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Impact of two new non-conventional yeasts, *Candida oleophila* and *Starmerella lactis-condensi*, isolated from sugar-rich substrates, on Frappato wine aroma

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A R T I C L E I N F O

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A B S T R A C T

The interest of non-*Saccharomyces* yeasts in wine fermentation increased constantly in last years. This study reports for the first time the enological potential of two strains *Starmerella lactis-condensi* MN412 and *Candida oleophila* YS209. In an innovative way, these strains were used in winemaking to improve floral and fruity aroma of Frappato red wine, which has not been explored. The enological performances of the two non-*Saccharomyces* strains were compared to a wine strain of *Starmerella bacillaris*, namely Cz3, previously characterized in winemaking conditions. In these three cases, the non-*Saccharomyces* strain was sequentially inoculated with *S. cerevisiae* wine strain NF213, used as control. The *St. lactis-condensi* MN412 was isolated from Sicilian manna, a sugar-rich matrix, extracted from *Fraxinus angustifolia* trees (Oleaceae). The strain *C. oleophila* YS209 was isolated from honey by-products. Microbiological counts showed the ability of MN412 and YS209 to maintain high counts up to 6 days of alcoholic fermentation. Regarding chemical parameters, Cz3 showed the highest glycerol production. Analysis of VOCs revealed that the trials with non-*Saccharomyces* yeasts were characterized by a higher concentration of esters that contributed positively to the fruity aroma of the wines. The sensory analysis confirmed that the use of MN412 and YS209 impacted positively the final wines in terms of fruity and floral intensity, respectively, while did not generate sensory defects. In conclusion, non-conventional yeasts represent strategy to improve floral-fruity freshness of wine aroma and sugar-rich matrices such as manna ash and honey might represent novel ecological niches as source of potential oenological yeast.

1. Introduction

Non-*Saccharomyces* yeasts are the largest microbial group present on grapes (Borren & Tian, 2021). In general, they play an important role during the first days of fermentation, when the ethanol content is quite low (Benito, Calderón, & Benito, 2019). During alcoholic fermentation, the composition of non-*Saccharomyces* yeast populations changes in relation to ethanol concentration. As ethanol levels increase, sensitive species decrease at the expense of resistant species (Zhao et al., 2021), such as yeasts belonging to the genus *Saccharomyces* (Mateus, Sousa, Coimbra, S Rogerson, & Simões, 2020). In the last decade, oenological microbiological studies have highlighted the key role of

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non-*Saccharomyces* yeasts in the aromatic expression of wines (Benito, Ruiz, et al., 2019; Romani et al., 2020; Varela, 2016).

Among the different non-*Saccharomyces* species, *Candida* and *Starmerella* species have recently been successfully used in mixed fermentation with *S. cerevisiae* to reduce ethanol content (Englezos, Rantsiou, et al., 2016), increase glycerol concentration (Giaramida et al., 2013) and generating pleasant esters in wine (Englezos, Torchio, et al., 2016). Currently, most of the *Candida* and *Starmerella* species used in winemaking are derived from oenological sources, mainly grapes and must (Di Maio et al., 2012). Recent studies proved that matrices with a high sugar content (e.g. by-products of honey production) are rich in microorganism, in particular *Saccharomyces* and non-*Saccharomyces* yeasts (Gaglio et al., 2017; Sinacori et al., 2014). Consequently, some of these strains present in these matrices have shown good aptitude for use in fermentation processes (Francesca et al., 2022). Moreover, Prestianni et al. (2022) applied *Saccharomyces cerevisiae* and *Hanseniaspora uvarum* isolate from honey by-product to improve and stabilize the quality of mead. Alfonzo et al. (2021) also tested the suitability of *S. cerevisiae* strains from non-oenological sources in winemaking and evidenced consistent differences with *S. cerevisiae* of grape origin. A previous study conducted by Guarcello et al. (2019) analysed the cultivable microbial ecology of Sicilian manna ash, a sugar-rich matrix, and isolated several non-*Saccharomyces* yeasts, including *Starmerella lactis-condensi* strains. *Starmerella lactis-condensi* were isolated from different oenological sources such as *Vitis labrusca* grapes (Čadež et al., 2020), botrytized Tokaj Essence wines (Csoma, Kállai, Antunovics, Czentye, & Sipiczki, 2021). Battistelli et al. (2021) have found a high presence of *St. lactis-condensi* investigating the cultivable microbiota of "mothers" of *Vino cotto*. Recently, Csoma, Kállai, Czentye, and Sipiczki (2023) clarified the fructosophilic role of the dominant species *St. lactis-condensi* in Essences, a typical sweet wine from the Tokaj wine region in Hungary.

Franco, Benavides, Valencia, Ramírez, and Urtubia (2021) isolated *C. oleophila* in spontaneous fermentations of grape musts, tested its fermentative capabilities, and conducted sequential fermentation with *S. cerevisiae* in laboratory bioreactors. The same authors found high acetic acid production by *C. oleophila* but did not investigate the impact of this yeast on the composition of volatile organic compounds (VOC) or the sensory profile of wines. Other authors, Lachance, Boekhout, Scorzetti, Fell, and Kurtzman (2011) found the ability of *C. oleophila* to metabolize glucose at various levels, and Aplin, White, and Edwards (2019) described *C. oleophila* under laboratory winemaking conditions finding high acetic acid production. Therefore, to date, *C. oleophila* has been not used as starter or co-starter in real winery conditions.

Sicily is among the main Italian regions active in the production of red and rosé wines. In 2020, about 2 million hL/year of red wines were produced in Sicily (ISTAT, 2020). Among red grapes, Frappato is an autochthonous cultivar mainly cultivated within the provinces of Ragusa and Trapani with a total surface of about 750 ha for the production of Controlled and Guaranteed Denomination of Origin wine "Cerasuolo di Vittoria" (Asciuto & Bacarella, 2008). Frappato wines are characterised by a light ruby red color, brilliant, vinous, fruity and floral notes (Leder, 2020), but very little is known about the evolution of physicochemical, microbiological and aromas parameters of these wines. Frappato wines are commonly produced with commercial strains of *S. cerevisiae*, the species that ensures fermentation reproducibility and wine balancing.

However, many other yeast species with secondary importance during fermentation persist for the entire process. The positive effect of non-*Saccharomyces* yeasts in developing high taste-olfactory complexities has been highlighted (Fazio et al., 2023). This aspect well encounters the current consumer demand for novel wine styles (Comitini, Canonico, Agarbati, & Ciani, 2023).

To our knowledge, however, no previous work has evaluated the effect of *St. lactis-condensi* and *C. oleophila* strains in sequential inoculation with *S. cerevisiae* during wine fermentation and investigated for their capability to improve aroma. Both *St. lactis-condensi* and *C. oleophila* strains have been isolated from novel ecological niches, such as manna ash and honey by-products with high sugar content.

Based on the above considerations, the present study aimed to: (i) to evaluate two non-conventional yeast strains (*St. lactis-condensi* MN412 and *C. oleophila* YS209) isolated from "natural environments" (manna and honey) and previously technologically characterised for their potential in Frappato winemaking using procedures commonly used in wineries; (ii) to deepen our knowledge on VOCs composition of Frappato red wine.

2. Materials and methods

2.1. Strain preparation, experimental plan and sample collection

Non-*Saccharomyces* strains *St. lactis-condensi* MN412 isolated from manna (Guarcello et al., 2019), *C. oleophila* YS209 isolated from honey by-product, and *S. cerevisiae* NF213 isolated from grape must (Settanni, Sannino, Francesca, Guarcello, & Moschetti, 2012) belong to the oenological yeast collection of the Department of Agricultural, Food and Forestry Sciences (SAAF; University of Palermo, Italy). All strains were reactivated from −80 °C stock in Yeast Peptone Dextrose (YPD; Condalab, Torrejón de Ardoz, Madrid, Spain) at 28 °C for 48 h and were reproduced in a concentrated liquid suspension by Bionova srl (Villanova sull'Arda, Piacenza, Italy). *St. bacillaris* Cz3 is a strain of oenological origin (Di Maio et al., 2012) deposited in the yeast collection of the Sicilian Regional Institute of Wine and Oil (IRVO, Palermo, Italy) and marketed by Bioagro srl (Thiene, Vicenza, Italy). Grape of "Frappato" cultivar were donated by the winery "Caruso & Minini srl" located in Marsala (Italy).

