Food Bioscience

Antioxidant effect of glutathione-rich specific inactivated yeast in pre-fermentative phase on organic Catarratto grape must.

--Manuscript Draft--

Manuscript Number:	
Article Type:	Research Paper
Keywords:	browning; colour development; glutathione; Metschnikowia pulcherrima; non-Saccharomyces yeast; wine oxidation.
Corresponding Author:	Nicola Francesca University of Palermo Department of Agricultural Food and Forest Sciences Palermo, Italy
First Author:	Enrico Viola
Order of Authors:	Enrico Viola
	Vincenzo Naselli
	Rosario Prestianni
	Michele Matraxia
	Antonino Pirrone
	Valentina Craparo
	Venera Seminerio
	Paola Vagnoli
	Antonella Porrello
	Natale Badalamenti
	Antonella Maggio
	Luca Settanni
	Giancarlo Moschetti
	Nicola Francesca
	Antonio Alfonzo
Abstract:	The aim of this research was to explore the possible effects of the use of a glutathione-rich specific inactivated yeast (GIY) on pre-fermentative stages of winemaking process. The research evaluated the single and combined antioxidant effects of GIY, with or without Metschnikowia pulcherrima, at different stages of grape pressing process. The antioxidant effect was evaluated by measuring soluble oxygen and colour browning of musts. The impact of different oenological protocols on the aromatic profile of the final wines was assessed through the volatile organic compounds (VOC) and sensory analysis. The inoculation treatment of M. pulcherrima during the pre-pressing phase, followed by the addition of GIY during the post-pressing stage, limited both O2 uptake (1.49 mg/L) and the browning phenomenon (0.093 OD at 420 nm) during the pre-fermentative phase. The analysis of VOC revealed a clear separation of aroma profiles between the treatments involving the addition of GIY and the controls. The protocol involving the addition of GIY during pressing showed the highest concentrations of ethyl octanoate (26.03 mg/L) and ethyl decanoate (26.87 mg/L). The sensory evaluation of the wines revealed the absence of off-odours and off-flavours in all treatments. However, the treatment involving the addition of M. pulcherrima and GIY received the greatest scores for the attributes smoothness and colour. In conclusion, the study found that GIY added treatments can be used in winemaking to enhance wine colour and counteract the detrimental effects of oxidation. These findings offer a potential alternative to reduce the use of SO2 in wine production.
Suggested Reviewers:	Piergiorgio Comuzzo

	piergiorgio.comuzzo@uniud.it
	Carien Coetzee coetzee@sun.ac.za
	Wessel Du Toit wdutoit@sun.ac.za
Opposed Reviewers:	

Università degli Studi di Palermo

DIPARTIMENTO SCIENZE AGRARIE, ALIMENTARI e FORESTALI

Il Direttore – Prof. Tiziano Caruso

SAAF DIPARTIMENTO SCIENZE AGRARIE ALIMENTARI FORESTALI

Palermo, 20/03/2024

Dear Editor,

I am pleased to submit the paper titled “Antioxidant effect of glutathione-rich specific inactivated yeast in pre-fermentative phase on organic Catarratto grape must” to your attention.

The paper dealt with the industrial production of experimental wines with the use of a glutathione enriched specific inactivated yeast (GIY). In addition, a treatment with a combination of *Metschnikowia pulcherrima* and the GIY was also studied. These bioprotection techniques are generally used to be added in the must before the inoculum of *Saccharomyces cerevisiae*. In this work, this GIY was tested as addition in different pre-fermentative stages to evaluate its antioxidant properties alone or together with the non-*Saccharomyces* yeast strain. The musts were produced starting from “Catarratto” grape variety and were analysed for several oxidation-related parameters. Moreover, VOCs and sensory analyses were performed in order to evaluate their possible effects on wine aroma.

In our opinion, the topic of this manuscript could be of interest for Food Bioscience readers and potentially falls within the aims of the journal.

We hope the manuscript could be revised by FB reviewers.

With my best personal regards,

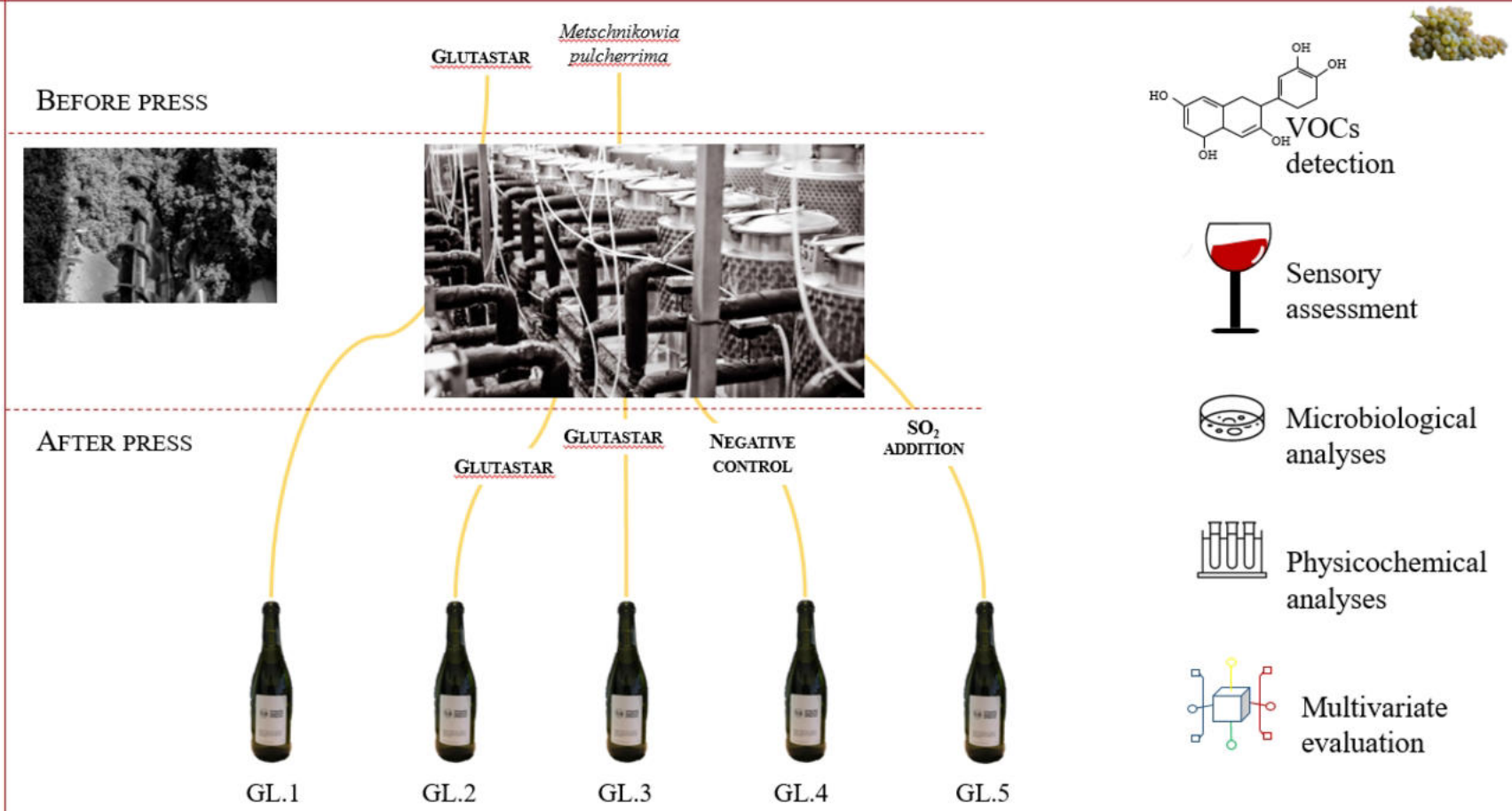
Nicole Francesca

Viale delle Scienze – Edificio 4 Ingr. B - 90128 Palermo (PA) Direzione Tel. 091.23861250
Responsabile Amministrativo Dott. Giovanni Rizzuto Tel. 091.23897200 mail: giovanni.rizzuto@unipa.it
mail: dipartimento.saaf@unipa.it - PEC: dipartimento.saaf@cert.unipa.it
sito web: www.unipa.it/dipartimenti/saaf

Highlights

- Glutathione rich inactivated yeast (GIY) addition in several prefermentative phases
- *Metschnikowia pulcherrima* was added in combination with GIY in a treatment
- The combination was able to limit dissolved O_2 levels and browning effects
- GIY-added trials showed the highest esters concentration through VOCs analysis

Graphical Abstract



Antioxidant effect of glutathione-rich specific inactivated yeast in pre-fermentative phase on organic Catarratto grape must.

Enrico Viola[a], Vincenzo Naselli[a], Rosario Prestianni[a], Michele Matraxia[a], Antonino Pirrone[a], Valentina Craparo[a], Venera Seminerio[a], Paola Vagnoli[b], Antonella Porrello[c], Natale Badalamenti[c,d], Antonella Maggio[c,d], Luca Settanni[a], Giancarlo Moschetti[a], Nicola Francesca[a,]*, Antonio Alfonzo[a]

[a] *Department of Agricultural, Food and Forest Sciences (SAAF), University of Palermo, Viale delle Scienze, Bldg. 5, 90128, Palermo, Italy*

[b] *Lallemand Italia, Via Rossini 14/B, 37060, Castel D'Azzano, Italy*

[c] *Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Viale delle Scienze, Bldg. 16, 90128, Palermo, Italy.*

[d] *NBFC-National Biodiversity Future Center, Piazza Marina 60, 90133, Palermo, Italy.*

*Corresponding authors. *E-mail address:* nicola.francesca@unipa.it (N. Francesca).

ABSTRACT

The aim of this research was to explore the possible effects of the use of a glutathione-rich specific inactivated yeast (GIY) on pre-fermentative stages of winemaking process. The research evaluated the single and combined antioxidant effects of GIY, with or without *Metschnikowia pulcherrima*, at different stages of grape pressing process. The antioxidant effect was evaluated by measuring soluble oxygen and colour browning of musts. The impact of different oenological protocols on the aromatic profile of the final wines was assessed through the volatile organic compounds (VOC) and sensory analysis. The inoculation treatment of *M. pulcherrima* during the pre-pressing phase, followed by the addition of GIY during the post-pressing stage, limited both O_2 uptake (1.49 mg/L) and the browning phenomenon (0.093 OD at 420 nm) during the pre-fermentative phase. The analysis of VOC revealed a clear separation of aroma profiles between the treatments involving the addition of GIY and the controls. The protocol involving the addition of GIY during pressing showed the highest concentrations of ethyl octanoate (26.03 mg/L) and ethyl decanoate (26.87 mg/L). The sensory evaluation of the wines revealed the absence of off-odours and off-flavours in all treatments. However, the treatment involving the addition of *M. pulcherrima* and GIY received the greatest scores for the attributes smoothness and colour. In conclusion, the study found that GIY added treatments can be used in winemaking to enhance wine colour and counteract the detrimental effects of oxidation. These findings offer a potential alternative to reduce the use of SO_2 in wine production.

Keywords: browning; colour development; glutathione; *Metschnikowia pulcherrima*; non-*Saccharomyces* yeast; wine oxidation.

1. Introduction

The tripeptide glutathione (GSH) is made up of glycine, l-glutamate, and cysteine. GSH can exist in cells in two different forms: reduced (GSH) and oxidized, which is known as glutathione disulfide (GSSG; Fahey, 2001). In most cases, the cell contains more than 90% of GSH in its reduced form, which results, after oxidation processes, in the formation of GSSG; however, glutathione reductase can convert GSSG back to GSH (Li et al., 2004).

Three key roles of GSH can be summed up as follows: detoxifier, immune enhancer, and antioxidant (Siyun et al., 2019). GSH is essential for enzyme activity, sulphur and nitrogen metabolism, endogenous toxic metabolite detoxification, bio reduction and, thus, defence against oxidative stress (Coetzee et al., 2016). As a result, it is regarded as a significant, adaptable, and potent self-generated defensive molecule (Li et al., 2004).

The role and use of glutathione in wine has attracted considerable scientific and commercial interest (Kritzinger, et al., 2013). In fact, oxidation is one of the main issues in wine production nowadays, it affects most wineries and can lead to various problems during the process. (Begum et al., 2023). This results in a number of defects in the final product, such as browning (Li et al., 2008; Nikolantonaki, & Waterhouse, 2012). In case of *Botritys cinerea* infections, there is a laccase release, an enzyme that could have a serious detrimental effect initially on musts and then on wines quality (Evli, & Uygun, 2023; Garcia et al., 2023). Moreover, dissolved oxygen in wines usually reacts with phenolic compounds, which presence is often associated with wine antioxidant properties. This reaction, especially in presence of metals acting as catalysers, results in chemical oxidations (Marchante et al., 2020). Oxidation results in a general loss of aromatic compounds and in the development of undesired ones (Zhang et al., 2023).

In order to find a solution to all these challenges and preserve wine quality, SO_2 is traditionally one of the most used additives in wine production because of its antimicrobial and antioxidant properties (Bahut et al., 2020). Indeed, it promotes *Saccharomyces cerevisiae* growth through the repressions of

other microorganisms, such as non-*Saccharomyces* yeasts or lactic acid bacteria, whose presence could lead to unwanted spontaneous fermentation (Guerrero, & Cantos-Villar, 2015). Such microbial control property also leads to reduced fermentation time, another key aspect that explains its wide use throughout the winemaking process (Delso et al., 2023). In addition, SO_2 exerts its antioxidant power through dissociation to bisulphite and sulphite ions, limiting the oxidation caused by Fenton reaction and leading quinones reduction that return in phenol form (Pelonnier-Magimel et al., 2023).

Despite these undiscussed advantages related to winemaking, several studies have highlighted some concerns about the potential SO_2 harmful effects on human health, especially in individuals susceptible to allergies (Gutiérrez- Escobar et al., 2023). Numerous studies have observed various reactions, including anaphylactic shock, gastrointestinal symptom and bronchospasm (EFSA, 2014). This increased scientific awareness of possible risks to consumers led World Healt Organization (WHO) and European Union (EU) to several recommendations to producers on limiting the use of sulphites in food industry. Furthermore, EU imposed a limit to the concentration of total SO_2 in wines (European Commission, 2009); and in an effort to maximize transparency to consumers, it has made it mandatory to indicate the presence of sulphites on the label of such products (European Commission, 2011).

Following scientific and authoritative alarm, the consumer community has also become increasingly concerned about the health effects of sulphites. Besides, there is now a clear common will to products that are as natural as possible and in which the use of chemical additives is definitely limited (De Vero et al., 2017; Gabrielli et al., 2017).

Given all of these factors, the food industry stated that alternative strategies that can lower or, perhaps, completely remove SO_2 from the production chain are required (Comuzzo et al., 2015).

