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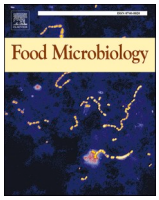

Enhancing the quality and safety of Nocellara del Belice green table olives produced using the Castelvetrano method

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ABSTRACT

The Castelvetrano method is the most widely used among the various table olive processing styles in Sicily. After debittering, the product is stored at low temperatures to prevent the growth of undesirable microorganisms. In an effort to enhance the production process, yeast isolates underwent genotypic characterization and technological screening. The screening process identified two yeast strains *Candida norvegica* OC10 and *Candida boidinii* LC1, which can grow at low temperatures and tolerate high pH values (up to 10) and salinity [10% (w/v)]. During the monitoring period, the inoculated trials showed limited presence of spoilage/pathogenic microorganisms. Additionally, the yeasts limited oxidative phenomena and softening of the drupes. The organic compounds detected were higher in the inoculated trials than in the control, and cold storage induced aromatic decay, which was less pronounced in the trial inoculated with *C. norvegica*. Sensory analysis revealed that the inoculated trials scored higher in sweetness, hardness and crispness.

1. Introduction

Table olives are a popular fermented food in the Mediterranean region (Perpetuini et al., 2020). The EU countries have had an average total table olive production of around 833,090 tonnes in the period 2011–2021 (IOC, 2022). Spain, Greece, and Italy are the countries with the highest production of table olives in the EU (Speranza et al., 2022). The Sevillian style in Spain and the natural style in Greece are the most commonly used methods to process table olives in the main producing countries (Ruiz-Barba et al., 2023). In Italy, the processing of table olives is typically related to local customs and traditions. Sicily is the leading region for table olive production, and in addition to the Seville and natural styles, table olives are also produced in western Sicily in the Castelvetrano style, with an average annual production of 31,000 tonnes (Zinno et al., 2017). The Castelvetrano method has enabled Sicily to gain a significant share of the national and international market by offering a product of excellent quality that is highly appreciated by consumers. The advantages of the Castelvetrano method are represented by the rapid debittering process, which typically takes two weeks (Zinno et al., 2017).

Table olives of Nocellara del Belice variety are debittered using sodium hydroxide at varying concentrations (2.5–3.5 °Bè) depending on the maturity of the drupes. After an overnight alkaline treatment, sea salt is added, which solubilises by gravity in the soda solution until a final NaCl concentration of 8% (w/v) is reached. However, storing the product at low temperatures is a limitation of this process. During spring, when average daily temperatures rise, the product is subject to deterioration due to undesirable fermentation (Lanza et al., 2012). In addition, the effect of the increase in temperature has a negative impact on the green color of the olives (Gandul-Rojas and Gallardo-Guerrero, 2018). To avoid these problems, the olives are stored in cold rooms (8 ± 1 °C) after debittering. Before commercialization, olives undergo multiple rinses to remove residual soda, immersion in acidified brine, pasteurization, and packaging (Catania et al., 2014). However, storing the product at low temperatures does not diminish the microbiological risks. Gas-pocket, off-flavours, and odours have been reported by several producers of Nocellara del Belice table olives processed according to the Castelvetrano style. The microbiological data available in the literature are quite limited; however, several microbial groups have been found associated with Castelvetrano style Nocellara del Belice table olives.

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Zinno et al. (2017) reported the presence of total aerobic mesophilic bacteria, yeasts, lactic acid bacteria (LAB), and streptococci at levels between 6 and 7 Log CFU/g three months after production. The same authors detected the presence of alkalitolerant bacteria (*Alkalibacterium*, *Natronobacillus* and *Marinilactibacillus*) in samples of Nocellara del Belice olives produced with the Castelvetrano style, using culture-independent techniques (denaturing gradient gel electrophoresis and next generation sequencing). Sabatini and Marsilio (2008) found high yeast populations (9.23 log CFU/mL) in brine. On the other hand, Romeo et al. (2012) highlighted that the Castelvetrano style negatively affected the survival of microbial populations. After 2 months of processing, the highest microbial concentrations were found for LAB (3.25 Log CFU/mL). These results show that there is variability in the microbiological data, which is probably related to different factors (environment, production, health status of the drupes, etc.). Furthermore, to our knowledge, there is no information on the dynamics of the physicochemical and microbiological parameters of Castelvetrano style Nocellara del Belice table olives during storage in cold rooms. To improve the safety and sensory quality of Castelvetrano style table olives, this study aimed to: (i) genotypically characterize the yeast strains collected; (ii) perform a technological selection of these strains; (iii) evaluate the bioprotective role of the yeasts by monitoring the populations of spoilage and/or potentially pathogenic microorganisms; (iv) determine the aromatic and sensory impact of the inoculated yeasts on the final product.

2. Materials and methods

2.1. Yeasts and growth conditions

The yeast cultures used in this study were obtained from brine samples of table olives of the Nocellara del Belice cultivar, produced in the Castelvetrano style during the most recent olive growing season (2021). All isolates belong to the yeast collection of the Department of Agricultural, Food and Forest Sciences (SAAF) at the University of Palermo and were stored in 2 mL vials containing Yeast Extract Peptone Dextrose (YEPD; Francesca et al., 2022) broth supplemented with 15% (w/v) glycerol at −80 °C. The isolates were refreshed in liquid culture using YEPD and incubated at a temperature of 30 °C for 48 h. After that, they were transferred to Petri dishes with YEPDA [1% (w/v) yeast extract; 2% (w/v) peptone; 2% (w/v) glucose; 2% (w/v) agar], and then incubated under aerobic conditions.

2.2. Genotypic characterisation of yeasts

Each yeast isolate was subjected to DNA extraction by cell lysis using the InstaGeneTM Matrix Kit (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's protocols (Matraxia et al., 2021). DNA extracted from the isolates was subjected to amplification of the 5.8S rRNA gene (Esteve-Zarzoso et al., 1999) using primers ITS1 (5′-TCCGTAGGTGAACCTTGCG-3′) and ITS4 (5′-TCCTCCGCTTATT GATATGC-3′). Each amplified product underwent RFLP analysis through overnight digestion (37 °C) by the endonucleases *Cfo*I, *Hae*III and *Hinf*I (Thermo Fisher Scientific, Monza, Italy). The restriction fragments of ITS amplicons were analysed in a two-step procedure with agarose at 1.5% and 3% (w/v) in 1 × Tris-Borate-EDTA (TBE) buffer (Guarcello et al., 2019). The visualisation of these fragments occurred in a UV transilluminator, (KODAK Gel Logic 100 system, USA). The GeneRuler 50 bp plus DNA ladder (MMedical s. r.l., Milan, Italy) was used as DNA ladder.

Strain typing was performed by Random Amplification of Polymorphic DNA (RAPD)-PCR analysis using the M13 primer (Andrighetto et al., 2000; Porru et al., 2018). Amplification reactions were performed in a 25 μL reaction volume containing 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 200 mmol/L each of dATP, dGTP, dCTP and dTTP, 1.5 mmol/L $MgCl_2$, 1 mmol/L primer M13, 80 ng DNA, and 2 U Taq DNA polymerase (Sigma Aldrich, Milan, Italy). Amplification was performed on a Swift max PRO thermal cycler (Esco Healthcare, Singapore) using the following program: 94 °C for 2 min, 35 cycles of 94 °C for 1 min, 42 °C for 20 s, 72 °C for 2 min and finally 72 °C for 10 min. RAPD profiles were visualised by UV light as reported above and images were acquired using the Gel Doc 1000 video gel documentation system (Bio-Rad, Richmond, VI, USA). The height of the bands was compared with the GeneRuler 100 bp DNA Ladder Plus marker (Thermo Fisher Scientific, Waltham, MA, USA). All RAPD profiles were analysed using Gelcompar II software, version 6.5 (Applied-Maths, Sint-Martens-Latem, Belgium; Alfonzo et al., 2023a). Each strain was then subjected to sequencing of the D1/D2 region of the 26S rRNA gene with primers NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGTG-TTTCAAGACGG-3′) following the protocol and amplification conditions reported by Kurtzman and Robnett (1998). DNA sequencing reactions were performed at BMR Genomics (Padua, Italy). Sequence verification was performed manually using Chromas 2.6.2 software (Technelysium Pty Ltd, Brisbane, Australia). The sequences obtained were compared with those deposited in GenBank using the Basic Local Alignment Search Tool (BLAST).

2.3. Yeast strains technology screening

2.3.1. Technological traits

All strains were grown in YEPD broth for 48 h at 30 °C, and tests were performed to evaluate the technological properties of each strain according to the methodology described by Bevilacqua et al. (2013), appropriately modified.