In order to evaluate the impact of non-*Saccharomyces* yeasts on Frappato wine, the experimental plan of the present study (Fig. 1) consisted of four treatments: N1, sequential inoculum of *St. lactis-condensi* MN412/*S. cerevisiae* NF213; N2, sequential inoculum of *C. oleophila* YS209/*S. cerevisiae* NF213; N3, sequential inoculum of *St. bacillaris* Cz3/*S. cerevisiae* NF213; N4, single inoculum of *S. cerevisiae* NF213. In trials N1–N3, *S. cerevisiae* NF213 was inoculated 72 h after the addition of non-*Saccharomyces* strains.

All vinification were conducted at Department SAAF of University of Palermo, Italy and samples were collected at different stages of vinification: after grape pressing, after yeast inoculation, during alcoholic fermentation at day 1, 2, 3, 6, and at the end of fermentation (14 days). All analyses were performed in triplicate.

2.2. Winemaking

Grapes were stemmer-crushed and supplemented with 2 g/hL of potassium metabisulphite (Chimica Noto s.r.l., Partinico, Italy). Bulk grape must was used to fill three test tanks (250 L each) for a total of 12 vats. Before yeast inoculation, 20 g/hL of diammonium phosphate (Chimica Noto s.r.l., Partinico, Italy) and 20 g/hL of Fermaid E™ (Lallemand, Castel D'Azzano, Italy) were added to each vat. All strains in concentrated liquid suspension [approx. 7.00×10^{10} colony-forming units (CFU)/g] were inoculated (20 mL/hL) according to the experimental plan; the alcoholic fermentation was conducted at 22 °C. At the end of alcoholic fermentation, 5 g/hL of potassium metabisulphite was added. The wines were aged in steel tanks 18 °C for two months. The cap was punched down manually three times a day. At the end of fermentation, the pomace was pressed using a pneumatic press (Puleo spa, Marsala, Italy) with scalar operating pressures increasing to a maximum of 1000 mbar.

At bottling, free sulphur dioxide was adjusted to an approximate concentration of 30 mg/L, a value recommended during the ageing and storage of wine and sufficient to guarantee, at the pH values measured in the wines described in this work, an amount of molecular SO_2 greater than 0.35 mg/L, the minimum value necessary to ensure the protection of bottled wines (Stocley et al., 2021). Bottled wines were kept at 15 °C.

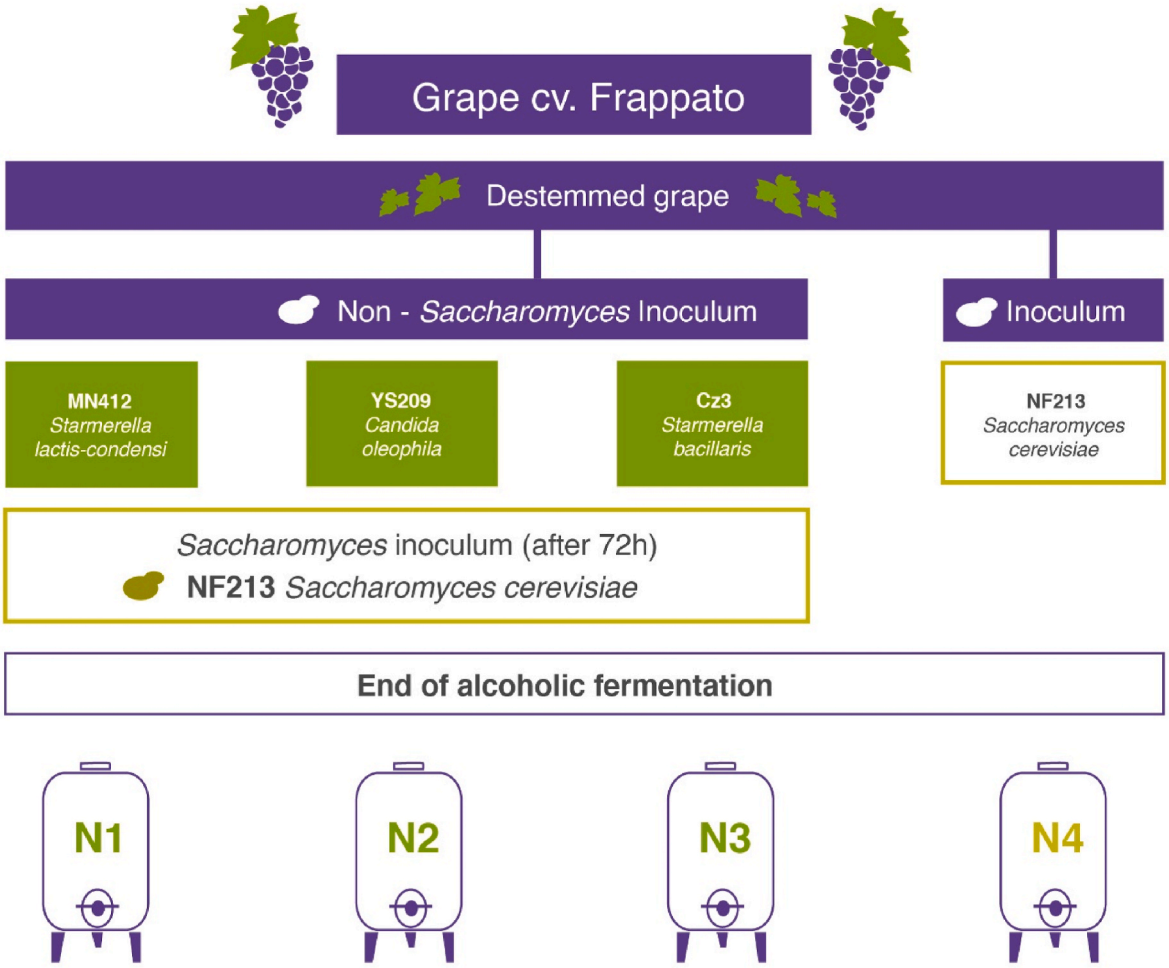

Fig. 1. Experimental design of Frappato wines vinified with different non-*Saccharomyces* yeast strain.

The winemaking process followed an oenological protocol used extensively by several wine companies. The process was performed at experimental wine cellar of University of Palermo based in Palermo city, Sicily (Italy).

2.3. Microbiological analysis

All samples collected during alcoholic fermentation were analysed for yeast colonies forming units, using various selective/differential culture media. Ten milliliters of each must sample were diluted in 90 mL of Ringer's solution (Sigma-Aldrich, Milan, Italy) and plated on Wallerstein Laboratory (WL; Condalab, Torrejón de Ardoz, Madrid, Spain) nutrient agar (incubated at 28 °C for 72 h) for *Saccharomyces* yeast quantification, and on lysine agar (Likson, Vicari, Palermo, Italy; incubated at 28 °C for 5 days) for non-*Saccharomyces* (Di Maio, Polizzotto, Planeta, & Oliva, 2011).

2.4. Yeast isolation, molecular identification and strain typing

The dominance of the three non-*Saccharomyces* strains selected for this study was verified after three days of alcoholic fermentation, while that of *S. cerevisiae* was investigated at the end of alcoholic fermentation. At least five colonies of each yeast group with different morphology were selected from the respective culture media using the morphological criteria described by Cavazza, Grando, and Zini (1992) and Pallmann et al. (2001). All isolates were purified by successive sub-cultures on YPD agar (Lai et al., 2022) and their purity was verified by light microscopy (Carl Zeiss LTd, Berkochen, Germany). Three isolates with the same morphology from a given sample were then subjected to genetic characterization.

Genomic DNA for PCR assays was extracted by InstaGene Matrix kit (Bio-Rad, Hercules, CA, USA) following the protocol provided by the manufacturer. Yeasts differentiation was performed by RFLP using the region spanning the internal transcribed spacers (ITS1 and ITS2) and the 5.8 S rRNA gene (Esteve-Zarzoso, Belloch, Uruburu, & Querol, 1999). One isolate per group was further analysed by sequencing the D1/D2 region of the 26 S rRNA gene to confirm the preliminary identification obtained by RFLP analysis as indicated by Alfonzo et al. (2020). DNA sequencing reactions were performed at AGRIVET (University of Palermo, Italy). 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).

The dominance of *S. cerevisiae* NF213 was confirmed by comparing the interdelta profile of the isolates from the highest cell dilution of musts with that of the pure strain. Interdelta analysis was conducted as described by Legras and Karst (2003). The persistence of non-*Saccharomyces* was carried out by comparing randomly amplified polymorphic DNA(RAPD)-PCR patterns of the isolates with those of the pure strains. RAPD-PCR was performed with primers M13 (Francesca et al., 2014) and XD5 (Di Maro, Ercolini, & Coppola, 2007). PCR products were visualised and compared as reported by Alfonzo et al. (2021).

