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Reducing energy demand in Castelvetrano-style table olive production through *Candida*-based bioprotection approach

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ABSTRACT

The traditional Castelvetrano method for producing Nocellara del Belice green table olives relies on prolonged refrigeration, often up to 180 days, to ensure microbiological stability and preserve desirable sensory attributes, resulting in substantial energy consumption. This study evaluated whether the use of two bioprotective yeasts, *Candida boidinii* LC1 and *Candida norvegica* OC10, could enable a 50% reduction in refrigeration time without compromising product quality. Olives were processed under nine experimental conditions combining different temperature regimes and inoculation strategies. Microbiological, physicochemical, volatile, and sensory parameters were monitored for 180 days. Refrigeration remained the primary barrier against spoilage microorganisms. However, yeast inoculation significantly reduced populations of Enterobacteriaceae, Pseudomonadaceae, and Staphylococcaceae, thereby enhancing microbiological stability under the tested conditions. Under refrigerated or combined treatments, the inoculated strains dominated the microbial community (>94%). Inoculated olives exhibited increased pulp firmness (up to 23.12 kg/cm^2), a more intense green colour, and more complex aromatic profiles mainly associated with esters and aromatic alcohols. Sensory panels indicated higher perceived sweetness, crispness, and overall acceptance. Importantly, shortening refrigeration from 180 to 90 days did not adversely affect the quality of inoculated olive batches. Targeted use of *C. boidinii* LC1 and *C. norvegica* OC10 therefore emerges as a promising bioprotective strategy that supports microbiological stability, defined as controlled microbial proliferation, while preserving sensory quality under the tested conditions, and suggests the potential for reduced energy use and environmental impact in the Castelvetrano method. The reduction in refrigeration time implies possible energy savings and lower CO_2 emissions. However, these estimates are based on a simplified modelling approach and should be considered indicative. Overall, the proposed strategy aligns with cleaner production goals and contributes to SDG 12 by reducing refrigeration duration and associated energy demand within the limits of a model based assessment.

1. Introduction

Global production of table olives is expected to reach nearly 4 million tonnes in the 2024/25 season, with the European Union contributing roughly half of this volume (IOC, 2025). Italy ranks among the leading producers, and Sicily, together with Apulia, represents one of the country's main cultivation regions. Within this context, the Nocellara del Belice cultivar stands out for its agronomic value and distinctive sensory attributes; it is predominantly grown in western Sicily, particularly in the Valle del Belìce (Consonni & Cagliani, 2019; Sgroi et al., 2024). Despite its economic relevance, Nocellara del Belice is highly susceptible to fungal diseases and insect damage, which can compromise post-harvest quality and reduce shelf life (Lamendola et al., 2024; Malheiro et al., 2015). Ensuring microbiological and sensory stability during storage therefore remains a major challenge for producers, particularly under production systems aimed at minimising

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environmental impact.

Debittering is an essential step in table olive processing, as oleuropein, the primary phenolic compound, imparts intense bitterness (Soler-Rivas et al., 2000). This process can be achieved either chemically, through alkaline hydrolysis, or biologically, via enzymatic hydrolysis mediated by β-glucosidase (Nikolaivits et al., 2017). In the Castelvetrano method, however, debittering relies exclusively on alkaline hydrolysis under high-pH and high-salinity conditions that do not support fermentation. Consequently, microbiological stability must be ensured through alternative preservation strategies. In Sicily, three traditional olive-processing methods are used: Greek-style (natural), Spanish-style (Sevillian), and the Castelvetrano method (Zinno et al., 2017). The latter, applied exclusively to Nocellara del Belice cultivar, enables rapid debittering and yields sweet, bright-green olives ready for market in about 20 days (Sabatini & Marsilio, 2008). After debittering, olives are typically stored at 8 ± 1 °C for up to six months prior to washing, acidification, and packaging (Kailis & Harris, 2007).

From an energy perspective, refrigerated storage has received limited attention in studies specifically addressing the Castelvetrano process. Nonetheless, research on the food cold chain clearly indicates that refrigeration is among the most energy-intensive stages in food preservation systems, with energy demand strongly influenced by storage duration and operating conditions. Marchi and Zanoni (2022) demonstrated that refrigeration makes a substantial contribution to total energy consumption across food and beverage cold chains, while Karacan et al. (2023) reported that energy use in cold-storage facilities can increase by up to 30–50%, depending on operational practices and storage conditions.

Although refrigeration effectively limits microbial growth, it represents one of the most energy-demanding phases of the Castelvetrano process. Prolonged cold storage increases electricity consumption and associated greenhouse-gas emissions, raising concerns about the environmental impact of cold chains (Heard & Miller, 2016). Similar conclusions have been drawn from life-cycle assessments of olive products, which identify storage-related energy use as a major environmental hotspot (Espadas-Aldana et al., 2019). Reducing reliance on refrigeration without compromising product safety or quality is therefore a key priority for lowering the environmental footprint of table-olive production and aligns with cleaner-production strategies aimed at minimising resource use in food processing (Galanakis, 2020; Marques et al., 2025).

Traditionally, yeasts associated with table olives have been considered spoilage organisms due to their association with off-flavours and pulp softening. However, recent studies have shown that selected yeast strains can act as bioprotective agents by inhibiting spoilage and pathogenic microorganisms, limiting oxidative processes, and preserving colour and texture without inducing fermentation (Alfonzo et al., 2024; Bencresciuto et al., 2023; Caridi et al., 2019; Rus-Fernández & Fuentes, 2024). This shift in perspective creates new opportunities for the Castelvetrano method, where fermentation is undesirable but microbiological stability and reduced energy demand are essential. Despite this growing recognition, most studies to date have focused on fermentative systems or pilot-scale conditions. Consequently, the technological validation of yeasts under non-fermentative, industrially realistic conditions, such as the Castelvetrano method, where stability relies primarily on refrigeration rather than acidification, remains limited.

Evidence from Spanish-style olives indicates that selected yeasts can also modulate volatile profiles and enhance sensory attributes (Ruiz-Barba et al., 2024), while integrated omics approaches are increasingly being applied to optimise yeast-based bioprotection strategies (Giavalisco et al., 2024). More broadly, microbial bioprotection has been recognised as a promising approach for reducing dependence on energy-intensive preservation technologies in food systems (Ben Said et al., 2019). Nevertheless, industrial adoption remains limited, largely due to the scarcity of studies evaluating the scalability and technological feasibility of yeast-based bioprotection under realistic storage conditions and reduced refrigeration regimes.

Recent studies suggest that bioprotective yeasts may enhance the technological stability and sensory quality of Castelvetrano-style olives while enabling shorter refrigeration periods, thereby reducing energy consumption and environmental impact (Alfonzo et al., 2024). However, further research is required to assess their performance under different storage conditions, as factors such as NaCl concentration and microbial ecology also influence volatile compound formation and overall aroma development (Alvanoudi et al., 2024).

Building on this emerging evidence, the present study investigates the application of two selected yeast strains, *Candida boidinii* LC1 and *Candida norvegica* OC10, in the processing of Nocellara del Belice olives using the Castelvetrano method. Olives inoculated with these strains were stored under three temperature- and time-dependent regimes to evaluate their ability to maintain product quality and microbiological control under the tested conditions while significantly reducing refrigeration requirements.

The novelty of this study lies in its integrated evaluation of selected bioprotective yeasts under non-fermentative Castelvetrano-style processing conditions, providing the first process-oriented assessment of targeted yeast inoculation under industrially relevant conditions, together with a preliminary evaluation of potential energy-reduction benefits. The main hypothesis is that targeted yeast bioprotection can support a more energy-efficient and environmentally sustainable production process without compromising the technological or sensory characteristics of the final product. Accordingly, this work aims to identify strategies that simultaneously enhance product quality, improve microbiological stability, and reduce the environmental impact of table-olive processing.

2. Materials and methods

2.1. Experimental model, table olive production process and sampling

The experimental productions were carried out using table olives of the Nocellara del Belice variety, hand-harvested from olive groves located in Castelvetrano (TP, Italy) in October 2023. All experimental trials were conducted under industrial processing conditions at the facilities of Geolive Belice S.r.l., following the standard operational parameters of the Castelvetrano method.

Following calibration, the olives were processed according to the Castelvetrano method. Twenty-seven high-density polyethylene (HDPE) drums, each with a capacity of 210 L (Altereko, Anagni, Italy), were filled with 140 kg of olives and 70 L of a 3.5 °Bé sodium hydroxide solution (approximately 2.4% w/w at 15.6 °C). After an eight-hour soaking period, 7 kg of sea salt (SA.NI.MA. S.A.S. di Daidone Massimo & C., Trapani, Italy) was added to a floating net positioned at the top of each drum. The salt dissolved by gravity over a four-hour period. Subsequently, the pH of the solution in each drum was adjusted to 10 through the gradual addition of lactic acid (Greensistem Sas, Foggia, Italy).

Nine treatments were established (Fig. 1), each performed in triplicate: (i) CB-8, inoculated with *C. boidinii* LC1, stored at 8 ± 1 °C for 180 days; (ii) CN-8, inoculated with *C. norvegica* OC10, stored at 8 ± 1 °C for 180 days; (iii) C-8, uninoculated stored at 8 ± 1 °C for 180 days; (iv) CB-AT, inoculated with *C. boidinii* LC1, stored at ambient temperature for 180 days; (v) CN-AT, inoculated with *C. norvegica* OC10, stored at ambient temperature for 180 days; (vi) C-AT, uninoculated, stored at ambient temperature for 180 days; (vii) CB-AT/8, inoculated with *C. boidinii* LC1, stored for 90 days at ambient temperature followed by 90 days at 8 ± 1 °C; (viii) CN-AT/8, inoculated with *C. norvegica* OC10, stored for 90 days at ambient temperature followed by 90 days at 8 ± 1 °C; (ix) C-AT/8, uninoculated, stored for 90 days at ambient temperature followed by 90 days at 8 ± 1 °C.