The resistance of the strains to salt was evaluated by growth tests in tubes containing 5 mL of YEPD with NaCl added at the following concentrations: 2, 4, 6, 8 and 10% (w/v). Each tube was individually inoculated with the respective yeast strain at a concentration of 6 Log CFU/mL. pH tolerance was determined by evaluating the growth of strains in tubes containing YEPD (5 mL) at the following pH values: 3, 4, 5, 7, 9, 11, and 12.5. HCl or NaOH (1 mol/L) was added to acidify or alkalise the YEPD broth. Growth tests at different temperatures were carried out by inoculation of each strain (6 Log CFU/mL) into tubes containing YEPD (5 mL) and then incubated under static conditions at the following temperatures 5, 10, 15, 25 and 37 C°. Finally, the ability to grow in the presence of lactic acid and acetic acid was assessed by acidifying the culture broth (YEPD) to pH 3.93 by adding lactic acid (1.2% v/v) or acetic acid (0.9% v/v), respectively, as reported by Bevilacqua et al. (2013). Broth cultures for salt and pH tests were incubated at 25 °C for 7 d. For growth tests at different temperatures, these cultures were incubated at 5 and 10 °C for 14 d, at 15 and 25 °C for 7 d and at 37 °C for 3 d. The CO_2 production of the strains was verified by applying the methodology described by Caridi et al. (2019). Each yeast strain (6 Log CFU/mL) was inoculated into a test tube containing 10 mL of YEPD broth and a Durham tube (inserted upside down) to assess gas production by the yeasts. All yeasts capable of producing gas were discarded. Each test was performed in triplicate, including a negative control represented by uninoculated test tubes.

2.3.2. Enzymatic activities

The yeast strains with the desired characteristics were then evaluated for the following activities: (*i*) polysaccharolytic (pectolytic and xylanolytic), lipasic, and catalase enzyme activity, according to the methodology reported by Hernández et al. (2007); (*ii*) β-glucosidasic activity, evaluated according to the procedure described by Parafati et al. (2022). Pectinolytic enzymes were assessed by measuring the growth of yeast strain colonies spot inoculated in Petri dishes containing Yeast Nitrogen Base Agar (YNBA, BD Difco™, Milan, Italy) supplemented with 1.25% apple pectin (w/v; Sigma-Aldrich, Milan, Italy) and acidified (pH 4) with a 1 N HCl solution. Xylanase production was evaluated by measuring the extent of the halo formed around the colony after growth at 25 °C for 10 d in Yeast Extract Agar (YEA; Condalab, Torrejón de Ardoz, Spain) supplemented with xylan (0.50% w/v),

peptone (0.50% w/v) and NaCl (0.50% w/v). Petri dishes were incubated at 25 °C for 10 d to assess polysaccharolytic activities (in triplicate for each strain). Lipase activity was determined by inoculating the yeast strains into Petri dishes containing rhodamine olive oil agar and incubated at 25 °C for 5 d in three replicates. The strains with lipase activity showed orange fluorescent halos when illuminated with UV light at 350 nm. The presence of the enzyme catalase was determined by pipetting a solution containing 3% (v/v) hydrogen peroxide (Sigma-Aldrich, Milan, Italy) onto yeast colonies plated on YEPD after 72 h incubation at 25 °C. Yeast colonies showing effervescence were considered positive. β-glucosidase activity was assessed by plating the yeast strains in a culture medium with the following composition: 6.7 g/L Yeast Nitrogen Base (YNG; BD Difco™, Milan, Italy), 5 g/L arbutin (Sigma-Aldrich, Milan, Italy), 0.2 g/L ferric ammonium citrate (Sigma-Aldrich, Milan, Italy), 20 g/L bacterial agar (Condalab, Torrejón de Ardoz, Spain) and adjusted to pH 5 b y the addition of a 1 N HCl solution. The inoculated plates were incubated for 5 d at 25 °C. The yeast strains that were positive in the test showed a brown colony color of varying intensity.

2.3.3. Preservative resistance

The resistance of the yeasts to the main preservatives used to stabilise the olives during packaging was determined. Each strain was inoculated into tubes containing 5 mL of YEPD and was incubated at 25 °C for 72 h. After this period, 1 mL of broth culture was sterile removed and centrifuged (Neya 16R, Remi Elektrotechnik LTD, Vasai, India) at 9000×*g* for 10 min. After removal of the supernatant, the pellet was washed three times with sterile saline containing 9 g/L NaCl. After a final centrifugation (9000×*g* for 10 min), the pellet was resuspended in 500 μL of sterile saline (0.9% w/v NaCl), ready for inoculation. The tests were performed in test tubes containing YEPD (2 mL) with the addition of Zinc chloride (0, 20, 30, 50, 70, 80, 100, 120, 140, 150, 170, 180, 200, 220 and 250 mg/L; Sigma-Aldrich, Milan, Italy), potassium sorbate (0, 10, 25, 50, 75, 100, 150, 250, 500, 1000, 1500 and 2000 mg/L; Agrovin, Ciudad Real, Spain), sodium benzoate (0, 150, 200, 250, 300, 350 and 400 mg/L; Tuto Chimica, Rossano Veneto, Italy), potassium metabisulphite (0, 10, 25, 50, 75, 100, 150, 250, 500, 1000, 1500 and 2000 mg/L; Chimica Noto, Partinico, Italy), citric acid (0, 0. 15, 0.30 and 0.45% w/v; Chimica Noto, Partinico, Italy) and natamycin (0, 30 and 60 mg/L; Sigma-Aldrich, Milan, Italy) (Arroyo-López et al., 2012a;

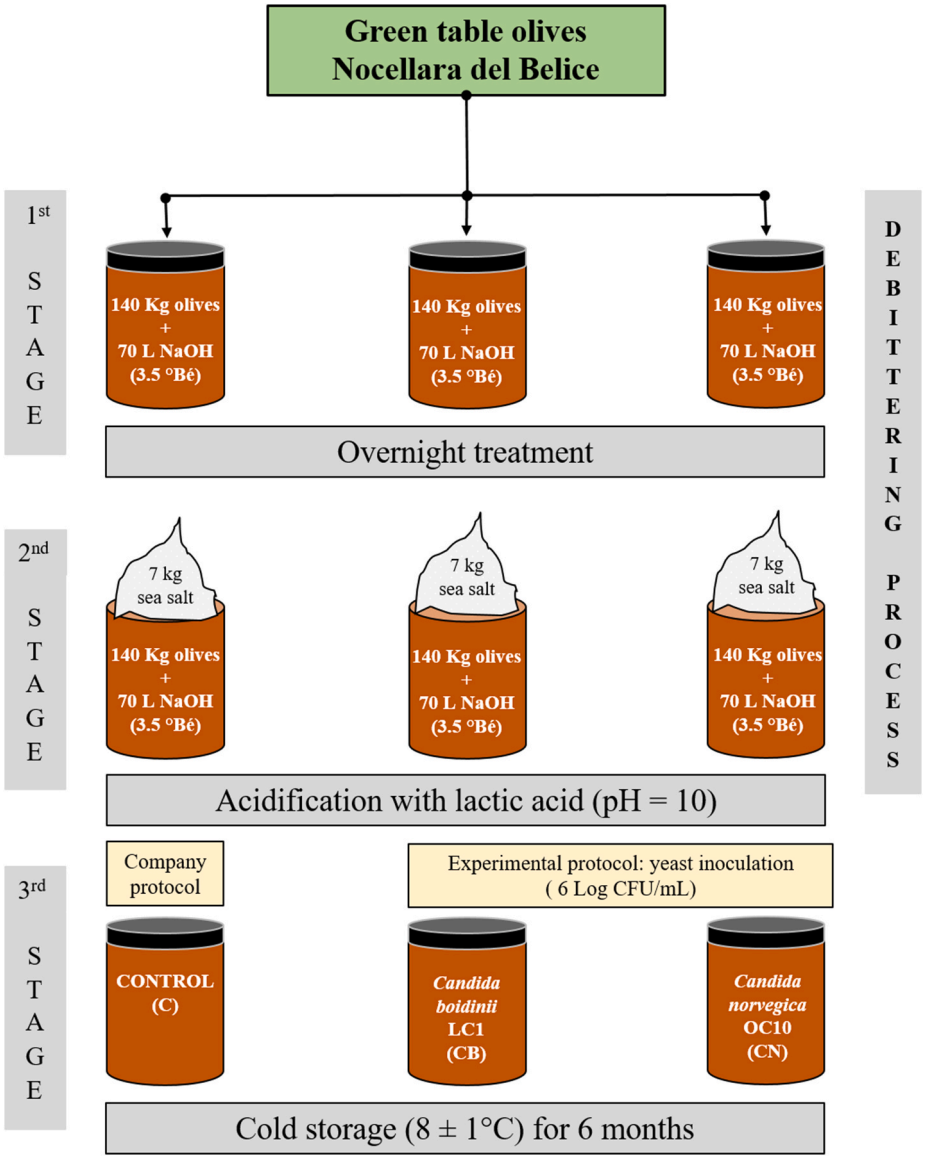

Fig. 1. Experimental design for the production of green table olives; abbreviations: °Bé, Baumé degree; the code C refers to the control trial; the codes CB and CN refer to the experimental trials. Each production was carried out in triplicate.

