32 ± 0.04 <sup>b</sup>	3.12 ± 0.04 <sup>de</sup>	3.23 ± 0.04 <sup>bcd</sup>	3.48 ± 0.04 <sup>a</sup>	3.25 ± 0.04 <sup>bc</sup>	3.14 ± 0.04 <sup>cde</sup>	3.16 ± 0.04 <sup>cde</sup>	3.06 ± 0.04 <sup>e</sup>	***	***
	F-Fruity	7.45 ± 0.20 <sup>a</sup>	7.04 ± 0.19 <sup>ab</sup>	6.55 ± 0.18 <sup>bc</sup>	5.97 ± 0.16 <sup>cd</sup>	7.54 ± 0.20 <sup>a</sup>	6.29 ± 0.17 <sup>d</sup>	6.82 ± 0.18 <sup>bc</sup>	5.81 ± 0.16 <sup>d</sup>	***	***
	F-Intensity	8.61 ± 0.13 <sup>a</sup>	8.57 ± 0.13 <sup>a</sup>	6.76 ± 0.10 <sup>b</sup>	6.47 ± 0.10 <sup>bc</sup>	8.64 ± 0.13 <sup>a</sup>	6.41 ± 0.09 <sup>c</sup>	6.74 ± 0.10 <sup>b</sup>	6.23 ± 0.09 <sup>c</sup>	***	***
	F-Persistence	7.12 ± 0.18 <sup>a</sup>	7.32 ± 0.19 <sup>a</sup>	6.21 ± 0.16 <sup>b</sup>	6.03 ± 0.15 <sup>b</sup>	7.18 ± 0.18 <sup>a</sup>	5.92 ± 0.15 <sup>b</sup>	5.98 ± 0.15 <sup>b</sup>	5.83 ± 0.15 <sup>b</sup>	***	***
	F-Complexity	7.31 ± 1.85 <sup>a</sup>	7.76 ± 1.97 <sup>b</sup>	6.23 ± 1.58 <sup>a</sup>	6.36 ± 1.61 <sup>b</sup>	7.43 ± 1.88 <sup>a</sup>	6.42 ± 1.63 <sup>b</sup>	5.58 ± 1.42 <sup>c</sup>	6.14 ± 1.56 <sup>b</sup>	***	***
	F-Pineapple	7.80 ± 0.15 <sup>a</sup>	8.10 ± 0.16 <sup>a</sup>	5.90 ± 0.12 <sup>c</sup>	6.03 ± 0.12 <sup>c</sup>	7.90 ± 0.16 <sup>a</sup>	5.70 ± 0.11 <sup>c</sup>	7.20 ± 0.14 <sup>b</sup>	5.80 ± 0.11 <sup>c</sup>	***	***
	F-Sweet fruit	6.38 ± 0.02 <sup>a</sup>	6.16 ± 0.02 <sup>c</sup>	6.00 ± 0.02 <sup>d</sup>	5.80 ± 0.02 <sup>d</sup>	6.31 ± 0.02 <sup>b</sup>	5.96 ± 0.02 <sup>d</sup>	6.29 ± 0.02 <sup>b</sup>	5.30 ± 0.01 <sup>f</sup>	***	***
	Overall quality	8.69 ± 0.02 <sup>b</sup>	8.91 ± 0.02 <sup>a</sup>	7.53 ± 0.02 <sup>h</sup>	6.91 ± 0.02 <sup>g</sup>	8.59 ± 0.02 <sup>c</sup>	7.62 ± 0.02 <sup>e</sup>	7.78 ± 0.02 <sup>d</sup>	7.46 ± 0.02 <sup>f</sup>	***	***
	Odour	8.81 ± 0.16 <sup>a</sup>	8.76 ± 0.16 <sup>a</sup>	6.25 ± 0.11 <sup>bc</sup>	6.16 ± 0.11 <sup>bc</sup>	8.86 ± 0.16 <sup>a</sup>	6.26 ± 0.11 <sup>bc</sup>	6.54 ± 0.12 <sup>b</sup>	6.12 ± 0.11 <sup>c</sup>	***	***
	Taste	7.64 ± 0.22 <sup>a</sup>	7.36 ± 0.22 <sup>a</sup>	7.21 ± 0.21 <sup>a</sup>	7.18 ± 0.21 <sup>a</sup>	7.56 ± 0.22 <sup>a</sup>	7.18 ± 0.21 <sup>a</sup>	7.46 ± 0.22 <sup>a</sup>	7.36 ± 0.22 <sup>a</sup>	***	***
	Mouth-feel	7.46 ± 0.16 <sup>a</sup>	7.26 ± 0.16 <sup>a</sup>	7.18 ± 0.16 <sup>a</sup>	7.16 ± 0.15 <sup>a</sup>	7.36 ± 0.16 <sup>a</sup>	7.18 ± 0.16 <sup>a</sup>	7.34 ± 0.16 <sup>a</sup>	7.12 ± 0.15 <sup>a</sup>	***	***
	Flavour	7.48 ± 0.02 <sup>ab</sup>	7.43 ± 0.02 <sup>b</sup>	6.22 ± 0.02 <sup>d</sup>	6.00 ± 0.02 <sup>e</sup>	7.51 ± 0.02 <sup>a</sup>	5.94 ± 0.02 <sup>f</sup>	6.28 ± 0.02 <sup>c</sup>	5.8 ± 0.02 <sup>g</sup>	***	***
Finish	After-smell	8.46 ± 0.01 <sup>b</sup>	8.56 ± 0.01 <sup>a</sup>	7.26 ± 0.01 <sup>d</sup>	7.12 ± 0.01 <sup>f</sup>	8.48 ± 0.01 <sup>b</sup>	7.21 ± 0.01 <sup>e</sup>	7.45 ± 0.01 <sup>c</sup>	7.11 ± 0.01 <sup>f</sup>	***	***
	After-taste	8.23 ± 0.00 <sup>a</sup>	8.18 ± 0.10 <sup>a</sup>	7.18 ± 0.09 <sup>b</sup>	7.18 ± 0.09 <sup>b</sup>	8.36 ± 0.10 <sup>a</sup>	7.18 ± 0.09 <sup>b</sup>	7.35 ± 0.09 <sup>b</sup>	7.14 ± 0.09 <sup>b</sup>	***	***

