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Effects of different yeast strains, nutrients and glutathione-rich inactivated yeast addition on the aroma characteristics of Catarratto wines

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ABSTRACT

Catarratto is one of the most common non-aromatic white grape varieties cultivated in Sicily (Southern Italy). In order to improve the aromatic expression of Catarratto wines a trial was undertaken to investigate the effect of yeast strain, nutrition and reduced glutathione. Variables included two *Saccharomyces cerevisiae* strains, an oenological strain (GR1) and one isolated from honey by-products (SPF52), three different nutrition regimes (Stimula Sauvignon Blanc™ (SS), Stimula Chardonnay™ (SC) and classic nutrition practice), and a specific inactivated yeast rich in reduced glutathione to prevent oxidative processes [Glutastar™ (GIY)] ensuing in ten treatments (T1-T10).

Microbiological and chemical parameters demonstrated the aptitude of strain SPF52 to successfully conduct alcoholic fermentation. During fermentation, the *Saccharomyces* yeast populations ranged from 7 to 8 logarithmic CFU/mL. All wines had a final ethanol content ranging between 12.91 and 13.85% (*v*/v). The dominance of the two starter strains over native yeast populations was higher than 97% as estimated by interdelta analysis. The addition of nutrients SS or SC increased the aromatic complexity of the wines as reflected by volatile organic compounds (VOCs) composition and sensory profiles. In particular, 32 VOCs were identified; alcohols (62.46–81.1%), thiols (0.27–0.87%), ethers (0.09–0.16%), aldehydes (0–1.21%), ketones (0–2.28%), carboxylic acids (4.21–12.32%), esters (0–10.85%), lactones (0.9–1.49%) and other compounds (0.77–6.9%). Sensory analysis demonstrated a significant impact on wine aroma in relation to yeast starter strain used, the type of nutrition (SS, SC or classic nutrition) and the presence/absence of GIY. The wines produced with GR1 yeast strain and SS (T2), SPF52 with SC (T9) both in presence of GIY showed higher overall quality. Trials T2 and T9 showed the highest scores for 13 and 18 attributes, respectively. The different nutrition, addition of GIY and the yeast starter strains diversified and enhanced sensory expression of Catarratto wines.

1. Introduction

Sicily is the largest Italian wine region accounting for about 17.5% of the overall Italian wine production (Fracassetti et al., 2018). In this region, approximately 100,000 ha of cultivated land are vineyards. Furthermore, Sicily has an ancient wine tradition and contributes to make Italy one of the three leading European countries for wine production. Among the white grape varieties, Catarratto is the most cultivated grape cultivar in Sicily (Carimi et al., 2010) and the second most cultivated in Italy (Robinson et al., 2013). Catarratto wines have a moderate alcohol by volume and a significant total acidity with variable pH values (Sannino et al., 2013). These parameters are variable according to the altimetry of the viticultural areas, in particular in hilly zones Catarratto wines show high values of total acidity, malic acid and low pH. Wines produced with this grape variety have a sufficient olfactory intensity, particularly characterized by descriptors of orange blossom and citrus fruits (Leder, 2020). From a gustatory point of view, Catarratto wines are commonly sapid with a long finish (Sannino et al.,

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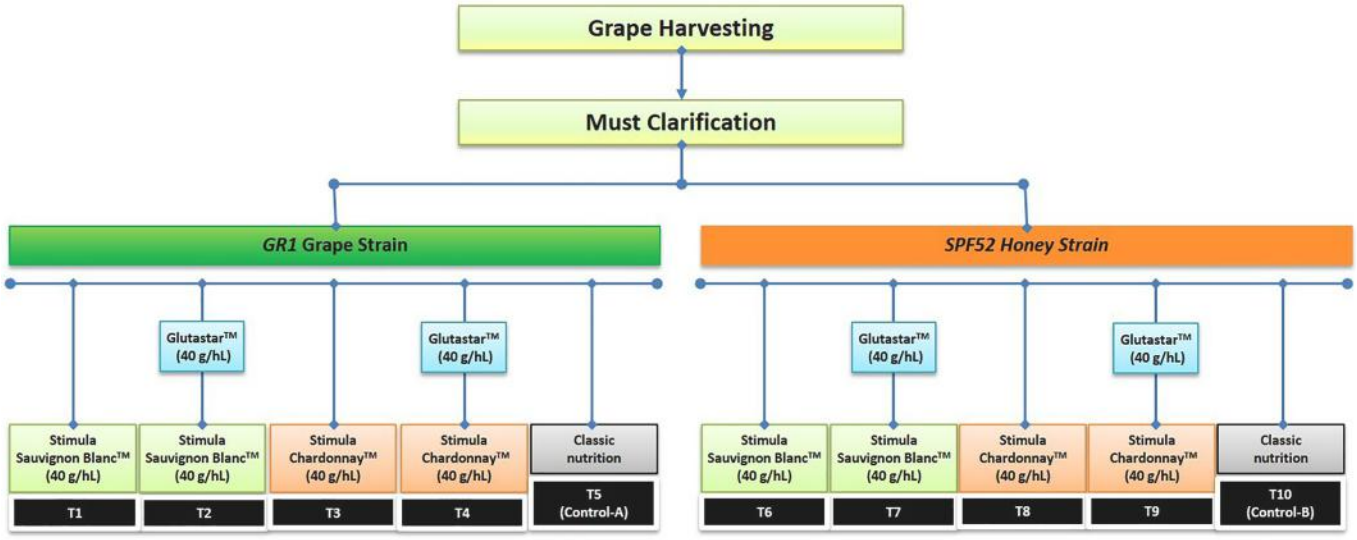

Fig. 1. Experimental design of Catarratto wines vinified with different yeast strains, nutrient regime and addition of an antioxidant.

2013). However, there is limited knowledge on Catarratto wine aroma, physic-chemical and microbiological characteristics (Fracassetti et al., 2018; Sannino et al., 2013).

Aroma is one of the principal wine attributes influencing wine consumer preferences (Mouret et al., 2015). The majority of fruity/floral aroma compounds are produced by yeast during alcoholic fermentation (AF) and their synthesis can be significantly influenced by oenological practices such as clarification, aeration, nutrient addition and fermentation temperature (Hernandez-Orte et al., 2006; Torrea et al., 2011). Moreover, the aromatic profile of wine is also influenced by the *Saccharomyces cerevisiae* strain used as a starter to conduct AF (Lambrechts and Pretorius, 2000). Indigenous yeast represents an important resource in winemaking; numerous *S. cerevisiae* strains isolated from grape berries and spontaneously fermented musts are used in winemaking (Cappello et al., 2004). In order to expand the choice of *S. cerevisiae* strains able to enrich the aromatic complexity of wines, their isolation from natural matrices not related with winemaking is becoming a common practice; some studies regarding the ecology of *S. cerevisiae* demonstrated that this species is present in natural sugar matrices such as manna (Guarcello et al., 2019), honey (Carvalho et al., 2005), honey by-products (Gaglio et al., 2017), fruits (Lee et al., 2011), and nectar (Dandu and Dhabe, 2011); *S. cerevisiae* isolated from honey have a high fermentative capacity and can be used for alcoholic mead production (Pereira et al., 2009). Several studies have evaluated the performance of oenological *S. cerevisiae* strains in mead production (Pereira et al., 2013; Pereira et al., 2014; Sottil et al., 2019), however, none have focused on using *S. cerevisiae* strains isolated from honey for winemaking.

Nitrogen is important for an efficient fermentation and the synthesis of various yeast-derived volatile compounds (Barbosa et al., 2012). Grape juice/must contains assimilable nitrogen in different forms, inorganic (ammonium) and organic (amino acids and peptides), which are assimilated differently by yeast (Ayestaran et al., 1995). Yeast nutrition management during fermentation is important for the wine aroma profile (Molina et al., 2009), and is commonly supplemented with diammonium phosphate, or yeast derivate nutrients to prevent problems related to nitrogen deficiency, such as slow/stuck fermentations and H_2S production (Vilanova et al., 2007).

To prevent loss of aroma, wine must be protected against oxidation at the earliest stages of the winemaking process, and can be achieved via the addition of natural antioxidant compounds, such as glutathione (L-g-glutamyl-L-cysteinyl-glycine) (Kritzinger et al., 2013). Glutathione is a tripeptide, which contains three constitutive amino acids, glutamate, cysteine and glycine, formed from the natural metabolism of yeast. In wine, glutathione can be present as a reduced (GSH) or oxidized form (GSSG). Glutathione is important in wine in its reduced form because it can scavenge orthoquinones responsible for browning and aroma loss due to oxidation mechanisms (Lavigne et al., 2007). It is well known that GSH is a more potent antioxidant than ascorbic acid (Cojocaru and Antoce, 2016). The International Organization of Vine and Wine (OIV) has recently adopted and incorporated a monograph (OIV-OENO 603-2018) on inactivated yeasts with guaranteed glutathione levels into the international oenological codex.

In order to better investigate of the effect of the nutritional management of yeasts during AF and the use of antioxidant compounds on wine aroma composition, in the present research, two yeast strains isolated from different ecological niches (grape and honey), two yeast nutritional managements and the addition of glutathione-rich inactivated yeast on the aroma composition and sensory quality of Catarratto wine were evaluated.

2. Materials and methods

2.1. Experimental design and sample collection

The experimental plan consisted of three variables: (i) addition of Glutastar™ inactivated yeast (GIY) as antioxidant; (ii) addition of Stimula Sauvignon Blanc™ (SS) and Stimula Chardonnay™ (SC) as yeast nutrient supplementation; and (iii) the inoculation of two yeast starters strains (GR1 and SPF52), conducted in duplicate (Fig. 1).

GIY is an inactivated yeast with a guaranteed glutathione level and also rich in other nucleophilic peptides (Bahut et al., 2020). SS and SC are organic nutrients, consisting of yeast autolysates formulated to provide optimal levels of amino acids, sterols, vitamins and minerals to promote the aromatic metabolism of yeasts; SS contains pantothenate, thiamine, folic acid, zinc and manganese and is formulated to improve volatile thiols, while SC contains biotin, vitamin B6, magnesium and zinc and is formulated to optimize the biosynthesis of volatile esters. GIY, SS and SC were provided by Lallemand Inc. (Castel D'Azzano, Verona, Italy).

Saccharomyces cerevisiae yeast strain GR1 and SPF52 belong to the oenological yeast collection of the Department of Agricultural, Food and Forest Sciences (SAAF) (University of Palermo, Italy). The strain GR1 was isolated from grapes (Francesca et al., 2010) and is used in industrial winemaking, while the strain SPF52 was isolated from fermented honey by-products (Gaglio et al., 2017) and selected for its high

performance to ferment grape must.