Hence, researches in the last decade have focused on finding a suitable alternative to SO_2. Among them there are lots of method that have been tried, each of them have some advantages, but there is not one of them that is able to totally replace must sulphating. However, lots of this studies have

highlighted that there is more than a possibility to, at least, reduce SO_2 concentration in musts and wines (Lisanti et al., 2019).

Among all these proposed methods, in recent years the use of glutathione attracted considerable interest, since its antioxidant activity has been known for several years (Singleton et al., 1985). Its use, however, has been mainly studied in wine-like solution and not enough in must and wine, especially at different stages of the winemaking process.

To our knowledge, no studies have been conducted comparing the addition of glutathione-enriched products at different pre-fermentation stages and in combination with *M. pulcherrima*. For this reason, the aim of this study was to evaluate the antioxidant activity of a glutathione-rich yeast derivative (GIY) added before and after pressing stage in a must from Catarratto grapes. In addition, a non-*Saccharomyces* yeast (*M. pulcherrima* MP1) was used in combination with the GIY to explore some possible interaction effect between them.

2. Materials and Methods

2.1 Experimental design and winemaking process

The experimental plan is reported in Fig. 1 and aimed to evaluate the effect of glutathione-rich specific inactivated yeast (GIY) (Glutastar™, Lallemand Inc. Italia, Castel D'Azzano, Italy) indicated to protect white and rose wines from oxidative phenomena during vinification process. Grape of Catarratto variety (San Giuseppe Jato, 37°59′20″ N; 13°11′34″ E, Palermo, Sicily, Italy) was processed at experimental cellar of Department of Agricultural, Food and Forest Sciences (SAAF) at University of Palermo.

Grapes were manually harvested, destemmed and crushed. Potassium metabisulphite (4 g/q; Esseco Group, Trecate, Italy) was added during destemming; the obtained must was split into 15 batches, each consisting of 100 L.

Five different trials were performed in order to evaluate the antioxidant effect of Glutastar™ during different timing of utilization in the pre-fermentative phase: GL.1 trial: GIY (30 g/q) was added in crushed-destemmed grapes before pressing; GL.2 trial: GIY (30 g/q) was added in the must at the exit of press; GL.3 trial: crushed-destemmed grapes was inoculated with 10 g/q of active dry yeast (ADY) of *M. pulcherrima* MP1. A and then with 30 g/q of GIY in must at the exit of press; GL.4 trial: (control) the treatment did not include the addition of either GIY or *M. pulcherrima*; GL.5 trial: an additional control was represented by adding 20 mg/L of SO_2, a practice commonly used in industrial winemaking (Arapitsas et al., 2018). Before clarification, 30 g/hL of Bactiless™ (Lallemand Inc. Italia, Castel D'Azzano, Italy) was added in each trial, to inhibit lactic and acetic bacteria population. Clarification lasted for 24 h at 5 °C in the presence of 2 g/hL of Lallzyme™ HC (Lallemand Inc. Italia, Castel D'Azzano, Italy).

The alcoholic fermentation was carried out using the *S. cerevisiae* NF213 strain belonging to the oenological yeast collection of the SAAF Department (University of Palermo, Italy; Francesca et al., 2024). The nutritional regime was adjusted according to the level of nitrogen in the must, in order to maintain at least the minimum amount of nitrogen suggested in the literature (120–140 mg/L) to avoid any kind of nutrient starvation issues (Kemsawasd et al., 2015). Hence, yeast assimilable nitrogen (YAN) was adjusted through two moments: before inoculum and at 1/3 of alcoholic fermentation (about 4 d). Yeasts were inoculated with 20 mL/hL of concentrated liquid suspension [about 7.00×10^{10} colony-forming units (CFU)/g]. Alcoholic fermentation took place at 18 °C. Temperature and residual sugars were daily checked. At the end of alcoholic fermentation, wines were added with further 20 mg/L of potassium metabisulphite, therefore removed from lees and transferred into clean glass carboy at 5 °C up to bottling phase.

Sample collection involved bulk grape must before and after clarification, must after yeast inoculum, must during alcoholic fermentation (25%, 50% and 75% of alcoholic fermentation), wine at the end of alcoholic fermentation. All samples were immediately subjected to analysis. Each experiment was performed in three replicates.

2.2. Microbiological analysis

All samples collected during wine production were analysed for yeasts and acetic bacteria populations. Must samples were diluted in Ringer's solution (Sigma-Aldrich, Milan, Italy) and analysed in triplicate for total yeasts (TY) on Wallerstein Laboratory (WL) nutrient agar (Pallmann et al., 2001) and acetic acid bacteria (AAB) on Kneifel agar medium (OIV, 2010). Presumptive *Saccharomyces* spp.; *Metschnikowia* spp. (brown/red pulcherrimin pigment production; Pallmann et al., 2001), and *Hanseniaspora* spp. (intense green; Martin et al., 2018), were inferred from yeasts count on WL nutrient agar. All media and the supplements used were purchased from Oxoid (Thermofisher, Milan, Italy).

2.3 Yeasts population dominance

In order to verify the *S. cerevisiae* dominance throughout the vinification process, five to seven colonies of each morphology were chosen from WL Petri plates and discriminated following the parameters reported by Pallmann et al. (2001). Colony morphology and colour were checked and sequentially propagated in order to obtain axenic cultures (Lai et al., 2022). In addition, a microscope observation was assessed to verify their cellular morphology (Carl Zeiss LTd, Oberkochen, Germany). Thereafter, a genetic characterization was used to identify the dominance of NF213 strain during winemaking process.

Extraction of genomic DNA to assay PCR analysis was obtained through InstaGene Matrix kit (Bio-Rad, Hercules, USA) and performed as reported by producer protocol. RFLP assay was used to assess yeast differentiation using the DNA region spanning the internal transcribed spacers (ITS1, ITS2) and the 5.8 rRNA gene as reported by Esteve-Zarzoso et al. (1999). In addition, one isolate for each group was evaluated through the sequencing of the D1/D2 region of the 26S rRNA gene (Alfonzo et al., 2020). Sequencing reactions of DNA were obtained and elaborated as reported by Francesca et al. (2024). The final confirmation of the dominance of *S. cerevisiae* strain applied in this work was obtained by the comparison of interdelta (Legras & Krast, 2003) profiles of the axenic cultures

isolated from musts with that of pure NF213 strain. Finally, the eventual persistence of *M. pulcherrima* MP1 in GL.3 trial was verified by randomly amplified polymorphic DNA(RAPD)-PCR analyses (Alfonzo et al., 2021; Francesca et al., 2014).

2.4. Physicochemical analysis

2.4.1. Wine composition

pH was determined by OIV-MA-AS313-15 method (OIV, 2020a) through a pH-meter (XS Instruments, Carpi, Italy), total acidity was determined by the methodology described by OIV-MAAS313-01 (OIV, 2020b). The ethanol content was assessed using the method described by Chawafambira (2015).

Enzymatic assays for glucose, fructose, glycerol, ammonia nitrogen, alpha-amino nitrogen, acetic acid, malic acid, lactic acid, total and free sulfur dioxide were conducted on a iCubio iMagic M9 (Shenzhen iCubio Biomedical Technology Co. Ltd. Shenzhen, China) as described by Matraxia et al. (2021). The reagents were purchased from R-Biopharm AG (Darmstadt, Germany). All chemical analyses were carried out in triplicate.

2.4.2. Oenological parameters

Soluble oxygen was monitored in order to evaluate the antioxidant effect of GIY during pre-fermentative phase. For this purpose, portable dissolved oxygen meter Mod. HQ30D, equipped with Intellical LDO101 Luminescence/Optical dissolved oxygen probe (Hach Lange S.r.l, Milan, Italy) were used. Musts were analysed for Optical Density (OD) at 420 nm (after press and after clarification phases) by a 6400 Spectrophotometer (Jenway Ltd., Sheung Wan, Hong Kong).

2.4.3. Colour analysis

The determination of colour characteristics of must after the pressing stage and wines at the end of alcoholic fermentation was performed using a Chroma Meter CR-400C (Minolta, Osaka, Japan) as previously described by Carvalho, Pereira, Pereira, Pinto, & Marques (2015). Colour of samples was determined basing on CIE *L*a*b** colour system [CIE *L*a*b**: lightness (*L**); redness/greenness

(*a**); yellowness/blueness (*b**)]. The colour parameters L*, *a**, *b** and hue angle (h) were measured, while Chroma (C*), values were calculated using the following equations:

$$C^*_{ab} = (a^2 + b^2)^{\frac{1}{2}} \quad (1)$$

where C* = Chroma, a = redness/greenness index and b = yellowness/blueness index.

2.4.4. Volatile organic compounds

Volatile compound composition was determined following the protocol reported by Alfonzo et al. (2021). Wine samples (10 mL) from each trial were mixed with MS SupraSolv® (Merck, Darmstadt, Germany) dichloromethane (10 mL) in a 100 mL conical flask, stirred at room temperature for 30 min, and centrifuged at 3000 rpm for 10 min by Low Speed Centrifuge (ScanSpeed 416) with Swing Rotor (LaboGene ApS, Vassingerød, Lynge, Denmark). The aqueous phase was removed and anhydrous sodium sulphate (1 g) was added before centrifuging at 3000 rpm for 10 min. The dichloromethane layer was removed, and dried under N_2 gas to 1 mL. Gas chromatographic analysis was performed with a GC–MS apparatus, an Agilent 7000C GC system, fitted with a fused silica Agilent DB-5MS capillary column (30 m × 0.25 mm i.d.; 0.25 μm film thickness), coupled to an Agilent triple quadrupole Mass Selective Detector MSD 5973; ionization voltage 70 eV; electron multiplier energy 2000 V; transfer line temperature, 295 °C. Solvent Delay: 5 min. Helium was the carrier gas (1 mL/min). The temperature was initially kept at 40 °C for first 5 min. Then gradually increased until 250 °C at 2 °C/min rate, and finally raised to 270 °C for 15 min. One μL of sample was injected at 250 °C automatically and in the splitless mode; transfer line temperature, 295 °C. The individual peaks were analysed using the GC MS Solution package, Version 2.72. Identification of compounds was carried out using Adams, NIST 11, Wiley 9 and FFNSC 2 mass spectral database. These identifications were also confirmed by other published mass spectra and linear retention indices (LRI). The LRI were calculated using a series of *n*-alkanes (C_8-C_{40}). In addition, some of the compounds were confirmed by comparison of mass spectra and retention times with standard compounds available at the Department STEBICEF – University of Palermo. For each VOC

identified, the (OAV) was evaluated as described by Butkhup et al. (2011) in order to identify which VOC was a significant contributor to the odour series characterising each wine.

2.5. Sensory evaluation

Sensory evaluation of experimental wines aimed to highlights differences between treatments. A quantitative descriptive analysis was performed according to Alfonzo et al. (2021).

Ten judges (6 men and 4 women, ranging from 26 to 45 years old) were recruited from University of Palermo. Everyone had a strong experience in winemaking and participated in previous studies as sensory judges. The judges were subjected to preliminary tests to determine their sensory performances on basic tastes and wine-associated aromas. The sensory analysis of wines was conducted following the methodology by Jackson (2016). The 10 panellists compared the five experimental wines during different sessions. They consensually generated 16 sensory descriptive attributes regarding appearance, odour, taste and overall quality. The set of attributes were: appearance (colour); odour (intensity, complexity, floral, fruity, spicy, overall odour quality); taste (intensity, persistence, sour, salty and smoothness, overall taste quality); overall rating. The panellists were also trained for the identification of wine off-odors and off-flavor: microbial (mouldy, corky, yeasty, buttery and cheesy); pungent (vinegary, alcoholic and sulfur); putrid (rancid, rotten egg and rubbery); petroleum (fusel, plastic and solvent), and other (Jackson, 2016; Issa-Issa et al., 2020). The panellists also generated a consensual descriptive ballot for the wines in which the descriptors were associated with a 9 cm unstructured scale anchored at the left and right extremes with the terms "none/weak" and "strong", respectively (Jackson, 2016; Biasoto et al., 2014). The five wine samples were evaluated in distinct tasting sessions carried out on successive days. Overall, each judge evaluated each of the five wines with three repetitions. For each repetition, a different wine bottle was opened. To control the contrast effect among the samples an incomplete balanced block design was used (Cochran & Cox, 1957).

2.6 Statistical and explorative multivariate analyses

Microbiological, physicochemical, oenological, sensory and VOC composition data were subjected to one-way analysis of variance (ANOVA). The Tukey's test was applied for pairwise comparison. Statistical significance was attributed to $p<0.05$. The hierarchical clustering analysis (HCA) and principal component analysis (PCA) of data were performed to investigate relationships among volatile organic compounds. All analyses were performed by XLStat software version 2020.3.1 for Excel (Addinsoft, New York, USA).

3. Results and Discussion

3.1 Microbiological monitoring

Yeast population during fermentation is reported in Fig. 2. On grape must, total yeasts (TY) were found at 3.6 Log CFU/mL. Yeasts ecology during winemaking first stages is often represented by non-*Saccharomyces* species, as reported by several authors (Varela et al., 2016; Di Maro et al., 2007); moreover, *Saccharomyces* spp. yeasts were not detected on grape must, in accordance with the study conducted by Romancino et al. (2008) due to their status named "viable but not cultivable" (Andorrà et al., 2010). However, Aponte and Blaiotta (2016) by using DNA method detection, found *Saccharomyces* spp. on grape and in grape must. Finally, no colony of acetic acid bacteria was found on Kneifel agar during all vinification stages.

M. pulcherrima MP1 was inoculated at 5.8 Log CFU/mL in GL.3 while in other treatments, presumptive native *Metschnikowia* spp. yeasts remained below 4 Log cycles. The addition of SO_2 in GL.5 resulted in the absence of presumptive *Metschnikowia* spp. Meanwhile, TY showed a decrease of approximately 1 log cycle during the post-pressing stage, confirming the study of King et al. (1981).

The NF213 strain of *S. cerevisiae* was inoculated in racked must and showed microbial counts ranging from 6.4 to 7.0 log CFU/mL and regular fermentation kinetics; a short delay in the exponential growth phase was observed in the GL.5 trial, probably due to the SO_2 addition (King et al., 1981).

265 *3.2 Dominance of inoculated strain*

266 A collection of 618 colonies isolated from WL Petri plates were analysed and a total number of 562
267 colonies were identified as yeasts belonging to presumptive *Saccharomyces.* This resulted from the
268 phenotypical observations and was further confirmed by the analysis of 5.8S-ITS amplicons that
269 revealed that each isolate coming from the respective colony shared a 5.8S-ITS region of 880 bp,
270 common to *S. cerevisiae* (Las Heras-Vazquez et al., 2003). Moreover, the restriction fragments
271 profiles found through *Cfo*I, *Hae*III and *Hinf*I was an additional confirmation that they were *S.*
272 *cerevisiae* (Guillamón et al., 1998). The remaining 56 isolates were almost all picked from Petri plates
273 belonging to GL.3 treatment (53/56) and, thus, they were classified as presumptive non-
274 *Saccharomyces* yeasts. In particular, they showed an amplicon size of 400 bp that is associated with
275 *M. pulcherrima* strains (Esteve-Zarzoso et al., 1999). Finally, the presence and dominance of NF213
276 strain in each treatment was confirmed by Interdelta analysis. Additionally, it showed a clear
277 dominance of NF213 strain, while in GL.3 treatment, the inoculum of *M. pulcherrima* determined a
278 dominance of this strain among the non-*Saccharomyces* yeasts. In order to complete yeast genotypic
279 analysis, the alignment of D1/D2 sequence of strains isolated throughout this study was performed
280 with the sequences of pure strains; they showed 100% alignment homology.