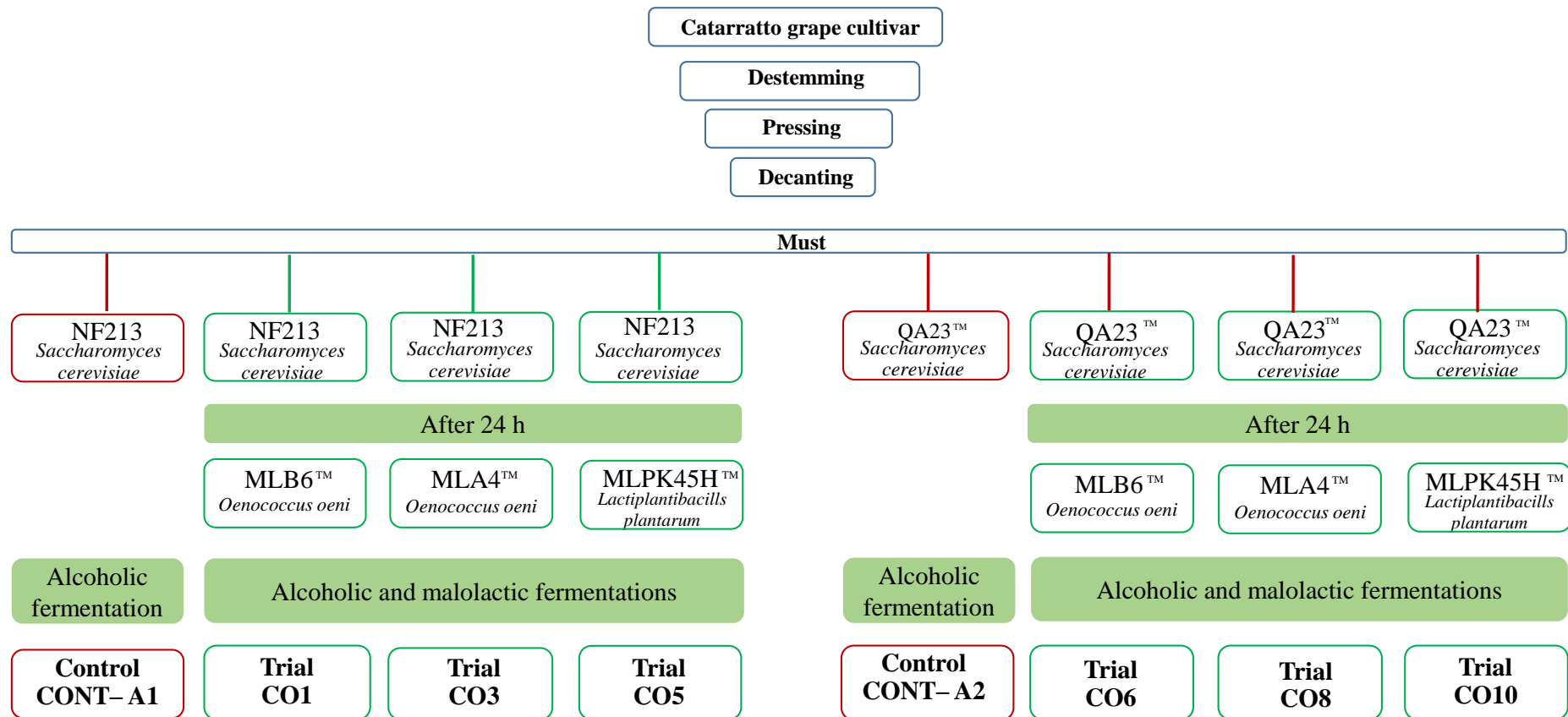
Results indicate mean value of three replicate sessions.

<sup>α</sup> Sensorial attribute;

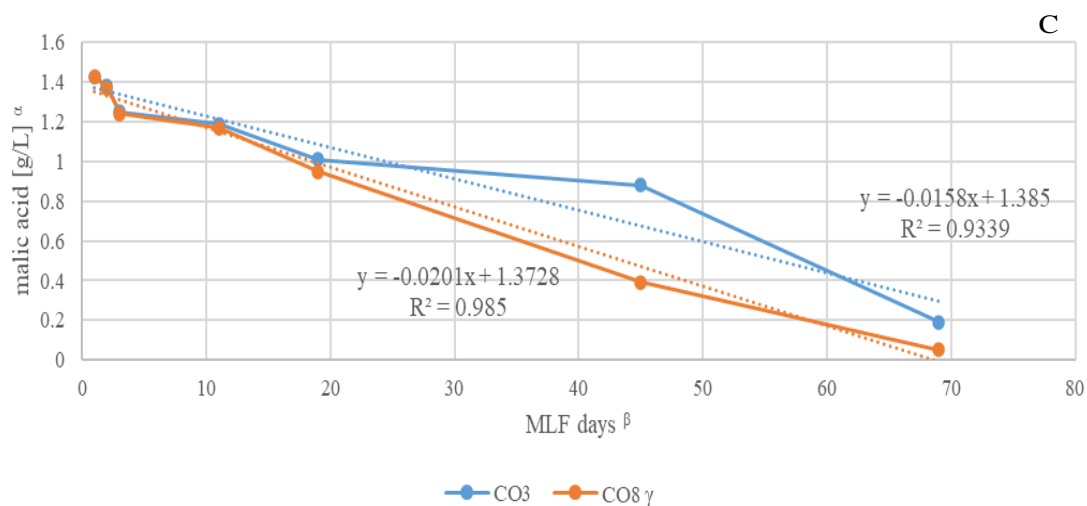
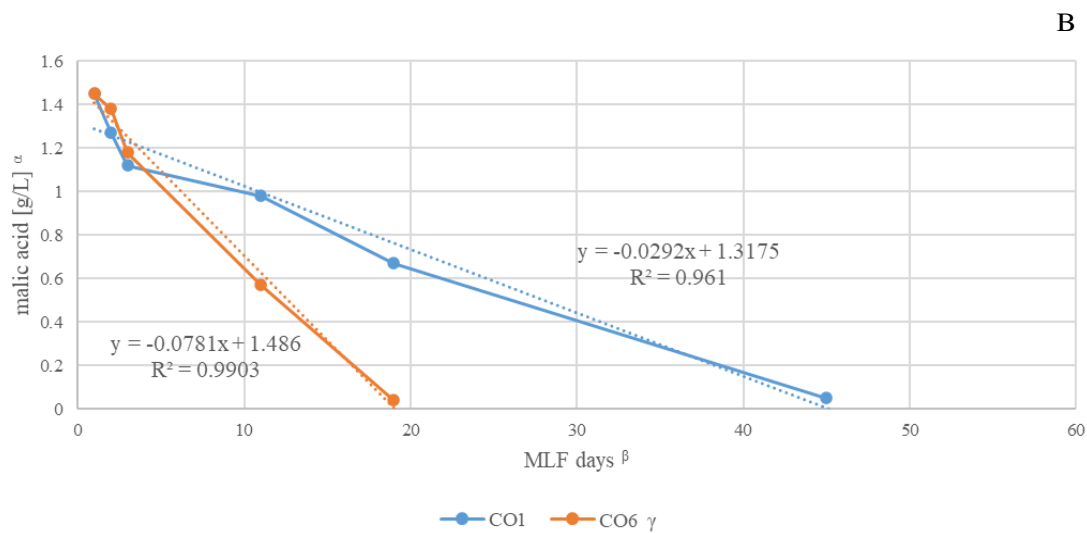
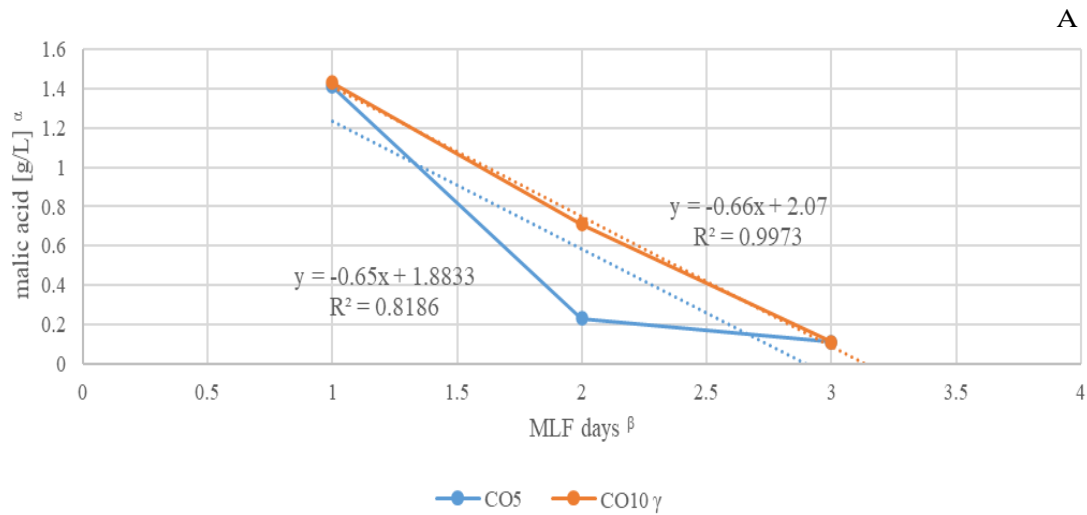
<sup>β</sup> Relative amounts expressed in on a numerical scale of 1 to 9;

<sup>γ</sup> 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.