The experimental design followed a completely randomised approach, with nine treatments carried out in triplicate. Each treatment

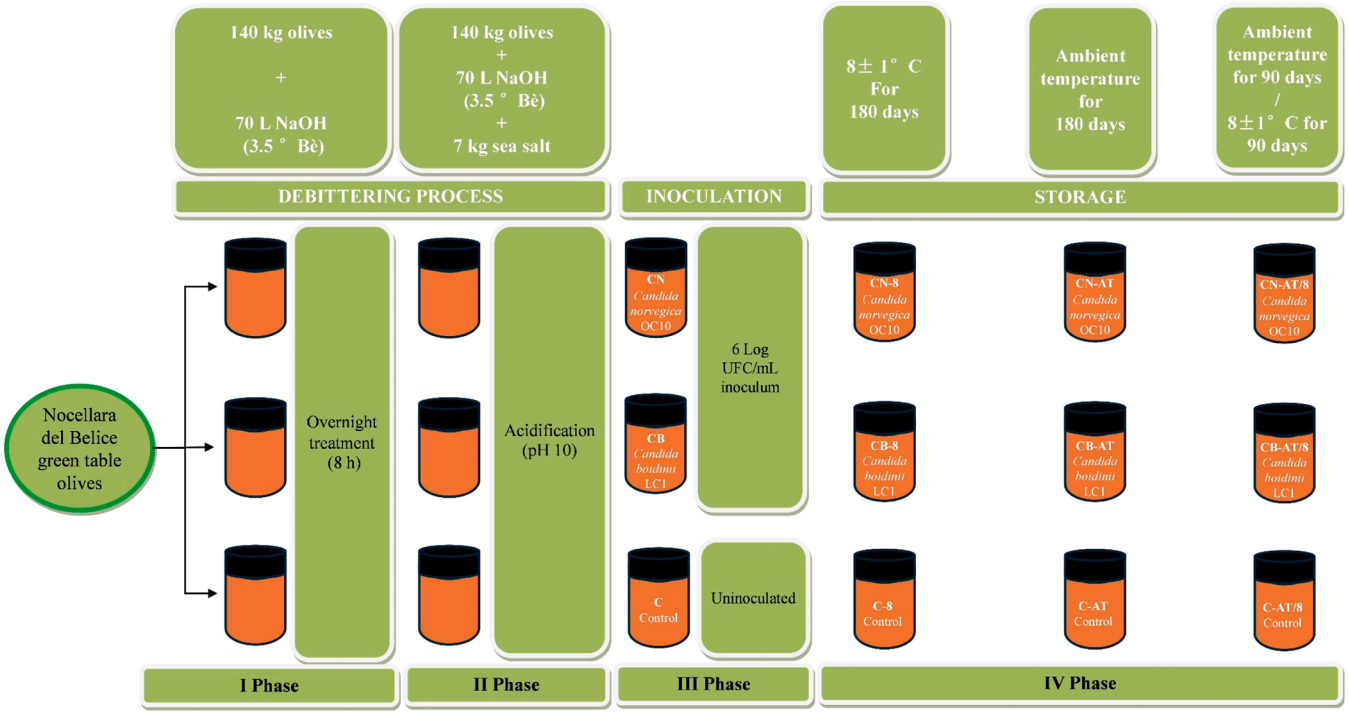

Fig. 1. Experimental design for the production of green table olives using the Castelvetrano method; Abbreviations: °Bé, Baumé degrees; C-8, control production not inoculated, stored at a temperature of 8 ± 1 °C for 180 days; CB-8, experimental production inoculated with *Candida boidinii* LC1, stored at a temperature of 8 ± 1 °C for 180 days; CN-8, experimental production inoculated with *Candida norvegica* OC10, stored at a temperature of 8 ± 1 °C for 180 days; C-AT, control production not inoculated, stored at ambient temperature for 180 days; CB-AT, experimental production inoculated with *C. boidinii* LC1, stored at ambient temperature for 180 days; CN-AT, experimental production inoculated with *C. norvegica* OC10, stored at ambient temperature for 180 days; C-AT/8, uninoculated control production, stored at ambient temperature for 90 days and then at 8 ± 1 °C for 90 days; CB-AT/8, experimental production inoculated with *C. boidinii* LC1, stored at ambient temperature for 90 days and then at 8 ± 1 °C for 90 days; CN-AT/8, experimental production inoculated with *C. norvegica* OC10, stored at ambient temperature for 90 days and then at 8 ± 1 °C for 90 days. Each experiment was carried out in triplicate.

was prepared and analysed independently, and samples were randomly assigned to the different storage conditions to minimise potential systematic error. For treatments stored at ambient temperature, environmental conditions were not actively controlled but were continuously monitored throughout the storage period to ensure realistic industrial conditions.

Yeast inocula (5 × 10^9 CFU/mL; Bionova Srl, Castel d'Azzano, Italy) were added at a rate of 20 mL per 100 kg of olives (28 mL per drum). Immediately after inoculation (day 0), yeast concentrations in the brine were approximately 6 Log CFU/mL. The sampling protocol involved the collection of both brine and olive samples for each treatment (n = 9) and each replicate (n = 3) using sterile disposable polypropylene jars (200 mL; Aptaca Spa, Canelli, Italy). Sampling was performed at 13 time points: day 0 and every 15 days up to day 180. Samples were transported under refrigerated conditions to the Department of Agricultural, Food and Forestry Sciences, University of Palermo, for microbiological and physicochemical analyses.

2.2. Microbiological analysis

Microbial populations were monitored throughout the 180-day storage period. Total mesophilic microorganisms were enumerated on Plate Count Agar (PCA) and incubated aerobically at 30 °C for 72 h (Tarantini et al., 2024). Lactic acid bacteria (LAB) were quantified using MRS agar for rod-shaped LAB and M17 agar for coccoid LAB, with incubation under anaerobic conditions at 30 °C for 48 h. Cycloheximide (0.1 g/L; Sigma-Aldrich, Milan, Italy) was added to the media to inhibit yeast and mould growth (Cirlincione et al., 2021). Yeasts were enumerated on Dichloran Rose Bengal Chloramphenicol (DRBC) Agar and incubated aerobically at 25 °C for 5 days (Beuchat, 1993). Enterobacteriaceae and coliforms were determined on Violet Red Bile Glucose Agar (VRBGA) and Violet Red Bile Agar (VRBA), respectively, following aerobic incubation at 37 °C for 24 h. Presumptive *Salmonella* and *Shigella* spp. were detected on Hektoen Enteric Agar (HEA) after incubation at 37 °C for 24 h (Busetta et al., 2023). Staphylococcaceae were enumerated on Mannitol Salt Agar (MSA) after aerobic incubation at 30 °C for 48 h (Fasolato et al., 2016). Pseudomonadaceae were cultivated on *Pseudomonas* Agar Base supplemented with CFC, incubated at 30 °C for 24 h (Aponte et al., 2012). Clostridia were assessed using the 3 × 3 Most Probable Number (MPN) method in Reinforced Clostridial Medium (RCM) (Sinacori et al., 2014).

All results were expressed as mean values of three independent replicates. All culture media and supplements were obtained from Condalab (Torrejón de Ardoz, Spain).

2.3. Dominance of *C. boidinii* LC1 and *C. norvegica* OC10

Yeast colonies exhibiting homogeneous macroscopic morphology were isolated and purified. Microscopic examination was performed to confirm morphological characteristics consistent with the genus *Candida*. Genomic DNA was extracted according to Leventdurur et al. (2016), and isolates were grouped based on restriction fragment length polymorphism (RFLP) analysis.

For RFLP analysis, PCR-amplified products were independently digested with the restriction endonucleases *Cfo*I, *Hae*III and *Hinf*I (Thermo Fisher Scientific, Vilnius, Lithuania). Each restriction reaction was performed in a final volume of 10 μL containing 3 μL of PCR product, 1 μL of 10 × Thermo Scientific™ Buffer R (Thermo Fisher Scientific, Vilnius, Lithuania), 0.1 μL of restriction enzyme (10 U/μL), and 5.9 μL sterile distilled water. Digestions were incubated at 37 °C overnight. Restriction fragments were separated by electrophoresis on 2.5% (w/v) agarose gels stained with SYBR™ Safe DNA Gel Stain

(Invitrogen™, Thermo Fisher Scientific, Carlsbad, CA, USA), using a GeneRuler™ 50 bp DNA Ladder (Thermo Fisher Scientific, Vilnius, Lithuania) as the molecular size marker.

Representative isolates from each RFLP group were further characterised by RAPD-PCR fingerprinting using the single primer M13 (5′-GAGGGTGGCGGTTCT-3′), following the protocols described by Bautista-Gallego et al. (2011) and Tofalo et al. (2013). PCR amplifications were performed in a final volume of 25 μL containing template DNA (2.5 ng/μL), PCR buffer supplemented with $MgCl_2$ (2.5 mM), dNTPs (0.2 mM each), M13 primer (2 μM), Taq DNA polymerase (0.625 U per reaction; Thermo Fisher Scientific, Vilnius, Lithuania), and sterile distilled water. The amplification programme consisted of an initial denaturation step at 94 °C for 2 min, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 42 °C for 20 s, and extension at 72 °C for 2 min, with a final extension at 72 °C for 10 min. RAPD-PCR amplicons were separated on 1.5% (w/v) agarose gels using a GeneRuler™ 100 bp Plus DNA Ladder (Thermo Fisher Scientific, Vilnius, Lithuania) as the molecular size marker.

Banding patterns were analysed using GelCompar II software (version 6.5; Applied Maths, Sint-Martens-Latem, Belgium) according to Guarcello et al. (2019). Dominance was calculated as the percentage of isolates exhibiting RAPD-PCR profiles identical to those of the inoculated strains.

2.4. Physicochemical parameters

Brine pH was measured using a Hanna Instruments HI98165 pH meter (Villafranca Padovana, Italy) by direct probe immersion (Alfonzo et al., 2023). Salinity in both brine and olive pulp was expressed as % NaCl and determined using a digital refractometer (DBS1, Giorgio Bormac Srl, Carpi, Italy) specifically designed for brine systems (Sidari et al., 2019). Brine temperature was recorded at each sampling point using a digital food thermometer (Euroxanty, Seville, Spain).

After six months of storage, olive colour was assessed using a Minolta Chroma Meter CR-400C (Osaka, Japan), following the method described by Martín-Vertedor et al. (2021). The colour parameters measured included L* (lightness), a* (red-green), b* (yellow-blue), chroma (C*) (Romeo et al., 2012), and total colour difference (ΔE*) (Sánchez et al., 2017). Pulp firmness was determined using a portable penetrometer (FT327), with results expressed as kg/cm^2 (Kaya et al., 2017). Measurements were taken at four equidistant points on 30 randomly selected olives (10 olives per replicate).

2.5. Analysis of volatile organic compounds

The analysis of volatile organic compounds (VOCs) was performed according to the methodology proposed by Martorana et al. (2015), with minor modifications as described below. Volatile compounds were isolated by headspace solid-phase microextraction (HS-SPME) and subsequently analysed by gas chromatography-mass spectrometry (GCMS–) using an Agilent 6890 gas chromatograph coupled to an Agilent 5973 N single-quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). For each analysis, 2 g of olive pulp were weighed into a 20 mL glass vial containing 1 g of sodium chloride, 8 mL of deionized water, and 0.20 mL of an internal standard solution (1-heptanol, 38.22 mg L^{-1}, prepared in 10% v/v absolute ethanol). Samples were equilibrated in a thermostatic water bath at 40 °C for 15 min. In parallel, the SPME fibre (50/30 μm DVB/CAR/PDMS; Supelco Inc., Bellefonte, PA, USA) was conditioned at 250 °C for 15 min. Extraction was performed by exposing the fibre to the headspace of the vial for 30 min at 40 °C. Subsequently, the analytes were thermally desorbed in the GC injection port, operated in splitless mode, at 250 °C for 2 min. Separation was achieved using a DB-WAX capillary column (Agilent Technologies; 30 m × 0.25 mm i.d., 0.25 μm film thickness), with helium as the carrier gas at a constant flow rate of 1 mL min^{-1}. The oven temperature programme was as follows: initial temperature of 40 °C held for 2 min; increase from 40 to 60 °C at 30 °C min^{-1} and held for 2 min; ramp from 60 to 190 °C at 2 °C min^{-1}; increase from 190 to 230 °C at 5 °C min^{-1}; and final holding at 230 °C for 15 min. The transfer line temperature was set at 230 °C. The mass spectrometer operated under electron ionization (EI) conditions at 70 eV, with data acquisition in scan mode over an m/z range of 30–350. Compound identification was achieved by comparing mass spectra with those contained in the W8N05ST mass spectral library. Semi-quantitative results were expressed as micrograms of 1-heptanol equivalents per kilogram of olive pulp.