281 *3.3. Physicochemical analysis*

282 *3.3.1. Wine composition*

283 Analysis of the principal chemical parameters (sugars, organic acids, nitrogen, pH, total acidity and
284 glycerol) on must and wine (end of alcoholic fermentation) are reported in Table 1.

285 Residual sugars at the end of alcoholic fermentation (14 d) ranged between 1.98 (GL.5) and 2.17
286 (GL.2) g/L with no statistical differences between trials. pH values increased during fermentation,
287 from 3.21 (must) to 3.32 on average at the end of alcoholic fermentation. In GL.5, the highest total
288 SO_2 value (55.2 mg/L) was determined at the end of alcoholic fermentation due to a further addition
289 of potassium metabisulphite compared to the other trials. The other treatments showed values

between 37.1 mg/L (GL.3) and 37.7 mg/L (GL.1). Acetic acid was detected only at the end of alcoholic fermentation, with values below 0.25 g/L in all trials, comparable to results reported by Alfonzo et al. (2021) on "Catarratto bianco lucido" cultivar and using autochthonous yeast strains. Such low concentrations of acetic acid, produced by *S. cerevisiae*, are reported in several study regarding wine fermentations driven by *S. cerevisiae* strains (Zamora et al., 2009; Mylona et al., 2016).

Total nitrogen (TN) in grape must was of 142.6 mg/L. At the end of alcoholic fermentation, total nitrogen values ranged between 143.88 (GL.5) and 149.66 (GL.2) mg/L. The higher values found at the end of alcoholic fermentation were justified by the addition of YAN prior to the *S. cerevisiae* inoculum and at 1/3 of alcoholic fermentation. As a matter of fact, in order to ensure a complete alcoholic fermentation conducted by *S. cerevisiae*, the minimum amount of nitrogen should be 120–140 mg/L (Kemsawasd et al., 2015).

No significant differences were found between trials and during all vinification process on pH, total acidity, acetic acid, malic acid and total nitrogen (data collected at 25%, 50% and 75% of alcoholic fermentation were not shown).

The highest glycerol content was reported for GL.3, which involved the use of *M. pulcherrima* MP1, showing a value of 7.3 g/L, comparable to those found in the study performed by Canonico et al. (2019), where a different strain of *M. pulcherrima* was used. Using non-*Saccharomyces* yeasts in combination with GIY, allows for larger amounts of compounds that contribute to improve the health conditions of yeast cells conducting fermentation (Matraxia et al., 2021; Lai et al., 2023). In addition, these molecules often have a positive impact on sensory aspects. For instance, glycerol improves the mouthfeel and overall taste of wine (Laguna et al., 2017).

3.3.2. Oenological parameters

Oxygen monitoring during clarification is represented in Fig. 3. The two values registered were obtained from post press and post clarification detection points. In trial GL.4, where no addition was made, the largest increase of O_2 (+95%) was registered from the technological phase of pressing to

316 the static clarification, reaching the highest O_2 value among trials, after this step (2.46 mg/L). In trial
317 GL.5, as expected (Coetzee, 2014) SO_2 addition contributed to record the lowest dissolved O_2
318 increase between the two steps (+62%). In both GIY-added trials (GL.1 and GL.2) the effect on
319 limiting O_2 uptake during clarification phase was marked, compared with negative control trial
320 (GL.4), with values of 1.94 and 1.90 mg/L, respectively. However, they registered higher values and
321 also a higher increase, compared to SO_2 added trial (GL.5) that showed an O_2 level of 1.59 mg/L at
322 the end of clarification. Our data are comparable to those found by Bustamante et al. (2024), who
323 tested the same GIY on a must-like solution. Finally, the treatment inoculated with the combination
324 of *M. pulcherrima* MP1 before press and Glutastar™ after that (GL.3), achieved the lowest dissolved
325 O_2 value (1.49 mg/L) in post clarification detection. Several authors (Catarino et al., 2014; Vidal et
326 al., 2004), reported that winemaking operations, such as crushing, pressing and low temperature
327 during the clarification process can lead to a large increase in dissolved O2 (up to 8 mg/L). The
328 addition of Glutastar™ before or after the pressing stage resulted in a lower increase in dissolved
329 oxygen levels in the next stages, especially when compared to the negative control. However, they
330 were not as effective as the SO_2-added trial in limiting oxygen uptake in musts.
331 The analysis revealed that the use of a non-*Saccharomyces* yeast, followed by the addition of a GIY,
332 resulted in lower dissolved oxygen values compared to the SO_2-added trial (GL.5). In addition, no
333 differences in dissolved oxygen were found between the GL.3 and GL.5 treatments in both post-
334 pressing and post-clarification. The effects of the different treatments on predisposition to browning
335 of the five musts in post pressing and after the clarification are reported in Fig. 4. The 420 nm OD
336 analysis revealed that GIY-added trials were capable to limit the browning effects on musts as
337 reported by Bustamante et al. (2024). The GL.2 (0.100), GL.3 (0.093) and GL.5 (0.106) trials showed
338 the lowest OD values, while the GL.4 treatment exhibited the highest value (0.187). Lyu et al. (2021)
339 also described a similar phenomenon where the musts that included the addition of GSH had the
340 lowest OD values (0.035), while the musts without GSH had higher observed values (0.268).
341 However, the addition of GIY during pre-pressing in GL.1 did not produce a comparable effect to the

results observed in the GL.2 and GL.3 trials or the GL.5 control. After clarification, the OD values were comparable to that observed during post-pressing. In particular, every treatment highlighted an increase in 420 nm OD values; GL.4 showed again the greatest values among treatments (0.216) and the highest increase (+15%), this result is easily explainable, because, as reported by Giménez et al. (2017), when glutathione or other compounds with antioxidant properties are not available, polymerization of the quinones leads to browning of the juice.

The trial GL.1 followed with 0.158, indicating that GIY addition made before the pressing stage was likely too early in order to exert its effect on the mitigation of browning. Finally, the two treatment in which the addition of Glutastar™ was made after the press stage (GL.2 and GL.3) and the control treatment (GL.5), showed the smallest increase (+8.0%, +5.4% and +6.6%). Hence, highlighting that the best effect on limiting browning potential can be achieved with the late GIY addition on must (after the pressing stage).

3.3.3. Colour characteristics of experimental wines

Colour analysis results are showed in Table 2. Colour of the musts was evaluated in order to understand how each experimental condition affected this important oxidation-related parameter. The highest L* (lightness) value in post-pressing stage was recorded from GL.2 (96.75), while the lowest was recorded from GL.4 (90.54). The parameter a* (redness/greenness) ranged from -1.93 (GL.2) to -1.45 (GL.1), while GL.2 showed also the highest value (8.17) of the parameter b* that measures yellowness/blueness. Moreover, C* followed the same trend than b* showing the highest value for GL.2 (8.40); lastly, hue angle (tonality) ranged from 101.00 (GL.1) to 104.12 (GL.5). This parameter is linked to absorbance differences and it is considered to represent colour qualitative attribute (Recamales et al., 2006). At end of alcoholic fermentation, GL.1, GL.2, GL.3 and GL.5 treatments showed similar L* values (from 95.40 to 96.05), the least bright treatment was again GL.4 (89.66). GL.2 resulted in the highest value of hue (103.40), followed by another GIY-added treatment (GL.1), the lowest was represented by the SO_2 free treatment (GL.4; 89.34). The higher values observed for GIY-added treatments in both lightness and hue angle values, indicate that these treatments gave

lighter and less brown musts (Gómez-Míguez et al., 2007) respect to negative control treatment (GL.4). In 2 years bottled wine, it can be observed that there was a general increase in C* values, that ranged from 12.37(GL.2) to 22.57 (GL.3). In addition, the opposite trend can be appreciated in hue values that went through a general decrease, they ranged from 86.66 (GL.4) to 99.95 (GL.2). These kind of changes, chroma increase and hue decrease, are characteristic of a white wine going through a storage period (Kallithraka et al., 2009). Moreover, the colour evolution was further confirmed by the rise of b* values that allowed the wine to pass from a pale yellow to a more intense one (Recamales et al., 2006). This analysis confirmed the positive impact of the glutathione enriched specific inactivated yeast and its combination with *M. pulcherrima* on wine colour.

3.3.4. Volatile organic compounds

Table 3 shows the levels of the VOCs detected through GC-MS analysis. Fifteen compounds were detected and grouped into four classes: alcohols, esters, carboxylic acids and other compounds. The most represented class was the esters, with 8 compounds detected. The class of VOCs with the highest concentrations is that of alcohols. The alcohol present in the highest quantities was 3-methyl-1-butanol in GL.1 (144.78 mg/L) and GL.5 (145.94 mg/L), followed by phenylethyl alcohol which was detected in the highest quantities in GL.2 (112.09 mg/L) and GL.5 (106.72 mg/L). The presence of phenylethyl alcohol is associated with the floral notes of wines as reported by Francesca et al. (2024). The trials GL.2 and GL.5 produced more alcohols than the other treatments. However, among alcohols, GL.3 registered the highest value for 4-Methyl-1-pentanol, while both GL.2 and GL.3 showed the highest amount of 3-methyl-1-pentanol (~ 2.6 mg/L each).

Hexanoic acid represented the carboxylic acids, with values below the perception threshold of 0.42 mg/L, only in the trials GL.4 and GL.5. Carboxylic acids, especially hexanoic acid, are responsible for unpleasant odours associated with rancidity and cheese (Francesca et al., 2024). The influence of esters on white wine aroma is well known and they are connected with fruity and flowery aromas

(Naselli et al., 2023). Interestingly, all the three treatments with GIY addition (GL.1, GL.2 and GL.3), showed the highest values in total esters. The higher values of ethyl decanoate were detected in GL.1, while ethyl octanoate was present in high concentrations in both GL.1 and GL.2. The OAV (Odor Activity Value) is the index that defines odour perception and it was higher than 1 for 7 out of 15 compounds. Thereafter, the sum of the OAVs was calculated (Welke et al., 2014) in order to determine the olfactory intensity of each wine and, in addition, an HCA and a PCA were performed to explore the relationships between VOCs and different treatments. The biplot graph (Fig. 5A) highlighted that 84.00% of the total variability is a function of factor 1 and factor 2 (50.41% and 33.59%, respectively). In the I quadrant there was an association between the ester with highest OAV (Ethyl octanoate) and the two Glutastar^{TM} -added treatments (GL.1 and GL.2), while GL.5 was associated with the main alcohols in the II quadrant. Moreover, in the IV quadrant GL.3 and GL.4 showed similar VOCs composition. These findings were further confirmed by the HCA (Fig. 5B), which highlighted a close correlation between GL.1 and GL.2. Interestingly, the HCA revealed a higher similarity between GL.4 and GL.5, than between GL.3 and GL.4 which were similar according to PCA.

Moreover, the biplot graph generated by principal component analysis revealed that several compounds are strictly and statistically linked to GL.1 and GL.2 trials. These compounds are mainly esters, they also showed the highest OAVs and, for this reason their contribution to wine odour intensity is significantly high. Since most of the aroma-related differences between the treatments could have been dependent on this class of compounds, we performed the HCA and the PCA taking only the class of esters into accounts. The biplot graph (Fig. 6A) correlated the analysed variables and highlighted that 84.71% of the total variability is a function of factor 1 for 63.51% and factor 2 for 21.20%. In the I quadrant, the wines from trial GL.1 and GL.2 are associated, respectively, with Ethyl octanoate and Ethyl decanoate, whereas in the IV quadrant, GL.3 is linked to the three esters remaining. In addition, Fig. 6B shows the bootstrap hulls, also produced by principal component analysis. The highest areas overlap was observed in treatments GL.2 and GL:3, indicating a similar

VOC composition. In contrast, GL.4 and GL.5 were the wines showing less overlaps and, thus, separating themselves from other trials. These findings were further confirmed by the HCA (Fig. 7), that revealed a clear grouping of the wines into two clusters: one is composed by GL.4 and GL.5, while the other one consists of the three remaining trials.

3.4. Sensory analysis

The quantitative sensory analysis showed slight differences in the organoleptic profiles of the wines (Fig. 8A). No descriptor related to unpleasant attributes (off-odour and off-flavour) or related to wine oxidation were listed. The analysis of variance highlighted a difference between the GL.3 and the other treatments regarding the colour and smoothness attributes. The higher score for colour given by the judges further confirms the antioxidant properties of this combination of non-*Saccharomyces* yeast and GIY on wine colour (Giménez et al., 2023). Furthermore, the wines also differed in smoothness attribute, with GL.3 showing the best evaluation by judges (5.60). The overall scores given to the wines by the tasters (Fig. 8B) showed no differences between the wines. This supports the fact that the protocols used resulted in a product with similar sensory characteristics to that produced with the use of SO_2. In fact, the use of bio-protection techniques during pre-fermentative phase could not highlights differences between treatments, due to a high matrix effect (Simonin et al., 2020).