Bautista-Gallego et al., 2012; Karaman et al., 2020; Mihyar and Yamani, 1997; Romero-Gil et al., 2016). Each tube was inoculated with the respective strain to be tested at a final concentration of 6 Log CFU/mL and incubated at 25 °C for 7 d. All preservative resistance tests were performed in triplicate for each strain and each preservative, and non-inoculated tubes were used as negative controls.

2.4. Olive processing, designing and sampling

The olives used for the experiment were harvested in October 2022 in olive groves located in the area of Castelvetrano (TP, Italy) and belonged to the Nocellara del Belice variety. After calibration, 1260 kg of XL category drupes were processed using the Castelvetrano method. Nine high-density polyethylene (HDPE) drums with a volume of 210 L (Altereko, Anagni, Italy) were used for the experiments. Each drum was filled in with 140 kg of olives and 70 L of a 3.5 °Bè NaOH solution. After 8 h, 7 kg of sea salt (Sosalt, Trapani, Italy) was added to the delimiting net at the upper part of each drum. After about 4 h, the salt will partially dissolve in the soda by gravity. Each drum of sodium solution and salt was adjusted to pH 10 by the addition of lactic acid (Interchem Italia SRL, Vigonza, Italy). The experimental design is shown in Fig. 1.

Three experimental productions were carried out: CB, table olives were inoculated with *Candida boidinii* LC1; CN, table olives were inoculated with *Candida norvegica* OC10; C, represents the control trial that was not inoculated. The strains *C. boidinii* LC1 and *C. norvegica* OC10 were used in liquid concentrated form [approximately 7.00×10^{10} colony forming units (CFU)/mL; Bionova srl, Villanova sull'Arda, Italy]. The dose of yeast strain used was 20 g per100 kg of olives. After inoculation, the drums were closed, coded and stored in cold room at 8 ± 1 °C. The samples were collected as follows: immediately after inoculation of the yeast strains, at 7 and 15 d and then fortnightly for six months (30, 45, 60, 75, 90, 105, 120, 135, 150, 165 and 180 d). Each sample, consisting of olives and brine with a total volume of 200 mL per replicate, was collected in sterile polypropylene containers (Medical-Market, Rome, Italy) and transported under refrigerated conditions to the Agricultural Microbiology Laboratories of the Department of Agricultural, Food and Forest Sciences of the University of Palermo.

2.5. Microbiological analyses

Brine samples were 1:10 serially diluted in Ringer's solution (Liofilchem srl, Roseto degli Abruzzi, Italy). The following microbial populations were monitored in this study: total mesophilic and psychrophilic microorganisms on Plate Count Agar (PCA) incubated for 3 d at 30 °C and 5 d at 15 °C (Lombardi et al., 2018), respectively; LAB rods and cocci on de Man-Rogosa-Sharpe (MRS) agar and M17 agar, respectively. Both media were supplemented with cycloheximide (10 mg/mL) and incubated anaerobically for 2 d at 30 °C (Cirlincione et al., 2021); yeasts on yeast peptone dextrose (YPD) agar, incubated aerobically for 5 d at 25 °C (Bevilacqua et al., 2013); Enterobacteriaceae on violet red bile glucose agar (VRBGA), incubated aerobically for 1 d at 37 °C (Martorana et al., 2015); total coliforms on Violet Red Bile Agar (VRBA), incubated aerobically for 1 d at 37 °C (Servili et al., 2006); *Salmonella* spp. and *Shigella* spp. on Hektoen enteric agar (HEA), incubated aerobically for 1 d at 37 °C (Busetta et al., 2023); Staphylococcaceae on mannitol salt agar (MSA), incubated aerobically for 2 d at 30 °C (Fontana et al., 2005); Pseudomonadaceae on Pseudomonas agar with CFC supplement (PCFC), incubated aerobically for 2 d at 30 °C (Campaniello et al., 2005). Clostridia were determined in Reinforced Clostridial Medium (RCM) using the 3 x 3 Most Probable Number (MPN; Sinacori et al., 2014) procedure. The microbial counts were expressed in Log CFU/mL as an average of three replicates. The media used in the study were supplied by Condalab (Torrejón de Ardoz, Spain).

2.6. Dominance of inoculated yeast strains

Yeast colonies with the same macroscopic morphology were purified to obtain axenic colonies. These colonies were then observed under a light microscope to determine their cell morphology. Yeasts with a cell morphology similar to the genus *Candida* were subjected to DNA extraction (Matraxia et al., 2021). After confirming that the isolates belonged to the genus *Candida* by RFLP analysis, isolates from each RFLP group were subjected to DNA (RAPD)-PCR analysis using primer M13 (Andrighetto et al., 2000; Porru et al., 2018). Amplification, band visualisation and result analysis by Gelcompar II software, version 6.5 (Applied-Maths, Sint-Martens-Latem, Belgium) were carried out as reported by Alfonzo et al. (2023b). The comparison of the polymorphic profiles of the isolates from the different experimental productions and the inoculated strains (*C. boidinii* LC1 and *C. norvegica* OC10) allowed us to determine the percentage of dominance.

2.7. Physicochemical analyses

Brine pH was measured according to the method described by Alfonzo et al. (2023a). The salt concentration of the brine, expressed as % NaCl, was determined using a digital refractometer DBS1 (Giorgio Bormac srl, Carpi, Italy) as described by Sidari et al. (2019). These parameters were measured according to the sampling times mentioned above and each parameter was expressed as the average of three replicates.

At the 6th month, the colorimetric measurement of the drupes was carried out using a Chroma Meter CR-400C (Minolta, Osaka, Japan) in accordance with Martín-Vertedor et al. (2022). Chroma (C) was calculated in accordance with Romeo et al. (2012).

For each trial, at the 6th month, 30 drupes were randomly sampled and the pulp hardness, expressed in kg/cm^2, was determined using a FT327 portable penetrometer (Facchini srl, Alfonsine, Italy), as reported by Kaya et al. (2017).

2.8. Volatile organic compounds

Esters and higher molecular weight alcohols were investigated, identified, and determined in olive samples using headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography-mass spectrometry (GC-MS) analytical instrument. The method is a simple and eco-friendly extraction and concentration analytical procedure. To identify and determine each compound, an experimental sequence of standard organic compounds was injected using the same extraction procedure. The identification of each compound was further aided by Kovats Indices (KI), which are derived from the retention time normalised to the adjacently eluted *n*-alkanes. KI values are independent of the analytical procedures and therefore useful for the identification of unknown compounds.

Gas-chromatographic analyses were performed using an Agilent 5890 GC system interfaced with a HP 5973 quadrupole mass spectrometer. A HP5-MS column was used (5% diphenyl - 95% dimethylpolysiloxane 30 m × 0.2 mm, 0.25 μm film, J & W Scientific, Folsom CA, USA). Ultra-high-purity helium was used as the carrier gas, and water and oxygen traps (Supelco, ITA) were installed on the carrier gas lines. The oven temperature was held at 40 °C for 5 min, then increased 5 °C/min to 220 °C and 10 °C/min to 280 °C, and maintained for 10 min. The carrier gas (He) was used with a flow rate of 1 mL/min. The molecular mass spectrum was recorded at an ionisation voltage of 70 eV and an ion source temperature of 220 °C (Catania et al., 2016). Samples were analysed by HS-SPME-GC-MS method with a PDMS-CAR-DVB fibre (Supelco, ITA). Extraction and purification procedure were performed by exposing the fiber to 3 of gently sliced olive in a 40 mL vial covered with a silica septum at 70 °C for 20 min. The fiber was manually inserted in a GC inlet port equipped with a specific glass liner for SPME injection (0.75 mm i. d.). Fibers were desorbed in the gas chromatograph inlet

port for 3 min at 250 °C by using splitless injection mode. The identified compounds were verified by comparison of the mass spectral data with those of authentic reference compounds. When standards were not available, the components were identified by mass spectrum matching using the NIST11 mass spectral library collection.