2.6. Sensory analysis

Sensory evaluation was performed by a trained panel of 16 assessors (8 males and 8 females, aged 21–46 years), in accordance with ISO 13,299:2016. A total of 20 sensory attributes were assessed following the methodology described by Ambra et al. (2017), including colour, odour, bitterness, texture, flavour, and overall appreciation. Evaluation sessions were conducted in the mid-morning in a temperature-controlled sensory analysis room (20–22 °C) under standardised lighting conditions. Sensory attributes were rated using a structured 0–9 intensity scale.

Microbiological monitoring revealed the presence of Enterobacteriaceae and the development of colonies on Hektoen Enteric Agar consistent with presumptive *Salmonella/Shigella* spp.. However, no confirmatory tests were performed. For precautionary reasons, samples stored at ambient temperature (C-AT, CB-AT, CN-AT) were therefore excluded from sensory evaluation. This resulted in an incomplete dataset, which should be considered when interpreting sensory comparisons across different treatments. Consequently, sensory data refer exclusively to refrigerated treatments (C-8, CB-8, CN-8) and combined ambient/refrigerated treatments (C-AT/8, CB-AT/8, CN-AT/8).

According to the Institutional Ethics Committee of the University of Palermo, this non-invasive sensory study, conducted exclusively with adult assessors, without the collection of personal, clinical, or sensitive data, and involving no foreseeable risk to participants, is exempt from formal ethical approval under current institutional regulations. All assessors were fully informed about the objectives and procedures of the study and provided written informed consent prior to participation. All procedures complied with the principles of the Declaration of Helsinki and with ISO 13,299:2016.

2.7. Statistical analysis

The assumptions of normality and homoscedasticity required for analysis of variance (ANOVA) were verified prior to statistical analysis. Microbiological, physicochemical, and sensory data were analysed by ANOVA, and mean comparisons were performed using Tukey's post hoc test. Statistical significance was set at $P < 0.001$. This was adopted adopting a conservative threshold to limit type I errors associated with multiple comparisons. This approach also reflects the variability inherent in biological datasets generated under different processing conditions. All statistical analyses were carried out using XLStat software (version 2019.2.2; Addinsoft, New York, USA).

Microbiological counts of rod-shaped and coccoid LAB, yeasts, Enterobacteriaceae, coliforms, presumptive *Shigella/Salmonella* spp., Staphylococcaceae, and Pseudomonadaceae were monitored over 180 days in Nocellara del Belice olives processed according to the Castelvetrano method under nine experimental conditions: C-8, CB-8, CN-8, C-AT, CB-AT, CN-AT, C-AT/8, CB-AT/8, and CN-AT/8. For each microbial group, the area under the curve (AUC) was calculated using the trapezoidal method, and values were normalised to allow comparison among treatments.

The normalised data were subsequently used for multivariate principal component analysis (PCA), performed with XLStat software using the correlation matrix and standardised variables. PCA was applied to nine microbiological variables (total mesophilic microorganisms, yeasts,

coccoid LAB, rod-shaped LAB, Enterobacteriaceae, coliforms, *Shigella/Salmonella*, Staphylococcaceae, and Pseudomonadaceae) to identify relationships among treatments and the main sources of variability. Results were visualised using a biplot (F1 vs F2) displaying both variable vectors and observations labelled by treatment.

Agglomerative hierarchical clustering (AHC) was conducted to evaluate similarities among the six treatments subjected to sensory evaluation (C-8, CB-8, CN-8, C-AT/8, CB-AT/8, and CN-AT/8), integrating data related to volatile organic compounds (VOCs) and sensory attributes. Prior to clustering, data were standardised using z-score transformation (mean = 0; standard deviation = 1, calculated with n–1) to minimise the influence of differing measurement scales. Variables with zero variance were excluded to avoid singularities. Clustering was performed using Ward's method with Euclidean distance as the measure of dissimilarity. The optimal number of clusters was automatically determined by XLStat software using the entropy criterion, and results were displayed as a dendrogram.

2.8. Energy efficiency and emission reductions

The following analysis is based on a simplified modelling approach aimed at estimating potential energy savings and the associated greenhouse gas emissions. The environmental implications of reducing the refrigeration period from 180 to 90 days were assessed by estimating the avoided electricity consumption (ΔE, kWh) and the corresponding avoided indirect greenhouse gas emissions (ΔCO_2-eq, kg) associated with purchased electricity, adopting a Scope 2, location-based accounting framework. The assessment draws on cold-storage energy-use benchmarks and life-cycle assessment (LCA) hotspot reasoning focused on the use phase of refrigerated facilities. National average electricity grid emission factors were applied, consistent with datasets reported by the IEA (2024/2025), ISPRA (2024), and the EEA (2025).

Cold-room electricity demand was parameterised using the specific energy consumption (SEC), expressed as kWh m^{-3} $year^{-1}$. A methodological SEC range of 30–70 kWh m^{-3} $year^{-1}$, with a reference value of 50 kWh m^{-3} $year^{-1}$, was adopted to reflect the typical variability in cold-storage performance arising from differences in chamber volume, geometry, insulation quality, and utilisation rate (Lawrence et al., 2019; Wang et al., 2025). Electricity consumption for a given refrigerated volume was calculated using Eq. (1):

$$E = \text{SEC} \times V \times \frac{t}{365}$$

where E is electricity consumption (kWh), SEC is specific energy consumption (kWh/m^3/year), V is refrigerated volume (m^3), and t is storage duration (days).

Avoided electricity resulting from a 90-day reduction in refrigeration was calculated using Eq. (2):

$$\Delta E = \text{SEC} \times V \times \frac{(180-90)}{365} = \text{SEC} \times V \times \frac{90}{365}$$

where ΔE is electricity avoided (kWh).

Avoided emissions were then estimated using Eq. (3):

$$\Delta CO_{2,eq} = \Delta E \times EF_{IT}$$

where $\Delta CO_{2,eq}$ is avoided emissions (kg CO_2-eq) and EF_{IT} is the Italy average grid emission factor (0.27 - 0.35 kg CO_2-eq/kWh).

Because the actual cold-room volume was not available, calculations were conservatively based on the minimum packed volume of the experimental drums (5.67 m^3). This assumption was deliberately precautionary in order to avoid overestimating potential energy savings and to ensure methodological transparency and reproducibility.

3. Results

3.1. Microbial population dynamics

The evolution of microbial populations over the 180-day monitoring period is illustrated in Table S1. Immediately after inoculation (day 0), total mesophilic microorganisms were detected only in the yeast-inoculated trials (CB-8, CN-8, CB-AT, CN-AT, CB-AT/8, and CN-AT/8), with densities ranging from 6.1 to 6.2 Log CFU/mL. In contrast, the uninoculated controls (C-8, C-AT, and C-AT/8) exhibited levels below the detection limit (<2.0 Log CFU/mL). A progressive increase was observed across all treatments throughout the storage period.

Between days 90 and 105, a decrease in mesophilic microorganisms was recorded in trials where the storage temperature was reduced from ambient to 8 ± 1 °C. This reduction was most pronounced in the C-AT/8 trial (−0.4 Log CFU/mL), followed by CN-AT/8 (−0.3 Log CFU/mL) and CB-AT/8 (−0.2 Log CFU/mL). In trials stored continuously at either ambient or refrigerated conditions, only minor increases (0.1–0.3 Log CFU/mL) were observed over the same interval. At day 180, mesophilic counts ranged from 8.1 to 9.7 Log CFU/mL.

LAB were detected from day 15 onwards in all trials. On day 15, rod-shaped LAB concentrations ranged from 3.7 Log CFU/mL (CB-8) to 7.5 Log CFU/mL (CB-AT/8), while coccus LAB ranged from 5.5 Log CFU/mL (CN-8) to 7.4 Log CFU/mL (CB-AT and CN-AT). Both LAB morphotypes exhibited an overall increasing trend, although a decline was observed in trials where the temperature was lowered after 90 days. The most marked reductions occurred in C-AT/8: −0.6 Log CFU/mL (rods) and −0.5 Log CFU/mL (cocci). At day 180, the highest rod-shaped LAB levels were found in CN-AT (8.9 Log CFU/mL), CB-AT (8.8), and C-AT/8 (8.8), while coccus LAB exceeded 9.0 Log CFU/mL in CB-8 and CB-AT.

Yeast concentrations at day 0 were highest in inoculated samples (6.1–6.2 Log CFU/mL). After 15 days, yeast populations increased by 0.5–1.7 Log CFU/mL, depending on the treatment. In uninoculated trials, concentrations ranged from 3.2 Log CFU/mL (C-8) to 3.7 Log CFU/mL (C-AT). Between days 90 and 105, yeast populations decreased in C-AT/8 (−0.4 Log CFU/mL), while inoculated trials showed slight increases (+0.1 Log CFU/mL). At day 180, CB-AT reached 7.4 Log CFU/mL, whereas C-AT/8 recorded 5.6 Log CFU/mL.

Pseudomonadaceae were detected from day 15, with initial concentrations ranging from 2.3 to 3.9 Log CFU/mL. In the control trials (C-8, C-AT, and C-AT/8), Pseudomonadaceae persisted until day 180. In inoculated trials, their presence was limited to day 15–105 (CB-AT, CN-AT), day 15–75 (CB-AT/8, CN-AT/8), and day 15–60 (CB-8, CN-8). At day 180, C-AT recorded the highest Pseudomonadaceae levels (4.6 Log CFU/mL), while C-8 and C-AT/8 showed lower values (2.0 and 2.2 Log CFU/mL, respectively).

Staphylococcaceae were first detected on day 15 (2.4–4.0 Log CFU/mL). A positive trend was observed only in C-AT, while all other trials showed a decrease. This group persisted until day 180 in C-8, C-AT, and C-AT/8. In inoculated trials, persistence varied: up to 150 days in CN-8, CN-AT, and CN-AT/8; 135 days in CB-8 and CB-AT; and 120 days in CB-AT/8. At day 180, the highest levels were found in C-AT (5.0 Log CFU/mL), with lower values in C-8 (2.2 Log CFU/mL) and C-AT/8 (2.4 Log CFU/mL). No colonies resembling *Staphylococcus aureus* were observed.

Enterobacteriaceae occurrence was influenced by both yeast inoculation and storage temperature. In C-8, they were detected up to day 30, with a decreasing trend (from 2.1 to 1.5 Log CFU/mL). In contrast, trials stored at ambient temperature showed a positive trend. In C-AT, Enterobacteriaceae persisted from day 15 to 180 (2.2–4.0 Log CFU/mL). CB-AT and CN-AT showed intermittent presence, particularly during the final stages of storage. Coliforms followed a similar pattern, albeit at lower counts.

Presumptive colonies consistent with *Salmonella* spp. (blue-green with black centres) were observed on HEA in CN-AT (day 135) and C-AT (day 165). As colonies observed on HEA should be considered presumptive for *Salmonella* and *Shigella*, and confirmatory tests (PCR or

serological) were not performed, definitive conclusions regarding microbiological stability under ambient storage conditions cannot be drawn. Presumptive *Escherichia coli*(salmon to orange colonies) appeared in C-AT (day 150), CN-AT (day 165), and in all ambient-stored trials by day 180. Green, translucent colonies resembling *Shigella* spp. were also frequently observed. No clostridia were detected during the monitoring period.

Multivariate analysis using PCA (Fig. 2) revealed a clear separation between treatments along the two principal axes, which together explained 87.69% of the total variability. The biplot shows that Enterobacteriaceae, coliforms, *Shigella/Salmonella*, Staphylococcaceae, and Pseudomonadaceae are strongly associated with the positive side of F1, where the C-AT and C-AT/8 treatments are located. This pattern indicates that the absence of inoculation and storage at ambient temperature favour the proliferation of undesirable and potentially pathogenic microorganisms. Conversely, treatments C-8 and CN-8, positioned in opposite quadrants, are negatively correlated with these variables, confirming that storage at 8 ± 1 °C effectively limits contamination.