2.5. Physicochemical analysis of musts and wines

The concentration of glucose, fructose, ethanol, glycerol, ammoniacal nitrogen, alpha-amino nitrogen, malic acid, lactic acid, and acetic acid were evaluated by means of the enzymatic analyser iCubio iMagic M9 (Shenzhen iCubio Biomedical Technology Co. Ltd., Shenzhen, China) as described by Matraxia et al. (2021). Samples were centrifuged (Remi Neya 16 R Giorgio Bormac s.r.l., Carpi, Modena, Italy; 5400×*g*, 10 min) and analysed following the manufacturer's protocol. All reagents were purchased from R-Biopharm AG (Darmstadt, Germany). The values of pH were 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 sulphur dioxide were measured in accordance with the methods described by OIV--MA-AS323-04 B (OIV, 2020c). All chemical analyses were carried out in triplicate.

2.6. Analysis of VOCs in wine samples

2.6.1. Liquid-liquid extraction

The volatile compound composition of the wine samples was determined by the method of Alfonzo et al. (2021) with appropriate modifications. Wine samples (10 mL) from all trials were mixed with MS SupraSolv® dichloromethane (5 mL) in a 50-mL conical flask (Merck, Milan, Italy), stirred at room temperature (20 ± 1 °C) for 30 min, and centrifuged at 3400×*g* for 10 min by low Speed Centrifuge (LaboGene ScanSpeed 416, Lillerød, Denmark) with Swing Rotor (LaboGene ApS, Vassingerød, Lynge, Denmark); the aqueous phase was removed, added with anhydrous sodium sulphate (Sigma-Aldrich, Milan, Italy; 1 g), and centrifuged at 3400×*g* for 5 min; dichloromethane layer was removed, and dried under N_2 gas to 0.2 mL.

2.6.2. Identification and quantification of VOCs by GC-MS

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

The temperature was initially maintained at 40 °C for 1 min, gradually increased to 250 °C at a rate of 3 °C/min for 30 min, and finally maintained at 250 °C at 10 °C/min. One microliter of each 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 (Adams, 2007; NIST, 2008). These identifications were also confirmed by other published mass spectra and linear retention indices (LRI). LRI were calculated using a series of n-alkanes (C8–C40). The quantifications of the individual metabolites were carried out using different standards, 1-Pentanol, 3-Ethoxy-1-propanol, Benzaldehyde, Hexanoic acid and Ethyl Hexanoate, used respectively to quantify, after an appropriate calibration line, the classes of alcohols, ethers, aldehydes, carboxylic acids and esters. Standard deviations were calculated by analysing samples in triplicate.

2.7. Sensory analysis

Sensory evaluation of experimental wines was performed by quantitative descriptive analysis. Fourteen judges (8 men and 6 women, ranging from 26 to 45 years old) were recruited from University of Palermo. All judges had experience in winemaking and participated in previous studies as members of panels judging wines. Besides, they were subjected to preliminary tests to determine their sensory performances on basic tastes and aromas of wines. Sensory analysis of wine was conducted as described by Jackson (2016). The 14 panellists compared the four experimental wines during different sessions. They consensually generated 16 sensory descriptive attributes regarding appearance (colour), odour (intensity, complexity, floral, fruity, spicy, balsamic, and overall odour quality), flavour, taste (intensity, persistence, sour, salty and smoothness, overall taste quality), and overall quality. The panellists also generated a consensual descriptive ballot (Biasoto, Netto, Marques, & da Silva, 2014; Jackson, 2016) and the descriptors were associated to a 9 cm unstructured scale (1 = extremely low, 5 = moderate intensity, 9 = extremely high). The four wine samples were evaluated in separate tasting sessions on consecutive days. In total, each judge rated each of the four wines in two sessions. Each replication was analysed separately and the results are expressed as the average of the three replicates.

2.8. Statistical analysis

ANOVA test was applied to identify significant differences among physicochemical parameters (pH, total acidity, acetic acid, residual sugars, glucose, fructose, alpha-amino nitrogen, ammoniacal nitrogen, ethanol, glycerol, malic acid, lactic acid, free and total SO_2), levels of *Saccharomyces* and non-*Saccharomyces,* VOCs concentration and sensory analysis. The post-hoc Tukey's method was applied for pairwise comparison of all data. Statistical significance was attributed to $P < 0.05$ (Mazzei, Francesca, Moschetti, & Piccolo, 2010).

Sensory Product Characterization Analysis (SPCA) was applied in order to determine the sensory differences of the wines produced by means of an analytical method based on the attributes describing each trial. For each session, the score was evaluated considering product, judge and session effect. A histogram chart of different colours was created for each wine. Blue is associated with coefficients that show a significant positive value and the red color with coefficients showing a significant negative value. Differences between trials were represented graphically with a sensory profile plot.

Statistical data processing and graphic construction were performed with the XLStat software version 2019.2.2 (Addinsoft, New York, USA) for Excel.

3. Results and discussion

3.1. Kinetics of yeast populations during fermentation

The growth of yeasts during the alcoholic fermentation is graphically shown in Fig. 2. The levels of non-*Saccharomyces* and *Saccharomyces* populations of Frappato must, at the beginning of monitoring, were 5.3 Log CFU/mL and <2.0 Log CFU/mL, respectively. Cell density of non-*Saccharomyces* increased at 6.0–7.0 Log CFU/mL just after inoculation; these densities are considered adequate to influence the sensory characteristics of wines (Du Plessis et al., 2017). After 48 h of non--*Saccharomyces* inoculation, the highest values were recorded for N3 (6.9 Log CFU/mL) and the lowest for N2 (6.60 Log CFU/mL). After 72 h, N3 reached values of less than 6 Log CFU/mL, in contrast to N1 and N3 which showed values of 6.6 and 6.4 Log CFU/mL respectively. In addition, the trials N1–N3 were inoculated with *S. cerevisiae* NF213 until 7.3 to 8.3 Log CFU/mL. After further 3 d, all trials showed a decrease of non-*Saccharomyces,* a trend already registered by Binati et al. (2020), who followed a sequential inoculum of *St. bacillaris* and *S. cerevisiae.* Specifically, after 6 d of alcoholic fermentation, only in N1 were the levels of non-*Saccharomyces* 6 Log CFU/mL, while a progressive decrease was observed in N2 (4.5 Log CFU/mL) and N3 (2.4 Log CFU/mL). The decrease of non-*Saccharomyces* populations in sequential inoculum with *S. cerevisiae* is determined by several events, mainly increased ethanol concentrations, secretion of inhibitory substances, and competition phenomena (Wang, Mas, & Esteve-Zarzoso, 2016). According to Binati et al. (2020), at the end of alcoholic fermentation (14 d), non--*Saccharomyces* populations were at levels lower than the detection limit.