Grapes of the "Catarratto bianco lucido" cultivar were harvested from a vineyard located in San Giuseppe Jato (37°59′20″ N; 13°11′34″ E, Palermo, Sicily, Italy) in the 2019 vintage. Wine production was conducted at "Cantina Sperimentale G. Dalmasso" of the Istituto Regionale del Vino e dell'Olio (IRVO) winery located in Marsala (Trapani, Sicily, Italy), Di Bella Vini soc. agr. a.r.l. winery (San Giuseppe Jato, Palermo, Italy) and Azienda Agricola Buonivini (Noto, Siracusa, Italy).

Samples were collected during grape harvest, from clarified bulk must, just after yeast starter inoculation, during AF (day 3, 6, 12 and 18), aging in steel vat (1, 3 and 5 months) and at bottling. All samples were transported at 4 °C in a portable fridge and subjected to analysis within 24 h from collection.

2.2. Winemaking process and monitoring

The grapes were manually harvested, and stemmer-crushed. Potassium metabisulphite (5 g/hL) was added to the bulk must and clarified into stain less-steel tank by cold settling for 24 h in presence of pectolytic enzymes (4 g/hL). The clarified bulk must was divided into twenty steel vats (2.5 hL each); each treatment consisted of two 2.5 hL tanks, for a total of 10 experimental treatments (T1 to T10; Fig. 1).

Prior to yeast inoculation, GIY (40 g/hL) was added to treatments T2, T4, T7 and T9; nutrient SS (40 g/hL) was added to treatments T1, T2, T6 and T7; nutrient SC (40 g/hL) was added to treatments T3, T4, T8 and T9. Yeast were inoculated in liquid concentrated form (approx. 7.00×10^{12} colony-forming units (CFU)/g) at 20 g/hL, T1 to T5 and T6 to T10 with *S. cerevisiae* strains GR1 and SPF52, respectively.

Treatments 5 and 10 were controls (control-A and control-B, respectively), with no addition of GIY, SS and SC, but received an addition of diammonium phosphate (15 g/hL; Chimica Noto s.r.l., Partinico, Italy). The AF was conducted at 18 °C. At the end of AF, the wines were settled, racked off lees, and transferred into stainless-steel tanks at 15 °C, and topped with nitrogen to avoid oxidation up to bottling stage.

2.3. Microbiological analysis

All samples collected during wine production were analysed for yeast and bacteria populations. Must samples were diluted in Ringer's solution (Sigma-Aldrich, Milan, Italy) and analysed in duplicate for total yeasts (TY) on Wallerstein Laboratory (WL) nutrient agar (Pallmann et al., 2001), mesophilic rod lactic acid bacteria (LAB) on de Man–Rogosa–-Sharpe agar (Capozzi et al., 2012), coccus LAB on glucose M17 agar (Francesca et al., 2014), acidophilic LAB on medium for *Leuconostoc oenos* agar (Caspritz and Radler, 1983) and acetic acid bacteria (AAB) on Kneifel agar medium (OIV, 2010). All media and supplements were purchased from Oxoid (Thermofisher, Milan, Italy).

2.4. Yeast isolation and genotypic identification

Yeasts were isolated from WL medium with at least five colonies per morphology randomly selected from the agar plates. The isolates were purified by successive sub-culturing on WL and their purity was verified under an optical microscope (Carl Zeiss Ltd.). Three isolates (from each sample) with the same morphology were then subjected to genetic characterisation (Cavazza et al., 1992).

Genomic DNA for PCR assays was extracted (Alfonzo et al., 2021) and yeast differentiation was by RFLP using the region spanning the internal transcribed spacers (ITS1 and ITS2) and the 5.8S rRNA gene (Settanni et al., 2012). One isolate per group was further analysed by sequencing the D1/D2 region of the 26S rRNA gene to confirm the preliminary identification obtained by RFLP analysis (Alfonzo et al., 2020a). DNA sequencing reactions were performed at AGRIVET (University of Palermo, Italy). The sequence identity was determined by BlastN search against the NCBI non-redundant sequence database (http://www.ncbi.nlm.nih.gov). Sequences were manually corrected using Chromas 2.6.2. (Technelysium Pty Ltd., Australia).

2.5. Strain typing of S. cerevisiae isolates

In order to verify the dominance of GR1 and SPF52 strains during AF, all isolates at the highest cell concentration were characterized by interdelta analysis. Genetic diversity within *Saccharomyces* isolates was assessed by interdelta analysis (Legras and Karst, 2003). Interdelta patterns were analysed using the GelCompar II software (v. 6.5. Applied-Maths, Sin Marten Latem, Belgium) and similarities among patterns were assessed; profiles showing more than 95% of similarity were considered identical.

2.6. Physicochemical analysis

2.6.1. Wine composition

Enzymatic assays for glucose, fructose, ethanol, glycerol, ammoniacal nitrogen, alpha-amino nitrogen and acetic acid were conducted on a iCubio iMagic M9 (Shenzhen iCubio Biomedical Technology Co. Ltd. Shenzhen, China) as described by Barbaccia et al. (2021). The reagents were purchased from R-Biopharm AG (Darmstadt, Germany).

Residual sugars were determined with a WineScan (FOSS, Hillerød, Denmark) calibrated following UNI CEI EN ISO/IEC 17025, 2018.

The pH was determined by OIV-MA-AS313-15 method (OIV, 2020a), total acidity was determined by the methodology described by OIV-MA-AS313-01 (OIV, 2020b), and free and total sulfur dioxide were measured in accordance with the methods described by OIV-MA-AS323-04B (OIV, 2020c). All chemical analyses were carried out in triplicate.

2.6.2. Oenological parameters

Wine samples were analysed for total extract, total phenols, total acidity and buffering power as described by CEE, 2676/90, ash alkalinity following the methodology of Usseglio-Tomasset (1995), flavans reactive to *p*-dimethylaminocinnamaldehyde (*p*-DACA) (Di Stefano et al., 1989), absorbance at 420 nm by spectrophotometer (UV-1601-Shimadsu) and the polyphenols oxidative medium (POM) test as described by Müller-Späth (1992). All analyses were carried out in duplicate.

2.6.3. Volatile organic compounds

Volatile compound composition was determined following the protocol described by Reddy and Dillon (2015). Wine samples (10 mL) from all trials were mixed with MS SupraSolv® 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 Industrivej 6–8, Vassingerød, DK-3540 Lynge, Denmark). The aqueous phase was removed and was added anhydrous sodium sulphate (1 g) before centrifuging at 3000 rpm for 10 min. The dichloromethane layer was removed, and dried under N_2 gas to 1 mL.

Gas chromatographic analyses were performed in two different GC–MS apparatus with two different columns. The first one was 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 second apparatus was a Shimadzu QP 2010 plus equipped with an AOC-20i autoinjector (Shimadzu, Kyoto, Japan) and with a Supelcowax 10 capillary column (30 m × 0.25 mm i.d.; 0.25 μm film thickness); ionization voltage 70 eV; transfer line temperature, 280 °C. Helium was the carrier gas (1 mL/min). For both columns, the temperature was initially kept at 40 °C for 5 min. Then gradually increased to 250 °C at 2 °C/min rate. Held for 15 min and finally raised to 270 °C at 10 °C/min. One μL of sample was injected at 250 °C

automatically and in the splitless mode; transfer line temperature, 295 °C.

The individual peaks were analysed using the GC MS Solution package, Version 2.72. Identification of compounds was carried out using Adams, NIST 11, Wiley 9 and FFNSC 2 mass spectral database. These identifications were also confirmed by other published mass spectra and linear retention indices (LRI). The LRI were calculated using a series of *n*-alkanes (C8-C40). 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.

2.7. Sensory evaluation

Sensory evaluation of experimental wines consisted of two steps: (*i*) sensory acceptance tests performed by consumers and (*ii*) quantitative descriptive analyses carried out by panellists to define aroma and sensory profiles. The sensory assessments were performed as described by Alfonzo et al. (2020b).

2.7.1. Acceptance test

Samples of experimental wines were evaluated for overall acceptability (Biasoto et al., 2014; Villanueva and Da Silva, 2009). A total of 87 consumers were recruited from the University of Palermo; lecturers, researchers, technicians and graduate students were invited to take part by filling in a recruitment form, and from a group of 25 habitual consumers of white wine 13 women and 12 men whose ages ranged from 21 to 42 years were selected. The selection criterion of the subjects was the consumption of at least one glass of white wine per week with no experience on wine sensory analysis.

All the consumers evaluated the overall acceptability of the 10 wine samples using a hybrid hedonic scale of 10 cm which included three points: dislike extremely (0), neither like or dislike (5) and like extremely (10). The ten wine samples were evaluated in two separate tasting sessions and carried out over two successive days. The effects of the presentation order and first-order carry-over of the samples were controlled using the crossover design (Wakeling and MacFie, 1995).

2.7.2. Quantitative descriptive analyses

Sixteen judges (9 women and 7 men, ranging from 23 to 41 years old) were recruited from Oenologist Associations: National Organization of Wine Taster (ONAV, Italy), Italian Sommelier Association (AIS, Italy) and University of Palermo. All had 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 the aromas associated with wines. The sensory profiles (ISO 13299, 2016) of the Catarratto wines were constructed using two selected panels (ISO/CD 8586, 2019) each of eleven judges trained over several sessions.

The sensory analysis of wine was conducted following the methodology by Jackson (2016).

The 16 panellists compared the ten experimental wines during different sessions. They consensually generated 50 sensory descriptive attributes regarding appearance, odour, flavour, taste, overall quality, and finish over several sessions. The set of attributes were: appearance (yellow colour, green reflexes); odour (intensity, persistence, floral, orange flowers, fruity, peach, apricot, plum, green apple, citrus fruit, grapefruit, tropical fruit, pineapple, banana, tamarind, small fruit, strawberry, liquorice, caramel, honey, wax, bread crust, box tree and cat pee); gustatory taste (sweet, sour, salty and bitter); mouth-feel (body and balance); flavour (intensity, persistence, floral, fruity, citrus fruit, tropical fruit, caramel, honey, box tree and cat pee), overall quality (overall quality, odour, taste, mouth-feel and flavour) and finish (after-smell and after-taste).

The panellists were also trained for the identification of wine off-odors and off-flavour: microbial (mouldy, corky, yeasty, buttery and cheesy); pungent (vinegary, alcoholic and sulfur); putrid (rancid, rotten egg and rubbery); petroleum (fusel, plastic and solvent), other (Issa-Issa et al., 2020; Jackson, 2016). 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 (Biasoto et al., 2014; Jackson, 2016).

The ten wine samples were evaluated in distinct tasting sessions carried out on successive days. Overall, each judge evaluated each of the ten 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 and Cox, 1957).

2.8. Statistical and explorative multivariate analyses

ANOVA test was applied to identify significant differences among chemical parameters determined during the winemaking process (pH, total acidity, volatile acidity, residual sugars, glucose, fructose, alpha-amino nitrogen, ammoniacal nitrogen, ethanol, glycerol, malic acid and lactic acid), microbiological analysis (*Saccharomyces* and non-*Saccharomyces* microbial counts), oenological parameters (total extract, total phenols, *p*-DACA flavans, absorbance, polyphenols oxidative medium test, buffering power and ash alkalinity) and sensory analysis (acceptance test and quantitative descriptive analyses). The post-hoc Tukey's method was applied for pairwise comparison of all data. Statistical significance was attributed to $P < 0.05$ (Mazzei et al., 2010).