Axis F2 was mainly correlated with total mesophilic microorganisms, rod-shaped LAB, coccus LAB, and yeasts, reflecting the typical olive microbiota. CB-AT and CN-AT were located on the positive side of this axis, highlighting that inoculation with *C. boidinii* and *C. norvegica* contributes to microbiological stability. Refrigerated inoculated treatments (CB-8 and CN-8) and combined treatments (CB-AT/8 and CN-AT/8) occupied intermediate positions, suggesting a balance between reduced contamination and limited metabolic activity of indigenous microorganisms.

Overall, PCA confirms that storage temperature is the main discriminating factor. Cold conditions reduce both microbial activity and contamination, while yeast inoculation at ambient temperature contributes partially to product stability. These results indicate that microbial instability is primarily driven by temperature, whereas the effect of yeast inoculation is secondary and becomes more relevant under controlled storage conditions. Taken together, the PCA suggests that yeast inoculation helps shape a more stable and predictable microbiota under non-fermentative Castelvetrano conditions. The absence of inoculation under ambient conditions increases the risk of contamination, while combined treatments show intermediate profiles, with a lower presence of pathogens than C-AT but a more limited microbial evolution than CB-AT and CN-AT.

3.2. Dominance of inoculated yeasts

A total of 818 yeast isolates were obtained from Petri dishes at the highest serial dilutions during microbiological monitoring of olive brine samples from the different experimental trials. Colonies were purified by streaking on the same culture medium (DRBC) and examined microscopically. Of the 818 isolates, 77 were obtained from the CB-8 trial, 84 from CN-8, 97 from CB-AT, 109 from CN-AT, 90 from CB-AT/8, and 101 from CN-AT/8. In addition, 81, 91, and 88 isolates were recovered from the control trials C-8, C-AT, and C-AT/8, respectively.

Amplicon sizes of the 5.8S-ITS region varied according to the experimental trial. In trials inoculated with *C. boidinii* LC1 (CB-8, CB-AT, and CB-AT/8), 241 isolates showed an amplicon size of approximately 700 bp, while the remaining 23 displayed different sizes (400 and 650 bp). In trials inoculated with *C. norvegica* OC10 (CN-8, CN-AT, and CN-AT/8), 260 isolates exhibited an amplicon size of 580 bp, whereas the remaining 34 showed distinct sizes (400, 650, and 850 bp). Amplicon sizes of 580 and 700 bp were also detected among isolates recovered from the control trials. However, subsequent RAPD-PCR analysis confirmed that none of these isolates shared a polymorphic profile with the inoculated strains.

Figure S1 illustrates the variation in dominance of the inoculated strains (*C. boidinii* LC1 and *C. norvegica* OC10) across the three

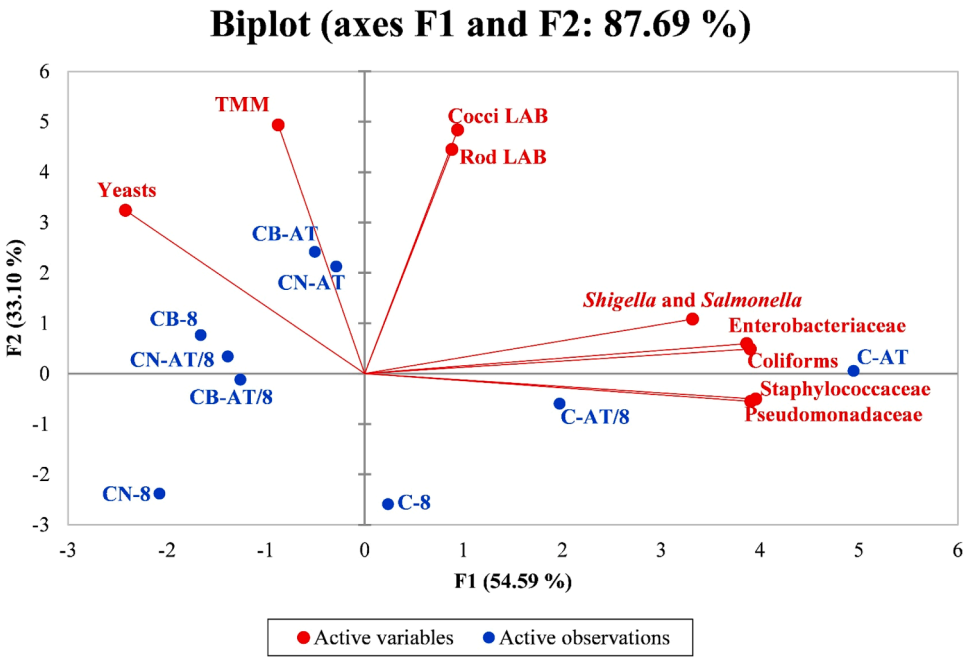

Fig. 2. Biplot of principal components (F1 vs F2) obtained from PCA analysis on normalised AUCs of microbial populations (F1 = 54.59%; F2 = 33.10%). Blue dots represent active observations (treatments), while red dots indicate active variables (microbial groups). The arrows indicate the direction and correlation of the variables with the principal axes. Abbreviations: TMM, Total mesophilic microorganisms; C-8, control production not inoculated, stored at a temperature of 8 ± 1 °C for 180 days; CB-8, experimental production inoculated with *Candida boidinii* LC1, stored at a temperature of 8 ± 1 °C for 180 days; CN-8, experimental production inoculated with *Candida norvegica* OC10, stored at a temperature of 8 ± 1 °C for 180 days; C-AT, control production not inoculated, stored at ambient temperature for 180 days; CB-AT, experimental production inoculated with *C. boidinii* LC1, stored at ambient temperature for 180 days; CN-AT, experimental production inoculated with *C. norvegica* OC10, stored at ambient temperature for 180 days; C-AT/8, uninoculated control production, stored at ambient temperature for 90 days and then at 8 ± 1 °C for 90 days; CB-AT/8, experimental production inoculated with *C. boidinii* LC1, stored at ambient temperature for 90 days and then at 8 ± 1 °C for 90 days; CN-AT/8, experimental production inoculated with *C. norvegica* OC10, stored at ambient temperature for 90 days and then at 8 ± 1 °C for 90 days.

temperature regimes examined (8 ± 1 °C, AT/8, and AT). Both strains showed high dominance under low-temperature conditions, with values exceeding 94% at 8 ± 1 °C (97.40% for *C. boidinii* LC1 and 94.05% for *C. norvegica* OC10). Under the AT/8 condition, dominance remained relatively stable, reaching 94.40% and 93.07%, respectively. In contrast, storage at ambient temperature for 180 days resulted in a marked reduction in dominance, which decreased to 83.51% for *C. boidinii* LC1 and 79.82% for *C. norvegica* OC10. Overall, these results indicate that storage temperature strongly affects the ability of the inoculated strains to dominate the olive microbiota, with greater stability achieved under refrigerated or combined storage conditions.

3.3. Salinity, temperature and pH

The results of physicochemical monitoring are shown in Fig. 3. Initial salt concentrations in the brine averaged 8.99 ± 0.23%, while slightly higher values were recorded in the pulp (9.39 ± 0.33%). No significant differences were observed among treatments. Salt levels fluctuated during the storage period, ranging from 7.0 to 9.2% in the brine and from 6.9 to 9.4% in the pulp.

Brine temperatures were recorded fortnightly to evaluate thermal variations from October 2023 to April 2024. Treatments stored under refrigeration (C-8, CB-8, and CN-8) maintained average temperatures of 8 ± 1 °C, as did treatments transferred to cold storage after 90 days (C-AT/8, CB-AT/8, and CN-AT/8). In contrast, samples stored at ambient temperature (C-AT, CB-AT, and CN-AT) exhibited a gradual temperature decrease during the first 90 days, from 22.23 ± 0.10 °C in October to 12.42 ± 0.34 °C in January. A slight increase was observed between days 105 and 120 (14.63 ± 0.42 °C), followed by a further decrease between days 120 and 150 (13.7 ± 0.20 °C). By day 180, the average brine temperature reached 17.7 ± 0.46 °C.

Acidification dynamics showed consistently lower pH values in ambient-stored treatments compared to refrigerated ones. Following an initial average pH of 10.04 ± 0.02, differences emerged after 15 days. In cold-stored trials (C-8, CB-8, and CN-8), pH values remained above 6 up to day 30, whereas ambient-stored samples ranged from 4.94 to 5.86. By day 135, brine pH in refrigerated treatments had decreased to 5.64 ± 0.35, while all other trials recorded values between 4.60 and 4.92. At day 180, pH values remained above 5 in refrigerated samples, while ambient-stored treatments averaged 4.75 ± 0.32. Overall, a gradual acidification trend was observed across all trials.

3.4. Colour and pulp firmness

The data relating to colour and firmness are reported in Table 1. Significant differences were observed for all colour parameters (L*, a*,