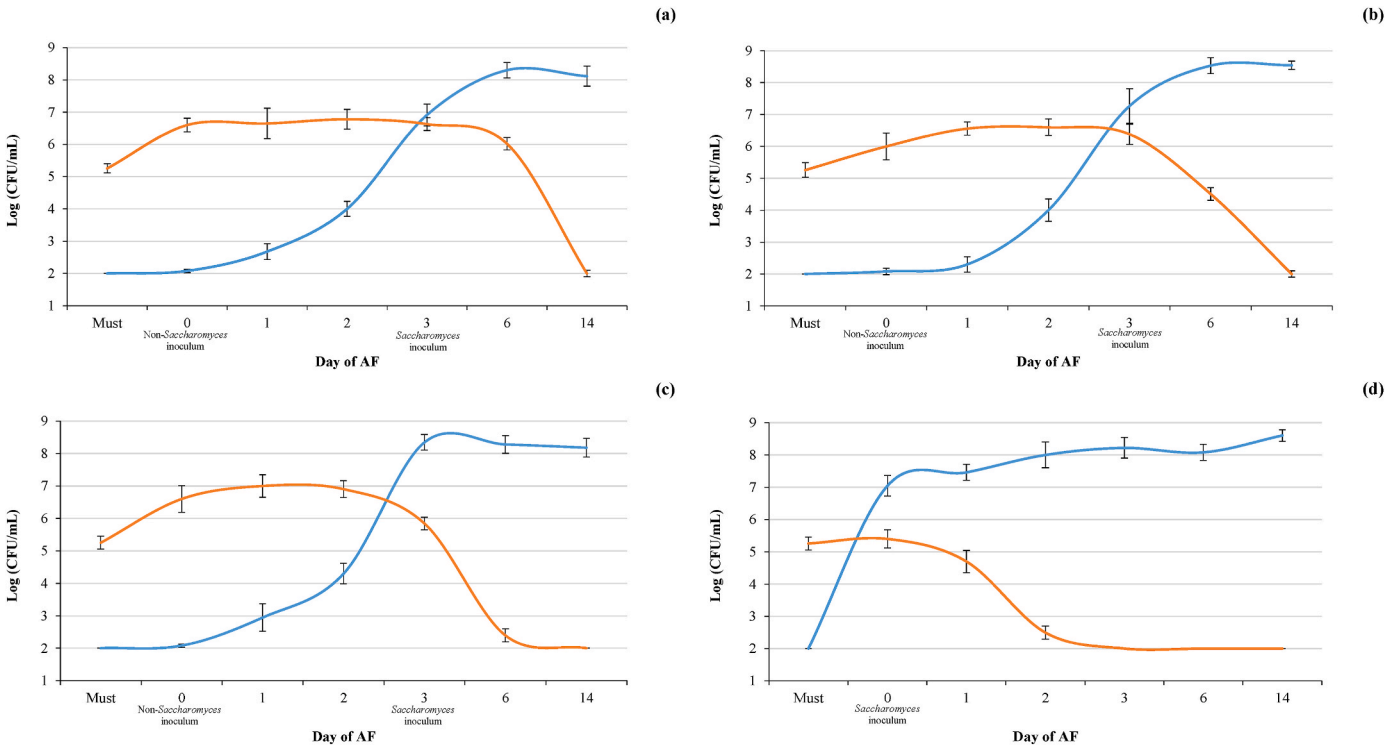

Fig. 2. Evolution of yeast populations of presumptive *Saccharomyces cerevisiae* and non-*Saccharomyces* populations during alcoholic fermentation: **(a)** sequential inoculum *Starmerella lactis-condensi* MN412/*Saccharomyces cerevisiae* NF213(N1); **(b)** sequential inoculum *Candida oleophila* YS209/*Saccharomyces cerevisiae* NF213 (N2); **(c)** sequential inoculum *Starmerella bacillaris* Cz3/*Saccharomyces cerevisiae* NF213(N3); **(d)** single inoculum *Saccharomyces cerevisiae* NF213 (N4; control). Legend: ■, presumptive *Saccharomyces*; ■, non-*Saccharomyces*.

On the contrary, *Saccharomyces* were in the range 7.9–8.6 Log CFU/mL for all trials.

3.2. Dominance of inoculated yeasts

A total of 1003 colonies that had grown on WL were isolated, sequentially re-propagated on WL and checked for their colony colour, colony topography and microscopic observations (Cavazza et al., 1992; Pallmann et al., 2001); 748 colonies were classified as *Saccharomyces*. The analysis of 5.8 S-ITS amplicons confirmed that all these isolates shared a 5.8 S-ITS region of 880 bp typical of *S. cerevisiae* and the profile of the restriction fragments obtained with *Cfo*I, *Hae*III and *Hinf*I confirmed that these isolates were *S. cerevisiae*. (Guillamón, Sabaté, Barrio, Cano, & Querol, 1998).

The other unclassified 255 isolates were assigned to the non-*Saccharomyces* yeast group.

Eighty-nine isolates were characterized by an ITS amplicon of 480 bp and were presumptively identified and *St. lactis-condensi*. In fact, the same ITS amplicon sizes were found by Solieri, Landi, De Vero, and Giudici (2006), who worked on *St. lactis-condensi*. Eighty-two isolates showed ITS amplicons of 630 and were considered presumptive *C. oleophila* (n = 82) while 67 were allocated to the species *St. bacillaris* (n = 67) based on the 430 bp amplicon (Gordún Quiles, Puig Pujol, Piñol, & Carbó Moliner, 2018; Wang, Wu, & Qiu, 2019). The remaining isolates (n = 17) showed an ITS amplicon between 750 (n = 11) and 760 (n = 6) bp with a colony morphology on WL agar similar to that of yeasts of the genus *Hanseniaspora*, which are very common in sicilian Frappato musts (Romancino, Di Maio, Muriella, & Oliva, 2008). RFLP profiles of non-*Saccharomyces* species confirmed what observed by other authors who identified yeasts (de Llanos Frutos, Fernández-Espinar, & Querol, 2004; Esteve-Zarzoso et al., 1999; Solieri et al., 2006; Wang et al., 2019).

Interdelta analysis confirmed the presence of three different strains of *S. cerevisiae*. The different interdelta profiles also indicated the presence of indigenous grape *S. cerevisiae* (Aponte, Romano, Villano, & Blaiotta, 2020). The direct comparison of the interdelta profiles showed that *S. cerevisiae* NF213 was the strain most frequently isolated (>96%). RAPD pattern (Fig. 3) comparison indicated that each non-*Saccharomyces* inoculated strains showed a dominance percentage higher than 90%. Yeast genotypic identification was completed by pairwise alignment of D1/D2 sequence of the 30% isolates with those of type strains (*C. oleophila* CBS2219^T, *S. cerevisiae* CBS 1171^T, *St. lactis-condensi* CBS 52^T and *St. bacillaris* CBS9494^T); D1/D2 sequence from the strains Cz3, MN412, NF213 and YS209, and used in this study showed 100% homology with type strains.

3.3. Chemical monitoring

The results of the chemical analyses are summarized in Table 1. The initial sugar content of Frappato grape must of this study was 231.83 g/L (114.18 g/L glucose and 117.65 g/L fructose), total acidity (TA) of 8.11 g/L tartaric acid, 2.13 g/L malic acid and pH 3.15.

After 72 h, the majority of chemical parameters showed significant differences among trials, while pH and concentrations of malic acid, lactic acid, free and total SO_2 were quite comparable. The trials inoculated with non-*Saccharomyces* strains (N1 and N2) showed the highest values of residual sugars (174.90 and 181.73 g/L, respectively) after 72 h. The trials N1 – N3 showed a higher consumption of fructose rather than glucose, compared to the trial N4. Fructose preference is a common characteristic of certain *Candida* strains (Englezos et al., 2019; Magyar & Tóth, 2011). The highest concentrations of ethanol and glycerol were registered for trial N4 [7.13% (v/v) and 6.68 g/L, respectively]. Among the sequential inoculation trials carried out, N2 was the trial containing the highest ethanol concentration [2.99% (v/v)] and the trial inoculated with *St. bacillaris* (N3) produced the lowest ethanol content, while trial N3 had the highest glycerol concentration (2.76 g/L). No decrease in the production of ethanol was found in the trial inoculated with *St. bacillaris*

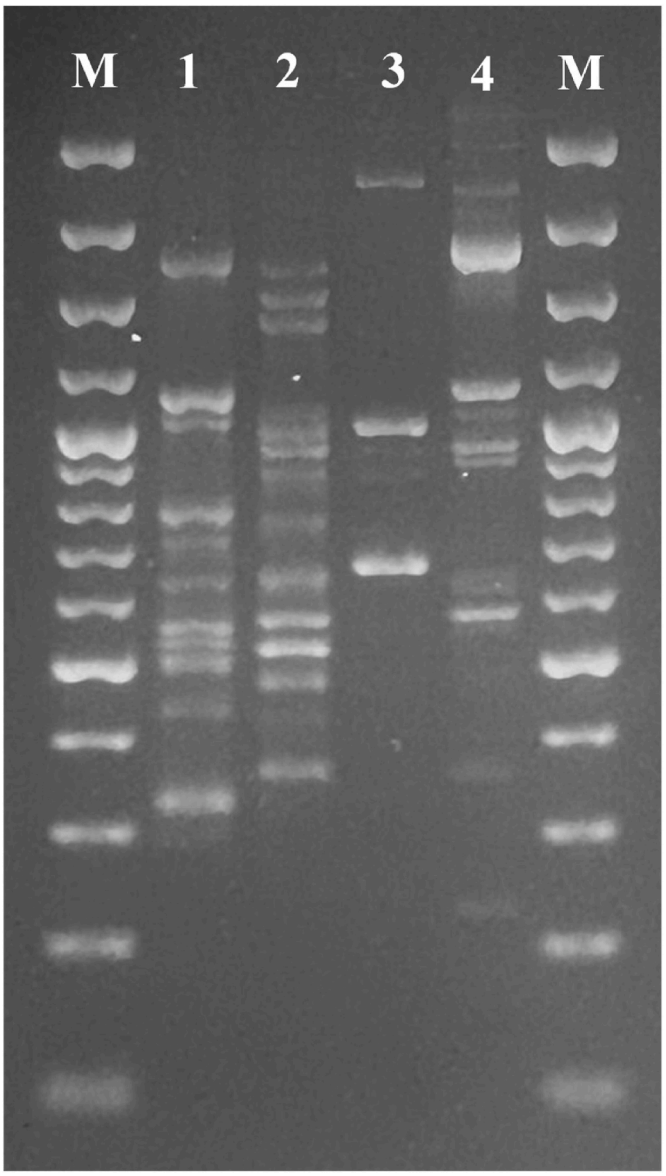

Fig. 3. RAPD profile generated with primer M13 (lanes 1–2) and primer XD5 (lanes 3–4); Abbreviations: M, 100 bp plus DNA marker; 1–3, *Starmerella lactis-condensi* MN412; 2–4, *Candida oleophila* YS209.