An explorative multivariate approach was employed to investigate relationships among data obtained during AF (ammoniacal nitrogen, alpha-amino nitrogen, ethanol, fructose, glucose, glycerol, malic acid, pH, residual sugars, total acidity and volatile acidity) from the different treatments.

The agglomerative hierarchical clustering (AHC) and principal component analysis (PCA) of data were performed to investigate relationships among treatments.

To graphically represent the VOCs concentrations, a heat map clustered analysis (HMCA), based on hierarchical dendrogram with heat map plot, was employed to show the individual content values contained in the data matrix as colours (Martorana et al., 2017). The relative values of VOCs concentration were depicted by colour intensity from yellow (lowest quantity) to red (highest quantity). Heat map analysis of the volatile levels was performed using the autoscaled data (Gaglio et al., 2017). The heat map was generated using ascendant hierarchical clustering based on Ward's method and Euclidian distance to show the similarities between VOCs and wine obtained with different yeast starter strains and nutrition regimes.

Multiple factor analysis (MFA) was performed on the data matrix consisted of 10 rows (trials) × 50 columns (50 attributes for sensory analysis) to explore the correlation between variables and different treatments, as well as discrimination among the treatments. Agglomerative hierarchical cluster analysis (AHCA) was also performed on the same data matrixes MFA to explore the variations and similarities of the treatments in relation to the sensory analysis.

Statistical data processing and graphic construction were performed with the XLStat software version 2020.3.1 (Addinsoft, New York, USA) for Excel.

3. Results and discussion

3.1. Dynamics of Saccharomyces spp. and non-Saccharomyces populations

The levels of *Saccharomyces* and non-*Saccharomyces* yeast populations are extremely important to understand the selective effect of the different nutrients used in the different treatments with starter yeasts in Catarratto wines. Adequate yeast nutrition contributes to improve the quality factors that can affect the value of wine (Bell and Henschke,

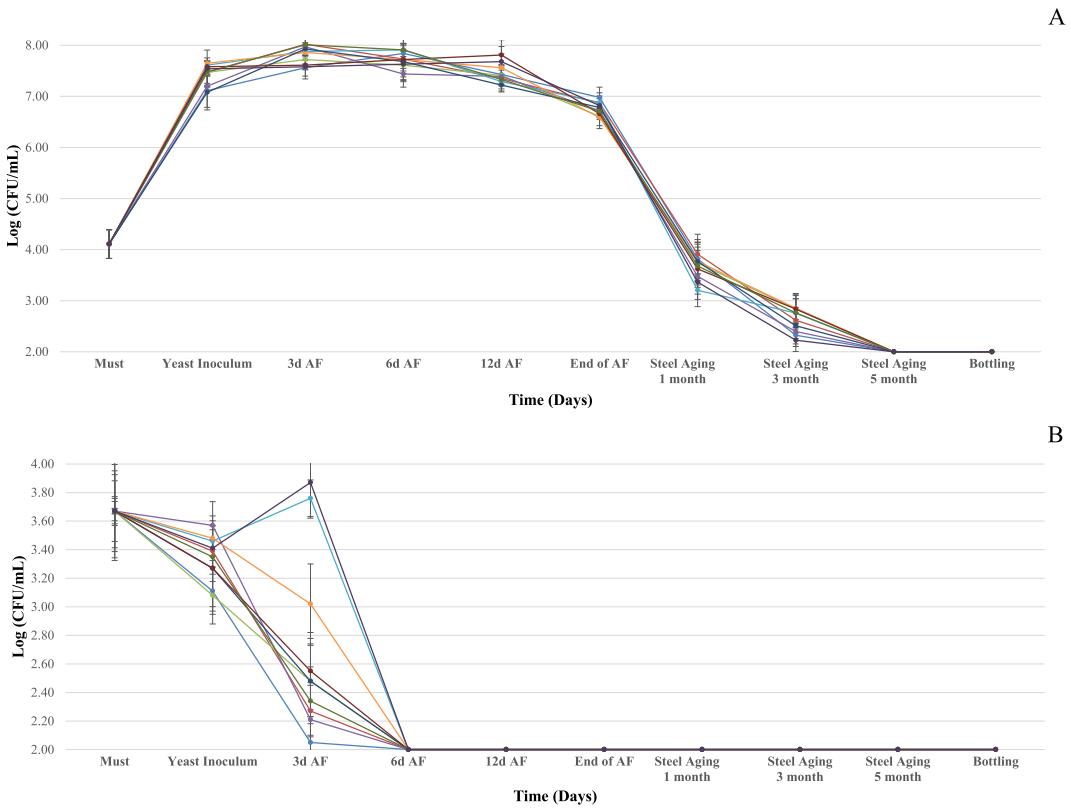

Fig. 2. Yeast population (Log CFU/mL) evolution during alcoholic fermentation and wine storage: (A) Presumptive *Saccharomyces*; (B) non-*Saccharomyces*. Legends: , T1; , T2; , T3; , T4; , T5 (Control-A); , T6; , T7; , T8; , T9; , T10 (Control-B).

2005).

The yeast populations during fermentation are shown in Fig. 2. Presumptive *Saccharomyces* (PS) and non-*Saccharomyces* (NS) yeast populations were 4.1 Log CFU/mL (Fig. 2a) and 3.7 Log CFU/mL (Fig. 2b), respectively, in the Catarratto must. These concentrations are comparable with those reported in other studies (Scacco et al., 2012). *S. cerevisiae* strains (GR1 and SPF52) were inoculated between 7.1 and 7.7 Log CFU/mL, and the initial ratio of PS/NS was between 2 and 2.5. Initial PS values were slightly higher than those reported by Scacco et al. (2012), where PS levels in Catarratto ranged between 5.8 and 6.3 Log CFU/mL with an initial *Saccharomyces*/non-*Saccharomyces* ratio between 1.7 and 4.6. After 3 days of AF, an increase of PS population upto 7.6–8.0 Log CFU/mL was observed for all treatments, whereas NS yeasts showed values in the range of 2.1–3.9 Log CFU/mL. Maximum PS levels were similar to those reported in the literature (7.9–8.1 Log CFU/mL), in fact, Scacco et al. (2012) in fermenting Catarratto musts observed the maximum concentration from 4 to 9 days after the start of AF. At the 6th day of AF, PS concentrations were observed at 7 Log CFU/mL and this trend was also observed until the 12th day. In this case, SS and SC showed typical growth kinetics for both starter yeasts. In contrast, NS populations were undetectable from the 6th day of AF onwards. This would be attributed to the increase in ethanol content, competition with *Saccharomyces* yeast and the reduction in growth factors (Morata and Loira, 2019). By the end of AF, a reduction of PS levels was observed for all treatments. At the end of AF, PS concentrations were between 6.6 and 7.0 Log CFU/mL, and continued to decline during aging in steel until bottling to 2.0 Log CFU/mL.

3.2. Molecular analysis of yeasts

A total of 3084 yeast colonies from WL media were isolated, purified to homogeneity and separated on the basis of WL colony morphology and 2767 isolates shared the morphological characteristics of *Saccharomyces* spp. Furthermore, analysis of 5.8S-ITS amplicons showed that all these isolates had the typical *Saccharomyces* spp. 5.8S-ITS region of 800–900 bp (White et al., 1990). The other isolates were assigned into the non-*Saccharomyces* yeast group, since their ITS amplicon sizes were different from 800 to 900 bp. All PS were further examined by restriction analysis of 5.8S-ITS region and directly identified as *S. cerevisiae* by comparing their restriction bands with those available in literature (Cordero-Bueso et al., 2011a, 2011b; Esteve-Zarzoso et al., 1999). For each RFLP group, one isolate was subjected to sequencing of D1/D2 domain the 26S rRNA gene that successful confirmed the species identification. Interdelta profiles indicated that 22 different *S. cerevisiae* strains were isolated at the highest cell densities from the ten treatments. The direct comparison of the interdelta profiles showed that *S. cerevisiae* GR1 and SPF52 were the strains most frequently (>97%) isolated, and thus demonstrated the dominance of the starter strains GR1 and SPF52 during AF. This is consistent with observations in the literature where the same approach to monitor the persistence and evaluate the dominance of the inoculated strains was used (Alfonzo et al., 2020b; Xufre et al., 2011).

3.3. Alcoholic fermentation

The conversion of grape sugars to alcohol by yeast is of course fundamental to winemaking and through their metabolism the production, various aromatic compounds the final wine quality and nuances

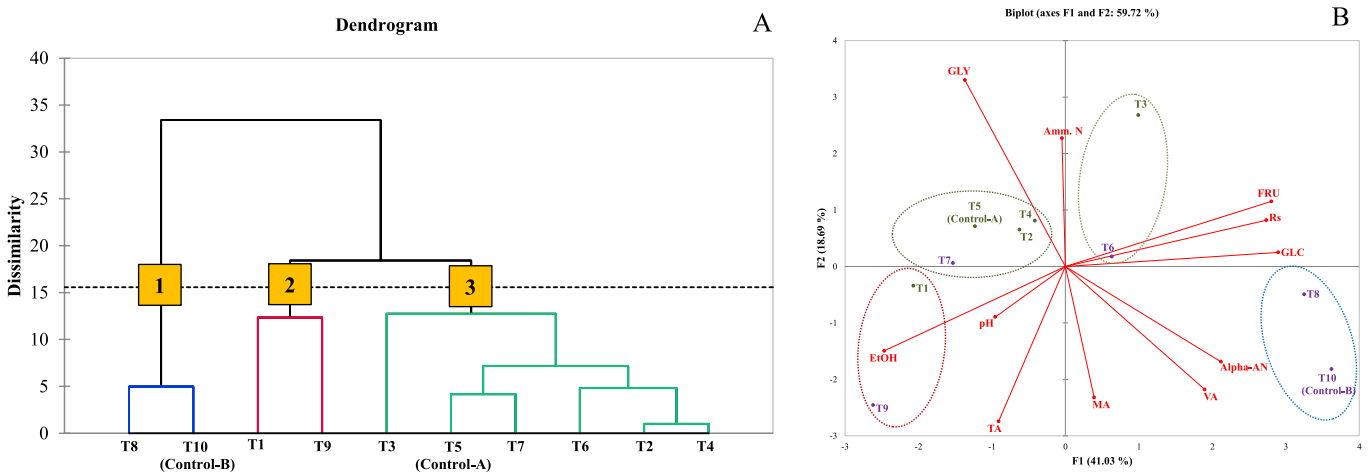

Fig. 3. Agglomerative hierarchical clustering (A) and biplot (B) using chemical parameters detected during alcoholic fermentation. Abbreviations: Amm. N, ammoniacal nitrogen; Alpha-AN, alpha-amino nitrogen; EtOH, ethanol; FRU, fructose; GLC, glucose; GLY, glycerol; MA, malic acid; Rs, residual sugars; TA, total acidity; VA, volatile acidity.