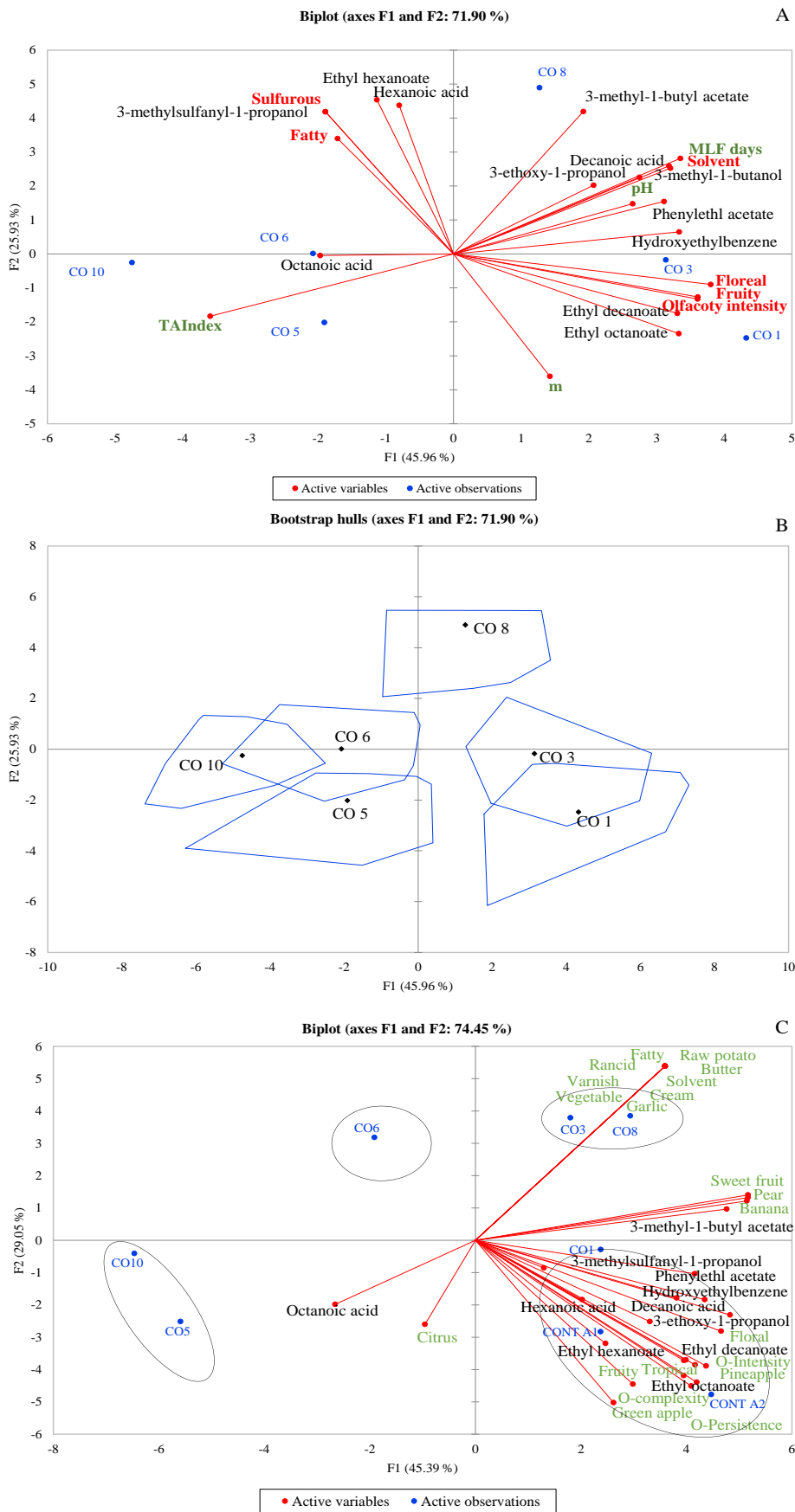
## Experimental plan



**Fig. 1.** Experimental plan.



**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*. Symbols: <sup>α</sup> malic acid [g/L]; <sup>β</sup> malolactic fermentation days; <sup>γ</sup> Trials.



**Fig. 3.** Principal component analysis (PCA) biplot: (A) OAV > 1 and TAIndex; m, malolactic fermentation days and pH; (B) Bootstrap hull's for OAV>1; (C) OAV > 1 and aroma attributes.



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**Supplementary Material**

[FC\\_Naselli\\_et\\_al\\_Supplementary\\_material.docx](#)



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

3

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6 Weber<sup>e</sup>, Paola Vagnoli<sup>f</sup>, Stéphanie Weidmann<sup>g</sup>, Raffaele Guzzon<sup>h</sup>, Luca Settanni<sup>a</sup>, Giancarlo  
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25 **ABSTRACT**

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

37

38 **Keywords**

39 Catarratto grape variety; Lactic acid bacteria; Malolactic fermentation; *Saccharomyces cerevisiae*;  
40 Technological affinity index; Wine aroma.

## 41 **1. Introduction**

42 The aroma of wine is the most important factor that influences consumer acceptance (Morata, 2018).  
43 The different aroma components in wine have different origins, including cultivars (Yang et al., 2021),  
44 agronomic techniques (Alem, Rigou, Schneider, Ojeda, & Torregrosa, 2019; Coletta et al., 2021), and  
45 geographic area.

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

48 Lactic acid bacteria (LAB) and yeasts produce a large number of secondary metabolites that create a  
49 sensory buffer in wine regulating the antagonisms and synergies of odor perceptions (Ferreira et al.,  
50 2016). LAB are microbial entities that most influence the organoleptic and technological framework  
51 of wine. For instance, the main technological function of LAB is the conversion of L-malic acid to  
52 L-lactic acid, which has deep implications on the gustatory, olfactory and microbial levels (Morata,  
53 2021). The loss of a carboxylic group by L-malic acid leads to a biological deacidification of the wine  
54 (Lasik, 2013), which is associated with an improvement in taste. Thus, many of these sensory effects  
55 are the direct result of increasing pH and decreasing total acidity. In red wine, this is the case with  
56 the decrease in the reactivity of phenolic hydroxyls to salivary proteins. As a result of malolactic  
57 fermentation, wines became less tannic and have greater smoothness characters (Costello, Siebert,  
58 Solomon, & Bartowsky, 2013).

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

67 of maintaining or improving the fruit and floral perceptions of wines while ensuring microbial  
68 stability.

69 The main interfering agent in the fruity and floral perceptions of wines is 2,3-butanedione, which is  
70 a by-product of malolactic fermentation. Bartowsky & Henschke (2004) pointed out several factors  
71 that lead to the production of 2,3-butanedione, including sulfur dioxide, dissolved oxygen, and  
72 temperature of malolactic fermentation performance. Furthermore, Olguín, Bordons, & Reguant  
73 (2009) reported that gene expression of the citric acid pathway, as well as 2,3-butanedione production,  
74 is stimulated by ethanol in the medium.

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

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

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

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

94 Conducting more in-depth studies on the effects that the LAB-*S. cerevisiae* consociation has on the  
95 aroma composition of wine could be worthwhile, as it could lead to novel knowledge in terms of  
96 aroma profiling of wine.