(N3), as also determined by Giaramida et al. (2013).

All the fermentations were completed in two weeks and the wines obtained were characterised by a residual sugar content of less than 1 g/L. *St. lactis-condensi* (N1) and *C. oleophila* (N2) did not cause any change in the oenological parameters in terms of acetic acid content. Also, no statistically significant differences were observed between trials in terms of glucose concentration, acetic acid, lactic acid and pH values.

Trial N2 inoculated with *C. oleophila*, contrary to what reported by Franco et al. (2021), produced little acetic acid (0.28 g/L). Aplin et al. (2019) tried to select *C. oleophila* as a co-starter, but the strain produced acetic acid higher than 0.8 g/L, for this reason it was discarded and never applied in vinification. To our knowledge, the present work is the first report on application of *C. oleophila* in wine fermentation under real winemaking condition, since the previous authors used strain of *C. oleophila* only in bioreactor and/or *in vitro* investigation (Aplin et al., 2019).

Significant differences were found for TTA values, which were lower for the trials inoculated with non-*Saccharomyces* (6.30–6.35 g/L tartaric acid). At the end of fermentation, ethanol concentrations ranged between 11.65% and 11.99% (v/v). The highest values in ethanol were observed in the control trial N4 and the use of non-*Saccharomyces* in sequential inoculation with *S. cerevisiae* can promote the reduction of ethanol content of wines. In this study, differences in ethanol content ranged from 0.21% to 0.34% (v/v). Benavides, Franco, Ceppi De Lecco, Durán, and Urtubia (2022) who tested different sequential inoculum combinations to lower ethanol content in wines observed similar results.

Malic acid concentration decreased slightly from the beginning of monitoring (2.13 g/L) to the end of fermentation for all trials (1.87–1.99 g/L). The negligible decrease of malic acid concentration observed during the alcoholic fermentation could be due to *Saccharomyces* spp. strains, which can degrade malic acid initially, present in must from 3% to 45% (Saayman & Viljoen-Bloom, 2006). Lactic acid was present in trace amount for all trials. Regarding glycerol, a much higher content than in the other trials was found in trial N3 inoculated with *St. bacillaris* (10.31 g/L), a similar increase was found by Giaramida et al. (2013). This compound influences wine sensory properties, especially in red wines where it positively contributes to smoothness, sweetness, and complexity (Comitini et al., 2011). The increase in glycerol content of wines produced with *C. oleophila* and *St. bacillaris* is a common phenomenon (Englezos et al., 2018; Franco et al., 2021). At bottling, chemical parameters changed insignificantly. For the first time, *C. oleophila* has been used in a grape must to obtain bottled wine according to commercial protocols. To our knowledge, *St. lactis-condensi* strains have only recently been selected for oenological applications and have shown greater efficiency in fructose utilisation and tolerance to sugar, alcohol and sulphur content compared to *St. bacillaris* (Csoma et al., 2023).

3.4. Volatile organic compounds of wines

The VOCs of wines are listed in Table 2. Quantitative differences were found among trials. The 29 identified compounds were grouped into alcohols, ethers, aldehydes, carboxylic acids, esters and other compounds.

Alcohols are dominant wine VOCs resulting from yeast fermentation (Kotseridis & Baumes, 2000). The compounds mainly detected in this study were phenylethyl alcohol and 1-pentanol with values varying from 2.08 mg/L (N2) to 40.63 mg/L (N4) and from 47.94 mg/L (N4) to 67.16 mg/L (N2), respectively. Phenylethyl alcohol is responsible for the floral notes (Cordente et al., 2021). Trial N3 inoculated with *St. bacillaris* differed from the others by high concentrations of 1-hexanol, 3-hexenol and 2-butanol, the first two correlating with herbaceous notes, while 2-butanol correlated with fruity notes (Escudero et al., 2004; Furdíková, Ševcech, Ďurčanská, Hronská, & Malík, 2014; Juan, Cacho, Ferreira, & Escudero, 2012; Komes, Ulrich, & Lovric, 2006; Malík, 2014). Among ethers, 3-Ethoxy-1-propanol, a compound that gives a fruity aroma (Velázquez, Zamora, Álvarez, Hernández, & Ramírez, 2015), was the only compound detected and the concentrations varied from 0.30 mg/L (N2) to 1.15 mg/L (N3).

Due to their rancid and cheesy smells (Călugăr et al., 2020), carboxylic acids are undesirable in wines and the experimental wines obtained in this study were characterized by very low concentrations (<0.06 mg/L).

Esters compounds are released during fermentation and directly influence the aromatic complexity of wines (Tempère et al., 2018). Within this class, ethyl acetate was significantly higher in wines processed with the sequential inoculum (N1–N2–N3) than single culture of *S. cerevisiae* (N4). In addition, ethyl hexanoate was found to be present at higher levels in the trial inoculated with *C. oleophila* (N2), while diethyl succinate was found to be present at higher concentrations in both the trial inoculated with *St. lactis-condensi* (N1) and the control (N4). These compounds are important because they are associated with the presence of fruity odours (Louw et al., 2010).

Ethyl acetate, which is also associated with the development of fruity flavours (Renault, Coulon, de Revel, Barbe, & Bely, 2015). Englezos

Table 1
Physicochemical parameters determined during the winemaking process.

Parameters	Musts	Vinification									
		3 d of alcoholic fermentation					End of alcoholic fermentation				
		N1	N2	N3	N4	S. S.	N1	N2	N3	N4	S. S.
Ammoniacal nitrogen[α]	249.17 ± 0.12	210.98 ± 0.14[c]	218.45 ± 0.09[a]	215.75 ± 0.13[b]	89.22 ± 0.18[d]	***	48.59 ± 0.14[d]	88.13 ± 0.09[a]	73.84 ± 0.17[b]	51.26 ± 0.18[c]	***
Alpha-amino nitrogen[α]	192.94 ± 0.15	192.48 ± 0.12[b]	192.51 ± 0.11[b]	207.92 ± 0.19[a]	59.61 ± 0.04[c]	***	97.86 ± 0.12[d]	105.68 ± 0.11[b]	102.58 ± 0.11[c]	116.47 ± 0.04[a]	***
Residual sugars[β]	231.83 ± 0.26	174.90 ± 0.12[ab]	181.73 ± 0.20[b]	172.31 ± 0.15[b]	90.01 ± 0.26[c]	***	0.14 ± 0.02[a]	0.07 ± 0.01[b]	0.07 ± 0.03[b]	0.12 ± 0.02[ab]	*
Glucose[β]	114.18 ± 0.10	112.49 ± 0.08[a]	109.55 ± 0.06[b]	110.86 ± 0.10[b]	29.32 ± 0.05[c]	***	0.03 ± 0.01[a]	0.02 ± 0.00 [a]	0.07 ± 0.03[a]	0.03 ± 0.01[a]	n.s.
Fructose[β]	117.65 ± 0.15	62.41 ± 0.21[a]	72.18 ± 0.12[a]	61.45 ± 0.08[b]	60.69 ± 0.14[c]	***	0.11 ± 0.02[a]	0.05 ± 0.02[ab]	0.00 ± 0.00[b]	0.09 ± 0.04[a]	**
Acetic acid[β]	0.02 ± 0.02	0.06 ± 0.02[b]	0.04 ± 0.03[b]	0.21 ± 0.04[a]	0.09 ± 0.02[b]	**	0.31 ± 0.02[a]	0.28 ± 0.04[a]	0.31 ± 0.06[a]	0.26 ± 0.01[a]	n.s.
Malic acid[β]	2.13 ± 0.03	2.08 ± 0.02[a]	2.02 ± 0.03[a]	2.11 ± 0.06[a]	2.10 ± 0.02[a]	n.s.	1.90 ± 0.02[b]	1.87 ± 0.03[b]	1.99 ± 0.04[a]	1.91 ± 0.02[b]	*
Lactic acid[β]	0.04 ± 0.01	0.02 ± 0.01[a]	0.04 ± 0.01[a]	0.05 ± 0.02[a]	0.03 ± 0.01[a]	n.s.	0.06 ± 0.02[a]	0.07 ± 0.01[a]	0.07 ± 0.02[a]	0.06 ± 0.02[a]	n.s.
Glycerol[β]	0.35 ± 0.01	0.75 ± 0.14[c]	0.70 ± 0.17[c]	2.76 ± 0.05[b]	6.68 ± 0.11[a]	***	7.90 ± 0.16[b]	8.26 ± 0.10[b]	10.31 ± 0.17[a]	8.29 ± 0.14[b]	***
Ethanol[γ]	0.01 ± 0.01	2.87 ± 0.02[c]	2.99 ± 0.01[b]	2.22 ± 0.06[d]	7.13 ± 0.01[a]	***	11.70 ± 0.06[bc]	11.78 ± 0.03[b]	11.65 ± 0.02[c]	11.99 ± 0.03[a]	***
pH	3.15 ± 0.01	3.16 ± 0.02[a]	3.15 ± 0.01[a]	3.17 ± 0.00[a]	3.15 ± 0.01[a]	n.s.	3.13 ± 0.02[a]	3.14 ± 0.01[a]	3.14 ± 0.00[a]	3.16 ± 0.01[a]	n.s.
Total acidity[δ]	8.11 ± 0.09	6.82 ± 0.12[b]	6.84 ± 0.10[b]	6.88 ± 0.10[b]	7.17 ± 0.10[a]	*	6.35 ± 0.10[b]	6.30 ± 0.10[b]	6.35 ± 0.10[b]	6.70 ± 0.10[a]	**
Free-$SO_2^α$	8.00 ± 0.00	8.00 ± 0.50[a]	8.50 ± 0.50[a]	8.00 ± 0.00[a]	8.75 ± 0.50[a]	n.s.	17.00 ± 0.50[a]	16.50 ± 0.50[a]	17.50 ± 1.00[a]	18.00 ± 0.50[a]	n.s.
Total-$SO_2^α$	9.50 ± 0.50	10.00 ± 1.50[a]	10.50 ± 1.00[a]	10.00 ± 0.50[a]	12.00 ± 1.00[a]	n.s.	32.00 ± 1.50[a]	28.00 ± 1.00[b]	30.00 ± 1.00[ab]	32.00 ± 1.00[a]	*