Table 1
Phenols and oxidation indicators of experimental wines.

Treatment	Total extract (g/L)	Total phenols (mg/L catechins)	*p*-DACA flavans (mg/L catechins)	Absorbance (420 nm)	test (%)	Buffering power (meq/L)	Ash alkalinity (meq/L)
T1	18.30 ± 0.13^{bc}	103.21 ± 1.66^{a}	21.65 ± 0.17^{b}	0.073 ± 0.02^{a}	6.49 ± 0.04^{b}	34.48 ± 0.59^{b}	13.83 ± 0.25^{b}
T2	18.00 ± 0.31^{bc}	102.26 ± 1.60^{a}	23.63 ± 0.32^{a}	0.074 ± 0.01^{a}	9.09 ± 0.13^{a}	37.04 ± 0.17^{a}	15.61 ± 0.19^{a}
T3	18.10 ± 0.05^{bc}	101.21 ± 0.63^{a}	17.23 ± 0.05^{d}	0.074 ± 0.00^{a}	2.59 ± 0.04^{d}	32.26 ± 0.58^{cd}	12.66 ± 0.08^{c}
T4	18.10 ± 0.09^{bc}	100.54 ± 1.00^{a}	19.22 ± 0.34^{c}	0.074 ± 0.01^{a}	4.81 ± 0.03^{c}	33.33 ± 0.57^{bc}	13.46 ± 0.07^{b}
T5 (Control-A)	16.80 ± 0.16^{d}	102.54 ± 1.40^{a}	19.18 ± 0.14^{c}	0.075 ± 0.01^{a}	4.76 ± 0.06^{c}	29.41 ± 0.57^{fg}	10.82 ± 0.05^{e}
T6	18.20 ± 0.14^{bc}	86.72 ± 0.39^{cd}	1.91 ± 0.03^{f}	0.101 ± 0.02^{a}	0.00 ± 0.00^{e}	31.25 ± 0.09^{de}	12.65 ± 0.10^{c}
T7	19.00 ± 0.10^{a}	88.48 ± 0.26^{bc}	2.81 ± 0.04^{e}	0.091 ± 0.02^{a}	0.00 ± 0.00^{e}	31.25 ± 0.37^{de}	12.65 ± 0.06^{c}
T8	18.40 ± 0.15^{b}	84.72 ± 0.64^{d}	1.47 ± 0.02^{f}	0.100 ± 0.01^{a}	0.00 ± 0.00^{e}	30.30 ± 0.18^{ef}	12.08 ± 0.05^{d}
T9	19.10 ± 0.13^{a}	83.39 ± 1.73^{d}	1.55 ± 0.02^{f}	0.085 ± 0.00^{a}	0.00 ± 0.00^{e}	33.33 ± 0.03^{bc}	13.41 ± 0.23^{b}
T10 (Control-B)	17.80 ± 0.38^{c}	91.48 ± 1.02^{b}	3.10 ± 0.03^{e}	0.105 ± 0.01^{a}	0.00 ± 0.00^{e}	28.57 ± 0.10^{g}	10.36 ± 0.19^{f}
Statistical significance	**	**	**	n.s.	**	**	**

Results indicate mean value ± standard deviation of two determinations.
Data within a column followed by the same letter are not significantly different according to Tukey's test.
P value: **, $P < 0.01$; n.s., not significant. Abbreviations: *p*-DACA, *p*-dimethylaminocinnamaldehyde; POM, polyphenols oxidative medium.

are achieved (Swiegers et al., 2005). Consequently, nutrients are key compounds both to support yeast growth and to ensure a regular and complete fermentation (Lambrechts and Pretorius, 2000).

The main wine composition parameter, are shown in Table S1. The final wine compositions are in agreement with that predicted from the initial grape must composition. The residual sugar varied between the treatments with *S. cerevisiae* SPF52 having slightly more than GR1. Final ethanol content was variable in the different treatments (13.64–14.02% (*v*/v)). Considering the initial sugar content of the must of Catarratto grapes (221.50 g/L) and the final ethanol content, these values are similar to those reported in the literature by Fracassetti et al. (2018) who predicted the use of the commercial strain of *S. cerevisiae* 20 CRU611 on musts with similar chemical characteristics. Glycerol produced by GR1 and SPF52 yeast strains ranged between 5.98 and 6.68 g/L: at these concentrations, glycerol contributes to the viscosity and softness of the wine, with a positive effect on its taste (Noble and Bursick, 1984). No statistical significance was found for total acidity in all treatments (5.36 to 5.40 g/L) and was slightly lower than that described by Fracassetti et al. (2018) and Scacco et al. (2012) who reported values more than 6 g/L of tartaric acid. The volatile acidity was variable between the treatments, but at the end of AF, values of 0.31 g/L were found for all the wines. This concentration is mainly due to the acetic acid produced by the yeast during AF, and the values were equivalent to those observed by other studies in Catarratto wines produced in Sicily (Fracassetti et al., 2018; Sannino et al., 2013; Scacco et al., 2012). Slight differences were observed for malic acid content (1.26 and 1.59 g/L). Lactic acid was not detected in any treatment. The free SO_2 and total SO_2 values ranged between 30 and 32 mg/L and 80–85 mg/L, respectively.

The effect of different strains of *S. cerevisiae* strains (GR1 and SPF52), chemical parameters (ammoniacal nitrogen, alpha-amino nitrogen, ethanol, fructose, glucose, glycerol, malic acid, residual sugars, total and volatile acidity), nutrient strategy (SS and SC) and the presence/absence of antioxidant compounds (Glutastar™) in the final wines was evaluated by a multivariate statistical analysis approach (Fig. 3).

Agglomerative hierarchical clustering (AHC) classified the trials in accordance with their mutual dissimilarity and relationships (Fig. 3a). This analysis classified the trials using ten variables selected on the basis of the results from chemical monitoring. All treatments were clearly separated into three clusters with a dissimilarity of 15%. The most numerous clusters was cluster 3 which included six treatments (T2, T3, T4, T5, T6 and T7). Whereas cluster 2 and cluster 1 were represented by the T8-T10 and T1-T9 trials, respectively. Cluster 1 was the most heterogeneous and this is confirmed by the Within-class variable. The variables that greatly impacted trial clusterization were different for each cluster. The trend of pH, total acidity, ethanol and malic acid during AF generated cluster 1. Glycerol was the variable that most discriminated against cluster 2, while cluster 3 was obtained by volatile acidity, residual sugars, glucose, fructose and alpha-amino nitrogen.

The biplot illustrated in Fig. 3b highlights the distribution of the different treatments in relation to the same chemical parameters used

Table 2
Volatile organic compounds (VOCs) of wines obtained from treatments T1-T10.

LRI[a]	LRI[b]	Ident.[c]	Compounds	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Σ alcohols													
1076		1. 2	2-Methyl-1-propanol	5.17	5.55	9.66	4.30	5.81	9.22	5.16	7.11	10.15	5.75
1190	736	1. 2	3-Methyl-1-butanol	46.36	52.17	50.18	59.87	43.86	43.06	42.49	41.62	36.99	35.34
1515	808	1. 2	2.3-Butanediol isomer	4.89	3.90	3.72	4.31	5.63	7.23	4.69	5.04	6.11	8.46
1553	913	1. 2	2.3-Butanediol isomer	1.13	0.71	0.82	0.81	1.43	1.74	1.01	0.99	1.34	2.85
1867	1122	1. 2	Phenethyl alcohol	20.34	16.57	13.39	15.62	15.72	10.17	6.93	7.25	8.50	8.50
1939	1387	1. 2	1-Dodecanol	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.07
2304	1103	1. 2. 3	Glycerin	0.00	1.19	1.74	0.00	3.60	0.00	2.43	4.07	2.21	0.38
2976	1444	1. 2	4-Hydroxyphenethyl alcohol	0.00	1.09	1.28	1.19	1.49	0.69	0.70	0.90	0.74	1.11
3427	3041	1. 2. 3	Cholesterol	t	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Σ thiols													
1677	988	1. 2	3-(Methylthio)-1-propanol	0.27	0.35	0.80	0.79	0.64	0.80	0.36	0.40	0.29	0.87
Σ ethers													
2171	1321	1. 2	4-Hydroxy-3-methoxys Tyrene	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.09	0.16	0.14
Σ aldehydes													
2323	2010	1. 2	Octadecanal	0.00	0.00	0.00	0.00	1.21	0.00	0.82	0.99	0.48	0.70
Σ ketones													
1259	–	1. 2	3-Hydroxy-2-butanone	0.00	0.00	0.00	0.00	0.00	0.51	0.00	0.00	2.28	0.00
Σ carboxylic acids													
1454	–	1. 2. 3	Acetic acid	0.82	0.00	0.00	0.00	0.00	1.61	0.00	1.26	3.46	4.27
1847	1028	1. 2	Hexanoic acid	0.28	1.08	0.72	0.00	1.40	0.00	1.39	1.44	1.17	1.83
2046	1206	1. 2	Octanoic acid	3.60	4.36	4.75	4.21	4.06	4.20	4.54	5.19	4.20	2.83
2283	1382	1. 2	Decanoic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2900	1387	1. 2	*n*-Hexadecanoic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.09
3123	2383	1. 2	Stearic acid	0.00	0.00	0.00	0.00	0.59	0.00	0.99	1.37	0.84	1.30
Σ EEFAs													
1213	996	1. 2	Ethyl hexanoate	0.37	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1427	1194	1. 2	Ethyl octanoate	0.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1611	1400	1. 2	Ethyl decanoate	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.21
Σ HAAs													
1111	873	1. 2	Isoamyl acetate	4.23	3.84	2.41	2.73	4.89	1.76	3.16	4.31	1.35	2.80
1778	1260	1. 2	Phenylethyl acetate	2.33	0.24	0.22	0.14	0.55	0.00	0.16	0.16	0.11	0.18
Σ EEBAs													
1319	814	1. 2	Ethyl 2-hydroxypropanoate	1.66	1.04	1.99	1.50	1.39	10.35	10.85	7.03	9.26	8.43
1486	936	1. 2	Ethyl 3-hydroxybutyrate	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Σ MEs													
1645	1181	1. 2	Ethyl succinate	0.26	0.17	0.22	0.14	0.22	0.43	0.65	0.30	0.55	0.13
1702	1108	1. 2	1.3-Propylene diacetate	0.23	0.39	0.34	0.00	0.57	0.00	0.22	0.29	0.00	0.00
2012	1272	1. 2	Ethyl dl-malate	0.37	0.47	0.38	0.32	0.19	0.07	0.24	0.26	0.18	0.27
Σ lactones													
1589	913	1. 2	Butyrolactone	0.99	1.08	0.83	0.65	0.91	0.75	0.97	1.13	0.79	1.17
2191	1305	1. 2	*γ*-Carboethoxy-*γ*-butyrolactone	0.34	0.41	0.41	0.43	0.26	0.15	0.42	0.34	0.22	0.19
Others													
1399	1254	1. 2	1.3-Di-tert-butylbenzene	0.73	0.41	0.91	0.71	0.90	0.72	0.91	1.10	0.83	1.34
1874	1508	1. 2	2.6-Di-tert-butyl-4-methylphenol	0.00	0.00	0.00	0.00	0.00	0.00	5.19	1.84	0.00	0.00
2270	1525	1. 2	2.4-Di-tert-butylphenol	0.29	0.36	0.83	0.51	0.78	0.51	0.80	0.75	0.66	1.19
Total compounds				95.39	95.38	95.60	98.23	96.10	96.47	95.08	95.23	92.87	92.40

Abbreviations: t, trace amount < 0.05%; EEFAs, ethyl esters of fatty acids; HAAs, higher alcohol acetates; EEBAs, ethyl esters of branched acids; MEs, miscellanea esters.