5. Conclusions

This research showed that the addition of GIY to Catarratto must at different stages along the pre-fermentation phases limited the effects of those factors that trigger the oxidation process. The use of GIY, both in the pre- and post-pressing stages, limited levels of dissolved O_2 during the pre-fermentative phases. Sensory analyses did not highlight any off-odours or off-flavours coming from the experimental wines without SO_2 addition. Moreover, the colour analysis confirmed the positive impact of GIY addition on both fermenting musts and bottled wine after two years of storage. The

multivariate statistical analysis performed on total OAVs and only on esters detected through VOCs analysis revealed a clear association between the most impactful fruity and flowery compounds and GIY-added trials. Interestingly, the interaction generated by the combination of *M. pulcherrrima* and GIY (GL.3) showed the least dissolved O_2 levels. Moreover, GL.3 obtained the best evaluation in smoothness attribute within the sensory assessment, probably due to its higher glycerol production. In conclusion, GIY demonstrated its suitability in potentially low or no added SO_2 wine production. It would be worthwhile to deeply study the mechanisms regulating the interactions between GIY and non-*Saccharomyces* yeasts (such as *M. pulcherrima*), as well to investigate their combined effects in different musts and with decreased amount of sulphites.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Acknowledgements

The research was conducted as part of the following research projects: (i) Supply Chain and District Contracts (5th call) project 'White Wine Identity: new horizons for an integrated and sustainable development of the Italian white wine supply chain', which was funded by the Ministry of Agriculture, Food Sovereignty and Forests. IRIS internal code PRI-1058; (ii) PSR - Rural Development Program Sicily 2014/2022-Sub-measure 16.01 "Support for the establishment and management of operational groups of the EIP on agricultural productivity and sustainability" - DDG. no. 5428 of 29/12/2021, project title: "Aromaticity and Longevity of Catarratto: Innovation in Field and Cellar based on

469 Biodiversity, Subproducts and High Sustainability Biotechnologies" - application no. 24250128113.

470 The authors are grateful to Lallemand Inc. (Montreal, Canada) for the supply of oenological products.

471

472

References

Alfonzo, A., Francesca, N., Matraxia, M., Craparo, V., Naselli, V., Mercurio, V., & Moschetti, G. (2020). Diversity of *Saccharomyces cerevisiae* strains associated to racemes of Grillo grape variety. *FEMS Microbiology Letters*, *367*(12), fnaa079. https://doi.org/10.1093/femsle/fnaa079

Alfonzo, A., Prestianni, R., Gaglio, R., Matraxia, M., Maggio, A., Naselli, V., Craparo, V., Badalamenti, N., Bruno, M., Vagnoli, P., Settanni, L., Moschetti, G., & Francesca, N. (2021). Effects of different yeast strains, nutrients and glutathione-rich inactivated yeast addition on the aroma characteristics of Catarratto wines. *International Journal of Food Microbiology*, *360*, 109325. https://doi.org/10.1016/j.ijfoodmicro.2021.109325

Andorrà, I., Esteve-Zarzoso, B., Guillamón, J. M., & Mas, A. (2010). Determination of viable wine yeast using DNA binding dyes and quantitative PCR. *International Journal of Food Microbiology*, *144*(2), 257–262. https://doi.org/10.1016/j.ijfoodmicro.2010.10.003

Aponte, M., & Blaiotta, G. (2016). Potential role of yeast strains isolated from grapes in the production of Taurasi DOCG. *Frontiers in Microbiology*, *7*, 809. https://doi.org/10.3389/fmicb.2016.00809

Arapitsas, P., Guella, G., & Mattivi, F. (2018). The impact of SO_2 on wine flavanols and indoles in relation to wine style and age. *Scientific Reports*, *8*(1), 858. https://doi.org/10.1038/s41598-018-19185-5

Bahut, F., Romanet, R., Sieczkowski, N., Schmitt-Kopplin, P., Nikolantonaki, M., & Gougeon, R. D. (2020). Antioxidant activity from inactivated yeast: expanding knowledge beyond the glutathione-related oxidative stability of wine. *Food Chemistry*, *325*, 126941. https://doi.org/10.1016/j.foodchem.2020.126941

Begum, P., Yang, L., Morozumi, T., Sone, T., & Kawaguchi, T. (2023). PANI sensor for monitoring the oxidative degradation of wine using cyclic voltammetry. *Food Chemistry*, *414*, 135740. https://doi.org/10.1016/j.foodchem.2023.135740

Biasoto, A. C. T., Netto, F. M., Marques, E. J. N., & da Silva, M. A. A. P. (2014). Acceptability and preference drivers of red wines produced from *Vitis labrusca* and hybrid grapes. *Food Research International*, *62*, 456–466. http://dx.doi.org/10.1016/j.foodres.2014.03.052

Bustamante, M., Giménez, P., Just-Borràs, A., Solé-Clua, I., Gombau, J., Heras, J. M., Sieczkowski, N., Gil, M., Perez-Navarro, J., Gómez-Alonso, S., Canals, J. M., & Zamora, F. (2024). Use of glutathione, pure or as a specific inactivated yeast, as an alternative to sulphur dioxide for protecting white grape must from browning. *Foods*, *13*(2), 310. https://doi.org/10.3390/foods13020310

Butkhup, L., Jeenphakdee, M., Jorjong, S., Samappito, S., Samappito, W., & Chowtivannakul, S. (2011). HS-SPME-GC-MS analysis of volatile aromatic compounds in alcohol related beverages made with mulberry fruits. *Food Science and Biotechnology*, *20*, 1021–1032. https://doi.org/10.1007/s10068-011-0140-4

Canonico, L., Comitini, F., & Ciani, M. (2019). *Metschnikowia pulcherrima* selected strain for ethanol reduction in wine: Influence of cell immobilization and aeration condition. *Foods*, *8*(9), 378. https://doi.org/10.3390%2Ffoods8090378

Cappello, M. S., Poltronieri, P., Blaiotta, G., & Zacheo, G. (2010). Molecular and physiological characteristics of a grape yeast strain containing atypical genetic material. *International Journal of Food Microbiology*, *144*(1), 72–80. https://doi.org/10.1016/j.ijfoodmicro.2010.08.013

Carvalho, M. J., Pereira, V., Pereira, A. C., Pinto, J. L., & Marques, J. C. (2015). Evaluation of wine colour under accelerated and oak-cask ageing using CIELab and chemometric approaches. *Food and Bioprocess Technology*, *8*, 2309–2318. https://doi.org/10.1007/s11947-015-1585-x

Catarino, A., Alves, S., & Mira, H. (2014). Influence of technological operations in the dissolved oxygen content of wines. *Journal of Chemistry and Chemical Engineering*, *8*, 390–394. https://doi.org/10.17265/1934-7375%2F2014.04.010

Chawafambira, A. (2021). The effect of incorporating herbal (*Lippia javanica*) infusion on the phenolic, physicochemical, and sensorial properties of fruit wine. *Food Science & Nutrition*, *9*(8), 4539-4549. https://doi.org/10.1002/fsn3.2432

Cochran, W. G.; Cox, G. M. (1957). *Experimental designs* (2nd ed.). John Wiley & Sons. ISBN: 978-0-471-54567-5

Coetzee, C. (2014). Oxidation treatments affecting Sauvignon blanc wine sensory and chemical composition (Doctoral dissertation, Stellenbosch: Stellenbosch University). http://hdl.handle.net/10019.1/86321

Coetzee, C., Van Wyngaard, E., Šuklje, K., Silva Ferreira, A. C., & du Toit, W. J. (2016). Chemical and sensory study on the evolution of aromatic and nonaromatic compounds during the progressive oxidative storage of a Sauvignon blanc wine. *Journal of Agricultural and Food Chemistry*, *64*(42), 7979–7993. https://doi.org/10.1021/acs.jafc.6b02174

Comuzzo, P., Battistutta, F., Vendrame, M., Páez, M. S., Luisi, G., & Zironi, R. (2015). Antioxidant properties of different products and additives in white wine. *Food Chemistry*, *168*, 107–114. https://doi.org/10.1016/j.foodchem.2014.07.028

De Vero, L., Bonciani, T., Verspohl, A., Mezzetti, F., & Giudici, P. (2017). High-glutathione producing yeasts obtained by genetic improvement strategies: a focus on adaptive evolution approaches for novel wine strains. *AIMS Microbiology*, *3*(2), 155. https://doi.org/10.3934/microbiol.2017.2.155

Di Maro, E., Ercolini, D., & Coppola, S. (2007). Yeast dynamics during spontaneous wine fermentation of the Catalanesca grape. *International Journal of Food Microbiology*, *117*(2), 201–210. https://doi.org/10.1016/j.ijfoodmicro.2007.04.007

Delso, C., Berzosa, A., Sanz, J., Álvarez, I., & Raso, J. (2023). Pulsed electric field processing as an alternative to sulfites (SO_2) for controlling *Saccharomyces cerevisiae* involved in the fermentation of Chardonnay white wine. *Food Research International*, *165*, 112525. https://doi.org/10.1016/j.foodres.2023.112525

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). (2014). Scientific Opinion on the evaluation of allergenic foods and food ingredients for labelling purposes. *EFSA journal*, *12*(11), 3894. https://doi.org/10.2903/j.efsa.2014.3894

Esteve-Zarzoso, B., Belloch, C., Uruburu, F., & Querol, A. (1999). Identification of yeasts by RFLP analysis of the 5.8 S rRNA gene and the two ribosomal internal transcribed spacers. *International Journal of Systematic and Evolutionary Microbiology*, *49*(1), 329–337. https://doi.org/10.1099/00207713-49-1-329

European Commission. (2009). Commission Regulation (EC) No 606/2009 of 10 July 2009 laying down certain detailed rules for implementing Council Regulation (EC) No 479/2008 as regards the categories of grapevine products, oenological practices and the applicable restrictions. *Official Journal of the European Union*, *193*, 1–59.

European Commission. (2011). Commission Regulation (EC). No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. *Official Journal of the European Union*, *12*, 1–177

Evli, S., & Uygun, D. A. (2023). Laccase modified GO/TiS2 nanocomposite based amperometric biosensor for (-)-epicatechin detection. *Microchemical Journal*, *188*, 108501. http://dx.doi.org/10.1016/j.microc.2023.108501

Fahey, R. C. (2001). Novel thiols of prokaryotes. *Annual Reviews in Microbiology*, *55*(1), 333–356. https://doi.org/10.1146/annurev.micro.55.1.333

Ferreira, V., López, R., & Cacho, J. F. (2000). Quantitative determination of the odorants of young red wines from different grape varieties. *Journal of the Science of Food and Agriculture*, *80*(11), 1659–1667. https://doi.org/10.1002/1097-0010(20000901)80:11%3C1659::AID-JSFA693%3E3.0.CO;2-6

Francesca, N., Sannino, C., Settanni, L., Corona, O., Barone, E., & Moschetti, G. (2014). Microbiological and chemical monitoring of Marsala base wine obtained by spontaneous fermentation during large-scale production. *Annals of Microbiology*, *64*, 1643–1657. https://doi.org/10.1007/s13213-014-0808-0

Francesca, N., Naselli, V., Prestianni, R., Pirrone, A., Viola, E., Guzzon, R., Settanni, L., Maggio, A., Vaglica, A., Bruno, M., Gristina, L., Oliva, D., Ferranti, G., Notarbartolo, G., Alfonzo, A., & Moschetti, G. (2024). Impact of two new non-conventional yeasts, *Candida oleophila* and *Starmerella lactis-condensi*, isolated from sugar-rich substrates, on Frappato wine aroma. *Food Bioscience*, *57*, 103500. https://doi.org/10.1016/j.fbio.2023.103500

Gabrielli, M., Aleixandre-Tudo, J. L., Kilmartin, P. A., Sieczkowski, N., & Du Toit, W. J. (2017). Additions of glutathione or specific glutathione-rich dry inactivated yeast preparation (DYP) to sauvignon blanc must: effect on wine chemical and sensory composition. *South African Journal of Enology and Viticulture*, *38*(1), 18–28. https://doi.org/10.21548/38-1-794

564 Garcia, L., Deshaies, S., Constantin, T., Garcia, F., & Saucier, C. (2023). Impact of phenolic composition and antioxidant
565 parameters on the ageing potential of Syrah red wines measured by accelerated ageing tests. *Food Chemistry*, 136613.
566 https://doi.org/10.1016/j.foodchem.2023.136613
567 Giménez, P., Just-Borras, A., Pons, P., Gombau, J., Heras, J. M., Sieczkowski, N., Canals, J. M., & Zamora, F. (2023).
568 Biotechnological tools for reducing the use of sulfur dioxide in white grape must and preventing enzymatic browning:
569 glutathione; inactivated dry yeasts rich in glutathione; and bioprotection with *Metschnikowia pulcherrima*. *European*
570 *Food Research and Technology*, *249*(6), 1491–1501. https://doi.org/10.1007/s00217-023-04229-6
571 Gómez-Míguez, M. J., González-Miret, M. L., Hernanz, D., Fernández, M. Á., Vicario, I. M., & Heredia, F. J. (2007).
572 Effects of prefermentative skin contact conditions on colour and phenolic content of white wines. *Journal of Food*
573 *Engineering*, *78*(1), 238–245. https://doi.org/10.1016/j.jfoodeng.2005.09.021
574 Guerrero, R. F., & Cantos-Villar, E. (2015). Demonstrating the efficiency of sulphur dioxide replacements in wine: a
575 parameter review. *Trends in Food Science & Technology*, *42*(1), 27–43. http://dx.doi.org/10.1016/j.tifs.2014.11.004
576 Guillamón, J. M., Sabaté, J., Barrio, E., Cano, J., & Querol, A. (1998). Rapid identification of wine yeast species based
577 on RFLP analysis of the ribosomal internal transcribed spacer (ITS) region. *Archives of Microbiology*, *169*, 387–392.
578 https://doi.org/10.1007/s002030050587
579 Guth, H. (1997). Quantitation and sensory studies of character impact odorants of different white wine varieties. *Journal*
580 *of Agricultural and Food Chemistry*, *45*(8), 3027–3032. https://doi.org/10.1021/jf970280a
581 Gutiérrez- Escobar, R., Aliaño- González, M. J., Marrufo- Curtido, A., Jiménez- Hierro, M. J., Puertas, B., & Cantos-
582 Villar, E. (2023). Sulfur dioxide- free Verdejo wines through the use of a pure stilbene extract: exploring possible
583 synergistic effect with glutathione. *Journal of the Science of Food and Agriculture*, *103*(3), 1152–1160.
584 https://doi.org/10.1002/jsfa.12209
585 Issa-Issa, H., Guclu, G., Noguera-Artiaga, L., López-Lluch, D., Poveda, R., Kelebek, H., Selli, S., & Carbonell-
586 Barrachina, Á. A. (2020). Aroma-active compounds, sensory profile, and phenolic composition of Fondillón. *Food*
587 *Chemistry*, *316*, 126353. https://doi.org/10.1016/j.foodchem.2020.126353
588 Jackson, R. S. (2016). *Wine Tasting: A Professional Handbook* (3rd ed.). Academic Press. ISBN: 978-0-12-801813-2
589 Kallithraka, S., Salacha, M. I., & Tzourou, I. (2009). Changes in phenolic composition and antioxidant activity of white
590 wine during bottle storage: Accelerated browning test versus bottle storage. *Food Chemistry*, *113*(2), 500–505.
591 https://doi.org/10.1016/j.foodchem.2008.07.083
592 Kemsawasd, V., Viana, T., Ardö, Y., & Arneborg, N. (2015). Influence of nitrogen sources on growth and fermentation
593 performance of different wine yeast species during alcoholic fermentation. *Applied Microbiology and Biotechnology*,
594 *99*, 10191–10207. https://doi.org/10.1007/s00253-015-6835-3

King Jr, A. D., Ponting, J. D., Sanshuck, D. W., Jackson, R., & Mihara, K. (1981). Factors affecting death of yeast by sulfur dioxide. *Journal of Food Protection*, *44*(2), 92–97. https://doi.org/10.4315/0362-028x-44.2.92

Kritzinger, E. C., Bauer, F. F., & du Toit, W. J. (2013). Role of glutathione in winemaking: a review. *Journal of Agricultural and Food Chemistry*, *61*(2), 269–277. https://doi.org/10.1021/jf303665z