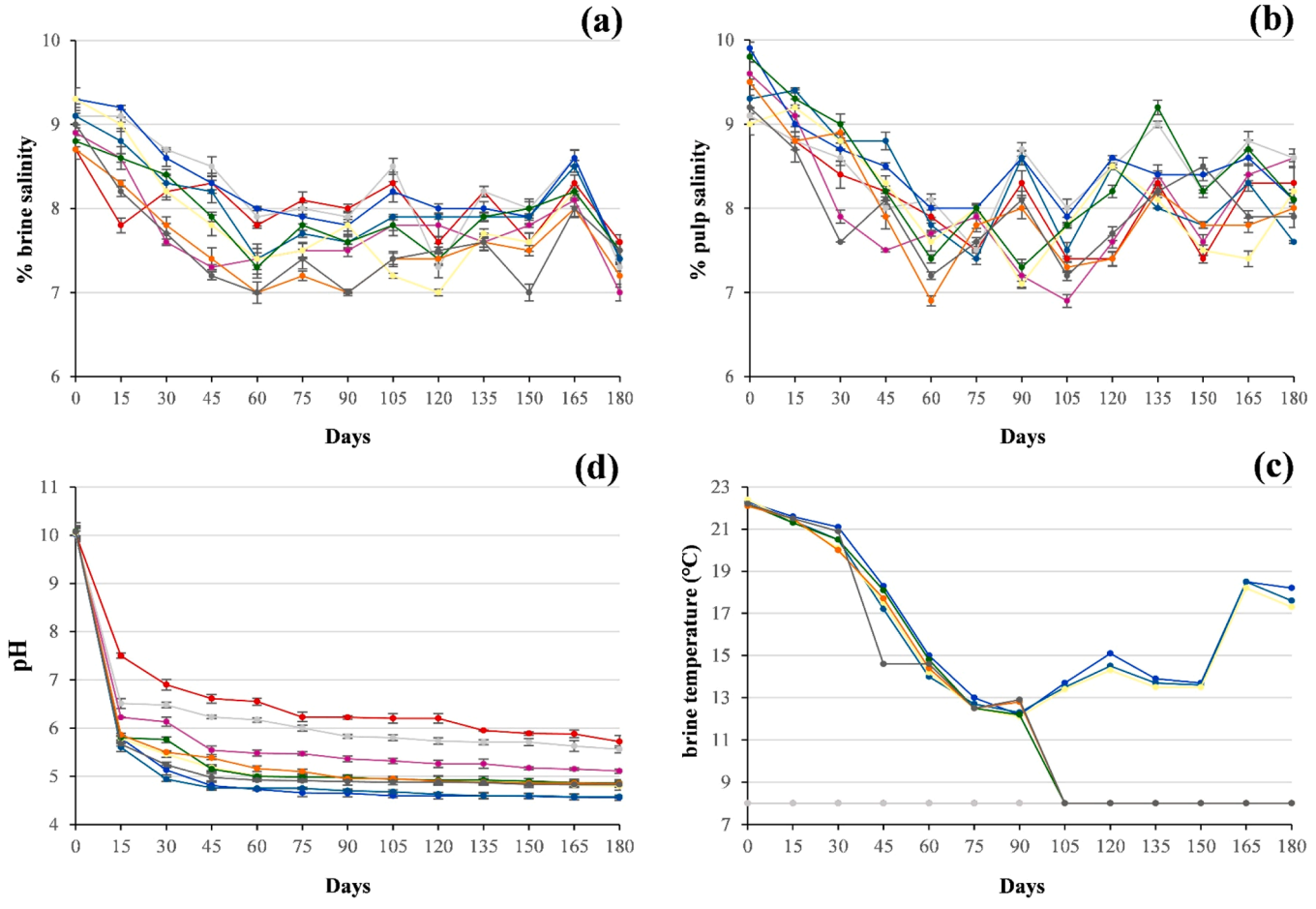

Fig. 3. Trend of the chemical-physical parameters of experimental productions of Nocellara del Belice table olives carried out according to the Castelvetrano method: **(a)** % salt detected in the brine; **(b)** % salt determined in the drupe pulp; **(c)** brine temperature; **(d)** pH. Symbols: ●, C-8, non-inoculated control production, stored at 8 ± 1 °C for 180 days; ●, CB-8, experimental production inoculated with *Candida boidinii* LC1, stored at 8 ± 1 °C for 180 days; ●, CN-8, experimental production inoculated with *Candida norvegica* OC10, stored at 8 ± 1 °C for 180 days; ●, C-AT, control production not inoculated, stored at ambient temperature for 180 days; ●, CB-AT, experimental production inoculated with *C. boidinii* LC1, stored at ambient temperature for 180 days; ●, CN-AT, experimental production inoculated with *C. norvegica* OC10, stored at ambient temperature for 180 days; ●, C-AT/8, control production not inoculated, stored at ambient temperature for 90 days and then at 8 ± 1 °C for 90 days; ●, CB-AT/8, experimental production inoculated with *C. boidinii* LC1, stored at ambient temperature for 90 days and then at 8 ± 1 °C for 90 days; ●, CN-AT/8, experimental production inoculated with *C. norvegica* OC10, stored at ambient temperature for 90 days and then at 8 ± 1 °C for 90 days.

Table 1
Drupe colour and pulp firmness determined on Nocellara del Belice table olives produced using the Castelvetrano method after 6 months of storage under different temperature conditions.

Treatment	Drupe colour					Pulp firmness (kg/cm^2)
	L*	a*	b*	C*	ΔE*	
C-8	40.13 ±1.51 d	−6.84 ±0.98 bcd	25.58 ±0.79 d	26.48 ±0.89 c	48.08 ±1.09 d	18.35 ± 0.25 c
CB-8	45.36 ±1.20 c	−8.21 ±0.43 cd	29.54 ±1.03 cd	30.66 ±0.73 b	54.75 ±0.89 c	22.86 ± 0.48 a
CN-8	46.55 ±1.17 c	−8.77 ±0.78 d	29.69 ±1.70 cd	30.96 ±1.24 b	55.90 ±1.22 c	23.12 ± 0.50 a
C-AT	52.41 ±0.72 a	−4.70 ±0.16 ab	30.61 ±1.27 bc	30.97 ±0.72 b	60.88 ±0.72 ab	17.79 ± 0.12 c
CB-AT	51.05 ±1.38 ab	−5.05 ±0.19 ab	33.25 ±1.69 abc	33.63 ±0.94 ab	61.13 ±1.09 ab	19.08 ± 0.41 c
CN-AT	53.08 ±1.06 a	−5.20 ±0.55 ab	33.38 ±1.59 abc	33.79 ±1.07 ab	62.92 ±1.07 a	21.44 ± 0.12 b
C-AT/8	48.01 ±1.31 bc	−4.26 ±1.14 a	31.84 ±1.06 abc	32.12 ±1.10 ab	57.77 ±1.17 bc	18.21 ± 0.59 c
CB-AT/8	52.28 ±0.66 ab	−7.26 ±0.95 bc	35.57 ±1.01 ab	36.30 ±0.98 a	63.65 ±0.87 ab	22.13 ± 0.62 ab
CN-AT/8	51.86 ±0.89 ab	−6.44 ±0.43 bc	35.26 ±1.30 a	35.85 ±0.87 a	63.05 ±0.88 a	22.25 ± 0.80 ab
S.S.	***	***	***	***	***	***

The results are expressed as the mean value (± standard deviation) of 30 drupes. Data in columns with the same letter are not significantly different by Tukey test. P value: ***, $P < 0.001$ (Tukey's test after ANOVA).
Abbreviations: C-8, control production not inoculated, stored at 8 ± 1 °C for 180 days; CB-8, experimental production inoculated with *Candida boidinii* LC1, stored at 8 ± 1 °C for 180 days; CN-8, experimental production inoculated with *Candida norvegica* OC10, stored at 8 ± 1 °C for 180 days; C-AT, control production not inoculated, stored at ambient temperature for 180 days; CB-AT, experimental production inoculated with *C. boidinii* LC1, stored at ambient temperature for 180 days; CN-AT, experimental production inoculated with *C. norvegica* OC10, stored at ambient temperature for 180 days; C-AT/8, non-inoculated control production, stored at ambient temperature for 90 days and then at 8 ± 1 °C for 90 days; CB-AT/8, experimental production inoculated with *C. boidinii* LC1, stored at ambient temperature for 90 days and then at 8 ± 1 °C for 90 days; CN-AT/8, experimental production inoculated with *C. norvegica* OC10, stored at ambient temperature for 90 days and then at 8 ± 1 °C for 90 days.

b*, C*, and ΔE*). Lightness (L*) values were higher in trials stored at ambient temperature (C-AT: 52.41; CN-AT: 53.08) and lower in control samples stored under refrigerated conditions (C-8: 40.13). Intermediate values were observed in the remaining treatments, ranging from 45.36 (CB-8) to 52.28 (CB-AT/8). The a* parameter (red-green axis) indicated a greener colour in products stored at low temperatures (e.g. CN-8: −8.77; CB-8: −8.21), whereas controls stored at ambient temperature exhibited less negative values (C-AT: −4.70; C-AT/8: −4.26), suggesting a slight shift towards red hues. However, inoculated samples subjected to the combined storage regime retained a relatively green colour (CB-AT/8: −7.26; CN-AT/8: −6.44). The b* parameter (yellow-blue axis) revealed progressive yellowing, with the lowest values observed in cold-stored controls (C-8: 25.58) and the highest values in inoculated trials (CB-AT/8: 35.57; CN-AT/8: 35.26), indicating that exposure to ambient temperature favoured yellow colour development. Colour saturation (C*) was higher in CB-AT/8 and CN-AT/8, suggesting more intensely coloured drupes, whereas C-8 appeared visibly paler (26.48). Total colour difference (ΔE*) showed marked variation among storage regimes: refrigerated trials exhibited lower average values (approximately 52.9; range 48.08–55.90), while samples stored under ambient or combined conditions showed greater differences (approximately 61.6 and 61.5, respectively). The combined storage regime displayed the widest variability (57.77–63.65).

Pulp firmness was highest in CN-8 (23.12 kg/cm^2) and CB-8 (22.86 kg/cm^2). Slightly lower values were recorded in CB-AT/8 (22.13 kg/cm^2), CN-AT/8 (22.25 kg/cm^2), and CN-AT (21.44 kg/cm^2). Significantly lower firmness values (17.79–19.08 kg/cm^2) were observed in all control samples (C-8, C-AT, and C-AT/8), as well as in CB-AT. *C. norvegica* OC10 consistently improved pulp texture across all storage regimes. *C. boidinii* LC1 produced comparable results under refrigerated conditions, although its effectiveness was reduced in samples stored at ambient temperature.

3.5. Volatile organic compounds

VOC profiles of Nocellara del Belice olives showed significant differences among the tested treatments, mainly driven by storage temperature and, to a lesser extent, by the inoculation of bioprotective yeasts (Table 2). Samples stored under refrigerated conditions and inoculated with yeasts exhibited higher relative levels of aromatic esters and alcohols, including isoamyl acetate and 2-phenylethanol. These compounds are typically associated with fresher and more pleasant aromatic notes and reflect selective metabolic activity favoured by low-temperature conditions and reduced oxidative phenomena. Conversely, storage at ambient temperature resulted in a pronounced accumulation of volatile acids, particularly acetic, propionic, and butyric acids, as well as phenolic derivatives and compounds linked to oxidative processes. The occurrence of these molecules suggests the activation of secondary metabolic pathways and increased microbial complexity, with potentially detrimental effects on sensory quality, often associated with fermentative notes or product defects.

The combined storage regime (AT/8) yielded intermediate VOC profiles, characterised by the coexistence of compounds formed during the initial ambient-temperature phase and those typically associated with refrigerated storage. In these treatments, the transition to refrigeration effectively limited the further accumulation of volatile acids, highlighting the stabilising role of low temperatures during the final stages of storage.

Overall, the results indicate that storage temperature represents the primary determinant of VOC composition and, consequently, of the aromatic profile of the product. The addition of bioprotective yeasts appears to modulate the balance among different classes of volatile compounds, contributing to a shift towards more stable and potentially higher-quality aromatic profiles, particularly under refrigerated conditions.

3.6. Sensory assessment

The sensory results are reported in Table 3. Sensory analysis was conducted only on refrigerated samples and those subjected to the combined treatment (AT/8), as samples stored at ambient temperature were excluded as a precautionary measure. Consequently, the sensory dataset was not fully complete. The analysis revealed significant differences among treatments for colour, olive odour, overall odour, sweetness, acidity, bitterness, pungency, chemical flavour, crispness, hardness, overall flavour, and overall appreciation.

The highest colour scores were recorded for CB-AT/8 (6.71), CN-AT/8 (6.61), and CN-8 (6.60), while the lowest value was observed in C-AT/8 (4.55). CN-AT/8 exhibited the most intense olive odour (5.57), which was also reflected in the overall odour scores. Sweetness was the most appreciated attribute, with values ranging from 7.47 to 7.99 in the inoculated samples, compared with 6.22 in C-8 and 5.74 in C-AT/8. Acidity was minimal in C-8 (1.83), whereas in the other treatments it ranged from 2.47 to 2.99. Bitterness generally showed low values, from

Table 2
Volatile organic compounds (VOCs) detected in Nocellara del Belice olives processed using the Castelvetrano method at the end of the monitoring period (180 days).