Parameters	Vinification Bottling				
	N1	N2	N3	N4	S.S.
Ammoniacal nitrogen[α]	n.d.	n.d.	n.d.	n.d.	n.d.
Alpha-amino nitrogen[α]	n.d.	n.d.	n.d.	n.d.	n.d.
Residual sugars[β]	0.04 ± 0.03[a]	0.02 ± 0.01[a]	0.03 ± 0.01[a]	0.05 ± 0.02[a]	n.s.
Glucose[β]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.03 ± 0.01[a]	0.02 ± 0.01[ab]	**
Fructose[β]	0.04 ± 0.03[a]	0.02 ± 0.01[a]	0.00 ± 0.00[a]	0.03 ± 0.01[a]	n.s.
Acetic acid[β]	0.33 ± 0.04[a]	0.29 ± 0.04[a]	0.35 ± 0.03[a]	0.28 ± 0.01[a]	n.s.
Malic acid[β]	1.84 ± 01[c]	1.83 ± 0.01[c]	1.91 ± 0.01[a]	1.88 ± 0.01[b]	***
Lactic acid[β]	0.06 ± 0.02[a]	0.08 ± 0.01[a]	0.07 ± 0.02[a]	0.07 ± 0.03[a]	n.s.
Glycerol[β]	7.67 ± 0.07[c]	8.02 ± 0.11[b]	10.13 ± 0.08[a]	8.00 ± 0.07[b]	***
Ethanol[γ]	11.75 ± 0.05[b]	11.74 ± 0.03[b]	11.68 ± 0.03[b]	11.94 ± 0.02[a]	***
pH	3.18 ± 0.02[a]	3.16 ± 0.01[a]	3.19 ± 0.00[a]	3.20 ± 0.01[a]	n.s.
Total acidity[δ]	6.20 ± 0.10[a]	6.20 ± 0.10[a]	6.30 ± 0.10[a]	6.40 ± 0.10[a]	n.s.
Free-$SO_2^α$	31.00 ± 1.50[a]	30.50 ± 1.00[a]	29.50 ± 1.50[a]	30.50 ± 1.00[a]	n.s.
Total-$SO_2^α$	55.00 ± 1.00[a]	49.00 ± 1.50[a]	50.00 ± 0.50[a]	51.00 ± 1.00[a]	n.s.

Result indicates mean value ± standard deviation of three determinations from three replicates. Data within a line followed by the same letter are not significantly different according to Tukey's test.
Symbols: [α], mg/L; [β], expressed in g/L; [γ], % v/v; [δ], tartaric acid g/L.
Abbreviations: S.S., statistical significance; P value: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s., not significant; n.d., not determined.
Frappato must fermented by: N1, sequential inoculum *Starmerella lactis-condensi* MN412/*Saccharomyces cerevisiae* NF213; N2, sequential inoculum *Candida oleophila* YS209/*Saccharomyces cerevisiae* NF213; N3, sequential inoculum *Starmerella bacillaris* Cz3/*Saccharomyces cerevisiae* NF213; N4, single inoculum *Saccharomyces cerevisiae* NF213.

Table 2
Volatile organic compounds detected in the four Frappato wines (all values in mg/L).

LRI	Compounds[a] (Common name)	Aroma description[b]	N1[c]	N2[c]	N3[c]	N4[c]	S.S.[d]
\	**Σ Alcohols**						
625	2-Methyl-2-butanol	Plastic, solvent, fly spray	0.71 ± 0.02^{b}	1.07 ± 0.03^{a}	0.76 ± 0.01^{b}	0.61 ± 0.02^{c}	***
664	2-Butanol	Alcoholic	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	8.28 ± 0.07^{a}	0.00 ± 0.00^{b}	***
760	1-Pentanol	Fusel	50.84 ± 1.14^{bc}	67.16 ± 1.23^{a}	51.71 ± 1.35^{b}	47.94 ± 1.21^{c}	***
796	2.3-Butanediol[f]	Buttery, creamy	0.17 ± 0.01^{a}	tr	tr	tr	***
796	2.3-Butanediol[f]	Buttery, creamy	tr	0.00 ± 0.00	tr	0.03 ± 0.01	n.s.[e]
829	3-Methyl-1-pentanol	Fusel	0.10 ± 0.01^{a}	tr	0.00 ± 0.00^{b}	0.11 ± 0.01^{a}	***
857	3-Hexenol	Grass, moss	0.06 ± 0.01^{b}	0.00 ± 0.00^{c}	0.15 ± 0.02^{a}	0.05 ± 0.01^{b}	***
872	1-Hexanol	Green	2.33 ± 0.04^{b}	2.25 ± 0.03^{c}	3.93 ± 0.02^{a}	1.30 ± 0.02^{d}	***
1039	Benzyl alcohol	Sweet, flower	0.00 ± 0.00^{b}	0.17 ± 0.01^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	***
1108	Phenylethyl alcohol	Floral, rose	28.33 ± 1.34^{b}	2.08 ± 0.02^{d}	14.18 ± 0.54^{c}	40.63 ± 1.76^{a}	***
1442	*p*-Thyrosol	Sweet, floral, fruity	0.28 ± 0.02^{b}	0.44 ± 0.03^{a}	0.06 ± 0.01^{c}	tr	***
	Σ Ethers						
816	3-Ethoxy-1-propanol	Fruit	0.86 ± 0.02^{b}	0.30 ± 0.01^{d}	1.15 ± 0.04^{a}	0.55 ± 0.03^{c}	***
	Σ Aldehydes						
960	Benzaldehyde	Bitter almond, nutty, smoky	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.06 ± 0.01^{a}	0.00 ± 0.00^{b}	***
	Σ Carboxylic acids						
875	3-Methyl-butanoic acid	Cheese, rancid	0.00 ± 0.00^{b}	0.03 ± 0.01^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	*
976	Hexanoic acid	Mild, fatty	0.00 ± 0.00^{b}	0.03 ± 0.01^{a}	tr	tr	*
–	4-Ethoxy-4-oxobutanoic acid	Unknown	0.06 ± 0.01^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	***
	Σ Esters						
613	Ethyl acetate	Ethereal, fruity	10.57 ± 0.23^{b}	13.91 ± 0.21^{a}	13.95 ± 0.18^{a}	4.56 ± 0.10^{c}	***
713	Propyl acetate	Pear	0.03 ± 0.01^{a}	0.04 ± 0.01^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	***
800	Ethyl butanoate	Apple	0.19 ± 0.01^{b}	0.25 ± 0.02^{a}	0.13 ± 0.01^{c}	0.14 ± 0.01^{c}	***
876	Isopentyl acetate	Banana, fruity tropical	0.30 ± 0.02^{a}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.20 ± 0.01^{b}	***
879	2-Methylbutyl acetate	Fruity	0.04 ± 0.01^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	***
937	Ethyl 3-hydroxybutyrate	Fruity, grape, green	0.19 ± 0.02^{a}	0.02 ± 0.01^{c}	0.13 ± 0.01^{b}	0.16 ± 0.02^{ab}	***
999	Ethyl Hexanoate	Sweet fruity, pineapple, green apple	0.77 ± 0.04^{b}	0.74 ± 0.03^{bc}	1.07 ± 0.05^{a}	0.66 ± 0.04^{c}	***
1153	Diethyl butanedioate (Diethyl succinate)	Fruit	0.82 ± 0.06^{a}	0.47 ± 0.05^{c}	0.68 ± 0.03^{b}	0.82 ± 0.01^{a}	***
1188	Ethyl octanoate (Ethyl caprylate)	Fruity, pear	0.90 ± 0.04^{b}	1.15 ± 0.06^{a}	0.69 ± 0.02^{c}	0.57 ± 0.03^{d}	***
1296	Ethyl nonanoate	Fruity, fatty	0.18 ± 0.02^{b}	0.24 ± 0.01^{a}	tr	0.05 ± 0.01^{c}	***
	Σ Others						
1245	1.3-Di-*tert*-butylbenzene	Unknown	0.90 ± 0.02^{c}	3.80 ± 0.04^{a}	1.57 ± 0.03^{b}	0.91 ± 0.04^{c}	***
–	Tryptophan	Unknown	0.06 ± 0.01^{c}	1.05 ± 0.07^{a}	0.49 ± 0.02^{b}	0.00 ± 0.00^{c}	***