Laguna, L., Sarkar, A., Bryant, M. G., Beadling, A. R., Bartolomé, B., & Moreno-Arribas, M. V. (2017). Exploring mouthfeel in model wines: Sensory-to-instrumental approaches. *Food Research International*, *102*, 478–486. https://doi.org/10.1016/j.foodres.2017.09.009

Lai, Y. T., Hsieh, C. W., Lo, Y. C., Liou, B. K., Lin, H. W., Hou, C. Y., & Cheng, K. C. (2022). Isolation and identification of aroma-producing non-*Saccharomyces* yeast strains and the enological characteristic comparison in wine making. *LWT*, *154*, 112653. https://doi.org/10.1016/j.lwt.2021.112653

Lai, Y. T., Hou, C. Y., Lin, S. P., Lo, Y. C., Chen, C. H., Hsieh, C. W., Lin, H. W., & Cheng, K. C. (2023). Sequential culture with aroma- producing yeast strains to improve the quality of Kyoho wine. *Journal of Food Science*, *88*(3), 1114–1127. https://doi.org/10.1111/1750-3841.16468

Las Heras-Vazquez, F. J., Mingorance-Cazorla, L., Clemente-Jimenez, J. M., & Rodriguez-Vico, F. (2003). Identification of yeast species from orange fruit and juice by RFLP and sequence analysis of the 5.8 S rRNA gene and the two internal transcribed spacers. *FEMS Yeast Research*, *3*(1), 3–9. https://doi.org/10.1111/j.1567-1364.2003.tb00132.x

Luchese, C. L., Sperotto, N., Spada, J. C., & Tessaro, I. C. (2017). Effect of blueberry agro-industrial waste addition to corn starch-based films for the production of a pH-indicator film. *International journal of biological macromolecules*, *104*, 11–18. https://doi.org/10.1016/j.ijbiomac.2017.05.149

Legras, J. L., & Karst, F. (2003). Optimisation of interdelta analysis for *Saccharomyces cerevisiae* strain characterisation. *FEMS Microbiology Letters*, *221*(2), 249–255. https://doi.org/10.1016/S0378-1097(03)00205-2

Li, H., Guo, A., & Wang, H. (2008). Mechanisms of oxidative browning of wine. *Food chemistry*, *108*(1), 1–13. https://doi.org/10.1016/j.foodchem.2007.10.065

Li, Y., Wei, G., & Chen, J. (2004). Glutathione: a review on biotechnological production. *Applied microbiology and biotechnology*, *66*, 233–242. https://doi.org/10.1007/s00253-004-1751-y

Lisanti, M. T., Blaiotta, G., Nioi, C., & Moio, L. (2019). Alternative methods to SO_2 for microbiological stabilization of wine. *Comprehensive Reviews in Food Science and Food Safety*, *18*(2), 455–479. https://doi.org/10.1111/1541-4337.12422

Lyu, X., Del Prado, D. R., Araujo, L. D., Quek, S. Y., & Kilmartin, P. A. (2021). Effect of glutathione addition at harvest on Sauvignon Blanc wines. *Australian Journal of Grape and Wine Research*, *27*(4), 431–441. https://doi.org/10.1111/ajgw.12495

626 Marchante, L., Márquez, K., Contreras, D., Izquierdo- Cañas, P. M., García- Romero, E., & Díaz- Maroto, M. C. (2020).
627 Impact of oenological antioxidant substances on the formation of 1- hydroxyethyl radical and phenolic composition
628 in SO_2 free red wines. *Journal of the Science of Food and Agriculture*, *100*(8), 3401–3407.
629 https://doi.org/10.1002/jsfa.10374
630 Martin, V., Valera, M. J., Medina, K., Boido, E., & Carrau, F. (2018). Oenological impact of the
631 *Hanseniaspora/Kloeckera* yeast genus on wines—A review. *Fermentation*, *4*(3), 76.
632 https://doi.org/10.3390/fermentation4030076
633 Matraxia, M., Alfonzo, A., Prestianni, R., Francesca, N., Gaglio, R., Todaro, A., Alfeo, A., Perretti, G., Columba, C.,
634 Settanni, L., & Moschetti, G. (2021). Non-conventional yeasts from fermented honey by-products: focus on
635 *Hanseniaspora uvarum* strains for craft beer production. *Food Microbiology*, *99*, 103806.
636 https://doi.org/10.1016/j.fm.2021.103806
637 Mylona, A. E., Del Fresno, J. M., Palomero, F., Loira, I., Bañuelos, M. A., Morata, A., Calderón, F., Benito, S., & Suárez-
638 Lepe, J. A. (2016). Use of *Schizosaccharomyces* strains for wine fermentation—effect on the wine composition and
639 food safety. *International Journal of Food Microbiology*, *232*, 63–72.
640 https://doi.org/10.1016/j.ijfoodmicro.2016.05.023
641 Naselli, V., Prestianni, R., Badalamenti, N., Matraxia, M., Maggio, A., Alfonzo, A., Gaglio, R., Vagnoli, P., Settanni, L.,
642 Bruno, M., Moschetti, G., & Francesca, N. (2023). Improving the aromatic profiles of Catarratto wines: Impact of
643 *Metschnikowia pulcherrima* and glutathione-rich inactivated yeasts. *Antioxidants*, *12*(2), 439.
644 https://doi.org/10.3390/antiox12020439
645 Nikolantonaki, M., & Waterhouse, A. L. (2012). A method to quantify quinone reaction rates with wine relevant
646 nucleophiles: A key to the understanding of oxidative loss of varietal thiols. *Journal of Agricultural and Food*
647 *Chemistry*, *60*(34), 8484–8491. https://doi.org/10.1021/jf302017j
648 OIV (International Organisation of Vine and Wine) (2010). Recueil des methodes internationales d'analyses des vins et
649 des moûts. https://www.oiv.int/fr/normes-et-documents-techniques/methodes-danalyse/recueil-des-methodes-
650 internationales -danalyse-des-vins-et-des-mouts-2-vol.
651 OIV (International Organisation of Vine and Wine) (2015) Compendium of international methods of analysis of wines
652 and musts (2 vol.) - chemical analysis: Alcohols - method OIV-MA-AS312–01a.
653 OIV (International Organisation of Vine and Wine). (2020a). Compendium of international methods of wine and must
654 analysis. In: OIV-MA-AS313–15. France, Paris. https://www.oiv.int/en/technical-standards-and-documents/methods-
655 of-analysis/com pendium-of-international-methods-of-analysis-of-wines-and-musts-2-vol.

OIV (International Organisation of Vine and Wine). (2020b). Compendium of international methods of wine and must analysis. In: OIV-MA-AS313–01. France, Paris. https://www.oiv.int/en/technical-standards-and-documents/methods-of-analysis/com pendium-of-international-methods-of-analysis-of-wines-and-musts-2-vol.

Pallmann, C. L., Brown, J. A., Olineka, T. L., Cocolin, L., Mills, D. A., & Bisson, L. F. (2001). Use of WL medium to profile native flora fermentations. *American Journal of Enology and Viticulture*, *52*(3), 198–203. http://dx.doi.org/10.5344/ajev.2001.52.3.198

Pelonnier-Magimel, E., Cameleyre, M., Riquier, L., & Barbe, J. C. (2023). Sensory specificities involving acetaldehyde and diacetyl in wines produced without added sulfur dioxide. *Journal of Agricultural and Food Chemistry*. https://doi.org/10.1021/acs.jafc.3c00757

Recamales, Á. F., Sayago, A., González-Miret, M. L., & Hernanz, D. (2006). The effect of time and storage conditions on the phenolic composition and colour of white wine. *Food Research International*, *39*(2), 220–229. https://doi.org/10.1016/j.foodres.2005.07.009

Romancino, D. P., Di Maio, S., Muriella, R., & Oliva, D. (2008). Analysis of non- *Saccharomyces* yeast populations isolated from grape musts from Sicily (Italy). *Journal of Applied Microbiology*, *105*(6), 2248–2254. https://doi.org/10.1111/j.1365-2672.2008.03894.x

Simonin, S., Roullier-Gall, C., Ballester, J., Schmitt-Kopplin, P., Quintanilla-Casas, B., Vichi, S., Peyron, D., Alexandre, H., & Tourdot-Maréchal, R. (2020). Bio-protection as an alternative to sulphites: impact on chemical and microbial characteristics of red wines. *Frontiers in Microbiology*, *11*, 1308. https://doi.org/10.3389/fmicb.2020.01308

Singleton, V. L., Salgues, M., Zaya, J., & Trousdale, E. (1985). Caftaric acid disappearance and conversion to products of enzymic oxidation in grape must and wine. *American Journal of Enology and Viticulture*, *36*(1), 50–56. https://doi.org/10.5344/ajev.1985.36.1.50

Siyun, L., Heping, C., Huan, Z., Khizar, H., Chengsheng, J., Shahzad, H., Muhammad, U. T., Xiaoming, Z., & Chi-Tang, H. (2019). Timely Addition of Glutathione for its interaction with deoxypentosone to inhibit the aqueous Maillard reaction and browning of glycylglycine–arabinose system. *Journal of Agricultural and Food Chemistry*, *67*(23), 6585–6593. https://doi.org/10.1021/acs.jafc.9b02053

Varela, C., Sengler, F., Solomon, M., & Curtin, C. (2016). Volatile flavor profile of reduced alcohol wines fermented with the non-conventional yeast species *Metschnikowia pulcherrima* and *Saccharomyces uvarum*. *Food Chemistry*, *209*, 57–64. https://doi.org/10.1016/j.foodchem.2016.04.024

Vidal, J. C., Toitot, C., Boulet, J. C., & Moutounet, M. (2004). Comparison of methods for measuring oxygen in the headspace of a bottle of wine. *Journal International des Sciences de la Vigne et du Vin*, *38*(3), 191–200.

686 Welke, J. E., Zanus, M., Lazzarotto, M., & Zini, C. A. (2014). Quantitative analysis of headspace volatile compounds
687 using comprehensive two-dimensional gas chromatography and their contribution to the aroma of Chardonnay wine.
688 *Food Research International*, *59*, 85–99. https://doi.org/10.1016/j.foodres.2014.02.002
689 Zamora, F. (2009). Biochemistry of alcoholic fermentation. In Moreno–Arribas, M. V., & Polo, M. C. (Eds.) *Wine*
690 *chemistry and biochemistry* (pp. 3–26). Springer. https://doi.org/10.1007/978-0-387-74118-5_1
691 Zhang, X., Blackman, J. W., & Clark, A. C. (2023). Ascorbic acid addition to rosé: impact on the oxidative and reductive
692 development of bottled wine. *Food Chemistry*, *424*, 136418. https://doi.org/10.1016/j.foodchem.2023.136418
693

Table 1. Chemical parameters determined during the winemaking process.

Parameters	Must	Vinification Post-pressing					
		GL.1	GL.2	GL.3	GL.4	GL.5	S.S.
pH	3.21 ± 0.01	3.24 ± 0.01 a	3.21 ± 0.01 a	3.22 ± 0.01 a	3.23 ± 0.01 a	3.25 ± 0.01 a	n.s.
TA$^{\Psi}$	5.70 ± 0.05	5.80 ± 0.02 a	5.60 ± 0.06 a	5.60 ± 0.03 a	5.60 ± 0.07 a	5.80 ± 0.02 a	n.s.
RS$^{\Psi}$	268.34 ± 1.98	267.47 ± 1.28 a	267.47 ± 1.01 a	267.99 ± 1.73 a	267.15 ± 1.31 a	267.91 ± 1.54 a	n.s.
Glycerol$^{\Psi}$	0.00 ± 0.00	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	n.s.
Acetic acid$^{\Psi}$	0.00 ± 0.00	0.00 ± 0.01 a	0.03 ± 0.00 a	0.03 ± 0.00 a	0.03 ± 0.00 a	0.03 ± 0.01 a	n.s.
L - Malic acid$^{\Psi}$	1.41 ± 0.02	1.37 ± 0.01 a	1.38 ± 0.01 a	1.39 ± 0.03 a	1.37 ± 0.02 a	1.36 ± 0.01 a	n.s.
L - Lactic acid$^{\Psi}$	0.00 ± 0.00	0.00 ± 0.0 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	n.s.
Amm. N$^{\alpha}$	68.57 ± 1.42	71.73 ± 0.11 d	75.26 ± 0.88 c	64.33 ± 0.94 e	86.53 ± 1.28 a	83.80 ± 1.24 b	***
Alpha-AN$^{\alpha}$	73.98 ± 1.30	91.84 ± 1.40 b	90.17 ± 1.20 c	86.22 ± 0.95 e	89.79 ± 1.20 d	98.50 ± 0.80 a	***
Free SO_2 $^{\alpha}$	1.3 ± 0.2	1.4 ± 0.2 b	1.1 ± 0.3 b	1.3 ± 0.5 b	1.2 ± 0.2 b	3.8 ± 0.4 a	***
Total SO_2 $^{\alpha}$	20.0 ± 0.1	19.5 ± 0.4 b	19.7 ± 0.6 b	19.6 ± 0.4 b	20.1 ± 0.2 b	40.0 ± 0.3 a	***
Ethanol$^{\beta}$	0.00 ± 0.00	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	n.s.

Parameters	Vinification Post-clarification					
	GL.1	GL.2	GL.3	GL.4	GL.5	S.S.
pH	3.27 ± 0.00 a	3.27 ± 0.00 a	3.26 ± 0.01 a	3.29 ± 0.02 a	3.30 ± 0.01 a	n.s.
TA$^{\Psi}$	5.77 ± 0.01 a	5.77 ± 0.02 a	5.78 ± 0.01 a	4.88 ± 0.03 b	5.05 ± 0.04 b	***
RS$^{\Psi}$	266.82 ± 0.45 a	266.21 ± 1.31 a	267.08 ± 0.24 a	266.73 ± 0.47 a	267.01 ± 1.13 a	n.s.
Glycerol$^{\Psi}$	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	n.s.
Acetic acid$^{\Psi}$	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	n.s.
L - Malic acid$^{\Psi}$	1.37 ± 0.01 a	1.37 ± 0.02 a	1.39 ± 0.03 a	1.39 ± 0.02 a	1.39 ± 0.01 a	n.s.
L - Lactic acid$^{\Psi}$	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	n.s.
Amm. N$^{\alpha}$	111.66 ± 0.85 d	104.68 ± 1.13 e	116.92 ± 1.11 c	119.18 ± 1.35 b	126.47 ± 0.79 a	***
Alpha-AN$^{\alpha}$	92.78 ± 0.42 c	100.93 ± 0.87 a	91.94 ± 0.66	92.60 ± 1.08 c	95.77 ± 0.74 b	***
Free SO_2 $^{\alpha}$	1.1 ± 0.2 b	1.3 ± 0.5 b	1.2 ± 0.1 b	1.4 ± 0.3 b	3.5 ± 0.3 a	***
Total SO_2 $^{\alpha}$	19.5 ± 0.5 b	19.5 ± 0.5 b	19.1 ± 0.3 b	19.4 ± 0.4 b	39.6 ± 0.3 a	***
Ethanol$^{\beta}$	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	n.s.