Volatile organic compounds	Experimental productions[1]									S. S.[2]
	C-8	CB-8	CN-8	C-AT	CB-AT	CN-AT	C-AT/8	CB-AT/8	CN-AT/8	
Acetic acid	1697.1 ± 330.6de	1239.3 ± 314.3de	643.4 ± 139.5e	8550.7 ± 980.2a	6134.8 ± 1155.7b	2796.8 ± 175.7cd	3844.2 ± 372.5c	5780.5 ± 197.2b	9730.7 ± 1025.3a	***
Propanoic acid	n.d. d	n.d. d	n.d. d	211.4 ± 17.9b	214.1 ± 9.4b	694.2 ± 79.8a	55.3 ± 6cd	57.4 ± 3.8cd	100.6 ± 13.6c	***
Butanoic acid	n.d. d	n.d. d	n.d. d	51.2 ± 2.3ab	59.8 ± 4.8a	37.7 ± 6.3bc	11.9 ± 1.9d	33.8 ± 9.4c	39.5 ± 9.6bc	***
∑ Acids	**1697.1 ± 330.6de**	**1239.3 ± 314.3e**	**643.4 ± 139.5e**	**8813.3 ± 1000.4a**	**6408.7 ± 1169.9b**	**3528.7 ± 261.8cd**	**3911.4 ± 380.5c**	**5871.8 ± 210.4b**	**9870.7 ± 1048.4a**	***
cis-2-heptenal	50.8 ± 10.4bcd	32±4.8cd	28.3 ± 0.1cd	67.8 ± 18.4b	100.9 ± 16.1a	38.5 ± 6.6bcd	105.3 ± 11.1a	58.9 ± 10.5bc	24.5 ± 4.9d	***
Nonanal	38.2 ± 4cd	93.4 ± 0.4a	90.2 ± 10a	40.7 ± 1.8cd	74.1 ± 20.7ab	51.8 ± 6.4bc	25.1 ± 1.2d	40.1 ± 0.9cd	56.6 ± 9.3bc	***
Benzaldehyde	71.1 ± 19.4f	155±1.6d	215.9 ± 6.2ab	171.8 ± 5.8cd	238.7 ± 15a	212.2 ± 10.4ab	223.8 ± 3.9ab	190.2 ± 21.1bc	118.3 ± 5.7e	***
∑ Aldehydes	**160.1 ± 33.8c**	**280.4 ± 6.8b**	**334.4 ± 16.4ab**	**280.3 ± 26.0b**	**413.7 ± 51.8a**	**302.4 ± 23.5b**	**354.1 ± 16.1ab**	**289.3 ± 32.5b**	**199.4 ± 19.9c**	***
Isoamyl acetate	29.6 ± 3.3de	28.2 ± 3.1de	165.2 ± 16.5a	23.8 ± 1.9de	27.9 ± 0.3de	60.5 ± 1.4c	12.5 ± 2.7e	37.5 ± 0.3cd	95.1 ± 19.1b	***
Ethyl hexanoate	n.d. d	7.9 ± 2.4bcd	5.2 ± 1.6cd	15.4 ± 1.5bc	10.0 ± 2.3bcd	10.5 ± 2.4bcd	7.2 ± 3.1bcd	18.2 ± 2.9b	36.3 ± 11.1a	***
Hexenyl acetate	68.7 ± 8.4de	76.5 ± 14.2de	47±6.7e	78.5 ± 23d	143.8 ± 8.3ab	127.2 ± 0.9ab	157.7 ± 10.7a	92.1 ± 1.3cd	116±5.4bc	***
Cyclohexyl isobutyrate	13.2 ± 0.9e	21.9 ± 2.8cde	20.3 ± 1.6de	44.1 ± 2.9abc	62.5 ± 19.8a	31.1 ± 2.8bcde	36.7 ± 10.2bcd	48.5 ± 1.4ab	36.2 ± 11.8bcd	***
Methyl octanoate	39.5 ± 4.8c	45.1 ± 5.5c	37.3 ± 5.1c	95.5 ± 5.1a	76.8 ± 15.4ab	39.9 ± 4.9c	59.0 ± 21.3bc	75.4 ± 4.9ab	65.0 ± 6.8bc	***
Ethyl octanoate	15.9 ± 1d	31.8 ± 0.1cd	18.0 ± 4.7cd	54.4 ± 10b	78.7 ± 3.9a	57.6 ± 8.5b	76.3 ± 4.5a	64.6 ± 8.7ab	34.3 ± 2.9c	***
Ethyl 7-octanoate	n.d. b	n.d. b	n.d. b	n.d. b	n.d.b	n.d. b	27.3 ± 1.5a	n.d. b	n.d. b	***
Benzyl acetate	n.d. e	n.d. e	n.d. e	43.9 ± 5.3cd	69.2 ± 4.1b	68.0 ± 8.8b	98.4 ± 15.6a	49.9 ± 12.3bc	22.4 ± 2.7d	***
∑ Esters	**166.8 ± 18.4c**	**211.4 ± 28c**	**292.9 ± 36.2bc**	**355.5 ± 49.8ab**	**468.9 ± 54.0a**	**394.9 ± 29.7ab**	**475.3 ± 69.5a**	**386.3 ± 31.9ab**	**405.3 ± 59.8ab**	***
6-methyl-5-heptene-2-one	n.d. e	52.7 ± 14.0ab	44.6 ± 14.4abc	34.9 ± 7.5bcd	n.d.e	16.5 ± 3.7de	18.3 ± 4.6cde	41.2 ± 6.2abcd	64.1 ± 15.4a	***
∑ Ketones	**n.d. e**	**52.7 ± 14.0ab**	**44.6 ± 14.4abc**	**34.9 ± 7.5bcd**	**n.d.e**	**16.5 ± 3.7de**	**18.3 ± 4.6cde**	**41.2 ± 6.2abcd**	**64.1 ± 15.4a**	***
2-butanol	19.8 ± 0.3ab	26.2 ± 0.4a	18.1 ± 5.3b	25.9 ± 2.9a	17.0 ± 2.9b	20.1 ± 1.6ab	18.0 ± 0.0b	14.5 ± 2.5b	26.7 ± 1.1a	***
1-butanol	8.0 ± 0.0abc	10.8 ± 0.6abc	13.9 ± 3.5a	5.3 ± 0.9c**	11.8 ± 0.1abc	7.0 ± 0.8bc	11.3 ± 1.3abc	9.4 ± 0.1abc	13.1 ± 5.5ab	**
Isoamyl alcohol	21.8 ± 0.9b	61.8 ± 13.1b	734.1 ± 148.8a	24.4 ± 2.7b	51.7 ± 16.5b	41.3 ± 4.1b	22±6.9b	36.5 ± 2.5b	117.3 ± 5.9b	***
1-hexanol	121.9 ± 14.2bcd	79.6 ± 0.5e	104.4 ± 0.4cde	144.6 ± 7.5bc	149.4 ± 35.6b	93.3 ± 2.5de	145.0 ± 4.6bc	134.8 ± 4.6bcd	215.4 ± 19.3a	***
(E)-3-hexenol	n.d. c	19.5 ± 2.8a	n.d. c	n.d. c	4.7 ± 0.6b	n.d. c	n.d. c	n.d. c	17.2 ± 1.7a	***
(Z)-3-hexenol	182.9 ± 15.3e	262.0 ± 1.1e	405.3 ± 43.0d	604.4 ± 33.2c	550.6 ± 19.5c	371.4 ± 8.9d	705.4 ± 2.9b	539.5 ± 16.9c	860.7 ± 80.4a	***
1-octanol	11.2 ± 1.3b	69.8 ± 5.2ab	117.1 ± 24.1a	87.1 ± 20.8ab	83.7 ± 10.5ab	76.8 ± 6.9ab	96.9 ± 2.1a	81.7 ± 12.0ab	59.6 ± 5.2ab	**
Benzyl alcohol	96.9 ± 21.1b	254.2 ± 36.1b	493.7 ± 110.2a	537.8 ± 78.6a	466.7 ± 36.5a	462.3 ± 21.9a	634.7 ± 14.0a	595.4 ± 99.5a	455.0 ± 82.2a	***
2-phenylethanol	586.8 ± 159.8e	1251.4 ± 193.1d	918.5 ± 212.9de	1323.6 ± 263.5cd	2040.2 ± 109.1a	1852.0 ± 130.6ab	1839.2 ± 10.3abc	1783.8 ± 304.2abc	1445.1 ± 208.3bcd	***
Homoguaiacol	n.d. d	n.d. d	n.d. d	4860.6 ± 1189.2b	6566.9 ± 333.8b	8471.4 ± 1064.7a	5054.2 ± 137.9b	5037.9 ± 773.5b	2587.9 ± 287.2c	***
∑ Alcohols	**1049.2 ± 212.9d**	**2035.3 ± 252.9d**	**2805.1 ± 548.3d**	**7613.5 ± 1599.3bc**	**9942.7 ± 565.2ab**	**11,395.7 ± 1242.1a**	**8526.6 ± 180.0b**	**8233.5 ± 1215.8bc**	**5798.1 ± 696.8c**	***
α-cubenene	172.2 ± 36.7cde	324.7 ± 10.2b	188.5 ± 10.6cd	121.4 ± 14.9e	399.9 ± 35.3a	204±30.1cd	212.5 ± 0.9c	227.4 ± 15.9c	140.4 ± 24.1de	***
Copaene	n.d. d	n.d. d	13.3 ± 3.0c	n.d. d	20.9 ± 0.7b	35.3 ± 5.2a	19.2 ± 0.1bc	17.3 ± 3.8bc	n.d. d	***
α-muurolene	39.3 ± 10.4cd	96.9 ± 24.7ab	55.6 ± 16.4cd	20.8 ± 4.2d	126.8 ± 15.6a	57.7 ± 3.8bcd	71±21.8bc	71.2 ± 6.4bc	19.9 ± 4.9d	***
∑Sesquiterpenes	**211.4 ± 47.1def**	**421.6 ± 34.9b**	**257.4 ± 30cde**	**142.2 ± 19.1f**	**547.6 ± 51.7a**	**296.9 ± 39cd**	**302.7 ± 22.8cd**	**315.9 ± 26.1c**	**160.3 ± 29ef**	***

Results are expressed as μg equivalents of 1-heptanol per kg of olives (mean ± standard deviation, n = 3).
n.d., not detected.
Statistical analyses were performed row-wise for each compound to assess significant differences among treatments. Different letters within a row indicate significant differences ($P < 0.001$).
Statistical analyses were performed row-wise for each compound to assess significant differences among treatments. Different letters within a row indicate significant differences ($P < 0.001$).

[1] Experimental productions: C-8 = uninoculated olives stored at 8 °C for 180 days; CB-8 = olives inoculated with *Candida boidinii* LC1 stored at 8 °C for 180 days; CN-8 = olives inoculated with *Candida norvegica* OC10 stored at 8 °C for 180 days; C-AT = uninoculated olives stored at ambient temperature for 180 days; CB-AT = olives inoculated with *C. boidinii* LC1 stored at ambient temperature for 180 days; CN-AT = olives inoculated with *C. norvegica* OC10 stored at ambient temperature for 180 days; C-AT/8 = uninoculated olives stored for 90 days at ambient temperature followed by 90 days at 8 °C; CB-AT/8 = olives inoculated with *C. boidinii* LC1 stored for 90 days at ambient temperature followed by 90 days at 8 °C; CN-AT/8 = olives inoculated with *C. norvegica* OC10 stored for 90 days at ambient temperature followed by 90 days at 8 °C.

[2] S.S., statistical significance; P value: ***, $P < 0.001$ **, $P < 0.01$.

0.71 (CB-AT/8) to 1.73 (C-8). Pungency was significantly higher in the inoculated samples (2.44–2.90) than in the controls (1.38). Conversely, chemical flavour scores were lower in the inoculated samples (0.42–0.75) than in the controls (1.83–1.93), indicating a reduced perception of undesirable notes.