[a] LRI: Supercowax10 column.

[b] LRI: DB5-MS column.

[c] Ident.: 1 = retention index identical to bibliography; 2 = identification based on comparison of MS; 3 = retention time identical to authentic compounds

for AHC analysis. Treatments T8 and T10 clustered into one group that was statistically correlated with alpha-amino nitrogen, malic acid and volatile acidity. Treatments T3 and T6 were associated with fructose, glucose and residual sugars. On the other quadrant of biplot, T2, T4, T5 and T7 grouping was driven by ammonium nitrogen and glycerol. Finally, treatments T1 and T9 were related with ethanol, pH and total acidity.

This behaviour is in agreement with several authors who observed that the use of different yeast strains, subjected to different nutritional regimes, can lead to variations in chemical parameters during the AF of must (Julien et al., 2001; Sablayrolles, 2009).

3.4. Oenological parameters

Results from oenological analysis of wines are shown in Table 1. Treatment wines T1 and T2, produced with GR1 yeast strain, SS and GIY additions, showed the lowest values of wine susceptibility to oxidation by POM test. A high value from POM test characterises the wine potential in preserving the wine phenolics and oxidation potential (Comuzzo et al., 2006). In this case, the highest values found in the POM test for T2 (9.09) demonstrates how this typology of wine is able to preserve a determined phenolic component (Voce et al., 2020). In fact, the total polyphenols content was higher in wines made with the GR1

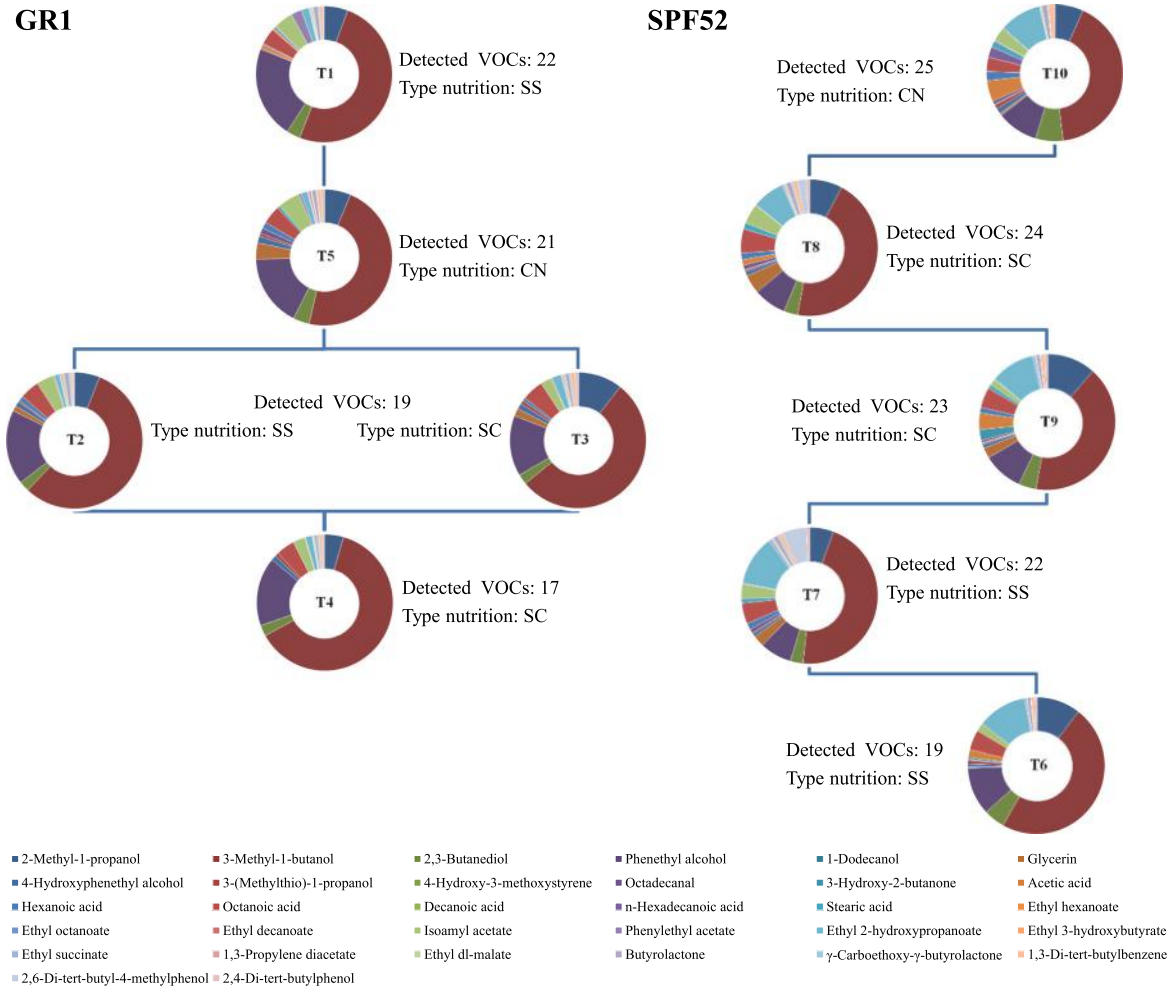

Fig. 4. Distribution of wines in relation to the number of volatile organic compounds (VOCs).

strain (100.54–103.21 mg/L catechins), with and/or without the addition of SS, SC and GIY compared to those obtained with the SPF52 strain (83.39–91.48 mg/L catechins). In this case, total polyphenol content was found to be nondependent on the presence/absence of glutathione, and the type of nutrition in T1-T5 treatments, whereas variations occurred in T6-T10 treatments fermented with SPF52. Most likely, as suggested by Grieco et al. (2019), the differences could be of microbiological nature. In fact, during the vinification process specific yeast strains are able to produce polysaccharides capable of establishing stable complexes with polyphenols (Brandolini et al., 2007). In addition, the p-DACA flavans content was higher in treatments T1-T5 made with GR1 yeast strain, and the highest values were observed in T1 (21.64 mg/L catechins) and T2 (23.63 mg/L catechins). The content of catechins is also an important quality parameter, to verify the level of oxidation of wine and the influence on colour (Katalinić et al., 2004).

Treatments T1 and T2, also stood out from the other experimental wines for their high content in buffering power corresponding to a higher amount of salified acids This might suggest a long gustatory perception and minerality/acidity taste perceived for these wines (Blouin and Peynaud, 2005). Based on these observation, the use of *S. cerevisiae* GR1 strain and/or GIY (mainly trials 3 and 4) did not reach the same values of trials T1 and T2, as well as with control thesis (T5; classic nutrition) the values obtained were the lowest compared to those observed in the trials T1-T4.

The experimental production obtained by strain SPF52 (T6-T10) showed a similar oenological characteristics and significant differences were found with respect to trials conducted with strain GR1. The oxidation POM-test showed no impact of GIY in these wines; there was no protection of oxidations of the phenolic compounds. Consequently, SPF52 strain treatment wines had lower levels of p-Daca flavans than GR1 wines, thus resulting in an increase of 420 nm optical density.

The highest content of total dry extract was in wines T7 and T9 (19.00 g/L and 19.10 g/L, respectively); all other treatments ranged from 16.80–18.40 g/L. These values are comparable with Catarratto wine studies (20.6–22.1 g/L) (Scacco et al., 2012).

Ash alkalinity was lower in the control wines (T5 and T10), with the highest value in T2 wine (15.61 meq/L). There was no correlation between the yeast strain, nutritional scheme nor the absence or presence of glutathione-rich inactivated yeast.

3.5. Volatile organic compound composition

The composition of VOCs of the ten samples is reported in Table 2. More quantitative than qualitative differences were observed in the composition of the ten wines. Thirty-two compounds of different chemical classes (alcohols, thiols, ethers, aldehydes, ketones, carboxylic acids, esters, lactones) were identified, representing more than 90% of total volatile wine components. The esters were classified into different chemical structure families ethyl esters of fatty acids (EEFAs), higher alcohol acetates (HAAs), ethyl esters of branched acids (EEBAs), and miscellaneous esters (MEs) (Puertas et al., 2018).

Alcohols were the most abundant compounds (52.44–80.60%), then carboxylic acids (4.21–12.32%), EEBAs (1.04–10.35%) and HAAs (1.46–6.56%). The most abundant alcohol in all samples (Fig. 4) is 3-

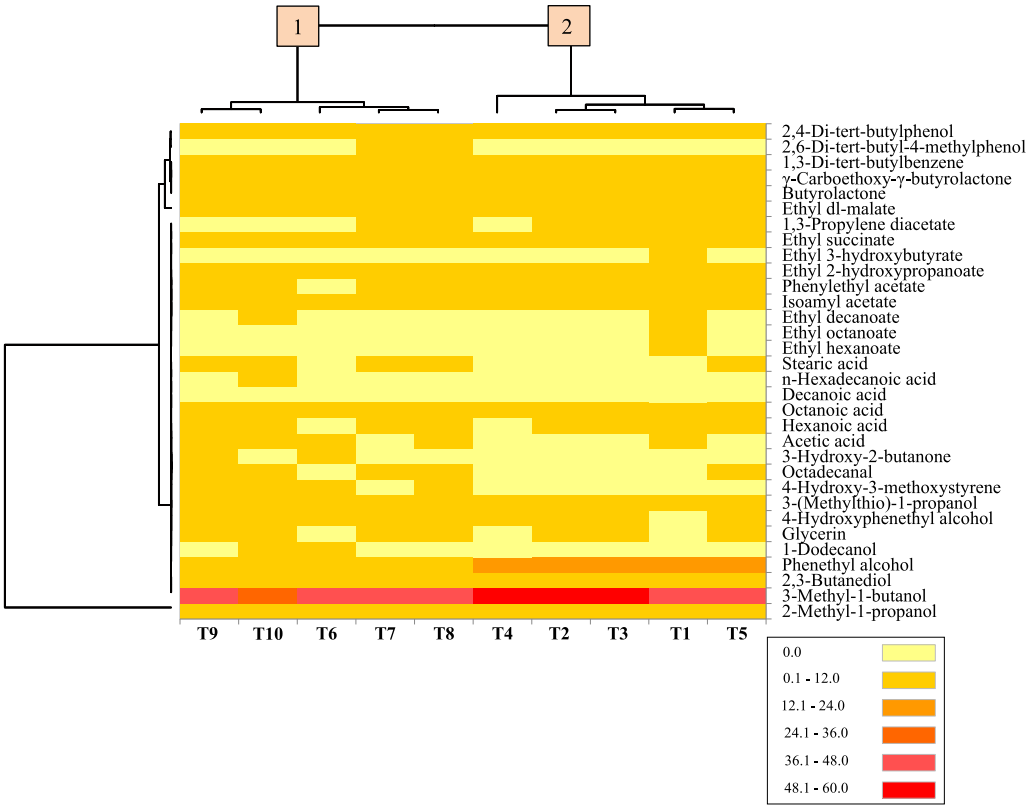

Fig. 5. Distribution of the volatile organic compounds (VOCs) from wines expressed as relative peak areas (peak area of each compound/total area) × l00. The hierarchical dendrogram is based on the values of VOCs. The heat map plot depicts the relative percentage of each compound within each wine.