Parameters	Vinification End of alcoholic fermentation					
	GL.1	GL.2	GL.3	GL.4	GL.5	S.S.
pH	3.32 ± 0.01 a	3.31 ± 0.00 a	3.31 ± 0.02 a	3.34 ± 0.01 a	3.33 ± 0.00 a	n.s.
TA$^{\Psi}$	7.90 ± 0.03 a	7.90 ± 0.01 a	7.90 ± 0.10 a	8.10 ± 0.02 a	8.00 ± 0.03 a	n.s.
RS$^{\Psi}$	2.10 ± 0.04 a	2.17 ± 0.05 a	2.09 ± 0.03 a	2.02 ± 0.02 a	1.98 ± 0.05 a	n.s.
Glycerol$^{\Psi}$	6.81 ± 0.04 c	6.77 ± 0.06 c	7.33 ± 0.02 a	6.92 ± 0.10 c	7.10 ± 0.08 b	***
Acetic acid$^{\Psi}$	0.25 ± 0.02 a	0.21 ± 0.01 a	0.23 ± 0.01 a	0.19 ± 0.01 a	0.25 ± 0.02 a	n.s.
L - Malic acid$^{\Psi}$	1.24 ± 0.02 a	1.26 ± 0.02 a	1.19 ± 0.04 a	1.24 ± 0.00 a	1.25 ± 0.02 a	n.s.
L - Lactic acid$^{\Psi}$	0.02 ± 0.00 a	0.01 ± 0.01 a	0.02 ± 0.00 a	0.01 ± 0.00 a	0.01 ± 0.01 a	n.s.
Amm. N$^{\alpha}$	106.82 ± 1.27 c	108.78 ± 0.94 a	107.25 ± 0.72 c	108.11 ± 0.97 b	105.81 ± 1.25 d	***
Alpha-AN$^{\alpha}$	40.18 ± 0.64 b	40.88 ± 1.14 a	39.71 ± 1.34 b	39.98 ± 0.97 b	38.07 ± 0.47 c	***
Free SO_2 $^{\alpha}$	19.3 ± 0.4 a	19.0 ± 0.3 a	19.2 ± 0.2 a	19.4 ± 0.5 a	19.1 ± 0.2 a	n.s.
Total SO_2 $^{\alpha}$	37.7 ± 0.4 b	37.2 ± 0.2 b	37.1 ± 0.3 b	37.4 ± 0.4 b	55.2 ± 0.1 a	***
Molecular SO_2 $^{\alpha}$	0.77 ± 0.1 a	0.77 ± 0.09 a	0.77 ± 0.08 a	0.78 ± 0.06 a	0.78 ± 0.08 a	n.s.
Ethanol$^{\beta}$	13.46 ± 0.07 a	13.51 ± 0.08 a	13.48 ± 0.05 a	13.52 ± 0.06 a	13.49 ± 0.06 a	n.s.

[1]Result indicates mean value ± standard deviation of two determinations from three replicates. Data within a row followed by the same letter are not significantly different according to Tukey's test. Tukey's test was not applied on must values. Ψ, expressed in g/L; α, expressed in mg/L; β, expressed as % (v/v).

Abbreviations: TA, total titratable acidity; RS, residual sugar; Amm. N, ammonia nitrogen; Alpha-AN, alpha-amino nitrogen; S.S., statistical significance; n.s.; not significant. P value: ***, $P < 0.001$.

700 **Table 2.** Evolution of CIELAB variables and red/green/blue (RGB) scale from post-pressing stage to two years of bottle ageing.

Parameters	Post-pressing					S.S.
	GL.1	**GL.2**	**GL.3**	**GL.4**	**GL.5**	
L*	93.61 ± 1.76 b	96.75 ± 0.49 a	95.21 ± 0.42 ab	90.54 ± 0.51 c	95.79 ± 0.62 ab	***
a*	-1.45 ± 0.13 a	-1.93 ± 0.03 b	-1.57 ± 0.10 ab	-1.61 ± 0.13 ab	-1.76 ± 0.26 ab	*
b*	7.07 ± 0.07 ab	8.17 ± 0.17 a	6.33 ± 0.49 b	6.44 ± 0.95 b	6.07 ± 0.41 b	**
C*	7.22 ± 0.05 ab	8.40 ± 0.17 a	6.53 ± 0.49 b	6.64 ± 0.93 b	6.33 ± 0.46 b	**
hue°	101.00 ± 0.56 b	103.48 ± 1.03 ab	103.74 ± 0.07 ab	102.54 ± 1.31 ab	104.12 ± 0.79 a	*
	End of alcoholic fermentation					
	GL.1	**GL.2**	**GL.3**	**GL.4**	**GL.5**	
L*	96.05 ± 0.25 a	95.78 ± 0.34 a	95.40 ± 0.38 a	89.66 ± 0.41 b	95.78 ± 0.78 a	***
a*	-1.23 ± 0.07 c	-0.96 ± 0.47 bc	-0.45 ± 0.08 ab	0.05 ± 0.09 a	-0.25 ± 0.10 a	**
b*	6.35 ± 0.63 a	5.30 ± 0.51 ab	3.96 ± 0.54 b	5.53 ± 0.58 ab	4.56 ± 0.6 ab	*
C*	6.47 ± 0.63 a	5.43 ± 0.70 ab	3.99 ± 0.55 b	5.53 ± 0.39 ab	4.57 ± 0.62 b	*
hue°	100.70 ± 0.57 b	103.04 ± 0.71 a	97.34 ± 0.72 c	89.34 ± 0.42 d	98.59 ± 1.02 bc	***
	2 years bottled wine					
	GL.1	**GL.2**	**GL.3**	**GL.4**	**GL.5**	
L*	95.98 ± 0.85 a	95.30 ± 0.87 a	95.37 ± 0.50 a	86.01 ± 0.77 b	95.55 ± 0.61 a	***
a*	-1.13 ± 0.08 a	-2.18 ± 0.04 bc	-2.54 ± 0.24 cd	-1.85 ± 0.13 b	-2.85 ± 0.11 d	***
b*	17.65 ± 1.60 b	12.34 ± 0.19 c	22.43 ± 0.64 a	14.20 ± 0.22 c	14.44 ± 0.02 c	***
C*	17.68 ± 1.59 b	12.37 ± 0.20 d	22.57 ± 0.71 a	14.32 ± 0.22 cd	14.72 ± 0.03 c	***
hue°	96.5 ± 0.28 b	99.95 ± 0.18 a	97.4 ± 0.41 b	86.66 ± 0.52 c	97.83 ± 0.59 b	***

701 Result indicates mean value ± standard deviation of two determinations from three replicates. Abbreviation: S.S., Statistical significance.
702 Data within a line followed by different letters are significantly different according to Tukey's test. P value:*, P<0.05; **, P < 0.01; ***, P
703 < 0.001.

704 **Table 3.** Volatile Organic Compounds detected in wines.

LRI[a]	Compounds	OPT	GL.1	OAV	GL.2	OAV	GL.3	OAV	GL.4	OAV	GL.5	OAV	S.S.
	Σ Alcohols		**236.18 ± 6.54 b**		**262.23 ± 7.79 a**		**220.20 ± 5.40 bc**		**209.22 ± 6.74 c**		**259.90 ± 7.38 a**		*******
1210	3-methyl-1-butanol	[1] 30 mg/L	144.78 ± 4.01 a	4.83	142.36 ± 3.74 ab	4.75	132.17 ± 3.15 bc	4.41	125.83 ± 4.08 c	4.19	145.94 ± 3.99 a	4.86	***
1317	4-methyl-1-pentanol	Unknown	0.41 ± 0.01 c		0.78 ± 0.02 b		0.87 ± 0.02 a		0.81 ± 0.02 b		0.80 ± 0.02 b		***
1329	3-methyl-1-pentanol	Unknown	1.76 ± 0.06 c		2.62 ± 0.08 a		2.61 ± 0.07 a		2.32 ± 0.06 b		1.87 ± 0.06 c		***
1354	1-hexanol	[2] 8 mg/L	3.86 ± 0.11 b	0.48	4.38 ± 0.13 a	0.55	3.10 ± 0.10 c	0.39	2.94 ± 0.09 c	0.37	4.57 ± 0.13 a	0.57	***
1859	Phenylethyl alcohol	[2] 14 mg/L	85.37 ± 2.35 b	6.10	112.09 ± 3.82 a	8.01	81.45 ± 2.06 bc	5.82	77.32 ± 2.49 c	5.52	106.72 ± 3.18 a	7.59	***
	Σ Carboxylic acids		**0.00 ± 0.00 a**		**0.00 ± 0.00 a**		**0.00 ± 0.00 a**		**0.03 ± 0.02 a**	**0.07**	**0.09 ± 0.04 a**	**0.21**	**n.s.**
1885	Hexanoic acid	[2] 0.42 mg/L	0.00 ± 0.00 a		0.00 ± 0.00 a		0.00 ± 0.00 a		0.03 ± 0.02 a	0.07	0.09 ± 0.04 a	0.21	n.s.
	Σ Esters		**63.42 ± 1.81 a**		**59.49 ± 1.80 ab**		**54.36 ± 1.57 c**		**57.25 ±1.75 bc**		**45.13 ± 1.37 d**		*******
891	Ethyl acetate	[1] 7.5 mg/L	2.33 ± 0.06 a	0.31	2.26 ± 0.06 a	0.30	2.03 ± 0.05 b	0.27	1.93 ± 0.05 b	0.26	1.90 ± 0.05 b	0.25	***
1040	Ethyl butanoate	[1] 0.02 mg/L	0.26 ± 0.01 b	13.00	0.29 ± 0.01 a	14.50	0.27 ± 0.01 ab	13.50	0.26 ± 0.01 b	13.00	0.25 ± 0.01 b	12.5	**
1123	3-methyl-1-butanol acetate	[2] 0.03 mg/L	2.62 ± 0.08 a	87.33	2.67 ± 0.09 a	89.00	2.31 ± 0.07 b	77.00	2.31 ± 0.08 b	77.00	0.71 ± 0.02 c	23.67	***
1238	Ethyl hexanoate	[2] 0.014 mg/L	4.88 ± 0.16 a	348.6	5.04 ± 0.17 a	360.0	4.74 ± 0.16 a	338.6	4.76 ± 0.15 a	340.0	2.41 ± 0.08 b	172.1	***
1269	Hexyl acetate	Unknown	0.33 ± 0.01 d		0.48 ± 0.01 b		0.57 ± 0.02 a		0.44 ± 0.01 c		0.09 ± 0.00 e		***
1453	Ethyl octanoate	[2] 0.005 mg/L	26.03 ± 0.72 a	5206	25.09 ± 0.73 a	5018	19.32 ± 0.50 c	3864	18.25 ± 0.70 b	3650	21.69 ± 0.71 b	4338	***
1639	Ethyl decanoate	[2] 0.2 mg/L	26.87 ± 0.77 a	134.3	23.53 ± 0.73 b	117.6	25.30 ± 0.76 ab	126.5	17.21 ± 0.75 ab	86.05	17.93 ± 0.50 c	89.65	***
1853	3-methylbutyl decanoate	Unknown	0.10 ± 0.00 a		0.13 ± 0.00 a		0.09 ± 0.00 a		0.09 ± 0.00 a		0.15 ± 0.00 a		n.s.
	Σ Others		**3.33 ± 0.09 c**		**3.41 ± 0.10 c**		**4.09 ± 0.11 b**		**3.61 ± 0.11 c**		**7.51 ± 0.26 a**		*******
1266	Styrene	Unknown	3.33 ± 0.09 c		3.41 ± 0.10 c		4.09 ± 0.11 b		3.61 ± 0.11 c		7.51 ± 0.26 a		***

705
706 [1]Guth, 1997; [2] Ferreira, López, and Cacho, 2000. Result indicates mean value ± standard deviation of two determinations from three replicates. Abbreviation: n.s., not significant; OPT, Odour perception
707 threshold; OAV, Odor activity value; S.S., Statistical significance. Data within a line followed by the same letter are not significantly different according to Tukey's test. P value:**, $P < 0.01$; ***, $P <$
708 0.001.

Fig. 1. Experimental design of Catarratto Organic wine vinified with the use of GIY and Bio-protective *M. pulcherrima* MP1 strain.

Fig. 2. Yeast population (Log CFU/mL) evolution during alcoholic fermentation: (A) total yeasts levels; (B) presumptive *Metschnikowia* spp. concentration. Abbreviations: AF, Alcoholic fermentation.

Fig. 3. Histogram of dissolved O_2 detected in post-press and post-clarification. For each phase, different letters indicate statistically significant values determined by using Tukey's test ($P < 0.05$).

Fig. 4. Histogram of optical density detected in post-press and post-clarification. For each group, different letters indicate statistically significant values determined by using Tukey's test ($p \leq 0.05$).

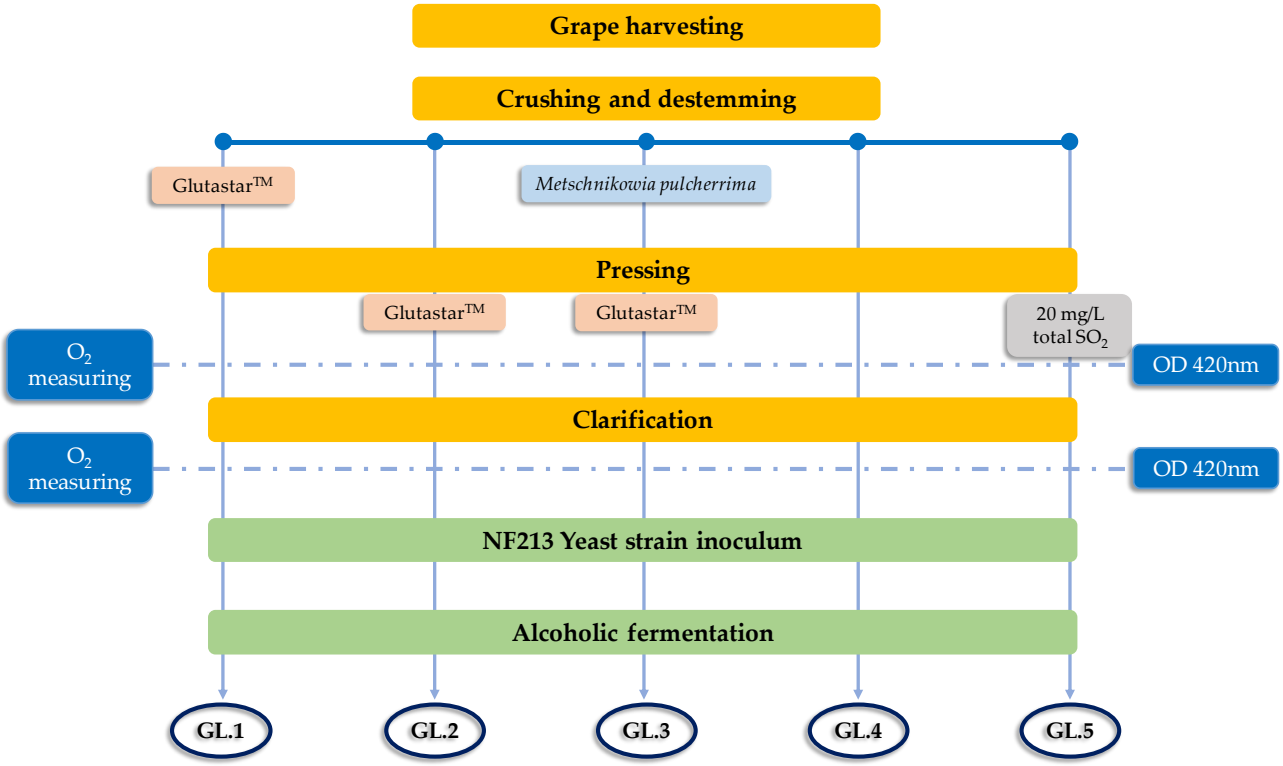
Fig. 5. Multivariate analyses performed on VOCs exceeding an OAV of 1: **(**A**)** Principal Component Analysis (PCA); (B) Hiercarchical Clustering Analysis (HCA).