Textural attributes also differed among treatments. Crispness and hardness were higher in the inoculated samples (crispness: 3.89–4.55; hardness: 5.03–5.97) compared with the controls (crispness: 1.90–2.11; hardness: 2.05–2.31). The highest overall flavour score was recorded in CN-8 (7.74), while the lowest value was observed in C-AT/8 (4.98). Overall appreciation confirmed a clear preference for the inoculated samples, with the highest score recorded in CN-8 (6.61) and the lowest in C-AT/8 (5.05). Notably, reducing the refrigeration period from 180 to 90 days did not negatively affect sensory quality.

Other attributes, including colour homogeneity, unpleasant odour, salinity, bitter aftertaste, persistence of bitterness, olive flavour, foreign flavours, and juiciness, did not show significant differences among treatments.

3.7. Influence of storage conditions and inoculation on quality and aromatic-sensory profile

The agglomerative hierarchical clustering (AHC), illustrated in Fig. 4, revealed distinct clustering patterns among treatments based on similarities in VOC and sensory profiles. This analysis identified three well-defined clusters, as confirmed by the distances between the centroids (9.297 between clusters I and II; 9.920 between clusters I and III; and 7.650 between clusters II and III) and by a cophenetic correlation coefficient of 0.721, indicating a good representation of the hierarchical structure. The inter-cluster variance (30.2%) further highlights that a substantial portion of the total variability is explained by the classification.

The centroids of the VOC and sensory variables provide clear insight into the trends characterising each cluster. Cluster I, consisting of the control C-8, exhibited negative values for most volatile compounds and sensory attributes, such as olive odour, overall odour, and overall appreciation, while showing positive values for acidity, outlining a less expressive and less appreciated sensory profile. Cluster II, which includes CB-8 and CN-8, was characterised by higher centroid values for several esters (isoamyl acetate, ethyl hexanoate, hexenyl acetate, cyclohexyl isobutyrate, ethyl octanoate, and benzyl acetate) and for the ketone 6-methyl-5-heptene-2-one, compounds associated with fruity and fresh notes. At the sensory level, this cluster showed positive trends for sweetness, pungency, and overall flavour, together with negative trends for chemical flavour.

Cluster III, comprising samples subjected to the combined storage regime (AT/8), displayed markedly positive centroids for aromatic alcohols such as 2-phenylethanol and benzyl alcohol, as well as for (Z)-3-hexenol, along with higher values for olive odour and overall appreciation. This profile suggests greater aromatic complexity and higher sensory acceptance. Overall, the results indicate that both the thermal regime and yeast inoculation are associated with significant differences in aromatic and sensory profiles. The combined regime is linked to greater aromatic intensity and complexity, whereas inoculation under refrigeration (8 ± 1 °C) is associated with increased ester and ketone production and a more favourable sensory perception compared to the control.

Although descriptive in nature, these findings suggest that strategies combining inoculation and thermal modulation may represent a promising approach to improve table olive quality, while also reducing refrigeration times and enhancing process sustainability.

3.8. Quantification of energy savings and CO_2-eq avoided by reducing refrigeration

Reducing the refrigeration duration from 180 to 90 days is associated with potential energy and emission benefits. At the unit scale, estimated electricity savings ranged from 7.40 to 17.26 kWh/m^3, based on the simplified modelling approach and the assumed SEC range of 30–70 kWh/m^3/year. Applying the average Italian electricity grid emission factor (0.27–0.35 kg CO_2-eq/kWh), this corresponds to approximately 2.0–6.0 kg CO_2-eq potentially avoided per cubic metre of refrigerated volume.

Using the conservative minimum packed volume of the experimental drums (5.67 m^3), the avoided electricity consumption was estimated at 42–98 kWh, with associated avoided emissions of 11–34 kg CO_2-eq. These values are likely to underestimate the actual benefit, as the operational volume of industrial cold rooms generally exceeds the minimum packed volume.

Because the intervention halves the refrigeration duration, it is expected to reduce refrigeration-related electricity consumption and associated Scope 2 emissions. However, the relationship between storage time and energy use is not necessarily linear and depends on specific system characteristics and operating conditions.

At an industrial scale, assuming a cold-room capacity of approximately 40 m^3 storing around 100 t of olives, the estimated savings may become more substantial. Modelled reductions in electricity consumption range from 296 to 690 kWh per batch, with associated avoided emissions of 80–241 kg CO_2-eq. These indicative values align with sustainability-oriented optimisation strategies by reducing energy demand at the source. Based on an average Italian electricity price of €0.137/kWh (January 2026), the estimated economic benefit per batch ranges from €40 to €95, corresponding to annual savings of approximately €405–945 for a facility processing ten batches per year, excluding indirect benefits such as reduced compressor wear and maintenance.

Table 4 summarises avoided electricity consumption and emissions at the experimental scale, where reducing refrigeration from 180 to 90 days resulted in electricity savings of 41.96–97.90 kWh and avoided emissions of 11.33–34.27 kg CO_2-eq. When combined with the demonstrated quality preservation and microbiological control of inoculated batches under the tested conditions, these results suggest that bioprotective yeast inoculation may represent a viable strategy for lowering energy use in Castelvetrano-style olive processing.

4. Discussion

The application of bioprotective yeasts in the Castelvetrano method emerges as a promising strategy not only for improving microbiological stability and product quality, but also for reducing the environmental footprint of the process. In a non-fermentative system, where maintaining structural integrity and hygienic safety is essential, targeted inoculation with *Candida* strains provides a competitive biological barrier against undesirable microorganisms without inducing lactic acid fermentation (Alfonzo et al., 2024; Campus et al., 2018; Rus-Fernández & Fuentes, 2024). Crucially, this biological support reduces reliance on

Table 3
Quantitative sensory analysis of Nocellara del Belice table olives at the end of the monitoring period (180 days).

Attributes	Trials						S. S.
	C-8	CB-8	CN-8	C-AT/8	CB-AT/8	CN-AT/8	
Colour							
Colour	5.48 ± 0.21 b	6.03 ± 0.23 ab	6.60 ± 0.11 a	4.55 ± 0.19 c	6.61 ± 0.29 a	6.71 ± 0.34 a	***
Colour homogeneity	5.08 ± 0.10 a	5.75 ± 0.66 a	5.61 ± 0.51 a	5.52 ± 0.22 a	5.11 ± 0.05 a	5.37 ± 0.28 a	N. S.
Odours							
Olive odour	2.54 ± 0.14 c	4.15 ± 0.30 b	3.59 ± 0.28 bc	4.55 ± 0.30 b	4.07 ± 0.22 b	5.57 ± 0.80 a	***
Overall odour	3.57 ± 0.20 d	4.37 ± 0.51 bcd	3.96 ± 0.39 cd	4.69 ± 0.38 bc	4.97 ± 0.22 b	5.73 ± 0.36 a	***
Unpleasant odour	0.15 ± 0.13 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.11 ± 0.08 a	0.00 ± 0.00 a	0.00 ± 0.00 a	N. S.
Taste & flavour							
Sweetness	6.22 ± 0.37 b	7.63 ± 0.36 a	7.99 ± 0.39 a	5.74 ± 0.10 b	7.47 ± 0.08 a	7.69 ± 0.26 a	***
Saltiness	3.81 ± 0.12 a	3.61 ± 0.43 a	3.63 ± 0.49 a	3.75 ± 0.10 a	3.83 ± 0.45 a	3.78 ± 0.31 a	N. S.
Acidity	1.83 ± 0.09 a	2.95 ± 0.04 b	2.86 ± 0.10 b	2.99 ± 0.27 b	2.86 ± 0.28 b	2.47 ± 0.23 b	***
Bitterness	1.73 ± 0.29 a	1.41 ± 0.15 ab	1.37 ± 0.12 ab	1.21 ± 0.19 ab	0.71 ± 0.36 b	0.97 ± 0.24 ab	**
Bitter aftertaste	1.61 ± 0.11 a	1.26 ± 0.11 a	1.85 ± 0.21 a	1.41 ± 0.32 a	0.93 ± 0.46 a	1.25 ± 0.39 a	N. S.
Bitter persistence	1.23 ± 0.21 a	1.20 ± 0.11 a	1.33 ± 0.20 a	1.87 ± 0.16 a	1.55 ± 0.35 a	1.67 ± 0.34 a	N. S.
Pungency	1.38 ± 0.06 c	2.90 ± 0.14 a	2.51 ± 0.21 b	2.44 ± 0.18 b	2.57 ± 0.14 b	1.70 ± 0.01 c	***
Chemical flavour	1.93 ± 0.23 a	0.48 ± 0.30 b	0.75 ± 0.16 b	1.83 ± 0.17 a	0.42 ± 0.42 b	0.63 ± 0.31 b	***
Olive flavour	3.51 ± 0.37 a	4.58 ± 0.81 a	4.01 ± 0.49 a	4.32 ± 0.13 a	4.65 ± 0.55 a	4.89 ± 0.49 a	N. S.
Off-flavour	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.11 ± 0.09 a	0.00 ± 0.00 a	0.00 ± 0.00 a	N. S.
Texture							
Crispness	2.11 ± 0.14 b	3.89 ± 0.34 a	4.32 ± 0.26 a	1.90 ± 0.41 b	4.05 ± 0.29 a	4.55 ± 0.17 a	***
Hardness	2.31 ± 0.35 b	5.03 ± 0.24 a	5.24 ± 0.57 a	2.05 ± 0.25 b	5.78 ± 0.36 a	5.97 ± 0.30 a	***
Juiciness	4.00 ± 0.54 a	4.33 ± 0.40 a	4.56 ± 0.25 a	3.94 ± 0.23 a	4.69 ± 0.03 a	4.15 ± 0.66 a	N. S.
Overall attributes							
Overall flavour	5.82 ± 0.10 bc	6.79 ± 0.46 ab	7.74 ± 0.36 a	4.98 ± 0.21 c	6.77 ± 0.25 ab	6.81 ± 0.70 ab	***
Overall appreciation	5.15 ± 0.13 b	6.52 ± 0.08 a	6.61 ± 0.34 a	5.05 ± 0.34 b	6.28 ± 0.41 a	5.97 ± 0.29 a	**

Results show mean ± standard deviation of values assigned by 16 panellists. Data in lines with the same letter are not significantly different by the Tukey test. P value: ***, $P < 0.001$; **, $P < 0.01$.
Abbreviations: S.S., statistical significance; N.S., not significant; C-8, control production not inoculated, stored at 8 ± 1 °C for 180 days; CB-8, experimental production inoculated with *C. boidinii* LC1, stored at 8 ± 1 °C for 180 days; CN-8, experimental production inoculated with *Candida norvegica* OC10, stored at 8 ± 1 °C for 180 days; C-AT, control production not inoculated, stored at ambient temperature for 180 days; CB-AT, experimental production inoculated with *C. boidinii* LC1, stored at ambient temperature for 180 days; CN-AT, experimental production inoculated with *C. norvegica* OC10, stored at ambient temperature for 180 days; C-AT/8, non-inoculated control production, stored at ambient temperature for 90 days and then at 8 ± 1 °C for 90 days; CB-AT/8, experimental production inoculated with *C. boidinii* LC1, stored at ambient temperature for 90 days and then at 8 ± 1 °C for 90 days; CN-AT/8, experimental production inoculated with *C. norvegica* OC10, stored at ambient temperature for 90 days and then at 8 ± 1 °C for 90 days.

prolonged refrigeration.