2.9. Sensory analysis

The sensory profiles of table olives produced using the Castelvetrano method were evaluated by a panel of 10 tasters (five men and five women aged between 29 and 62) according to the guidelines of ISO 13299:2016. The sensory analysis was carried out by trained tasters who are regularly contacted by Geolive Belice S. r.l. To identify the sensory attributes describing the products, the judges were subjected to two preliminary tasting sessions, one week apart. The product subjected to the preliminary tasting was commercial samples of Nocellara del Belice green table olives produced using the Castelvetrano method. Once the sensory characteristics had been defined, the experimental product was tasted. Prior to tasting, the experimental olives were rinsed with low mineral content water to remove excess salt and served in ceramic dishes. Each sample was identified by a three-digit code that could not be traced back to the codes used for the experimental productions.

According to Ambra et al. (2017a), the sensory profiles of table olives were evaluated using 18 attributes: bitter, bitter aftertaste, bitter persistency, chemical flavour, color, color homogeneity, crispness, hardness, juiciness, olive flavour, olive odour, overall flavour, overall odour, pungency, salty, sour, and sweetness. Sessions were conducted in the morning, simultaneously among all tasters, in a room with a temperature between 20 and 22 °C and adequate lighting. The intensity of each attribute was rated using a scale with values ranging from 0 (no perception) to 9 (strong perception). During each session, each judge was given water and salt-free crackers at the end of each tasting session.

2.10. Statistical analysis

Data of microbial growth, pH, salinity, color, drupe hardness, VOC composition and quantitative sensory analysis were subjected to analysis of variance (ANOVA) and compared pairwise using Tukey's test. Statistical significance was considered at $P \leq 0.001$. The graphical representation of the distribution of the VOCs of the different experimental productions was performed using heat map analysis, represented by a hierarchical dendrogram and a map to represent the individual values of the relative peak areas in the data matrix as colours. The amount of each compound was represented by an intensity scale ranging from −1 (blue) to >1 (red). All analyses were performed using XLStat software ver. 2019.2.2 (Addinsoft, New York, USA).

3. Results and discussion

3.1. Yeast species detected in brine

A total of 157 yeast isolates were separated into eight groups based on their RFLP profile. The results of the RFLP analysis are shown in Table 1. Each group was represented by the following species: *Debaromyces hansenii*, *Candida norvegica*, *Saccharomyces cerevisiae*, *Candida boidinii*, *Komagataella pastoris*, *Wickerhamomyces anomalus*, *Candida aaseri* and *Meyerozyma guilliermondii*. RAPD analysis allowed all yeast isolates to be characterized at the strain level ($n = 43$). Sequencing of the D1/D2 region of the 26S rRNA gene on each strain confirmed the preliminary identification obtained by RFLP. Except *K. pastoris*, all identified species have been isolated with different frequencies from brines of table olives produced with different styles (Bautista-Gallego et al., 2011; Mougiou et al., 2023; Pereira et al., 2015). However, their presence has never been reported in the brine of table olives produced with the Castelvetrano style.

According to some studies, certain strains of *D. hansenii* are known to be resistant at low pH, in high salinity environments (Psani and Kotzekidou, 2006). Perpetuini et al. (2020) have described the application of *D. hansenii* to improve the organoleptic characteristics of table olives. *Candida boidinii* and *S. cerevisiae*, unlike *C. norvegica*, have been used in mixed inoculum with *Lactiplantibacillus plantarum* as a starter to improve the fermentation process of table olives (Bencresciuto et al., 2023; Tufariello et al., 2015; Zhu et al., 2019). Recently, *W. anomalus* has been used in coinoculum with *L. plantarum* as an oleuropeinolytic killer yeast for the production of low-salt table olives (Bencresciuto et al., 2023). *Candida aaseri* and *M. guilliermondii* are species commonly detected in fermented table olives (Sidari et al., 2019; Simões et al., 2021).

3.2. Technological traits of yeast strains

According to the results of the study, 35 out of 43 strains tested (Table S1) were able to tolerate 10% (w/v) NaCl, eight strains were able to grow at pH 10, 18 strains showed the ability to grow at 5 °C, and 38 strains tolerated the presence of lactic acid. On the other hand, 40 strains were inhibited by acetic acid, and only two strains showed the ability to produce CO_2 from glucose. The results indicate that most of the strains are able to tolerate the presence of NaCl and lactic acid, while they are susceptible to the presence of acetic acid. However, the levels of acetic acid found in the brine of Castelvetrano style table olives are usually <2 g/L. The ability of all strains to adapt to different conditions is a strain

Table 1
Molecular identification of yeasts isolated from the brine of Nocellara del Belice table olives produced using the Castelvetrano method.

Number of isolates	Size amplicons 5.8S-ITS (bp)	Size of restriction fragment (bp)			Number of strains[a]	Species	Range size of the PCR products (bp)	Acc. No. (range % similarity)
		*Cfo*I	*Hae*III	*Hinf*I				
93	650	300 + 280	400 + 130+80	320 + 320	21	*Debaromyces hansenii*	564–582	OR227807-827 (99.48–100)
48	580	500	370 + 200	300 + 250	12	*Candida norvegica*	562–582	OR227795-806 (99.65–100)
6	850	380 + 340	320 + 230+175 + 135	365 + 360+115	2	*Saccharomyces cerevisiae*	573–574	OR227830-831 (100)
5	700	340 + 300	700	380 + 185+150	4	*Candida boidinii*	556–574	OR227791-794 (100)
2	400	360	400	250 + 150	1	*Komagataella pastoris*	346	OR227828 (100)
1	650	550 + 100	640	330 + 320	1	*Wickerhamomyces anomalus*	580	OR227832 (100)
1	650	270 + 270+100	430 + 110+90	350	1	*Candida aaseri*	537	OR227790 (100)
1	630	310 + 270+40	110 + 400+100	310 + 300	1	*Meyerozyma guilliermondii*	573	OR227829 (100)

[a] Strain typing was performed with RAPD-PCR.

specific characteristic. The study detected 17 strains with psychotropic properties, which is of considerable importance, since in the processing of table olives according to the Castelvetrano method, the olive drums are normally stored in cold rooms (8 °C). Furthermore, most of the strains were not able to produce CO_2, a characteristic that is essential for the reduction of gas pockets, a fairly frequent alteration in this type of product, due to the presence of gasogenic microorganisms (Arroyo-López et al., 2008).

The analysis of the results allowed the selection of eight strains (LC1, LC15, LC25, LC32, OC10, OC16, OC20 and OC72) for subsequent enzymatic characterization and preservative resistance tests, the results of which are reported in Table S2. The enzymatic characterization of the eight strains verified the absence of pectolytic and xylanolytic activity; these characteristics are considered undesirable in yeast strain selection (Bevilacqua et al., 2012). On the other hand, in terms of desired enzymatic activities, all strains were positive for the presence of catalase and lipolytic activity (Rodríguez-Gómez et al., 2012). The presence of the enzyme catalase represents an important aspect as it prevents oxidative phenomena in drupes, while lipase activity positively affects the aroma of table olives (Sidari et al., 2019). Beta-glucosidase activity was absent in two strains (LC1 and LC32), low in one strain (OC20), and present in the remaining five strains. This characteristic is an important aspect for yeasts inoculated in table olives produced with the Seville or Natural style, since beta-glucosidase activity allows the potential application of these strains in the debittering process (Restuccia et al., 2011). However, it is a marginal aspect for table olives produced with the Castelvetrano method, since debittering is carried out exclusively with sodium hydroxide (Ambra et al., 2017b). Finally, strains LC1 and OC10 showed the highest resistance to six and four preservatives, respectively. Although this aspect is of particular interest for probiotic yeasts, resistance to the main preservatives is an important factor in limiting the development of spoilage microorganisms in the product containing acidified brine and not subjected to pasteurization, characterized by a limited shelf-life.

3.3. pH and salinity

The pH and salinity trends are shown in Fig. 2. Initially (0 d), the average pH of the brine was 10 ± 0.08. The value found is due to acidification with lactic acid in order to lower the high pH values that are normally recorded in the initial phase. This condition provided a suitable environment for the inoculation of the yeast strains *C. boidinii* LC1 and *C. norvegica* OC10. During the 180 days of observation, the pH values decreased to a final value between 5.29 (CN) and 5.81 (C). These pH values, around 5.0–6.0, represent one of the limitations of the method. However, the measured pH values are lower than those reported by Romeo et al. (2012) and higher than those recorded by Ambra et al. (2017b). The pH of the brine in trial C was higher during the 180 d compared to the inoculated trials (CB and CN). The differences in pH found between the different trials could be due to fermentation metabolism and the level of LAB populations present (Hurtado et al., 2012). In addition, the presence of yeasts in the inoculated trials promotes LAB growth (Chytiri et al., 2020) and this could explain the lower pH found in trials CB and CN compared to trial C.