Fig. 6. Principal Component Analysis (PCA) performed on esters detected by VOCs analysis: (A) Biplot graph; (B) Bootstrap hulls.

Fig. 7. Dendrogram generated by Hiercarchical Clustering Analysis (HCA) performed on esters detected by VOCs analysis.

Fig. 8. Results from sensory analysis: (A) Histogram of overall quality rating of each trial; (B) Spider plot with each descriptor. Data followed by asterisks are significantly different according to Tukey's test. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$. Abbreviations: n.s.; not significant.

728 **Fig. 1.**



729

730

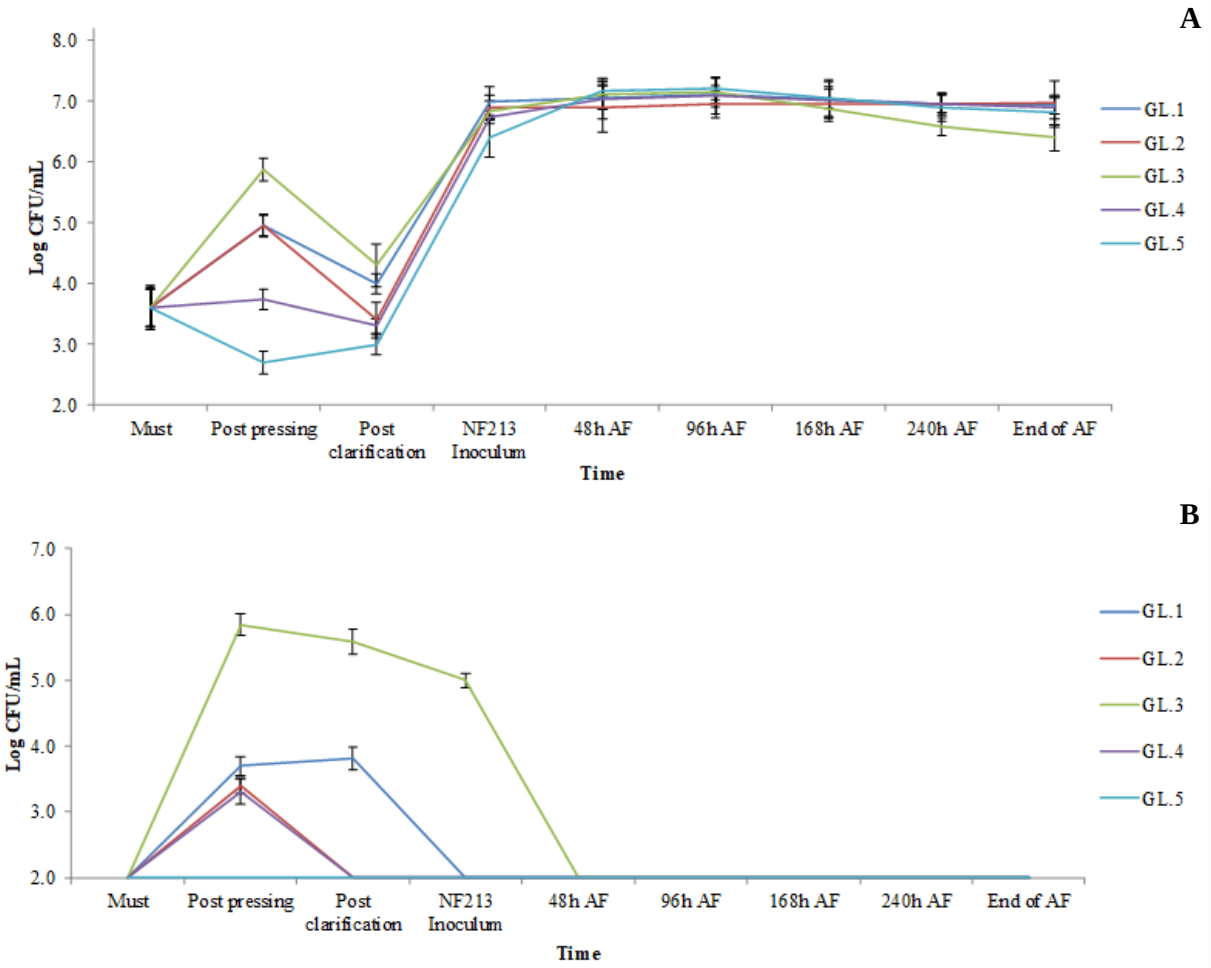
731

732

733

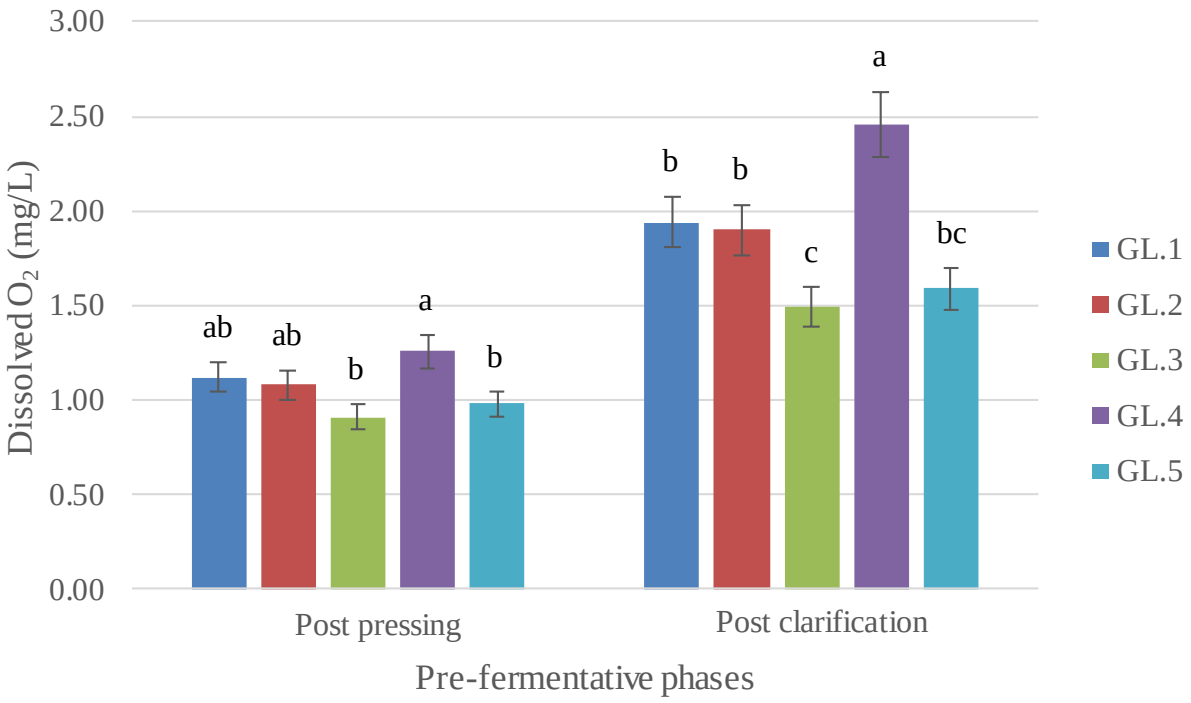
734

735 **Fig. 2.**


A
8.0
7.0
6.0
5.0
4.0
3.0
2.0
Log CFU/mL
Must
Post pressing
Post clarification
NF213 Inoculum
48h AF
96h AF
168h AF
240h AF
End of AF
Time
GL.1
GL.2
GL.3
GL.4
GL.5
B
7.0
6.0
5.0
4.0
3.0
2.0
Log CFU/mL
Must
Post pressing
Post clarification
NF213 Inoculum
48h AF
96h AF
168h AF
240h AF
End of AF
Time
GL.1
GL.2
GL.3
GL.4
GL.5