97 This study proposes a technological affinity index (TAIndex) for this purpose. In particular, in the  
98 present research, three commercial LAB strains (two *Oenococcus oeni* and one *Lactiplantibacillus*  
99 *plantarum* strains) were used in different co-inoculation with two *S. cerevisiae* strains. The research  
100 activity aimed to evaluate the effects of the LAB-*S. cerevisiae* consociation on: (i) kinetics of  
101 malolactic fermentation in terms of duration and yield; (ii), improvement of high olfactory impact  
102 VOC concentration; (iii) sensory characterization of aroma of Catarratto after malolactic  
103 fermentation; and (iv) TAIndex calculation.

104

## 105 **2. Material and methods**

### 106 *2.1. Experimental design and sampling*

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

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

117 The QA23™, MLB6™, MLA4™ and MLPK45H™ strains were used as described by the  
118 manufacturer (Lallemand Inc. Italia, Castel D'Azzano, Verona, Italy). The strain NF213, which  
119 belongs to the strain collection of the Department of Agriculture, University of Palermo, was used at  
120 a dose of 20 g/hL. Before yeast inoculation, total nitrogen levels were adjusted as reported by  
121 Kemsawasd, Viana, Ardö, & Arneborg (2015) using an organic nutrient. Additionally, in the control  
122 trials CONT A1 and CONT A2, 10 g/hL of lysozyme (Esseco s.r.l. San Martino, Novara, Italy) were  
123 added before inoculation of *S. cerevisiae* to prevent the development of indigenous LAB.  
124 Samples were taken for analysis from the clarified must before, after *S. cerevisiae* yeast inoculation,  
125 after LAB inoculation and at the end of alcoholic fermentation (days 1, 2, 3 and 11).  
126 The samples were collected in triplicate and transported under refrigerated conditions, placed in a  
127 climate chamber at 4 °C, and analytically processed within 24 h. Samples for VOCs analysis were  
128 collected at the end of sugar or malic acid depletion (days 11, 19, 45, 69).  
129 For brevity of the manuscript and ease of reference of the data by the reader, only data collected at  
130 significant sampling points have been reported in the manuscript.

131

## 132 2.2. Winemaking process

133 The grapes were destemmed and crushed, and 4 g/q of metabisulfite (Laffort, France) was added.  
134 Two g/hL of pectolytic enzyme LALLZYME HC™ (Lallemand Inc. Italy, Castel D'Azzano, Verona,  
135 Italy) was added to the must during the static settling stage. In addition, a temperature of 10 °C was  
136 maintained for 24 h to facilitate the catalytic action of the pectins. Then, the must was aliquoted into  
137 24 (1 hL each) steel tanks to constitute eight different trials, each of which was conducted in  
138 triplicates. The trials were inoculated as described in the experimental plan when they reached a  
139 temperature of 15 °C. Fermentation took place inside a climatic cell in such a way as to allow the  
140 constant and uniform maintenance of 20 °C for the fermentation activities to take place. The addition  
141 of potassium metabisulfite (Esseco s.r.l., San Martino, Novara, Italy) was carried out one week after

142 the complete degradation of malic acid. Therefore, microbial inactivity was ensured by 0.8 mg/L  
143 molecular SO<sub>2</sub> (Tomasset, 1978).

144

### 145 *2.3. Microbial counts and identification of yeasts and LAB strains*

146 During alcoholic fermentation, plate counts were performed to estimate the levels of total yeasts  
147 (Pallmann et al., 2001), which were differentiated into *Saccharomyces* and non-*Saccharomyces*  
148 colonies as described by Varela (2016). LAB population was monitored according to the procedure  
149 described by Tristezza et al. (2016).

150 Yeast isolates were purified and phenotypically grouped as reported by Alfonzo et al. (2020). The  
151 selection and molecular identification of yeast isolates at species level (Francesca et al., 2024) and  
152 genetic strain characterization (Alfonzo et al., 2021) were also performed. The isolation and genetic  
153 characterization of LAB strains were conducted as reported by Solieri et al., 2010.

154

### 155 *2.4. Physico-chemical analysis*

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

164

### 165 *2.5. Analysis of VOCs in wine samples*

#### 166 2.5.1. Standard solutions

167 Standards for each compound were purchased individually from Sigma-Aldrich (82024 Taufkirchen,  
168 Germany). 2,3-butanediol was used as standard for the alcohol fraction, acetoin as standard for the  
169 carboxyl-function fraction and ethyl lactate as standard for the ester fraction. In addition, n-alkane  
170 standards (C8 to C40) were purchased from Aldrich Chemical Co. (St. Louis, Mo., USA). Standard  
171 solutions of each compound were prepared at five different concentrations: 2,3-butanediol, 53.25  
172 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,  
173 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  
174 mg/L, 326.00 mg/L, 477.00 mg/L.

#### 175 176 2.5.2. Extraction, identification and quantification of VOCs by GC-MS

177 To determine the volatile compound composition, wine samples (10 mL) from all trials were mixed  
178 with MS SupraSolv® dichloromethane (5 mL) in a 50-mL conical flask. The mixture was stirred at  
179 room temperature for 30 min and then centrifuged at 4000 rpm for 10 min using a Low Speed  
180 Centrifuge (ScanSpeed 416) with Swing Rotor (LaboGene ApS Industrivej 6–8, Vassingerød, DK-  
181 3540 Lyngø, Denmark). The aqueous phase was removed, and anhydrous sodium sulphate (1 g) was  
182 added before centrifugation at 4000 rpm for 5 min. The dichloromethane layer was removed and dried  
183 under N<sub>2</sub> gas to 0.3 mL.

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

189 The temperature was initially maintained at 40 °C for 1 min. Then it was gradually increased to 250  
190 °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  
191 was injected at 250 °C automatically and in the splitless mode; transfer line temperature, 295 °C. The  
192 individual peaks were analysed using the GC MS Solution package, Version 2.72. Identification of

193 compounds was carried out using Adams, NIST 11, Wiley 9 and FFNSC 2 mass spectral database.  
194 These identifications were also confirmed by other published mass spectra. Quantification was carried  
195 out using the three calibration lines. For compounds belonging to other classes than the standards,  
196 similarity was used for quantification. A dilution factor was used for the reported data.  
197 To determine which VOCs were actively contributing to the wine's aroma profile, the detected  
198 concentrations were transformed into odour activity units (OAV) using the method described by  
199 Butkhup et al. (2011). The method reported by Butkhup et al. (2011) was also used to calculate the  
200 aroma series per individual wine (fruity, floral, fatty, solvent and sulphurous). The sum of the  
201 individual odourant active values (OAV) determined for each volatile organic compound per trial  
202 defined the olfactory intensity of the test wine. The VOCs with  $OAV > 1$  were then grouped and  
203 reported in tables for ease of reading and processing.

204

#### 205 *2.6. Sensory analysis*

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

214

#### 215 *2.7. Determination of technological affinity index*

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

218  $TAIndex = \{ [V_i / (-m)] / t_c \}$ , expressed in “t<sup>-1</sup>”;

219 where:

220 - “ $V_i$ ”: is the instantaneous speed of the reaction for the conversion of malic acid to lactic acid (malo-  
221 lactic fermentation), expressed in “[malic acid] g/L × day<sup>-1</sup><sub>MLF</sub>”;

222 - “ $m$ ”: is the degradation rate of malic acid in the unit of time, expressed in [malic acid] g/L × day<sup>-1</sup>  
223 <sub>MLF</sub>”;

224 - “ $t_c$ ” is the time of the reaction for the conversion of malic acid to lactic acid (malo-lactic  
225 fermentation) corrected by the graphical method modified of Di Stefano & Cravero (1989).