The salt content in pulp and brine averaged 7.9–10.6% and 7.8–10.4%, respectively. After seven days, an increase in salt content was observed in both pulp and brine for all treatments due to complete solubilisation of the salt. From the 15th to the 180th d, the salinity values fluctuated with average values of 8.35 ± 0.28% in the pulp and 8.21 ± 0.29% in the brine. However, the salt content was comparable to that estimated in other productions performed with Castelvetrano processing method (Ambra et al., 2017a; Zinno et al., 2017).

3.4. Microbial population dynamics

Fig. 3 shows the results of the counts of the microbial populations

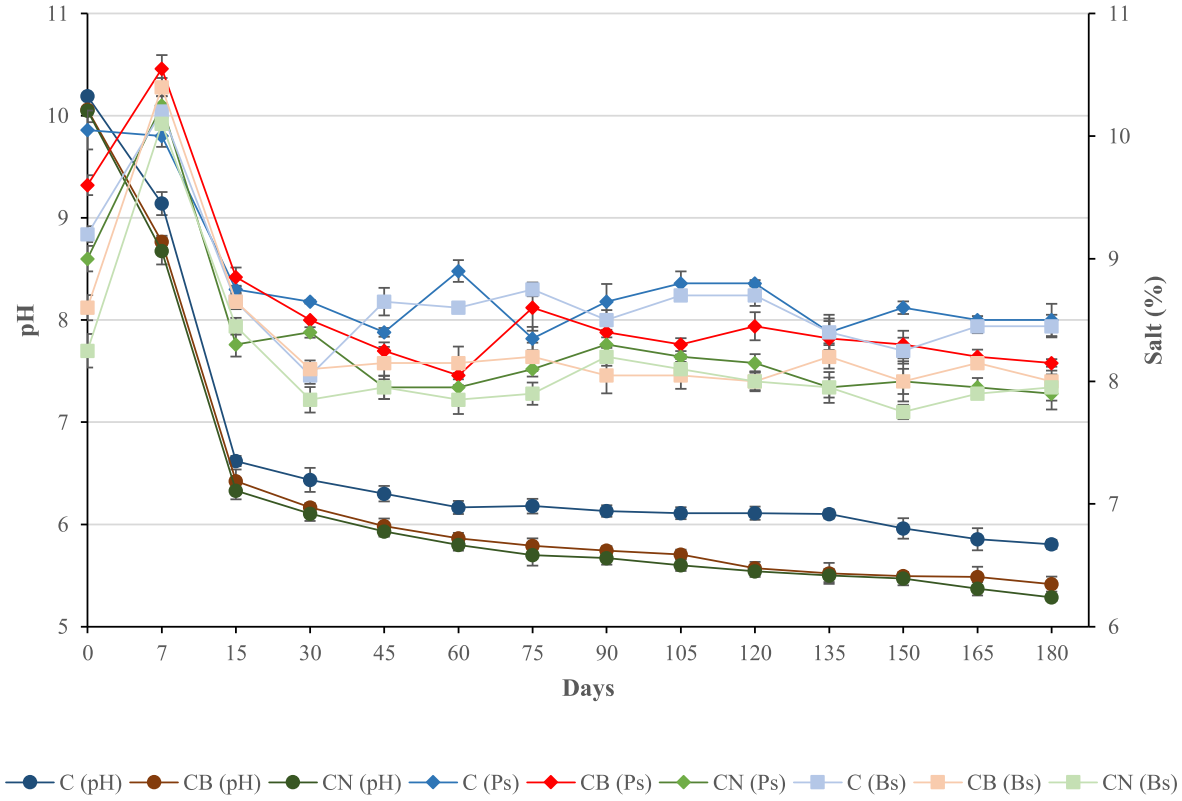

Fig. 2. Variation in pH and salt content of the brine and pulp of Nocellara del Belice table olives produced by the Castelvetrano method. Abbreviations: C, control trial; CB, *Candida boidinii* LC1; CN, *Candida norvegica* OC10; Ps, pulp salt content; Bs, brine salt content.

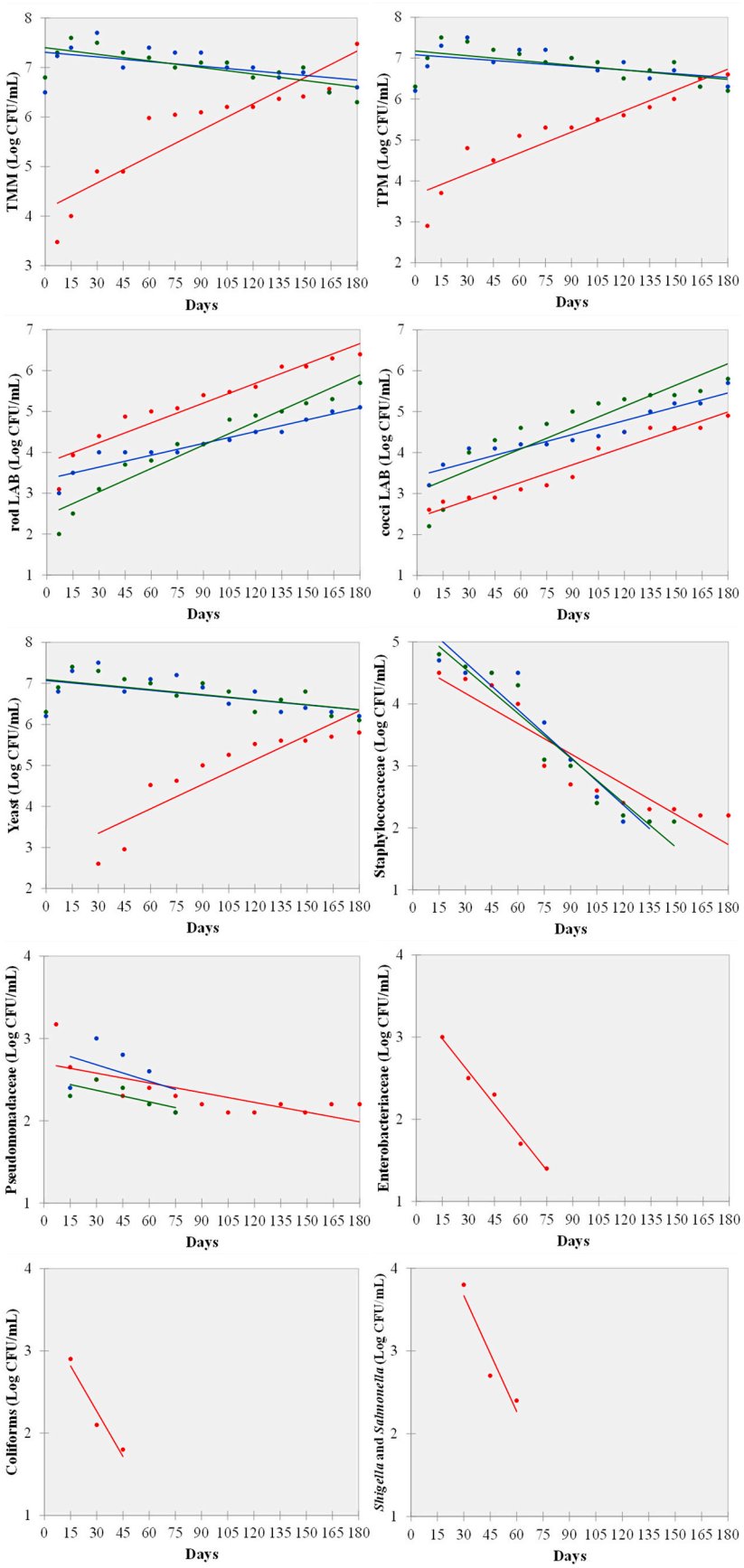

Fig. 3. Trend of microbial groups monitored during the production process of Nocellara del Belice table olives produced by the Castelvetrano method. Abbreviations: TMM, total mesophilic microorganisms; TPM, total psychrophilic microorganisms; LAB, lactic acid bacteria. Symbols: ●, C (control trial); ●, CB (*Candida boidinii* LC1); ●, CN (*Candida norvegica* OC10); —, trendline C; —, trendline CB; —, trendline CN.

monitored. The trend observed for total mesophilic microorganisms (TTM) and total psychrophilic microorganisms (TPM) was quite similar in the inoculated trials (CB and CN) compared to the control trial which showed an opposite trend. The higher levels of TTM and TPM found at time 0 in trials CB and CN can be attributed to the presence of the yeasts in the inoculated trials, but during monitoring there was a reversal of the trend, probably due to the action of the inoculated yeasts on the indigenous microbial community (Arroyo-López et al., 2008).