Abbreviations: tr: trace amount <0.01 mg/L.
Data within a line followed by the same letter are not significantly different according to Tukey's test.
P value: *, P < 0.05; ***, P < 0.001.
Frappato must fermented by: N1, sequential inoculum *Starmerella lactis-condensi* MN412/*Saccharomyces cerevisiae* NF213; N2, sequential inoculum *Candida oleophila* YS209/*Saccharomyces cerevisiae* NF213; N3, sequential inoculum *Starmerella bacillaris* Cz3/*Saccharomyces cerevisiae* NF213; N4, single inoculum *Saccharomyces cerevisiae* NF213.
[a] Compounds are classified in order of retention time.
[b] Aroma descriptions are reported in the online database of Good Scents Company Information (http://www.thegoodscentscompany.com/), Flavornet (http://www.flavornet.org/) and LRI & Odour Database (http://www.odour.org.uk/).
[c] Relative amounts, expressed as mg/L.
[d] Statistical significances; [e] not significative; [f] stereoisomers not identified.

et al. (2019) found a similar increase in ethyl acetate content during mixed fermentation of *St. bacillaris*/S. *cerevisiae*.

Among the treatments, N1 and N2 were distinguished from the others by higher levels of ethyl octanoate, 0.90 mg/L and 1.15 mg/L respectively.

For the first time the impact on VOCs by *C. oleophila* (N1) was studied. Previously, Franco et al. (2021) used this species as a co-starter *in vitro* winemaking experiment, analysing only the basic chemical-physical parameters. This is the first paper to report a study of VOCs associated with *C. oleophila* for oenological use as a co-starter. Its previous use has been in agriculture as a biocontrol agent (Raspor, Miklič-Milek, Avbelj, & Čadež, 2010). The growing interest in non-*Saccharomyces* strains with must bio-protective action (Naselli et al., 2023), offers interesting insights into the selection of new co-starter strains. Further investigations will be necessary to verify the possible bio-protective action of the strain YS209 *C. oleophila*. Matraxia et al. (2021) applying non-*Saccharomyces* strains isolated and selected from honey by-products, also found an increase in the ester content of beers. To our knowledge, strains of *St. lactis-condensi* have only recently been selected for oenological applications (Csoma et al., 2023).

3.5. Sensory evaluation

Among the 15 attributes that defined the sensory profile of each wine, SPCA indicated that the highest discriminating power was represented by odour overall quality, flavour overall quality and overall quality, while the lowest discriminating power was shown by colour. The definition of the sensory characteristics of each wine, expressed in model coefficients for each product-descriptor combination is shown in Fig. 4. Trials N1 and N2 showed a number of attributes with significant positive effect of 7 and 9, respectively. Treatment N3, showed 6 attributes with significant negative effect. The coefficients defining the complexity, fruity and spicy odours of wine from trial N1 (Fig. 4a) produced with sequential inoculation of *St. lactis-condensi* showed the highest coefficients when compared to all other trials. The highest colour, intensity and floral coefficients were obtained from trial N2 (Fig. 4b), produced by sequential inoculation with *Candida oleophila*. The high floreal values in N2 are possibly due to the presence of *p*-Thyrosol. However, the olfactory threshold of this compound is not known (Valera, Olivera, Boido, Dellacassa, & Carrau, 2021).

The wine from trial N3 was characterized by a strong smoothness on the palate (Fig. 4c). The high smoothness values could be related to the amount of glycerol of wine (Ciani & Ferraro, 1998). The activity of *St.*