226 The parameter  $V_i$  was calculated using the following formula:

$$227 \quad V_i = \frac{\Delta[\text{malic acid}](\text{g/L})}{\Delta[t_c]}$$

$$228 \quad V_i = \frac{[\text{malic acid}]_{\text{inoculum LAB}}(\text{g/L}) - [\text{malic acid}]_{\text{end malo\_lactic fermentation}}(\text{g/L})}{t_c \text{ end malo\_lactic fermentation LAB inoculum} - t \text{ inoculum LAB}}$$

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

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

$$233 \quad t_c = d(A, B) = \sqrt{(X_2 - X_1)^2 + (Y_2 - Y_1)^2}$$

234 where:

235 -  $X_1$ , coordinate point referring to the day malolactic fermentation started (LAB inoculum)

236 -  $X_2$ , coordinate point referring to the day of the end of malolactic fermentation

237 -  $Y_1$ , coordinate point referring to the concentration of malic acid detected at the end of malolactic  
238 fermentation

239 -  $Y_2$ , coordinate point referring to the malic acid concentration detected at the start of malolactic  
240 fermentation (LAB inoculum)

241

## 242 2.8. Statistical analysis

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

249

## 250 3. Results and discussion

### 251 3.1. Microbial growth dynamics and strain monitoring

252 The trends of yeast monitoring during the course of fermentation are shown in Fig. S1a, b, c, d. At  
253 the beginning of the trial, the detection of *S. cerevisiae* and non-*Saccharomyces* populations in  
254 Catarratto must were 4.11 (Fig. S1a, b) and 3.67 Log CFU/mL (Fig. S1c, d), respectively. After yeast  
255 inoculation, the cell density of the first experimental set using *S. cerevisiae* NF213 strain ranged from  
256 7.20 and 7.47 Log CFU/mL between trials. The levels of *S. cerevisiae* detected after adding QA23  
257 strain in the second test set were comparable, with a degree of variability between trials of 7.21 and  
258 7.51 Log CFU/mL. The population of non-*Saccharomyces* decreased correspondingly with the  
259 increase in *S. cerevisiae*. These decreases were at values of  $< 2.0$  Log CFU/mL on the second day of  
260 fermentation. The monitoring of non-*Saccharomyces* was consistent with the findings of Wang, Mas,  
261 & Esteve-Zarzoso, (2016).

262 Before inoculation of the *S. cerevisiae* strains, LAB values of 3.1 Log CFU/mL were detected (Fig.  
263 S1e, f). After inoculation of the LAB, which occurred 24 h after the addition of the yeast strain, the  
264 bacterial populations in the CO1 and CO3 and CO6 and CO8 trials (inoculated with *O. oeni* strains)  
265 were between 5.41 and 5.51 Log CFU/mL. These values were similar to those reported by Celik et  
266 al. (2018). In the trials involving the addition of the 24-h MLPK45H strain of *S. cerevisiae*, a cell  
267 density of 7.71 Log CFU/mL was found. The highest levels of LAB were 5.88 – 5.93 Log CFU/mL

268 for *O. oeni* and 8.0 Log CFU/mL for *L. plantarum*, respectively. At the end of alcoholic fermentation,  
269 which occurred for both experimental sets on the 11<sup>th</sup> fermentation day, the yeasts were at a cell  
270 density of 7.39 Log CFU/mL. At the 19th fermentative day, the CO5 and CO10 trials resulted in a  
271 lower concentration in LAB than the CO1, CO3 and CO6, and CO8 trials (Fig. S1e, f). This  
272 phenomenon is imputable to the depletion of malic acid and to the addition of potassium  
273 metabisulphite. At the 19th and 45th fermentation days, corresponding to the end of malolactic  
274 fermentation for CO1 and CO6 trials, the levels of LAB were 5.21 and 5.00 Log CFU/mL,  
275 respectively. On the 69th day fermentation, the CO3 and CO8 trials completed malic acid degradation  
276 and LAB reached densities of 4.43 and 3.76 Log CFU/mL, respectively.  
277 The microbial population of each trials was dominated by all inoculated strains, as determined by the  
278 DNA fingerprinting method.

279

### 280 3.2. Kinetics of the main oenological parameters

281 Table S1 shows the technological parameters of the starting wort.

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

285 The higher speed of malic acid depletion could be attributed to the TAIndex found when using the  
286 LAB strain MLPK45H with the two *S. cerevisiae* strains NF213 and QA23 ( Table 1) respectively.  
287 TAIndex values ranging from 0.348 to 0.351 (Tab. 1) microbiologically stabilized the must before  
288 the end of alcoholic fermentation that occurred in 11 days (Table S3).

289 The kinetics of malic acid degradation were influenced by the association of LAB strain MLB6 with  
290 the two different yeasts, QA23 and NF213. In fact, the CO6 trial, (MLB6-QA23) terminated malic  
291 acid degradation within day 19 of winemaking (Table S4), compared to the CO1 trial, (MLB6-  
292 NF213), that terminated at day 45 (Fig. 2b; Table S5). The difference in the timing of malolactic  
293 fermentation of the two different consociations, MLB6-QA23 and MLB6-NF213, leads to the

294 hypothesis of a different requirement of the two yeasts to assimilate metal cofactors, such as Mn<sup>2+</sup> or  
295 Mg<sup>2+</sup> elements, which are essential to trigger the endogenous process of enzymatic decarboxylation  
296 in *O. oeni* (Lonvaud-Funel, 2022). On the other hand, *S. cerevisiae* may respond differently to  
297 counteract the co-presence of LAB in the medium through the production of volatile catabolites  
298 (Alexandre, Costello, Remize, Guzzo, & Guilloux-Benatier, 2004). In contrast, CO3 and CO8 trials  
299 (MLA4-NF213; MLA4-QA23) required 69 days to complete malolactic fermentation. (Fig. 2c; Table  
300 S6).

301 Table S6 shows the best technological performances were recorded by the trials involving the use of  
302 LAB strain MLPK45H, specifically the CO5 and CO10 trials. In spite of the consumption of malic  
303 acid, the total acidity values were the highest in each of the trial batteries compared to the trials  
304 involving the use of *O. oeni*; 4.98 g/L H<sub>2</sub>T versus 4.61 and 4.64 g/L H<sub>2</sub>T (for the CO5 trials versus  
305 CO1 and CO3, respectively); 4.95 g/L H<sub>2</sub>T versus 4.59 and 4.58 g/L H<sub>2</sub>T (for the CO10 trials versus  
306 CO6 and CO8, respectively). The higher lactic acid yield of *L. plantarum* strains and their low  
307 production of acetic acid contributed to these values.

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

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

315

### 316 *3.3. Volatile organic compound composition*

#### 317 3.3.1. Higher alcohols

318 Alcohols were identified as the most abundant aromatic fraction in the wines object of investigation  
319 (Table 2). The trials that predicted LAB - *S. cerevisiae* microbial consociations showed lower

320 amounts of higher alcohols than the control trials (CONT A1 and CONT A2), with the exception of  
321 the CO3 trial (Table 2). These results confirm the findings of Knoll et al. (2012). The CO3 trial stood  
322 out by registering a higher total amount of alcohols than the CONT A1 control (Table 2). The  
323 production of three different enantiomers of 2,3-butanediol in comparison to the CONT A1 control  
324 trial contributed to this result (Table 2). This peculiarity was exclusively recorded in the MLA4 -  
325 NF213 consociation (CO3 trial), indicating that this microbial combination has a higher conversion  
326 rate of 2,3-butanediol than the corresponding CO8 trial (MLA4 -QA23) and all other microbial  
327 interaction trials. Furthermore, the microbial consociation MLB6-NF213 (CO1 trial) exhibited a  
328 peculiarity in its favor, recording a significant production of 3-ethoxy-1-propanol in comparison to  
329 the CONT A1 trial (Table 2). This peculiarity was attributed to the *S. cerevisiae* yeast strain used in  
330 the microbial combination, as it was not produced in the corresponding CO6 trial (MLB6-QA23;  
331 Table 2).