The detection of rod LAB populations only occurred after 7 days and showed an increasing trend during monitoring. This phenomenon is imputable to high pH values (8.68–9.14) registered at the 7th d, which are considered sub-optimal for the growth of LAB populations (Saeed et al., 2023). The levels of rod LAB detected in the inoculated trials (CB and CN) were lower than those found in the control trial. Although yeasts are known to promote the growth of LAB populations (Psani and Kotzekidou, 2006), in this case, the inoculated yeast strains seem to have exerted a competitive effect, reducing the levels of rod LAB (Panagou et al., 2008).

The trends of the cocci LAB populations were similar to those observed for the rod forms. Cocci LAB levels in trial C were always lower than those registered for the trials CB and CN during the entire observation period. The differences in cocci LAB levels between treatments could be due to the interaction between yeasts and LAB; in particular, yeast metabolism would allow the availability of different growth factors that promote LAB population growth (Hurtado et al., 2012). However, the levels of LAB populations observed were lower by about one log cycle compared to Zinno et al. (2017). This phenomenon could be due to the cold storage of the productions.

During the 180 days monitoring period, the yeast populations in trial C showed an increasing trend, whereas a slight decreasing trend was observed in the inoculated trials (CB and CN). The presence of yeasts could be caused by the decrease in pH (Campus et al., 2018). In fact, it was only after 30 d, in trial C, that yeasts above the detection limit were detected. Indeed, the high pH conditions limited the development of indigenous yeasts. The yeast count values in trial C were less than 1 log cycle compared to those of Zinno et al. (2017), while higher than those reported by Romeo et al. (2012), determined on table olives produced in the Castelvetrano style.

Staphylococcaceae are a group of bacteria that find a favourable environment for growth in table olives due to their tolerance to salinity, temperature and pH (Benítez-Cabello et al., 2019). A decreasing trend was observed for Staphylococcaceae in all treatments. The presence of this microbial group was recorded from the 15th d to the 180th d for trial C, and from the 15th d to the 135th d and 150th d for trials CB and CN, respectively. Romeo et al. (2012) reported the presence of this bacterial group at relatively low densities in table olives produced using the Castelvetrano method. Their absence after about five months of monitoring in inoculated trials is an important parameter to define the safety standard of the final product. No yellow colonies surrounded by yellow areas, ascribable to the presence of *Staphylococcus aureus*, were found during monitoring (Kateete et al., 2010).

The study observed a similar trend for Pseudomonadaceae. In trial C, the presence of pseudomonads was detected after 7 d and until the end of the process. In the inoculated trials (CB and CN), pseudomonads were observed from the 15th to the 75th d. Although pseudomonads are among the spoilage microorganisms of table olives (Botta and Cocolin, 2012), their presence was only detected up to 6 months in the control trial. The low levels observed in Castelvetrano olives do not represent a concern for the quality of the final product. However, their presence could lead to the presence of biogenic amines and to swelling of the olives, linked to a decrease in the acidity of the brine (Campaniello et al., 2005).

In the uninoculated trial (C), Enterobacteriaceae, coliforms, *Shigella* and *Salmonella* spp. were detected with a decreasing trend. This group of bacteria represents one of the main risks associated with the Castelvetrano method, because pH conditions are ideal for their development (Romeo et al., 2012). Enterobacteriaceae were detected from day 15–75, coliforms from day 15–45 and *Shigella* spp. from day 30–60. Macroscopic analysis of the colonies observed on HEA excluded the presence of *Salmonella* spp. The exposure of the olives to low temperatures severely limited their development compared to what was observed by Zinno et al. (2017). However, some species are able to grow at a temperature of 8 °C (Bagamboula et al., 2002; Ridell and Korkeala, 1997). The absence of potentially pathogenic bacterial groups in the trials inoculated with *C. boidinii* (CB) and *C. norvegica* (CN) is due to the dominance and persistence of yeasts, as also observed by Bonatsou and Panagou (2022). There were no detectable levels of clostridia throughout the monitoring period.

3.5. Yeast dominance

A total of 318 yeast isolates were taken from Petri dishes from the highest serial dilutions during the microbiological monitoring of olive brine samples from the different experimental productions. The collected yeast colonies were subjected to a purification streak in the same culture medium (YEPDA) and characterised microscopically. Of the 318 yeast isolates, 75 were from the trial CB, 70 from the trial CN and 173 from the control trial. The isolates showed variable amplicon size of the 5.8S-ITS region in relation to experimental production. In trial CB, 70 isolates had an amplicon size of 700 bp, while the other five isolates had a different size (650 bp). The totality of isolates from trial CN trial were characterised by an amplicon size of 580 bp. The amplicon sizes of the 5.8S-ITS region of the yeast isolates collected in the inoculated trials were comparable to those of *C. boidinii* LC1 and *C. norvegica* OC10. Amplicon sizes were similar to those reported by Sinigaglia et al. (2010) for *C. boidinii* and Pham et al. (2011) for *C. norvegica*. Furthermore, the 70 isolates from the trials CB and CN showed a RFLP profile identical to that of the strains inoculated in the respective experimental trials. The RFLP profiles of the isolates were similar to those reported in the literature and confirmed the two inoculated yeast species (Pham et al., 2011; Sun and Liu, 2014). None of the 173 yeast isolates collected from the control trial showed an amplicon size of the 5.8S-ITS region between 620 and 850 bp. RAPD-PCR analysis was applied to compare the isolates collected and determine the percentage of dominance of *C. boidinii* LC1 and *C. norvegica* OC10. Specifically, in trial CB, 93.33% of the isolates share the same *C. boidinii* LC1 RAPD profile, while in trial CN, 100% of the yeasts share the same *C. norvegica* OC10 RAPD profile. None of the 173 yeast isolates from control production shared the same polymorphic profile with the inoculated strains.

3.6. Physical parameters

The color of table olives is a crucial factor in determining the final product's pleasantness (Conte et al., 2020). The color of olives can vary from green to yellow, brown to black depending on the processing style used (Ramírez et al., 2015). Olives that are partially debittered with soda (Seville style) or completely debittered (Castelvetrano style) tend to retain a more intense green color compared to untreated olives (natural style; Rocha et al., 2020). This phenomenon can be attributed to the inactivation of the enzyme polyphenol oxidase by lye treatment, which prevents the oxidation of *o*-diphenolic compounds in drupes (Ramírez et al., 2015). Among the four parameters evaluated to describe the olive color (Table 2), only lightness showed no difference between the different treatments. The parameter a*, which defines the red/green variation, was more negative in the inoculated trials (CB and CN) than control trial, showing that there is a clear difference in the green hue. The values of parameter a* were similar in trial C, but slightly lower in trials CB and CN when compared to those reported by Catania et al. (2014) and Romeo et al. (2012). A similar situation was observed for the parameter b*, which measures the blue/yellow variation. The range of values observed for b* (31.51–36.32) shows that the olives did not suffer from excessive yellowing. This is also demonstrated by the color

Table 2
Physical parameters determined on Nocellara del Belice table olives produced using the Castelvetrano method after 6 months in cold storage.

Trials	Drupe color				Pulp hardness (kg/cm^2)
	L	a*	b*	C	
C	51.74 (±0.96) ^{a}	−3.44 (±1.22) ^{a}	31.51 (±1.89) ^{b}	32.86 (±1.05) ^{b}	14.30 (±0.77) ^{b}
CB	54.22 (±1.06) ^{a}	−9.23 (±0.92) ^{b}	36.32 (±1.17) ^{a}	37.51 (±0.91) ^{a}	18.65 (±1.38) ^{a}
CN	53.60 (±0.73) ^{a}	−8.73 (±1.13) ^{b}	35.51 (±1.06) ^{ab}	36.68 (±1.02) ^{a}	16.67 (±1.44) ^{ab}
Statistical significance	N.S.	**	*	**	*

Result indicate mean value (± standard deviation) of 30 olives.
Data in the same column followed by the same letter are not significantly different according to Tukey's test. P value: **, P < 0.01; *, P < 0.05; N.S., not significant.
Abbreviations: C, control trial; CB, *Candida boidinii* LC1; CN, *Candida norvegica* OC10.

saturation (C) values, which were higher in trials CB and CN than control trial. Consequently, trials CB and CN were less prone to browning than control trial. The comparison of the observed C values with those reported by Romeo et al. (2012) shows that this parameter is similar for the control trial, while it was higher for the experimental productions (CB and CN). The presence of inoculated yeasts probably resulted in greater preservation of the green color of the drupes, limiting oxidation processes.