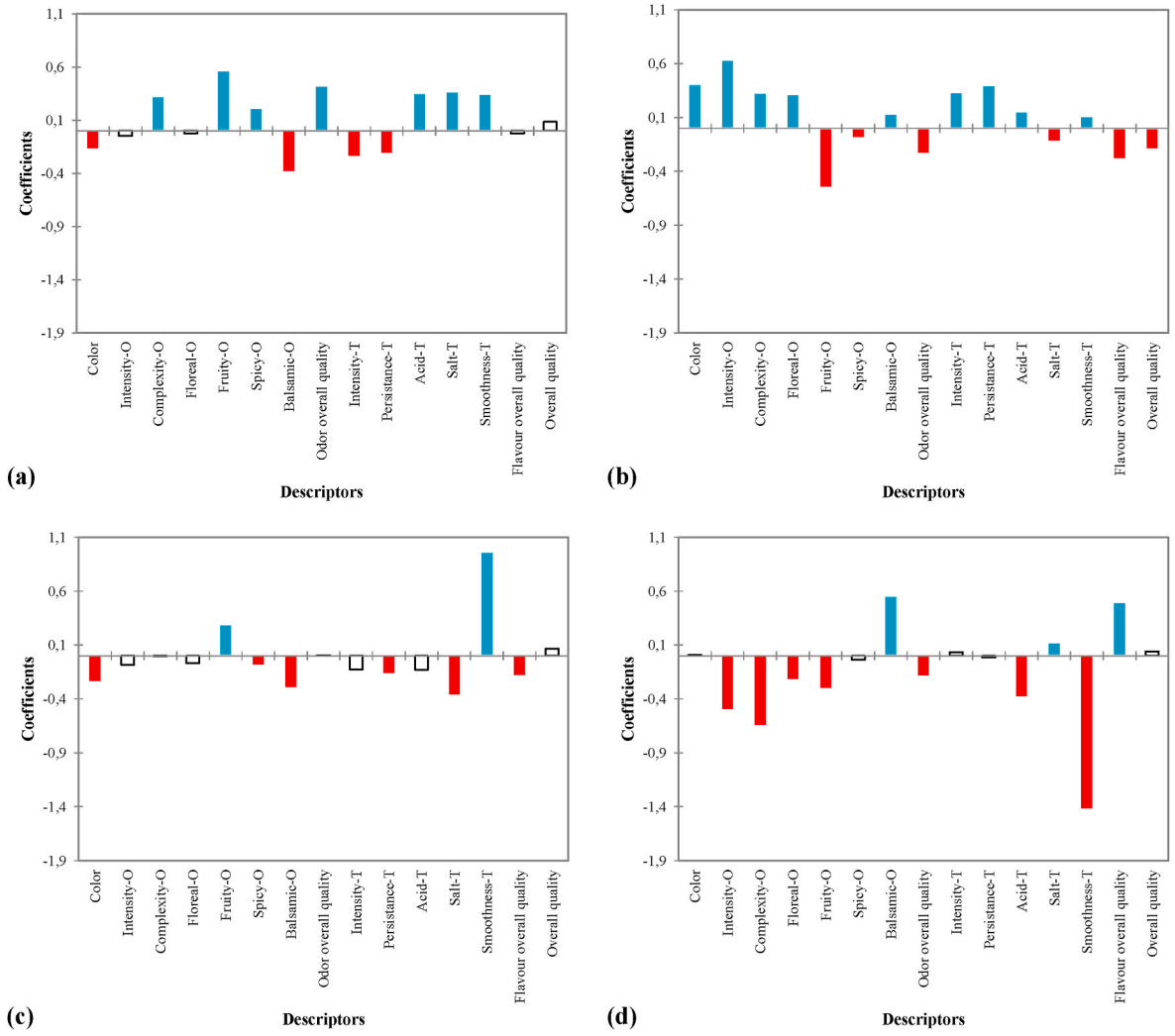

Fig. 4. Sensory profiles of Frappato wines obtained with sequential inoculation of: (a) N1, sequential inoculum *Starmerella lactis-condensi* MN412/*Saccharomyces cerevisiae* NF213; (b) N2, sequential inoculum *Candida oleophila* YS209/*Saccharomyces cerevisiae* NF213; (c) sequential inoculum *Starmerella bacillaris* Cz3/*Saccharomyces cerevisiae* NF213; (d) N4, single inoculum *Saccharomyces cerevisiae* NF213. The blue colour is associated to coefficients that have a significant positive value and the red colour is associated to coefficients that have a significant negative value. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

bacillaris before addition of *S. cerevisiae* was sufficient to increase the glycerol until values defining for the smoothness of this wine, a similar behaviour was found by Giaramida et al. (2013). The final wine of the control trial (N4) was characterised by balsamic odours, low intensity and complexity of odour, the taste was neither acidic nor smooth and it was also characterised by a higher flavour overall quality. (Fig. 4d). The higher flavour overall quality scores in N4 wine could be due to the presence of phenylethyl alcohol and diethyl butanedioate, which are responsible for fruity and floral notes (Scutaraș;u et al., 2022; Zhang, Luan, Duan, & Yan, 2018). However, none of the wines analysed showed off-odour.

In order to better evaluate the differences among Frappato wines, the data of the sensory analysis performed were illustrated in the sensory profile graph (Fig. 5). The biplot graph correlates the attributes of wines variables that explained 85.05% of the total variability as function of factor 1 (45.92%) and 2 (39.13%). This graph reveals a clear grouping of the wines into 3 clusters. In the first quadrant, the wines from the trial N1 and N3 were correlated with the attributes of taste (acid and smoothness), odour (complexity, fruity and spicy) and odour overall quality. In the third quadrant, trial N4 wine was associated with balsamic odour attribute. In the last quadrant, trial N2 wine was strongly associated with colour, intensity, and persistence of taste, also for floreal and intensity odour. In all trials, Frappato wines showed different sensory profiles. The wine from trial N1 and N2 wine produced with *St. lactis-condensi* and *C. oleophila* were of considerable interest and showed high scores for most of the descriptors of sensory evaluation.

4. Conclusions

The impact of sequential inoculation of *St. lactis-condensi*, *C. oleophila*, and *St. bacillaris* with *S. cerevisiae* was determined in final wines with different aromatic profiles. The application of sequential inoculation strains led to an increase in esters compared to wines fermented with *S. cerevisiae* alone, which resulted in an increase in floral and fruity notes in the wines compared to the non-*Saccharomyces* inoculated strain. Differences were observed in the trials inoculated with *C. olephila*, particularly with regard to ethyl acetate and ethyl octanoate, whereas *St. lactis-condensi* showed a higher amount of isopentyl acetate. The wines produced using *St. bacillaris* revealed a high concentration of ethyl acetate, as in the case of *C. oleophila*, and ethyl hexanoate. The wine produced through a single inoculation of *S. cerevisiae* was characterised by a higher presence of diethyl butanedioate. However, in the aromatic expression of the wine as assessed by sensory analysis, the flavour-olfactory differences between the wines were related to the

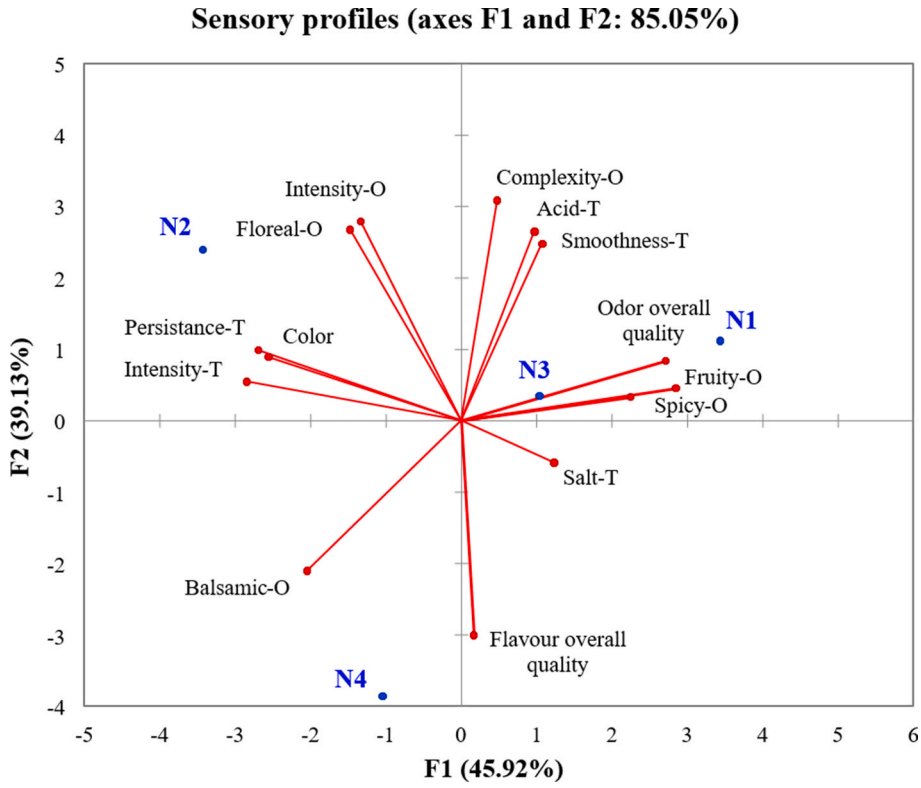

Fig. 5. Sensory profiles graph showing the distribution of different Frappato wine in relation to the taste and odour attributes. Codes: N1, *sequential inoculum Starmerella lactis-condensi* MN412/*Saccharomyces cerevisiae* NF213; N2, sequential inoculum *Candida oleophila* YS209/*Saccharomyces cerevisiae* NF213; N3, sequential inoculum *Starmerella bacillaris* Cz3/*Saccharomyces cerevisiae* NF213; N4, single inoculum *Saccharomyces cerevisiae* NF213.

fruity aroma in wines produced with *St. lactis-condensi* due to the presence of esters, floral for wines produced with *C. oleophila* in relation to the concentration of alcohols, in particular *p*-thyrosol. The wine produced with *St. bacillaris* was characterised by smoothness due to its high glycerol content. In contrast, the wine acquired from *S. cerevisiae* fermentation exhibited a distinct sensory profile, less floral and fruity, but a superior overall flavour, as determined by its phenylethyl alcohol and diethyl butanedioate content. The study showed that the use of non-*Saccharomyces* strains from non-oenological matrices can be successfully used to improve the quality and aroma profiles of wines, providing consumers with a wine with a diversified aromatic component.

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Ethical statement

Ethics approval was not required for this research.

CRediT authorship contribution statement

Nicola Francesca: Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Vincenzo Naselli:** Methodology, Formal analysis. **Rosario Prestianni:** Writing – original draft, Software, Methodology, Formal analysis. **Antonino Pirrone:** Methodology, Investigation, Data curation. **Enrico Viola:** Formal analysis, Software. **Raffaele Guzzon:** Formal analysis, Conceptualization. **Luca Settanni:** Writing – review & editing, Writing – original draft, Visualization. **Antonella Maggio:** Writing – review & editing, Writing – original draft, Resources, Data curation. **Alessandro Vaglica:** Software, Formal analysis. **Maurizio Bruno:** Writing – review & editing, Writing – original draft. **Luciano Gristina:** Supervision, Resources, Project administration, Funding acquisition. **Daniele Oliva:** Writing – review & editing, Writing – original draft, Visualization, Methodology. **Giuseppe Ferranti:** Methodology. **Giuseppe Notarbartolo:** Methodology. **Antonio Alfonzo:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Data curation, Conceptualization. **Giancarlo Moschetti:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no conflict of interest with the research topic.

Data availability

Data will be made available on request.

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