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

341

### 342 3.3.2. Esters

#### 343 3.3.2.1. Acetate esters

344 Trial showed significant differences in terms of acetate ester amount and composition (Tab. S7, S8).

345 The total acetate ester values were higher in CO8 trials (3.77mg/L) than the CONT A2 control

346 (3.34mg/L). The lowest values were found in the CO6 and CO10 trials (2.68 and 2.19 mg/L,  
347 respectively). 3-methyl-1-butyl acetate, phenylethyl acetate, and 2-methyl-1-butyl acetate were the  
348 VOCs that contributed the most to the increase in acetate ester content in the CO8 trial compared to  
349 the CONT A2 control (Table 2). These increases observed under experimental pH conditions contrast  
350 with the findings of Costello, Siebert, Solomon, & Bartowsky, 2013. This phenomenon suggests that  
351 wine limiting conditions (Costello, Siebert, Solomon, & Bartowsky, 2013) or those created by the  
352 coexistence of LAB and yeasts during fermentation are crucial in activating some biosynthetic  
353 processes (Liu et al., 2017). The hypothesis is supported by the varying recorded production of  
354 phenylethyl acetate in the CO8 trials and 2-methyl-1-butylacetate in the CO6, CO8 and CO10 trials  
355 compared to the control, CONT A2. In such cases, the esterification process due to LAB activity can  
356 decrease hydroxybutylbenzene concentration, thereby reducing the potential toxicity against bacterial  
357 cells (Table 1, Corre, Lucchini, Mercier, & Cremieux, 1990; Romano, Ciani, & Cocolin,  
358 2022). Indeed, Romano, Ciani, & Cocolin (2022) have reported that hydroxybutylbenzene produced  
359 by yeasts is well known to inhibit the transport of sugars and amino acids within the bacterial cell.  
360 MLPK45H strain co-inoculated with different strains of *S. cerevisiae* (CO5 and CO10 trials) resulted  
361 in the lowest concentration of acetate esters compared to the other consociated trials and the related  
362 CONT A1 and CONT A2 controls (Table S7 and S8).

363

#### 364 3.3.2.2. Ethyl Esters

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

369 Therefore, the timing of malolactic fermentation is a key factor in increasing the aromaticity of a  
370 wine. Therefore, it is possible to hypothesise that the prolongation of the malolactic fermentation time  
371 positively influenced the recording of the higher total ester content due to the greater synthesis of

372 monoethyl succinate and diethyl succinate in the CO3 trials (Table 2, Table S7). Most likely, these  
373 esters represent the metabolic response of the LAB - *S. cerevisiae* (MLA4 -NF213) consociation to  
374 the deleterious effects exerted by free succinate on bacterial cells during co-fermentation (Torres-  
375 Guardado, Rozès, Esteve-Zarzoso, Reguant, & Bordons, 2022)

376

### 377 3.3.3. Carboxylic acids

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

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

398 (Alexandre, Costello, Remize, Guzzo, & Guilloux-Benatier, 2004; Rossouw, Du Toit, & Bauer, 2012)  
399 toward LAB represents a synthesis of aromatic precursors.

400

#### 401 3.3.4. Aldehydes

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

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

419

#### 420 3.3.5. The 2,3-butanedione, 2,3-butanediol and 3-hydroxy-2-butanone compounds

421 2,3-butanedione, also known as diacetyl, is a diketone whose production has always been attended to  
422 by technicians because of its olfactory perceptions. In fact, aromas resulting from the production of  
423 this compound can also result in off-flavors depending on the concentration produced. In wine, LAB

424 play a key role in the production of diacetyl, which is synthesized due to the degradation of citric  
425 acid.

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

434

#### 435 *3.4. Active volatile compound analysis*

436 The aroma profiles of the wines were characterized by VOCs from the metabolic processes of the  
437 biotechnology used. The study found that the active volatile component, represented by VOCs above  
438 the perception threshold ( $OAV > 1$ ), was composed of 11 compounds for the trials involving the  
439 combination of the LAB MLB6, MLA4, MLPK45H strains with the *S. cerevisiae* NF213 (CONT A1,  
440 CO1, CO3 and CO5 trials) (Table 3). For the test set in which the same LAB were combined with *S.*  
441 *cerevisiae* QA23 strain for the initiation of fermentation (trials CONT A2, CO6, CO8 and CO10) the  
442 active volatile component was composed of 12 compounds (Table 3). According to Ferreira et al.  
443 (2016), the sensorial buffer of wine is composed of a total of three higher alcohols (methyl-1-butanol,  
444 3-ethoxy-1-propanol, hydroxyethylbenzene), three medium chain fatty acids (hexanoic acid, octanoic  
445 acid and decanoic acid), two acetate esters and three ethyl esters (3-methyl-1-butyl acetate,  
446 phenylethyl acetate, ethyl hexanoate, ethyl octanoate and ethyl decanoate). 3-methylsulfanyl-1-  
447 propanol was the VOC that significantly differentiated trials in addition to the amount of OAVs  
448 detected (Table 2 and 3).

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

459 The increase in TAIIndex found among the trials associated with the MLPK45H, CO5 and CO10 trials  
460 (Table 1) resulted in a significant decrease in active volatile components (Table 3) and, thus, in  
461 olfactory intensity. This is probably due to the rapid degradation of malic acid that occurred when the  
462 culture medium had low limiting factors, low concentrations of ethanolic substrate (Table S2) and,  
463 presumably, low concentrations of medium-chain fatty acids by the metabolism of *S. cerevisiae*.  
464 The limited concentrations of these two compounds in solution during the first three days of  
465 vinification would not have led LAB to catalyse the octanoic and decanoic acid esterification  
466 reactions. Thus, the failure of LAB to contribute to esterification resulted in a decrease in octane  
467 intensity. This hypothesis formulation would also explain the positive correlation of octanoic acid  
468 with the CO5 and CO10 trials (Fig. 3a).

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

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

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

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

487 The technological affinity between LAB and *S. cerevisiae* is calculated by using data from the  
488 relationship between malic acid degradation as a function of time. The resulting trend reveals the  
489 extent to which the decarboxylative activity of LAB is affected by the activity of the *S. cerevisiae*  
490 strain during the co-fermentation phase (Alexandre, Costello, Remize, Guzzo, & Guilloux-Benatier,  
491 2004).

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

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

506

#### 507 3.4.1. The fruity olfactory perception of the wines

508 Different microbial associations were used to create aromatic profiles that had a significant presence  
509 of fruit components (Table 4). The fruity perceptions of the wines were mainly due to the presence  
510 of ethyl octanoate, ethyl hexanoate, 3-methyl-1-butyl acetate and 3-ethoxy-1-propanol. These  
511 compounds were detected above the threshold of sensory perception (Table 3; Fig. S4a, b).

512 The microbial consociations MLB6-NF213 and MLA4 -QA23 (CO1 and CO3 trials) resulted in the  
513 highest fruit aromas, with 1870.50 and 1440.56 OAV, respectively (Table 3). In the CO1 trial, MLB6  
514 was used to create wine with prominent pineapple and pear aromas, both of which were due to the  
515 synthesis of ethyl octanoate, 1610 olfactory units (Table 3; Fig. S4a). In contrast, CO8 trial had a  
516 more varied fruit component, including pineapple, pear, green apple and banana, which were  
517 attributable to the synthesis of ethyl octanoate, ethyl hexanoate, and 3-methyl-1-butyl acetate for  
518 1.005, 316 and 102.67 olfactory units, respectively (Table 3; Fig. 4b).