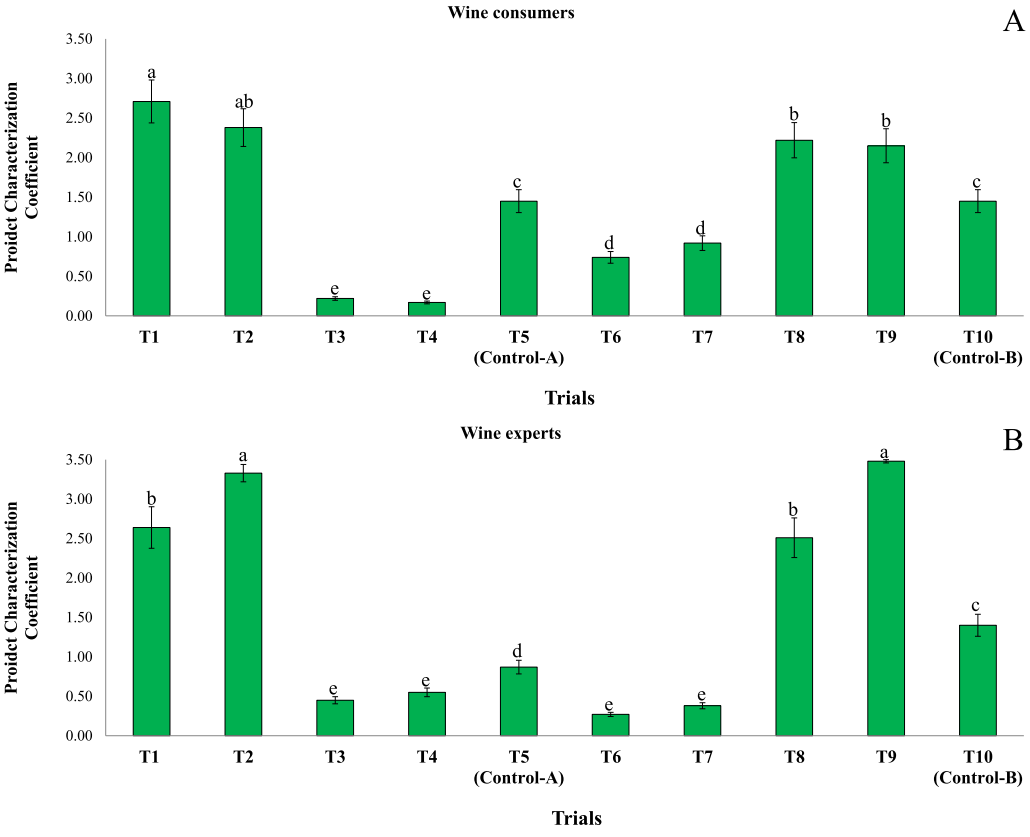

Fig. 6. Sensory analysis based on product characterisation for overall acceptability of wines (T1–T10): (A) wine consumers; (B) wine experts.

Table 3
Sensory attributes of the experimental Catarratto wines.

Attributes	Trial										SEM	Statistical significance	
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10		Judges	Wine
Appearance													
Yellow colour	6.85^{f}	6.70^{h}	6.85^{f}	6.80^{g}	6.78^{g}	7.39^{a}	7.20^{d}	7.28^{c}	7.15^{e}	7.35^{b}	0.02	*	*
Green reflexes	3.36^{e}	3.46^{d}	3.83^{b}	3.89^{a}	3.74^{c}	3.12^{fg}	3.01^{h}	3.09^{g}	3.19^{f}	3.18^{f}	0.03	*	*
Odour													
Intensity	7.15^{d}	8.30^{a}	7.80^{b}	5.25^{f}	5.28^{f}	6.25^{e}	7.56^{c}	7.22^{d}	8.19^{a}	7.35^{d}	0.09	*	*
Persistency	7.38^{d}	8.10^{b}	6.82^{f}	5.01^{h}	4.10^{i}	5.98^{g}	7.65^{c}	7.11^{e}	8.64^{a}	7.68^{c}	0.11	**	***
Floral	6.88^{b}	7.30^{a}	2.15^{f}	3.25^{e}	3.25^{e}	6.29^{c}	6.10^{c}	2.20^{f}	3.20^{e}	5.38^{d}	0.16	***	***
Orange flowers	7.20^{b}	7.70^{a}	1.00^{d}	1.00^{d}	1.00^{d}	1.00^{d}	2.90^{c}	1.00^{d}	1.00^{d}	1.00^{d}	0.21	**	***
Fruity	6.70^{d}	6.20^{e}	7.70^{b}	4.25^{f}	4.50^{f}	6.71^{d}	3.10^{g}	7.30^{c}	8.02^{a}	2.50^{h}	0.16	*	**
Peach	1.00^{e}	1.00^{e}	7.12^{a}	3.85^{d}	1.00^{e}	6.65^{b}	1.00^{e}	1.00^{e}	5.25^{c}	1.00^{e}	0.20	***	***
Apricot	1.00^{d}	1.00^{d}	7.08^{a}	1.00^{d}	1.00^{d}	6.41^{b}	1.00^{d}	1.00^{d}	4.25^{c}	1.00^{d}	0.20	***	***
Plum	1.00^{c}	1.00^{c}	1.00^{c}	1.00^{c}	1.00^{c}	1.00^{c}	1.00^{c}	6.82^{a}	5.58^{b}	1.00^{c}	0.17	***	***
Green apple	1.00^{b}	3.20^{a}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	0.05	*	**
Citrus fruits	6.20^{b}	7.90^{a}	3.54^{c}	3.25^{c}	1.00^{d}	1.00^{d}	1.00^{d}	1.00^{d}	1.00^{d}	1.00^{d}	0.20	**	***
Grapefruit	4.35^{b}	7.70^{a}	2.65^{c}	1.00^{d}	1.00^{d}	1.00^{d}	1.00^{d}	1.00^{d}	1.00^{d}	1.00^{d}	0.18	**	**
Tropical fruits	1.00^{c}	1.00^{c}	8.30^{a}	1.00^{c}	5.25^{b}	1.00^{c}	1.00^{c}	8.12^{a}	7.98^{a}	1.00^{c}	0.27	***	***
Pineapple	1.00^{c}	1.00^{c}	7.77^{a}	1.00^{c}	4.00^{b}	1.00^{c}	1.00^{c}	1.00^{c}	1.00^{c}	1.00^{c}	0.18	**	**
Banana	1.00^{c}	1.00^{c}	7.62^{a}	1.00^{c}	4.80^{b}	1.00^{c}	1.00^{c}	1.00^{c}	1.00^{c}	1.00^{c}	0.18	**	***
Tamarind	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	7.75^{a}	1.00^{b}	1.00^{b}	0.17	*	**
Small fruits	3.50^{c}	3.98^{b}	4.20^{b}	4.01^{b}	1.00^{d}	5.12^{a}	4.12^{b}	1.00^{d}	1.00^{d}	1.00^{d}	0.13	**	***
Strawberry	1.00^{b}	1.00^{b}	6.75^{a}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	0.14	**	*
Licorice	6.50^{a}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	0.03	*	*
Anice	6.87^{a}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	0.02	*	*
Caramel	1.00^{d}	1.00^{d}	1.00^{d}	1.00^{d}	1.00^{d}	6.10^{c}	7.20^{b}	7.35^{ab}	7.52^{a}	7.12^{b}	0.14	**	***
Honey	1.00^{d}	1.00^{d}	1.00^{d}	1.00^{d}	1.00^{d}	5.87^{c}	7.75^{a}	7.85^{a}	7.86^{a}	7.01^{b}	0.15	**	***
Wax	1.00^{d}	1.00^{d}	1.00^{d}	1.00d	1.00^{d}	6.15^{c}	6.98^{b}	6.18^{c}	6.58^{bc}	7.45^{a}	0.25	***	***
Bread crust	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	7.10^{a}	1.00^{b}	6.98^{a}	1.00^{b}	0.27	***	***
Box tree	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	7.12^{a}	0.15	**	**
Cat pee	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	8.70^{a}	0.19	**	**
Taste													
Sweet	2.40^{g}	2.54^{f}	2.97^{c}	2.58^{f}	2.34^{g}	2.98^{c}	2.72^{e}	3.48^{b}	3.59^{a}	2.91^{d}	0.03	*	*
Sour	7.86^{a}	7.42^{b}	6.65^{d}	6.75^{d}	6.12^{e}	5.15^{g}	6.80^{d}	5.38^{f}	5.37^{f}	6.98^{c}	0.07	**	*
Salty	6.28^{c}	7.10^{a}	5.50^{f}	5.15^{g}	4.45^{h}	5.25^{g}	6.50^{b}	5.70^{e}	5.85^{d}	6.58^{b}	0.06	*	*
Bitter	2.15^{b}	2.38^{a}	1.80^{e}	2.05^{c}	1.92^{d}	1.38^{f}	1.22^{g}	1.10^{h}	1.25^{g}	1.38^{f}	0.04	*	*
Mouth-feel													
Body	7.15^{d}	7.88^{b}	6.82^{e}	6.15^{g}	6.35^{f}	6.31^{f}	7.51^{c}	7.80^{b}	8.42^{a}	6.75^{e}	0.06	*	*
Balance	6.80^{c}	8.32^{a}	6.25^{e}	5.98^{f}	5.00^{g}	6.17^{e}	6.89^{c}	6.50^{d}	7.49^{b}	6.20^{e}	0.07	*	*
Flavour													
Intensity	6.93^{c}	7.50^{b}	6.26^{d}	5.14^{f}	5.25^{f}	5.71^{e}	7.45^{b}	7.80^{a}	7.85^{a}	7.10^{c}	0.08	*	**
Persistency	6.82^{e}	8.00^{b}	5.44^{f}	5.68^{f}	4.82^{g}	5.58^{f}	7.15^{d}	7.70^{c}	8.78^{a}	6.87^{e}	0.10	**	**
Floral	6.12^{a}	5.10^{b}	2.25^{h}	3.25^{f}	2.50^{h}	3.15^{f}	4.12^{d}	3.52^{e}	4.82^{c}	2.20^{h}	0.10	**	**
Fruity	6.25^{d}	6.90^{c}	7.62^{a}	4.08^{f}	5.12^{e}	5.12^{e}	1.96^{h}	6.92^{c}	7.25^{b}	2.80^{g}	0.15	**	**
Citrus fruits	6.65^{b}	7.20^{a}	1.60^{c}	1.00^{d}	1.00^{d}	1.00^{d}	1.00^{d}	1.00^{d}	1.00^{d}	1.00^{d}	0.20	**	***
Tropical fruits	2.25^{d}	2.98^{c}	8.10^{a}	1.00^{e}	4.62^{b}	1.00^{e}	1.00^{e}	8.12^{a}	7.87^{a}	1.00^{e}	0.25	***	***
Caramel	1.00^{d}	1.00^{d}	1.00^{d}	1.00^{d}	1.00^{d}	3.85^{c}	6.25^{b}	7.10^{a}	7.02^{a}	4.25^{c}	0.21	***	***
Honey	1.00^{d}	1.00^{d}	1.00^{d}	1.00^{d}	1.00^{d}	6.27^{b}	7.64^{a}	7.85^{a}	7.35^{a}	3.88^{c}	0.25	***	***
Box tree	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	7.15^{a}	0.15	**	**
Cat pee	1.00^{c}	1.00^{c}	1.00^{c}	1.00^{c}	3.02^{b}	1.00^{c}	1.00^{c}	1.00^{c}	1.00^{c}	8.75^{a}	0.19	**	***
Overall quality	7.58^{b}	8.38^{a}	6.89^{c}	6.10^{d}	4.32^{e}	6.27^{d}	7.70^{b}	7.50^{b}	8.57^{a}	2.20^{f}	0.16	*	**
Odour	7.68^{c}	8.40^{b}	7.03^{e}	5.15^{g}	4.10^{h}	6.17^{f}	7.50^{cd}	7.20^{de}	8.86^{a}	1.50^{i}	0.18	**	**
Taste	7.25^{c}	7.89^{a}	6.92^{e}	6.12^{f}	5.30^{h}	5.78^{g}	7.10^{d}	7.01^{de}	7.54^{b}	6.12^{f}	0.07	*	**
Mouth-feel	7.10^{cd}	7.55^{b}	6.50^{e}	5.89^{g}	5.90^{g}	6.37^{f}	6.97^{d}	7.20^{c}	8.32^{a}	5.80^{g}	0.06	*	**
Flavour	7.41^{c}	8.20^{b}	6.80^{e}	6.32^{f}	4.39^{g}	6.32^{f}	7.22^{cd}	6.98^{de}	8.81^{a}	1.93^{h}	0.16	**	***
Finish													
After-smell	7.31^{b}	8.00^{a}	6.80^{c}	6.21^{d}	4.10^{e}	6.15^{d}	7.11^{bc}	6.80^{c}	8.15^{a}	1.59^{f}	0.16	**	***
After-taste	7.11^{b}	8.10^{a}	6.30^{c}	6.32^{c}	5.10^{d}	6.11^{c}	7.35^{b}	7.10^{b}	7.96^{a}	1.38^{e}	0.15	**	***