Pulp hardness is also defining for the sensory characteristics of table olives (Lanza et al., 2021). After 180 d, trial CB showed the highest pulp hardness values (18.65 kg/cm^2), followed by trial CN (16.67 kg/cm^2) and control C (14.30 kg/cm^2). Although in some cases, *C. boidinii* species can cause softening of the pulp (Lanza et al., 2021), in this case, the trial inoculated with the same yeast species gave the highest pulp hardness values. This phenomenon is due to the inability of the strain used in the present study to produce pectolytic enzymes. The different viable levels of TMM and TPM populations for the inoculated and uninoculated trials might explain the different pulp hardness values. Indeed, some authors attributed lower pulp hardness to the development of pectinolytic microorganisms, mainly yeasts, but also bacteria of the genus *Bacillus* or other Gram-negative groups (Lanza, 2013).

3.7. Volatile organic compound composition

The headspace of the three different experimental productions was subjected to chromatographic analysis at two different times (90 and 180 d). The analysis revealed a total of 49 compounds, distributed differently according to the time of detection and the yeast strain inoculated. At the 90th day of cold storage (Table S3), 34 compounds were detected, 18 of which in the control trial, 24 in trial CN and 25 in trial CB. The compounds with the highest relative peak area percentage values were *n*-decanoic acid (22.62) in control trial and α-copaene in both CB (20.41) and CN (22.25) trials. Volatile organic compounds (VOCs) present only in the products were five for control trial [2-nonen-1-ol, (E); octanoic acid, methyl ester; 1,2-ethanediol, 1-phenyl-; D-limonene and undecanal], four for trial CB [*cis*-2-nonene; benzenemethanol, α-(2-methylenecyclopentyl), (R*,R*); pyridine, 5-ethyl-2-methyl], and two for trial CN (4-nonene and nonanoic acid). In contrast, 1-nonanol, 4,8-dimethyl was detected in both control and CN productions. After 180 d, the total number of VOCs decreased to 31, seven of which were present in control trial and 17 compounds in both CB and CN trials (Table S4). The compounds with the largest relative peak area percentage values were α-copaene in control (42.96) and CN (28.13) trials and α-farnesene in CB trial (35.17). α-farnesene was detected in both control and CB trials, but not in CN production. Their presence has already been detected in fermented table olives produced with different processing styles (Bleve et al., 2015; Dabbou et al., 2012), but they have never been reported in table olives produced with the Castelvetrano method. In contrast, four compounds (ylangene, benzene propanoic acid, ethyl ester, and benzene acetaldehyde) were detected only in the inoculated trials. These compounds do not seem to be necessarily generated by the metabolism of the inoculated yeasts, since their presence has been reported by several authors in Nocellara Etnea, Giarraffa and Grossa di Spagna table olives processed in the natural style and inoculated with different LAB strains (Randazzo et al., 2014; Vaccalluzzo et al., 2022).

The aromatic profiles of the experimental productions were compared and the results showed that three compounds were present only in the control production [oxime-, methoxy-phenyl; 1-hexanol, 5-methyl-2-(1-methylethyl) and benzeneethanol, 4-hydroxy], 10 in trial CB [phenyethyl alcohol; pyridine, 3-ethyl-4-methyl; 3-octene, (E); acetic acid, chloro-, 2-phenylethyl ester; benzenepropanoic acid, methyl ester; octanoic acid, ethyl ester; *p*-tolylhydroxylamine; camphenol, 6; 2-decen-1-ol, (E)- and Octanoic acid, methyl ester], and 11 in trial CN [phenylethyl Alcohol; benzyl alcohol; cycloheptane, methyl-; 3-hexen-1-ol, (Z); 1-octanol; acetic acid, phenylmethyl ester; pyridine, 4-ethyl; 3-hexen-1-ol, acetate, (Z); acetic acid, 2-phenylethyl ester; benzaldehyde and 6-heptenoic acid, ethyl ester]. Although the number of VOCs detected was reduced from 90 to 180 d, a greater diversification of aroma profiles was observed between the productions inoculated with *Candida boidinii* LC1 and *Candida norvegica* OC10 and the uninoculated trial after 6 months. Sabatini and Marsilio (2008) conducted a study on the VOC composition of Nocellara del Belice table olives processed by the Castelvetrano method and identified 22 substances and only four (1-hexanol, 5-methyl-2-(1-methylethyl); 3-hexen-1-ol, (Z); nonal; acetic acid) were also detected in the present study. These differences could be due to the different storage temperatures, since Sabatini and Marsilio (2008) followed the productions in drums kept at room temperature and not at low temperatures.

The dendrogram generated by the hierarchical cluster analysis, combined with the heat map, showed that the inoculated yeast strains were able to diversify the aromatic profile of each experimental production as a function of the time variable. The graphical representation of the VOC composition in relation to the aroma profiles of the 90 and 180-d trial productions is shown in Fig. 4. The dendrogram revealed the presence of three distinct clusters: (*i*) represented by $C_{(90)}$; (*ii*) formed by $CB_{(90)}$, $CN_{(90)}$ and $CN_{(180)}$ productions; (iii) consisting of the $C_{(180)}$ and $CB_{(180)}$ experiments. Cluster 1 was distinguished from the other clusters by *n*-decanoic acid, cluster 2 b y phenylethyl alcohol and cluster 3 b y α-copaene. *n*-decanoic acid, phenylethyl alcohol and α-copaene are compounds commonly found in table olives fermented through Greek and Sevillian styles, but they have never been reported in table olives produced by the Castelvetrano method (Cortés-Delgado et al., 2016; Mikrou et al., 2021; Ruiz-Barba et al., 2023; Sánchez et al., 2018). This comparison shows that the VOC profiles of the $CB_{(90)}$ and $CN_{(90)}$ trials were quite similar, but after 180 d, only the $CN_{(180)}$ production maintained a VOC profile comparable to that of the 90-d production. In fact, 24 compounds were detected at 90 d in the CN study, which was reduced to 17 compounds at 180 d. Of these 17 compounds, 12 were detected at both 90 and 180 d.

3.8. Sensory evaluation

According to the sensory analysis results presented in Fig. 5 (Table S5), the attributes with the highest scores (>7) were color homogeneity for CB and CN, overall flavour for trials CB and CN, crispness

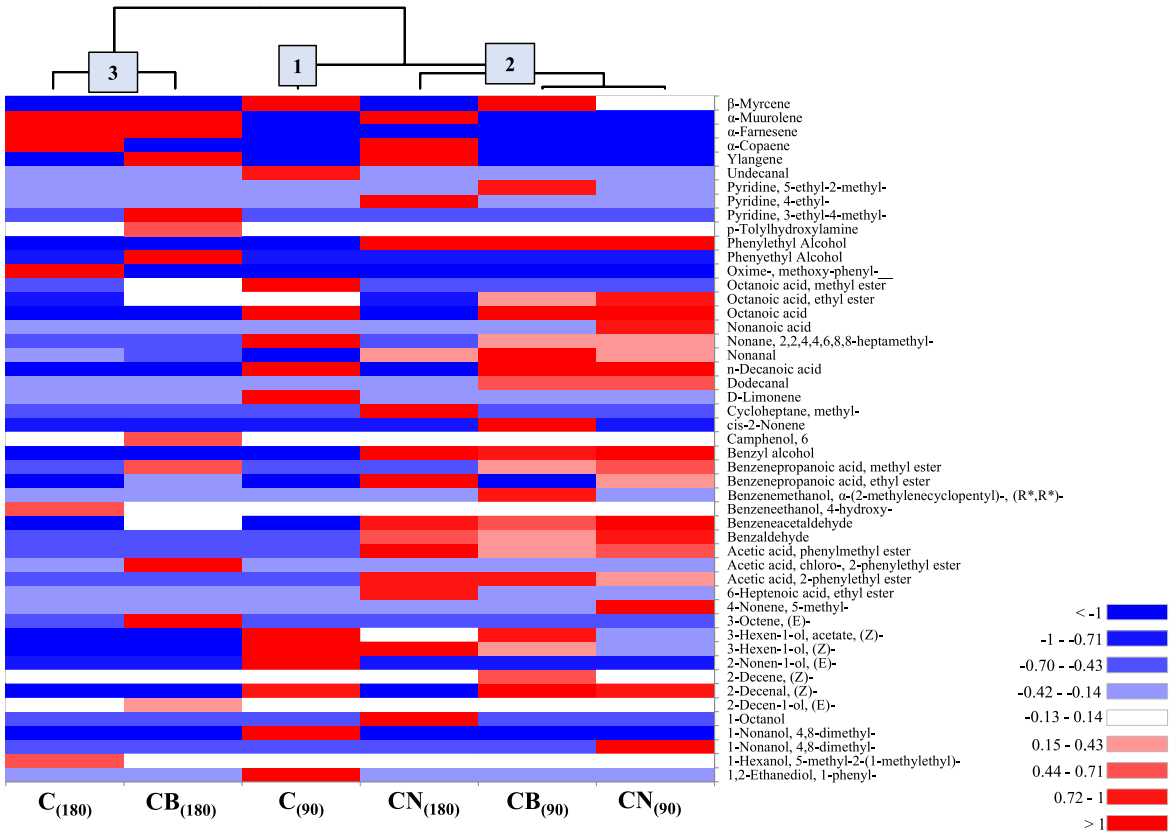

Fig. 4. Distribution of volatile organic compounds among different experimental productions of Nocellara del Belice table olives, detected at 90 and 180 d of cold storage. Experimental productions: $C_{(90)}$, 90-day control; $C_{(180)}$, 180-day control; $CB_{(90)}$, *Candida boidinii* LC1 at 90 days; $CB_{(180)}$, *Candida boidinii* LC1 at 180 days; $CN_{(90)}$, *Candida norvegica* OC10 at 90 days; $CN_{(180)}$, *Candida norvegica* OC10 at 180 days.