519

#### 520 3.4.2. The floral olfactory perception of the wines

521 The floral component of the wines showed varying values between the trials, but the results were  
522 consistent in both experimental sets. In the CO1 trial, the microbial association LAB - *S. cerevisiae*  
523 recorded higher floral intensity values than the control CONT A1 (Table 4). The compound ethyl  
524 ester decanoate contributed to this trend, with an OAV of 6.20 in the CO1 trial compared to 4.20 in  
525 the CONT A1 trial (Table 3; Fig. S4a). In both experimental sets (CO3 and CO8 trials), the use of the  
526 *O. oeni* MLA4 strain allowed for the repeatability of the results regarding floral intensity when

527 varying the *S. cerevisiae* strain used in the microbial association. The OAV was 8.58 in the CO3 trial  
528 and 8.26 in the CO8 trial (Table 4).

529

### 530 3.4.3 The fatty olfactory perception of wines

531 The effects of microbial consociation on olfactory fatty descriptors are significant (Table 4). The  
532 trials that included different LAB - *S. cerevisiae* associations recorded the lowest odour activity value  
533 (OAV) levels compared to the control trials. The CO6, CO8 and CO10 trials (MLB6-QA23; MLA4  
534 -QA23; MLPK45H-QA23) (Table 3) stood out for the decrease in off-flavours from medium-chain  
535 fatty acids. On the contrary, in the set of trials in which NF213 was used as the fermentative starter  
536 (Table 4) only the CO1 trial (MLB6-NF213) followed the same trend. The CO3 trial showed no  
537 significant differences compared to the CONT A1 control (12.18 Vs 13.58 OAV), while the CO5  
538 reported a higher level of fatty OAV than the latter (13.58 Vs 19.03 OAV; Table 3).

539

### 540 *3.4.4 The olfactory perception of solvent and sulfur wines*

541 The VOCs that contributed to the olfactory descriptors of solvent and sulphur were 3-methyl-1-  
542 butanol and 3-methylsulphanyl-1-propanol. These compounds were detected above the perception  
543 threshold (Table 3) and were closely dependent on the *S. cerevisiae* strain starter used to initiate  
544 alcoholic fermentation in the two different experimental sets (Fig. S2 and S3). The *S. cerevisiae* QA23  
545 strain showed a particular propensity to produce 3-methylsulfanil-1-propanol compared to the NF213  
546 strain (Table 2 and Table 3).

547 The different microbial associations of LAB - *S. cerevisiae* used in the two experimental sets resulted  
548 in a significant reduction in OAVs compared to the CONT A1 and CONT A2 controls, which can be  
549 traced back to the negative olfactory perceptions of solvents and sulphur (Table 4). The trials that  
550 stood out the most were those in which the *L. plantarum*, MLPK45H strain, CO5 and CO10, was  
551 used in combination with the two yeasts NF213 and QA23 (Table 4). The decreases recorded in the  
552 sulphur OAVs could be due to suppression effects by the LAB against the yeast. In fact, a partial

553 deactivation of the biosynthetic pathway leading to the production of 3-ethoxy-1-propanol within the  
554 yeast cell cannot be excluded (Irwin, 1992).

555

#### 556 3.4.5 The olfactory intensity of wines

557 The olfactory intensity of the wines was found to be heterogeneous in the two different test sets. The  
558 olfactory intensity (Fig. S4c, d) was found to be a function of the LAB - *S. cerevisiae* consociation  
559 implanted in the must. In the CO1 trial, which was microbiologically consociated with LAB strain  
560 MLB6 (*O. oeni*) and *S. cerevisiae* NF213 strain, no significant differences were observed with the  
561 control CONT A1 (Fig. S4c), 1892 vs 2023 OAV, respectively (Table 3). Within the same  
562 experimental set, the CO3 and CO5 trials produced no significant differences in the aromatic intensity  
563 of the wine (1462 vs 1350 OAV; Table 3). The trend was different for the trials conducted with the  
564 starter yeast QA23 strain. The trials in which the presence of different microbial associations, LAB -  
565 *S. cerevisiae* was predicted (CO6 CO8 and CO10 trials) showed significant drops in total OAV (Fig.  
566 S4d).

567

#### 568 *3.5. The sensory analysis*

569 Table 5 presents the data from the sensory measurements. The microbial consociation trials in the  
570 two experimental sets resulted in significantly different sensory profiles. The appearance of the wine  
571 was influenced by the variability of colour attributes. The yellow colour showed a variability between  
572 the trials ranging from 7.12 and 7.47. This is consistent with the findings of Naselli et al. (2023).  
573 The green reflections in the trials were similar to each other, but had higher values than those  
574 described by Scacco et al. (2012). The CO1 trial stood out for having the highest value of green  
575 reflections compared to the control and compared to all trials in the two different experimental sets  
576 (Table 5). The wines were submitted to a panel, which outlined different olfactory profiles depending  
577 on the LAB - *S. cerevisiae* microbial consociation used. Among the microbial consociation trials, the  
578 CO1 trial (MLB6 - NF213) stood out for its high values of intensity, persistence and tropical scents

579 with values of 8.69, 7.61 and 8.31, respectively (Table 5). These olfactory attributes appeared not to  
580 differ from the CONT A2 control. However, the panel detected higher levels of the fruitiness and  
581 pineapple than the CONT A2 control.

582 The OAV values (Table 3) showed a contrast with the sensory analysis results (Table 5). This could  
583 be explained by the suppressive effects carried out by 3-methyl-1-butanol and medium-chain fatty  
584 acids on fruitiness and pineapple hint (Cameleyre, Lytra, Tempere, & Barbe, 2015). However, no off-  
585 flavours attributable to hints of solvent, garlic, raw potato, rancid and varnish emerged in the trials of  
586 the two experimental sets. This lack of olfactory manifestations not picked up by the human nose  
587 suggests that the role played by 3-methyl-1-butanol and medium-chain fatty acids, while being above  
588 the threshold of perception, is to counterbalance olfactory perceptions with synergy or masking  
589 phenomena (Ferreira et al., 2016) within the sensory buffer (Ferreira, Escudero, Campo, & Cacho,  
590 2008).

591 The use of LAB - *S. cerevisiae* microbial associations is crucial in enhancing the fruitiness of the  
592 wine. This is due to their ability to significantly reduce the concentrations of 3-methyl-1-butanol and  
593 medium-chain fatty acids in the wines during fermentation (Table 2). The CO1 trial stood out from  
594 the CONT A1 control and other microbial association trials by recording the highest value of olfactory  
595 complexity (Table 5). This could be attributed to the higher floral perception recorded by the judges  
596 which was found to be comparable to that of the CONT A2 test. Additionally, the synergistic effect  
597 of the VOC mixture, which was found to be below the perception threshold, could have contributed  
598 to this result (Atanasova et al., 2005). The sensory analysis also revealed that the CO8 trial (MLA4-  
599 QA23) had the highest values of the olfactory descriptors banana, pear, and sweet fruit (6.97, 6.74,  
600 and 6.86, respectively) compared to the CONT A2 control and the other trials of the two experimental  
601 sets. These results are consistent with the findings of the OAV (Table 3). The taste fraction of the  
602 different trials was evaluated, and a further distinctive aspect emerged between the trials. Specifically,  
603 the CO1 and CO3 trials were characterized by a higher sweetness sensation than the CONT A1  
604 control. Meanwhile, trials CO5 and CO10 were the most acidic of the trials that included microbial