Results indicate mean value. Abbreviation: SEM, Standard Error of the Mean.
Data within a line followed by the same letter are not significantly different according to Tukey's test.
P value: ***, $P < 0.001$; **, $P < 0.01$; * $P < 0.05$.

methyl-1-butanol (isoamyl alcohol). Both yeast strains, GR1 (grape) and SPF52 (honey), both nutrients (SS and SC) and the presence of the antioxidant GIY, promoted the production of 3-methyl-1-butanol compared to controls. Furthermore, the presence of antioxidants (GIY) significantly increased the amount of 3-methyl-1-butanol in T2, T4 and T7, while T9 displayed only a small increase.

Esters influence wine aroma, not only directly but also via complex synergistic interactions. The fermentative strategy immensely affects the total ester content (Puertas et al., 2018). The total amount of esters generated by the honey strain SPF52 was higher than the GR1 grape strain. The most abundant class of esters was EEBAs, with ethyl 2-hydroxypropanoate as the unique compound of this family. It is present in higher quantities in the wines made with SPF52 honey strain, and could be responsible for the caramel and/or honey aroma noted in the sensory analysis of these wines. Among the various treatments, only the T8 showed lower amounts of ethyl 2-hydroxypropanoate than control.

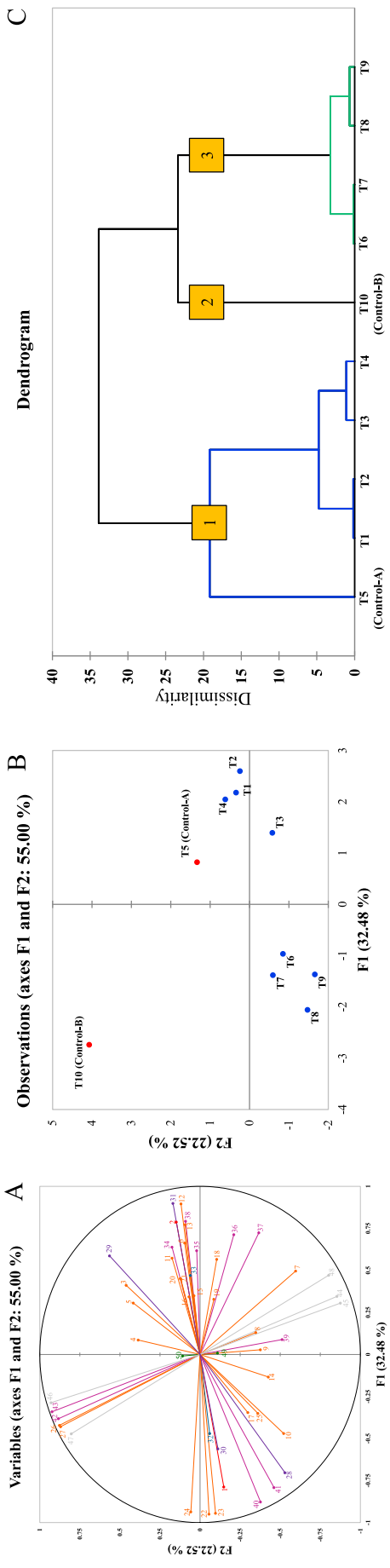

Fig. 7. Correlations of the sensory analysis and discrimination among different wines: (A) Variable loading plot of MFA: ■ appearance (1, yellow colour; 2, green reflexes), ■ odour (3, intensity; 4, persistence; 5, floral; 6, orange flowers; 7, fruity; 8, peach; 9, apricot; 10, plum; 11, green apple; 12, citrus fruit; 13, grapefruit; 14, tropical fruit; 15, pineapple; 16, banana; 17, tamarind; 18, small fruit; 19, strawberry; 20, licoric; 21, anice; 22, caramel; 23, honey; 24, wax; 25, bread crust; 26, box tree; 27 cat pee), ■ taste (28, sweet; 29, sour; 30, salty; 31, bitter), ■ mouth-feel (32, body; 33, balance), ■ flavour (34, intensity; 35, persistence; 36, floral; 37, fruity; 38, citrus fruit; 39, tropical fruit; 40, caramel; 41, honey; 42, box tree; 43, cat pee, ■ overall quality (44, overall quality; 45, odour; 46, taste; 47, mouth-feel; 48, flavour), ■ finish (49, after-smell; 50, after-taste); (B) sample scores of MFA analysis; (C) AHC dendrogram of trials based on their dissimilarity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The addition of GIY (T7 and T9) favours the formation of 2-hydroxypropanoate. It is clearly the opposite for the must fermented with GR1 grape strain: in this case, the absence of antioxidants stimulates a greater production of EEBAs. Regarding the acetates deriving from long chain alcohols (HAAs), GR1 strain produced higher amounts (2.63–6.56%) than the SPF52 strain (1.46–4.47%). Differences can also be noted regarding the impact of nutrients and antioxidant on the final composition of the wine. In fact, SS in absence of GIY, produced higher concentrations of HAAs; while a lower amount of the latter was present when the yeast was treated with SC. SPF52 honey strain, in the presence of SC and in the absence of GIY, favoured a higher content of HAAs in wine samples than those treated with GIY; in turn, the presence of antioxidants seemed to favour the production of HAAs with SS compared to T6. As reported by Renault et al. (2015), the EEFAs, compounds which contribute to fruity aromas, are present in minimal quantities in all samples (0.21–1.02%) and reflected in the sensory analysis.

The most abundant carboxylic acid in all wines was octanoic acid, except in T10 where it was acetic acid. GR1 strain produced wines, had minimal (T1) acetic acid.

Other volatile compounds identified in all samples were 2.4-DTBP and the corresponding aromatic compound without phenolic group. In T7 and T8 wines, 4-methyl-2.6-DTBP was also present. These bioactive secondary metabolites, produced by various groups of organisms, are reported in the literature (Zhao et al., 2019).

3.5.1. *Statistical multivariate analysis of VOCs composition*

The graphical representation of VOCs analysis is shown in Fig. 5. The double hierarchical dendrogram combined with heat map plot showed that all additions (yeast, nutrient and GIY) significantly affected VOCs composition of the wines. There are two distinct VOCs clusters, with the most important being the high quantity of ethyl esters of branched acids (EEBAs 7.04–10.85%) and a lower presence of alcohols (62.46–72.26%) in the T6-T10 group compared to the T1-T5 group (EEBAs 1.04–1.74%; alcohols 77.54–86.10%). Control A and control B are characterized by the lower quantity of alcohols in the two groups (77.54% in control A and 62.46% in control B).

Interestingly, the T1-T5 wines were grouped into one mega-cluster with the discriminator as yeast strain, GR1 grape strain. T4 showed the highest dissimilarity values, in contrast to T1 and T5, T2 and T3 with similar VOCs composition. T4 exhibited the highest alcohols content (86.10%) and the lowest carboxylic acids content (4.21%).

The second cluster (T6-T10) are the experimental wines produced with SPF52 honey strain. This cluster has three subclasses: T9 and T10, T7 and T8, and T6 which represented a separate subclass. It could be proposed that the SPF52 honey strain produces very different VOCs depending on the nutrition strategies (SS and SC) and antioxidant (GIY) addition. Furthermore, GR1 grape strain significantly affected wine composition independently by nutrition strategies and antioxidant addition.