and hardness for trial CB, juiciness, olive flavour and olive odour for trial CN. In the control trial, the highest scores were registered for sour (3.02), pungency (2.94), oxidised flavour (1.27) and chemical flavour (1.39). Although the scores given by the judges were weak, these descriptors are commonly associated with sensory defects in table olives (Ambra et al., 2017a). Color showed higher values in the inoculated trials (CB and CN) rather than in uninoculated control trial, confirming the data obtained from the color analysis of the drupes. It is also interesting to note that the hardness of the olives from the trials CB and CN was higher than that of the control trial, in agreement with pulp hardness. However, the hardness values were higher than those reported by Catania et al. (2015) and comparable to those of Ambra et al. (2017a). The inoculation of yeasts lacking pectinolytic and xylanolytic activity had a positive effect on the crispness and hardness attributes (Arroyo-López et al., 2012b). The high scores for the overall flavour and odour parameters in trials CB and CN could be justified by the lipolytic activity of *C. boidinii* LC1 and *C. norvegica* OC10 (Bevilacqua et al., 2015). Similarly, the oxidised flavour attribute, which was higher in the control table olives, could be due to the oxidation of unsaturated fats and the formation of peroxides. The catalase-positive inoculated yeasts (*C. boidinii* LC1 and *C. norvegica* OC10) have a strong activity in preserving the oxidation processes of the drupes Bautista-Gallego et al. (2011); Hernández et al. (2007). Furthermore, certain yeasts of the *Candida* genus in olives produce natural antioxidants (carotenoids, citric acid, tocopherols and glutathione) that can limit these processes (Arroyo-López et al., 2012b). The attributes bitter aftertaste, bitter persistency, olive odour and salty taste did not show significant differences between the treatments.

4. Conclusions

Until a few decades ago, yeasts in table olives were considered to be spoilage microorganisms, since their presence caused both the appearance of unpleasant odours and flavours and the softening of the drupes due to the enzymatic degradation of the olive cell walls. However, a wise technological selection of yeast strains and the use of the selected strains could potentially improve the final product without changing the process. The use of *C. boidinii* LC1 and *C. norvegica* OC10 resulted in a modification of the microbiota of table olives produced using the Castelvetrano method, creating an environment hostile to spoilage and/or potentially pathogenic microorganisms due to their bioprotective activity. This phenomenon has led to an improvement in the quality of the final product, protecting it from oxidative processes, maintaining both the green color of the drupes and the hardness of the pulp, richer in the desired volatile organic compounds and with sensory notes particularly appreciated by the panelists.

In the future, yeast strains with bioprotective activity will be used to produce Nocellara del Belice green olives processed by the Castelvetrano method. The cold storage period of the product will be excluded or reduced to reduce the production costs represented by the high energy consumption required to keep the product at a refrigerated temperature. This would increase the sustainability of the production process and obtain a product with physicochemical and organoleptic characteristics similar to those obtained using the traditional method.

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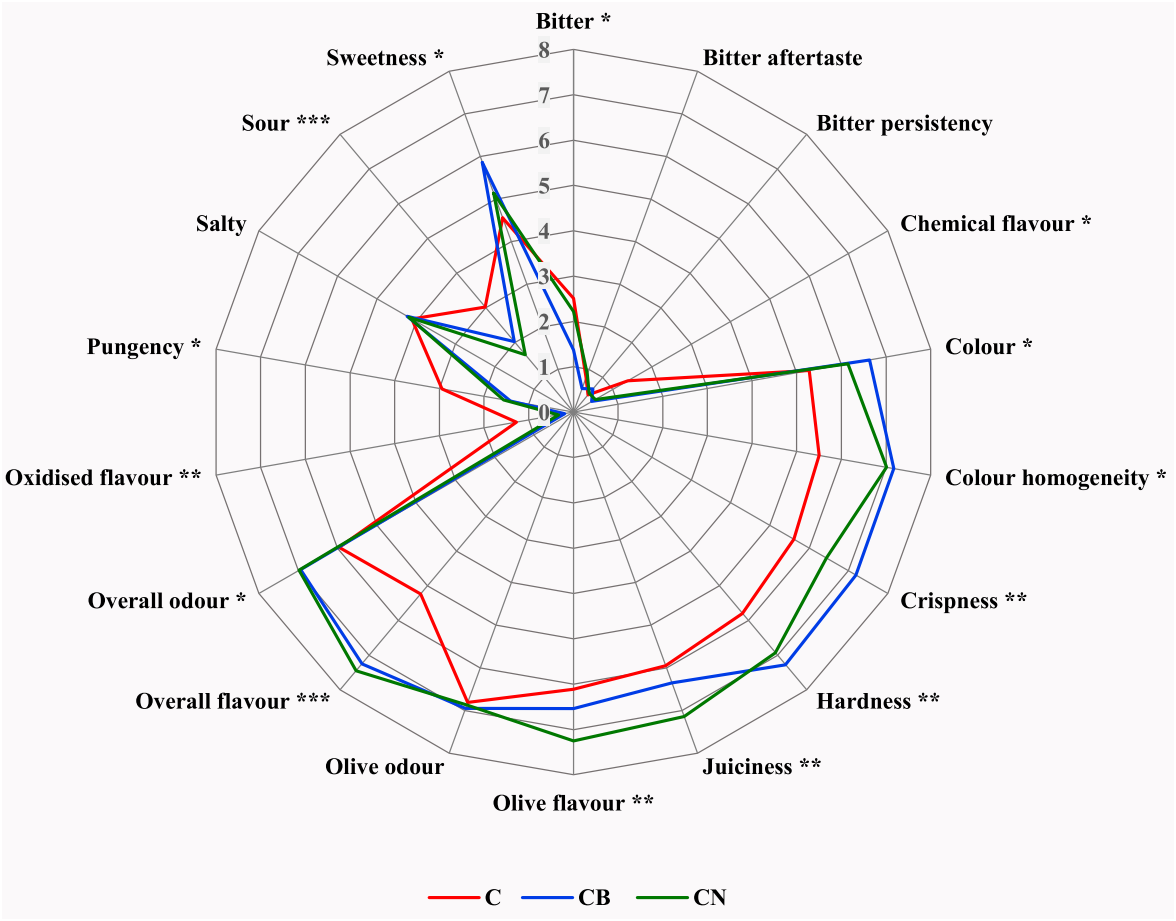

Fig. 5. Spider graph of the average scores for the attributes determined by the judges during the sensory analysis of Nocellara del Belice table olives inoculated with different yeast strains. Abbreviations: C, control trial; CB, *Candida boidinii* LC1; CN, *Candida norvegica* OC10. Symbols: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

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CRediT authorship contribution statement

Antonio Alfonzo: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Davide Alongi:** Visualization, Methodology, Formal analysis, Data curation. **Rosario Prestianni:** Software, Formal analysis, Data curation. **Antonino Pirrone:** Visualization, Formal analysis. **Vincenzo Naselli:** Formal analysis, Data curation. **Enrico Viola:** Data curation. **Claudio De Pasquale:** Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation. **Francesco La Croce:** Validation, Resources, Conceptualization. **Raimondo Gaglio:** Visualization, Investigation, Formal analysis, Data curation. **Luca Settanni:** Writing – review & editing, Writing – original draft, Validation, Methodology. **Nicola Francesca:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Giancarlo Moschetti:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that there is no conflict of interest for this research.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2024.104477.

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