605 association. This sensory effect could result from the increased lactic acid synthesis and lack of citric  
606 acid degradation recorded when the *L. plantarum* MLPK45H strain was used (Table S6).  
607 The judges perceived the bitter taste component differently in the different trials of the two  
608 experimental groups. The trials including microbial consortia were less bitter than the CONT A1 and  
609 CONT A2 controls. Specifically, the CO3 and CO8 trials reported the lowest bitterness value  
610 compared to the CONT A1 and CON A2 controls (4.28 and 4.24 vs 6.08 and 6.11) and the different  
611 microbial consociations CO1, CO5 and CO6, CO10 (4.28 and 4.24 vs 4.65 - 4.68, and 4.58 - 4.71,  
612 respectively). This phenomenon could be attributed to the protease enzymes synthesized by the LAB,  
613 which could promote yeast autolysis from the fermentation phase (de Andrade Bulos et al., 2023).  
614 The panel judges detected clear differences in the flavor of the wines between the trials. Among the  
615 trials inoculated with different microbial combinations, the CO1 trial showed the highest values of  
616 flavor intensity and persistence (Table 5). However, no difference was felt with the CONT A1 and  
617 CONT A2 controls. For taste sensations, the CO1 trial was shown to be different from the CONT A1  
618 and CONT A2 controls in complexity and for the pineapple descriptor (Table 5). The CO1 trial also  
619 differed from the other trials in aftertaste (8.56). Although the aftertaste (8.18) was higher among the  
620 sister trials, it did not differ from the CONT A1 control. The panel judges concluded that the CO1  
621 trial (MLB6-NF213) had the best overall quality with the distinction of 20 sensory descriptors. This  
622 result was higher than the CONT A1 control (15 sensory descriptors) and significantly different from  
623 the homologous CO6 trial (MLB6-QA23).

624

### 625 *3.6 Sensory profiles associated with volatile organic compounds*

626 To highlight the correlations between aromatic attributes and OAV values, the principal component  
627 analysis technique was used. The values of F1 and F2 explained 45.39% and 29.05% of the total  
628 variance, respectively (Fig. 3c). This analysis helped to distinguish olfactory active scents into 4  
629 different groups between trials of the two different experimental test sets.

630 The first group was defined by the CONT A1 and CONT A2 trials, and the CO1 trials. The sensory  
631 attributes of intensity, persistence, and olfactory complexity were correlated with these trials. In turn,  
632 the latter were closely dependent on the odor perceptions of green apple, tropical, and pineapple.  
633 These olfactory attributes detected by the panel of judges showed a correspondence with the OAVs  
634 (Table 3). In fact, trials CONT A1 and CONT A2, and CO1 were positively correlated with the higher  
635 alcohol 3-ethoxy-1-propanol and ethyl hexanoate and ethyl octanoate ethyl esters. Similar results  
636 were reported by Ugliano & Moio (2005) and Vilanova & Martínez (2007). The CONT A1, CONT  
637 A2, and CO1 groups showed further positive correlations between OAV and olfactory sensory  
638 attributes. Floral perception was found to be positively correlated with phenylethylacetate,  
639 hydroxyethylbenzene, hexanoic acid, decanoic acid, and 3-methyl-1-butanol. These findings are in  
640 agreement with the studies conducted by Cañas, Romero, Alonso, & Herreros (2008); Selli et al.,  
641 (2004) and Ferreira et al. (2016). However, no olfactory correspondence between 3-methyl-1-butanol,  
642 hexanoic acid, decanoic acid, and the corresponding scents of solvent, rancid, and fatty was detected.  
643 In fact, PCA delineated an inverse correlation between the sensory attributes and the VOCs. As a  
644 result, the judges were unable to discriminate the related scents despite being detected above the  
645 threshold of perception.

646 The second group was represented by the CO3 and CO8 trials (MLA4 -NF213 and MLA4 -QA23).  
647 The odour perceptions associated with these tests (rancid, grease, butter, paint, solvent, etc.) had no  
648 correspondence with the VOCs associated with them and found above the perception threshold.

649 It is likely that in a mixture characterized by low olfactory threshold VOCs and ethanol, the medium-  
650 chain fatty acids hexanoic and octanoic, and the higher alcohols 3-methyl-1-butanol and 3-  
651 methylsulfanyl-1-propanol, play a role in defining some synergy and antagonism relationships  
652 (Ferreira, 2012; Ferreira et al., 2016) or embedding (Naselli et al., 2023). These compounds may not  
653 be perceptible to the human nose. Although the odor perceptions of cream and buttery were positively  
654 correlated with trials CO3 and CO8, no identifications were observed either by the judges or by gas  
655 chromatographic analysis for 2,3-butanedione. However, the statistical PCA analysis showed

656 potential productivity of 2,3-butanedione by MLA4 strain, in association with both *S. cerevisiae*  
657 strains. Celik, Cabaroglu, & Krieger- Weber (2019) have already observed such evidence. With  
658 reference to the second group and, specifically, the CO8 trial (MLA4 -QA23), the latter showed a  
659 close relationship with banana, pear, and sweet fruity scents. These olfactory perceptions were  
660 consistent with the findings of the OAVs as they were attributable to 3-methyl-1-butyl acetate  
661 (Azudin, Sangaran, & Abd Shukor, 2020).

662 The third group was represented by the CO6 trial alone (MLB6-QA23). This test was negatively  
663 correlated with the fruity and floral scents attributable to both the first group and the second group  
664 above. Similar behavior can be attributed to the fourth group represented by the CO5 and CO10 trials  
665 (MLPK45H-NF213; MLPK45H-QA23). The panels citrusy hints to them, which were not in  
666 correspondence with what was detected with the OAVs.

667

#### 668 **4. Conclusions**

669 The volatile fraction of wine is a complex subject to investigate. The perceivable olfactory attributes,  
670 which derive from the different mixture of VOCs present in solution in wine, do not follow linear  
671 dynamics nor do they respond to additive phenomena. In fact, in the two different experimental sets,  
672 the OAV units readings for specific sensory perceptions did not always correspond with those  
673 measured by the panel judges. This phenomenon was found to be closely dependent on the microbial  
674 consociation used (Fig. 2b) and thus on the relationships established between LAB and *S. cerevisiae*  
675 during co-fermentations. According to this study, the diversity of malic acid degradation trends in  
676 LAB was found to be a function of the microbial consociation adopted. The trophic requirements of  
677 each individual and the antagonistic means (production of medium-chain fatty acids and higher  
678 alcohols) implemented by *S. cerevisiae* to colonise the culture medium led to interferences in LAB  
679 with regard to decarboxylation times (MLF day) (Table 1) and malic acid degradation rates per unit  
680 time (m). The quantification of these latter parameters also made it possible to clarify the relationships  
681 between microbial association and VOCs with a high olfactory impact by calculating the TAIndex.

682 TAIindex values tending towards 0 (0.009-0.013) were associated with wines with a higher production  
683 of the ethyl esters ethyl octanoate and ethyl decanoate. TAIindex values of close to 0.02 (0.019)  
684 indicate wines with olfactory VOCs mainly attributable to acetate esters and higher alcohols. In  
685 contrast, TAIindex values higher than 0.148 showed a negative correlation with the main olfactory  
686 VOCs, thus limiting the contribution of LAB to aroma production.

687 In conclusion, the wines obtained from the Catarratto cultivar after malolactic fermentation were  
688 found to have an olfactory profile mainly characterized by a fruity and floral component that  
689 contributed to olfactory complexity. No buttery hints attributable to the production of 2,3-butanedione  
690 were detected.

691 This aspect validates the microbial association technique as a biotechnological strategy capable of  
692 modulating the aromatic profiles of wines and guaranteeing their microbial stability.

693 Further investigations will be carried out with non-targeted metabolomics approaches to probe the  
694 effects on VOCs highlighted by the TAIindex. Finally, it cannot be ruled out that TAIindex can be  
695 integrated into artificial intelligence calculation software to predict and diversify the aroma profiles  
696 of wines according to consumer taste.

697

#### 698 **Declaration of competing interest**

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

701

#### 702 **Data availability**

703 Data will be made available on request.

704

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