3.6. *Sensory analysis*

The highest overall acceptability scores were found for T1 (2.71) based on wine consumers' response (Fig. 6a), whereas, T2 (3.33) and T9 (3.48) were rated highest by wine expert (Fig. 6b). It is worthy of note that wines T2 and T9 had very high overall acceptability scores from both wine consumers and wine experts. From the sensory acceptance test, wines produced with strain GR1, SS and GIY (T2) and strain SPF52, SC and GIY (T8) were the most appreciated. Probably, the aromatic behaviour of the strains and the type of nutrition in conjunction with antioxidants guaranteed a higher acceptability of the wine. None of the ten wines were judged as “unpleasant/unacceptable”.

The quantitative sensory analysis results are reported in Table 3. All the wines mainly showed differences related to the yeast strain used (GR1 or SPF52). This phenomenon has been extensively studied by numerous authors and especially with indigenous *S. cerevisiae* strains

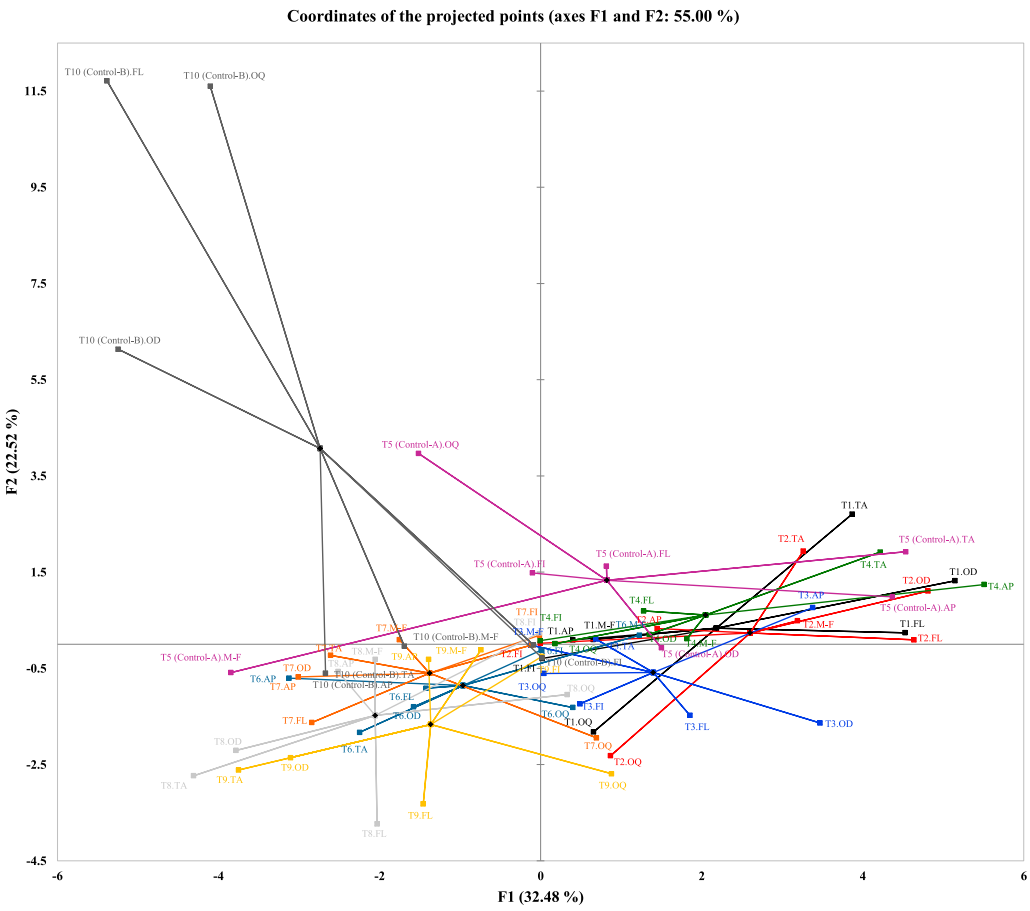

Fig. 8. Coordinates of the projected points of the groups of variables in the F1 and F2 axes of the multiple factor analysis (MFA). Abbreviations: AP, appearance; FI, finish; FL, flavour; M-F, mouth-feel; OD, odour; OQ, overall quality; TA, taste.

where it is possible to differentiate the wines by sensory characteristics (Liu et al., 2016; Puertas et al., 2018). A significant impact on the sensory characterisation of wines produced by the same yeast strain, and also by the nutrition type (SS, SC or classic nutrition) and the presence/absence of GIY. Differences in wine appearance were variable in the treatments with variations in yellow colour (6.7–7.39), and green reflexes (3.01–3.89). Treatments T2 and T9 showed the highest scores for 13 attributes (odour: intensity, floral, orange flowers, green apple, citrus fruit and grapefruit; taste: salty and bitter; mouth-feel: balance; flavour: citrus fruit; overall quality: taste; finish: after-smell and after-taste) and 18 attributes (odour: intensity, persistency, fruity, tropical fruit, caramel, honey and bread crust; taste: sweet; mouth-feel: body; flavour: persistency, tropical fruit, caramel and honey; overall quality: odour, mouth-feel and flavour; finish: after-smell and after-taste), respectively. These results confirmed the results of the wine experts reported for the sensory acceptance test. Consequently, the combination of the GR1 yeast strain with SS and GIY produced wines with a high overall quality (8.38). On the other hand, the combination of SPF52, SC and GIY obtained wines with overall quality values of 8.57. The use of different "Stimula" (specific nutrient), in combination with the yeast strain, were able to enhance particular aromas. Torrea and Henschke (2004) observed how the impact of three different concentrations of yeast assimilable nitrogen (YAN) on Chardonnay must can influence the composition of the descriptors of the final wine. Evidently, the different nutrition and addition of GIY in relation to the yeast strain resulted in different sensory expressions. Just as different yeast autolysates are able to influence the perception of wine aroma (Comuzzo et al., 2006).

It is interesting to note that in T1 and T2 wines, aromas of orange flowers were perceived (7.2 and 7.7, respectively) which were absent in wines produced using SC (T3 and T4) and classic nutrition (T5). On the other hand, the peach aroma attribute was detected in trials involving the addition of SC (T3 and T4) compared to those involving the use of SS (T1-T2) or classical nutrition (T5). The wines produced with SPF52 and nutrient SC (T8-T9) showed the presence of tropical fruit (8.12 and 7.98, respectively) and plum (6.82 and 5.58, respectively) aromas while no such aromas were detected for the wines produced with SS (T6-T7) and classic nutrition (T10). Wines T6 and T7, made with yeast SPF52, aromas of small fruits were perceived when the nutrition regime included SS, such aromas were not detected in wines produced with SPF52 and SC (T8-T9) and those with classic nutrition (T10). In some cases, the experimental wines produced aromas recognised exclusively in one treatment: green apple in T2, tamarind in T8, strawberry in T3, liquorice and aniseed in T1. Only in T10 wine, the attributes of box tree and cat pee were detected as odour and taste. The analysis of aromas revealed the presence of the caramel, honey and wax descriptors in the wines inoculated with SPF52 with variable scores in the T6-T10 wines, regardless of the nutritional scheme and the presence/absence of GIY. These attributes were not perceptible for wines produced with the GR1 strain. This tendency was also observed in the wine flavour (caramel and honey). The attributes describing the taste of the wines differed between the wines. High ratings were observed in T9 for sweet, T1 for sour, T2 for salty and bitter. Mouth-feel also showed statistically significant differences in almost all wines. The attribute body showed highest rating in T9 (8.42), whereas in T2 the highest rating of balance was found (8.32). For all wines, off-aromas and off-flavours were not detected. The addition of GIY in some cases increased flavour in terms of intensity and persistence, which was greater than in wines without antioxidants. In fact, treatment with GIY resulted in obtaining the improved wines (T2 and T9) with

more complexity of sensory profiles. Badea et al. (2017) demonstrated that doses of 40 mg/L of glutathione added in musts before AF helped to protect varietal aromas of wines and result in wines with sensory profiles highly appreciated by consumers.

3.6.1. Multiple factor analysis of sensory scores

Multiple factor analysis (MFA) was used to determine if there are any correlations between the winemaking variables in the sensory data. This led to the identification of four factors with eigen values higher than 1, indicating that the total number of variables (50) for the 10 wines could be grouped into only four factors which explained 86.46% of the total variance. The association between the variables and the MFA factor is indicated by the contribution and cos^2 value. Interestingly, specific aroma, taste and flavour descriptors were attributable to different factors. The aromas (orange flowers, citrus fruit, grapefruit, caramel, honey and wax), taste (sweet, sour, bitter) and flavours (intensity, persistency, floral, fruity, citrus fruit, caramel and honey) were associated with F1, whereas the aromas (fruity, plum, box tree and cat pee), flavours (box tree and cat pee) and overall quality (odour, taste, mouth-feel and flavour) were associated with F2, and the aromas (tropical fruit, pineapple and banana), taste (salty), mouth-feel (body and balance) and flavour (tropical fruit) were associated with F3. The aromas (intensity, floral, peach, apricot and strawberry) and finish (after-smell and aftertaste) a were associated to F4. As shown in Fig. 7a,b, the two-dimension model of MFA of variables explained 55% of the total variance, with F1 and F2 accounting for 32.48 and 22.52%, respectively. The variables loading plot of MFA (Fig. 7a) showed that 18 variables were located in the first quadrant, twelve in the second quadrant, twelve in the third quadrant and 8 in the fourth quadrant. Fig. 7b shows that the trials were grouped into three clusters. However, both MFA observation plot (Fig. 7b) and AHCA dendrogram (Fig. 7c) showed that the T5 (Control-A) grouped with wines T1, T2, T3, and T4. Interestingly, the wine T10 (Control-B) did not cluster with the group of wines made with yeast strain SPF2. Indeed, the wines T6, T7, T8 and T9 represented a different cluster. Within each cluster, wine pairs T1-T2, T3-T4, T6-T7 and T8-T9 showed low dissimilarity. This attribute-by-attribute comparison was also plotted from the MFA, showing the degree of similarity in sorting between the groups for each attribute. The groups of variables had different influences in each trial, as indicated in Fig. 8. The shorter the arm, the more similarly the groups sorted that attribute.

4. Conclusions

In this study, different experimental protocols were evaluated to obtain different aromatic expressions for a Catarratto cultivar classified as non-aromatic. The *S. cerevisiae* SPF52 strain, isolated from sugary matrices different from grape must, was proven to be suitable for wine production. The addition of nutrients Stimula Sauvignon Blanc™ or Stimula Chardonnay™ before the inoculation of starter yeasts allowed to increase the aromatic complexity of the final wines, as confirmed by VOCs and sensorial analysis. Finally, the addition of GSH-enriched inactivated yeast Glutastar™ was useful to prevent the chemical oxidation of musts and wines and to generate the highest aromatic intensity. The study focused on the aromatic evaluation of wines bottled after 5 months of stain-less steel tank aging. Further studies are needed to investigate the aromatic evolution of wines during the aging in bottle.

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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.

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