736

737 **Fig. 3.**

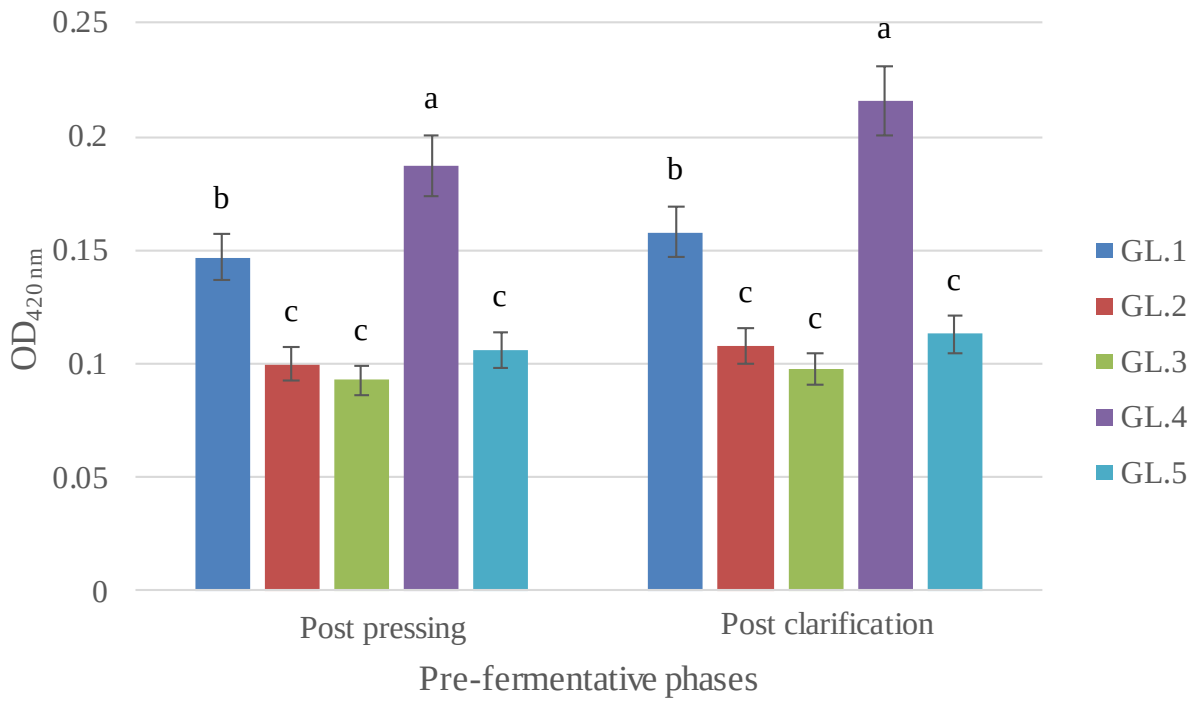


738

739

740

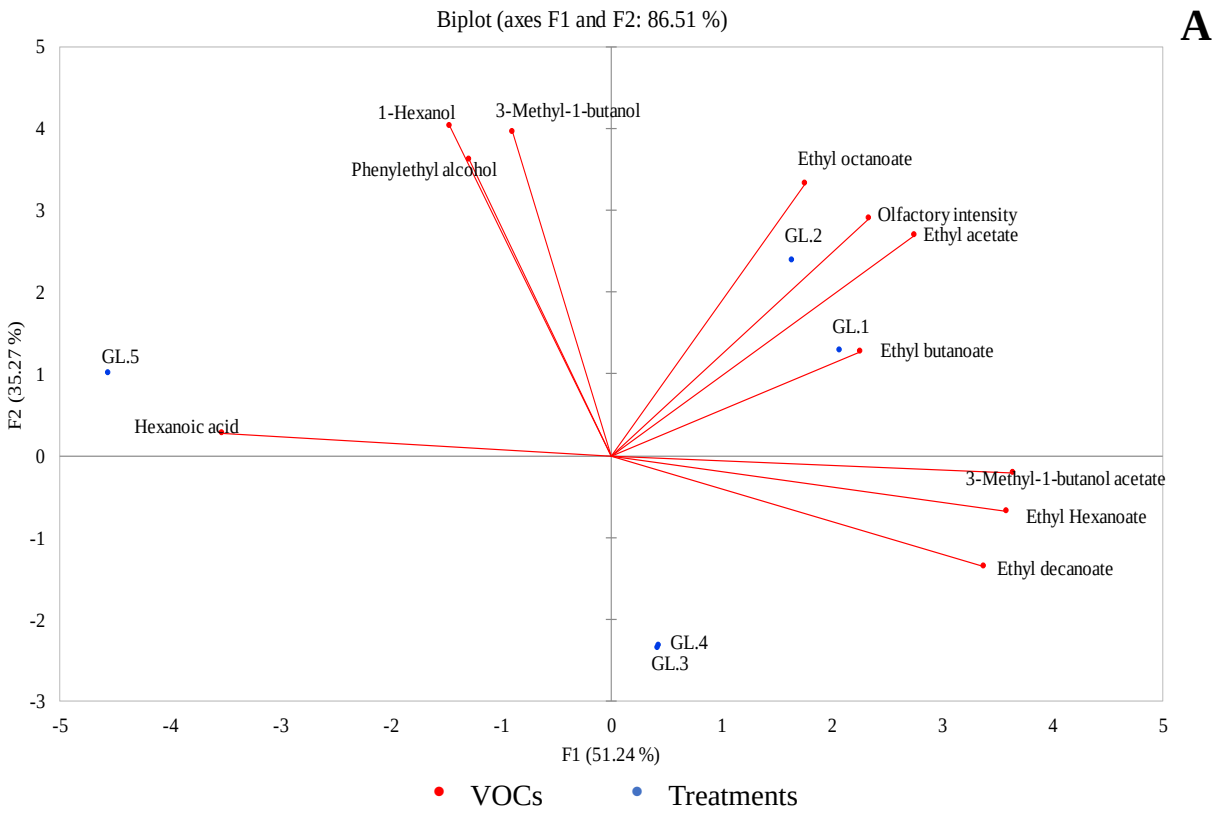
741 **Fig. 4.**

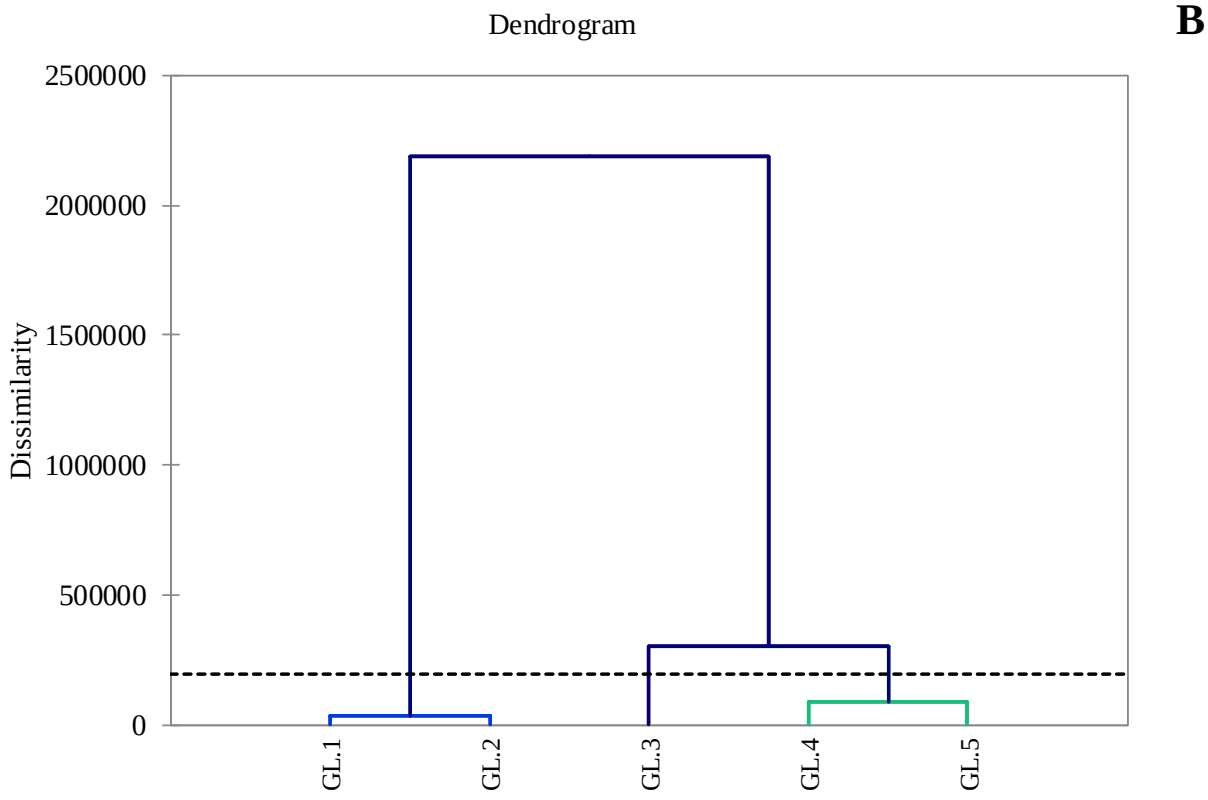


742

743

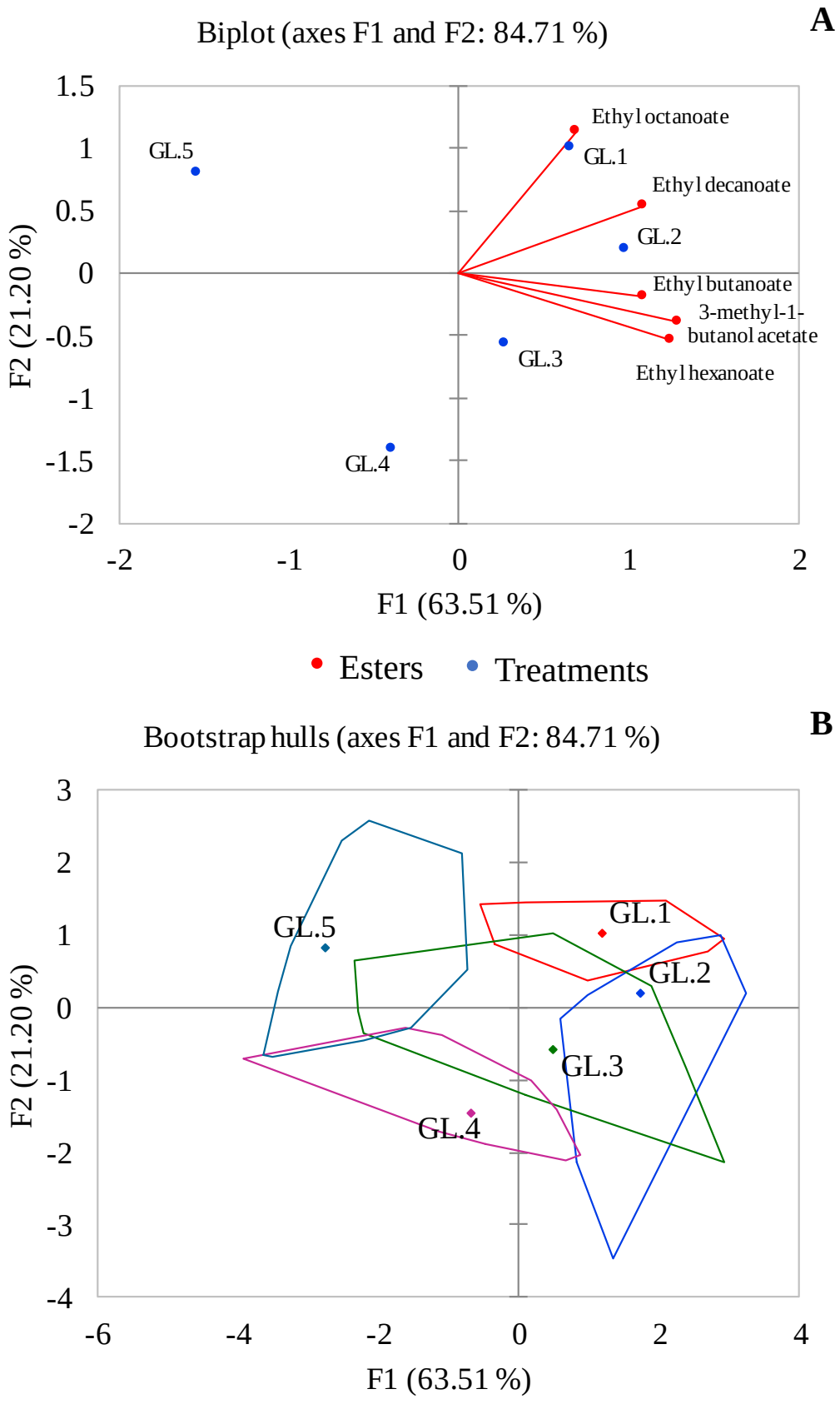
744 **Fig. 5.**


A
Biplot (axes F1 and F2: 86.51 %)
1-Hexanol
3-Methyl-1-butanol
Phenylethyl alcohol
Ethyl octanoate
Olfactory intensity
Ethyl acetate
GL.2
GL.1
Ethyl butanoate
GL.5
Hexanoic acid
3-Methyl-1-butanol acetate
Ethyl Hexanoate
Ethyl decanoate
GL.4
GL.3
F2 (35.27 %)
F1 (51.24 %)
VOCs
Treatments


B
Dendrogram
Dissimilarity
2500000
2000000
1500000
1000000
500000
0
GL.1
GL.2
GL.3
GL.4
GL.5

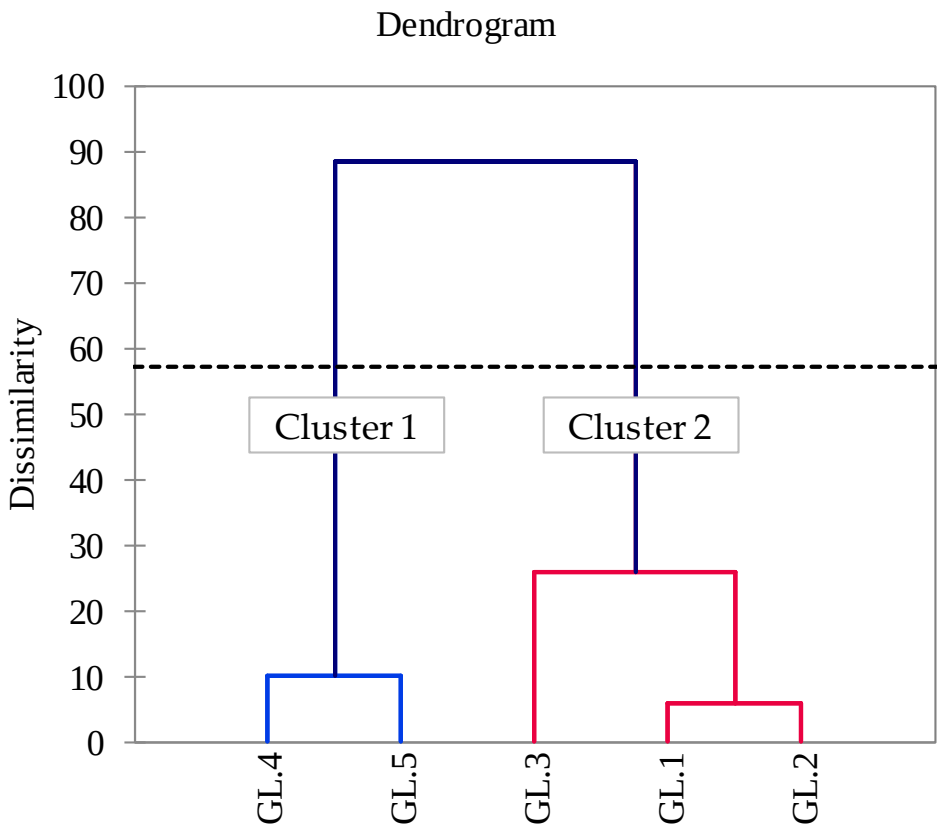
745

746 **Fig. 6.**



747
748

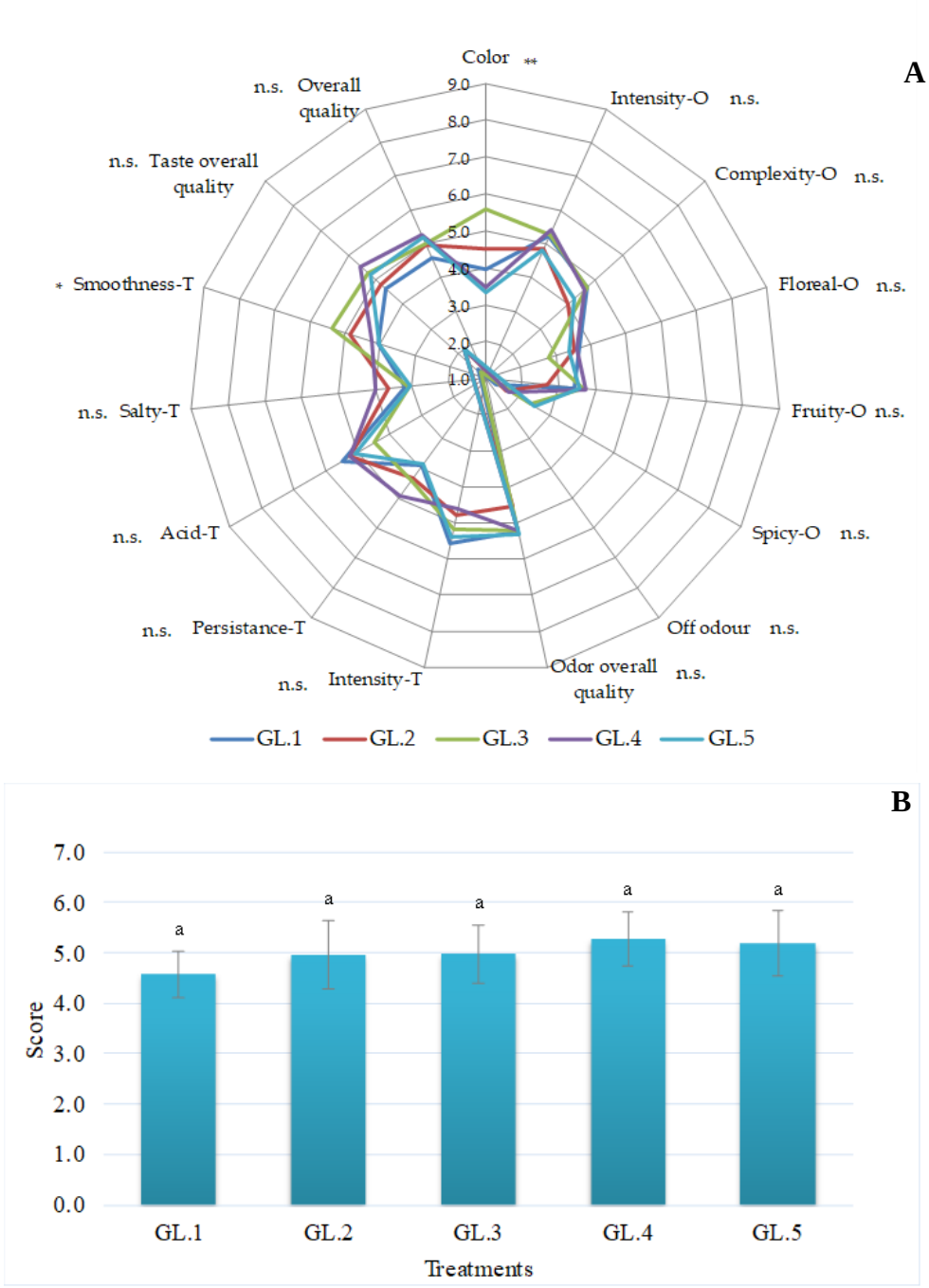
749 **Fig. 7.**



750

751

752 **Fig. 8.**


A
Color **
Intensity-O n.s.
Complexity-O n.s.
Floreal-O n.s.
Fruity-O n.s.
Spicy-O n.s.
Off odour n.s.
Odor overall quality n.s.
Intensity-T n.s.
Persistance-T n.s.
Acid-T n.s.
Salty-T n.s.
Smoothness-T *
Taste overall quality n.s.
Overall quality n.s.
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
GL.1 GL.2 GL.3 GL.4 GL.5
B
Score
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
a a a a a
GL.1 GL.2 GL.3 GL.4 GL.5
Treatments

753

CONFLICT OF INTEREST

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.