Temperature remained the dominant factor shaping microbial outcomes, with inoculation exerting a complementary stabilising effect. Continuous cold storage at 8 ± 1 °C effectively suppressed opportunistic and spoilage microorganisms, in line with previous observations in table-olive ecosystems (Abriouel et al., 2011). However, inoculation enhanced ecological steering under both refrigerated and combined regimes, promoting the persistence of desirable yeast populations without triggering fermentation. These yeasts do not play a fermentative role but act as bioprotective agents by competing for space and nutrients and by indirectly modifying the brine microenvironment in ways that are unfavourable to the growth of opportunistic and spoilage microorganisms. These results can be better understood by considering how the microbial community responds to inoculation and storage conditions. In non-inoculated treatments, particularly under ambient conditions, an increase in Enterobacteriaceae and other undesirable microorganisms indicates a reduced level of microbial control and a more heterogeneous, less structured microbiota. In contrast, inoculated trials exhibited more controlled microbial evolution, characterised by higher yeast populations and lower levels of undesirable microorganisms. This pattern suggests that the early establishment of a dominant yeast population limits the growth of competing microbial groups by reducing the availability of resources and space. This behaviour is further influenced by temperature. Under refrigerated conditions, reduced microbial growth rates favour the persistence of the inoculated strains, thereby reinforcing their stabilising effect on the system. Conversely, under ambient conditions, the lower dominance observed indicates a reduced capacity of the inoculated strains to maintain stability of the microbial community over time. These results therefore suggest that bioprotective activity in this system arises from the combined effect of inoculum establishment and temperature control, which together contribute to a more stable and predictable microbial ecosystem under Castelvetrano processing conditions.

The high dominance of the inoculated strains under cold and AT/8 conditions reflects their ecological fitness under alkaline and saline constraints, as well as their oxidative-protective activities (Arroyo-López et al., 2012; Bevilacqua et al., 2009; Hernández et al., 2007). This strong temperature-dependent dominance is consistent with the ecological origin of the yeast strains, which were isolated from olive brines stored under low-temperature conditions typical of the Castelvetrano process. Such prior exposure likely confers a competitive advantage under refrigeration, where selective pressure and reduced microbial turnover favour their persistence over less adapted indigenous microbiota. In contrast, ambient-only storage showed presumptive growth of *Salmonella/Shigella* and Enterobacteriaceae colonies, without confirmatory identification, underscoring the microbiological risks associated with unrefrigerated storage and justifying the exclusion of these batches from sensory evaluation (Romeo et al., 2012). The findings should be interpreted considering the methodological limitations, as the colonies

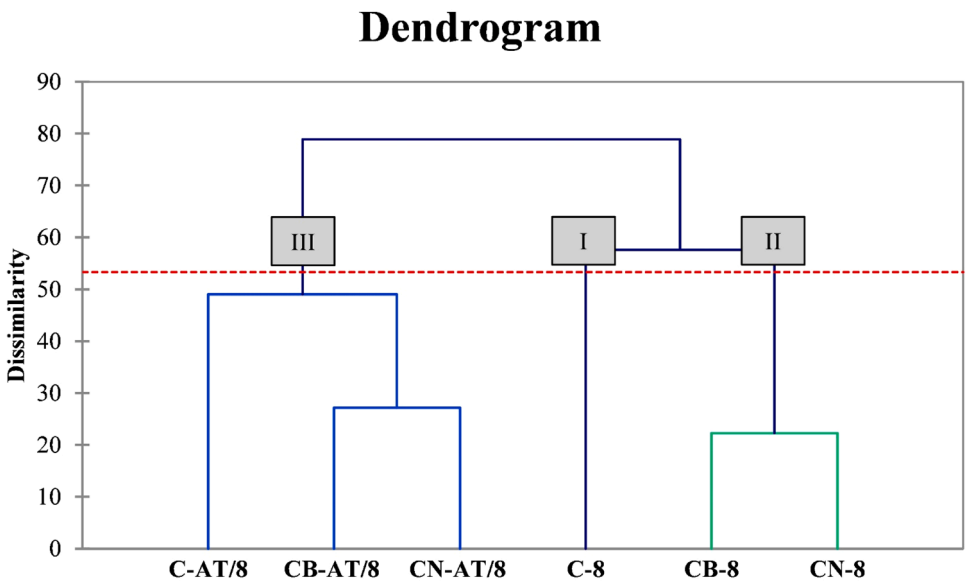

Fig. 4. Dendrogram obtained by agglomerative hierarchical clustering (AHC) on standardised data of volatile compounds (VOCs) and sensory attributes relating to the six treatments: C-8 (non-inoculated control, stored at 8 ± 1 °C for 180 days), CB-8 (*Candida boidinii* LC1 at 8 ± 1 °C for 180 days), CN-8 (*Candida norvegica* OC10 at 8 ± 1 for 180 days), C-AT/8 (non-inoculated control, 90 days at ambient temperature + 90 days at 8 ± 1 °C), CB-AT/8 (*C. boidinii* LC1, 90 days at ambient temperature + 90 days at 8 ± 1 °C), CN-AT/8 (*C. norvegica* OC10, 90 days at ambient temperature + 90 days at 8 ± 1 °C). Clustering was performed using Ward's method and Euclidean distance. The red dashed line indicates the level of dissimilarity used to define the three main clusters (I, II, III).

Table 4
Estimated electricity savings, avoided Scope 2 CO_2-eq emissions and percentage reduction by halving refrigeration time (from 180 days to 90 days).

SEC (kWh/m³/year)	Unit *ΔE* (kWh/m³)	Unit ΔCO_2-eq[1] (kg/m³)	Experiment *ΔE* (kWh)	Experiment ΔCO_2-eq[1] (kg)	Reduction (%)
30	7.40	2.00–2.59	41.96	11.33–14.69	50
50	12.33	3.33–4.32	69.93	18.88–24.48	50
70	17.26	4.66–6.04	97.90	26.43–34.27	50

Abbreviations: SEC, Specific Energy Consumption for cold rooms; *ΔE*, electricity avoided by reducing refrigeration from 180 to 90 days; ΔCO_2-eq, avoided emissions calculated using Italy's grid emission factor.
[1] Location-based Italy grid factor 0.27–0.35 kg CO_2-eq/kWh.

observed on selective media were not confirmed by molecular or biochemical analyses. Therefore, they cannot be considered as evidence of the presence of pathogens, but rather as indicators of microbiological instability under ambient storage conditions. From a technological perspective, their occurrence highlights the limitations of ambient storage without additional control measures and emphasises the need for combined preservation strategies. This observation highlights a key safety concern in non-fermentative Castelvetrano processing and reinforces the importance of refrigeration.

PCA highlights relationships among treatments that are consistent with the observed microbial trends. The separation of treatments particularly reflects the dominant effect of temperature on microbial composition. Ambient conditions are associated with higher levels of undesirable microorganisms, whereas refrigerated conditions are characterised by more stable and controlled microbial communities. Yeast inoculation appears to act as a secondary factor, reducing dispersion among samples and resulting in more consistent microbial compositions. Across all treatments, brines exhibited gradual acidification, more pronounced at ambient temperature (Martín-Vertedor et al., 2021). From a microbiological perspective, pH trends influence competitive interactions among microbial groups and thus contribute to microbial dynamics. However, under ambient storage conditions, the degree of acidification remains insufficient to ensure microbiological safety without additional barriers such as refrigeration or yeast inoculation. Seasonal fluctuations and the absence of refrigeration further complicates the control of opportunistic microbiota (Rodríguez-Gómez et al., 2017). Within the constraints of the Castelvetrano method, where salinity, temperature, and pH (typically 5.0–5.5) act as the main hurdles, bioprotective yeasts contributed to hygienic stability while preserving the characteristic sweetness and mild flavour profile valued by producers (Romeo et al., 2012). This targeted microbial intervention therefore offers a low-impact tool of enhancing stability without altering sensory identity.

Colour and texture data further support the benefits of inoculation. Greener hues and higher chromatic saturation were better preserved under cold or combined regimes, likely due to reduced oxidation of chlorophyll-related pigments and lipids, consistent with the oxidative-protective capacity of certain *Candida* strains (Campus et al., 2018; Ramírez et al., 2015). Higher pulp firmness in inoculated batches suggests limited pectinolytic activity and reduced degradation of the pectic matrix, aligning with previous findings on the detrimental effects of uncontrolled pectinolytic yeasts (Golomb et al., 2013). This is consistent with the technological profile of the selected strains, which exhibit no detectable pectolytic or xylanolytic activity and therefore do not contribute to enzymatic degradation of the olive cell wall (Alfonzo et al., 2024). Their inoculation may thus help preserve tissue integrity by limiting endogenous breakdown and the establishment of spoilage microorganisms.

Sensory descriptors such as crispness, hardness, and overall flavour corroborated instrumental measurements, with inoculated batches at 8 °C and AT/8 outperforming controls (Alfonzo et al., 2024; Bacceli et al., 2023). However, sensory interpretation is limited to refrigerated and combined regimes, as ambient-only treatments were excluded for safety reasons. Sensory attributes were closely linked to VOC composition and microbial activity. Higher scores for olive odour, overall odour, sweetness, and flavour in inoculated batches corresponded to VOC profiles characterised by greater contributions of esters and aromatic alcohols. Conversely, higher scores for chemical flavour in non-inoculated controls were associated with increased levels of volatile acids and oxidation-related compounds.

AHC provided an integrated overview of the relationships between

VOC profiles and sensory perception. The clustering pattern indicates that inoculated samples tend to group more closely together, suggesting a more consistent relationship between VOC composition and sensory attributes. In contrast, non-inoculated samples reveal greater variability, reflecting less controlled microbial dynamics. These patterns clearly distinguish inoculated samples from non-inoculated controls in terms of both aroma-related compounds and sensory attributes. From an industrial perspective, the positioning of AT/8 inoculated samples within clusters associated with positive sensory perception supports the feasibility of reducing refrigeration time without compromising quality. However, practical implementation requires careful monitoring of process conditions to ensure consistent performance across different production settings.

The VOC profiles, interpreted on a relative basis due to their semi-quantitative nature, confirmed that temperature is the primary factor driving volatile composition, while yeast inoculation modulates specific metabolic pathways rather than determining the overall VOC trajectory. In the non-fermentative Castelvetrano context, VOCs arise predominantly from oxidative pathways acting on lipid precursors, generating aldehydes and unsaturated alcohols (Sabatini and Marsilio, 2008; Sabatini, 2010). Rather than fermentation-derived metabolites, volatile compounds originate mainly from lipid oxidation processes, with yeast activity contributing to additional transformations such as aldehyde reduction and esterification.

These transformations yield compounds such as 2-phenylethanol, benzyl alcohol, isoamyl acetate, and benzyl acetate, whose formation is influenced by both matrix-associated enzymes and yeast metabolism. Under refrigerated conditions, yeast inoculation, particularly with *C. norvegica*, favoured the persistence and transformation of specific volatile intermediates, resulting in higher relative contributions of selected esters and ketones. In contrast, combined AT/8 storage promoted the accumulation of aromatic alcohols, reflecting a balance between oxidative processes active during ambient storage and reduced metabolic turnover during subsequent refrigeration. Overall, yeast inoculation acts as a modulatory factor, influencing the intensity and distribution of specific VOC classes within temperature-driven aroma trajectories.

Taken together, these results explain why the effectiveness of bioprotective yeasts is strongly dependent on processing conditions. Their performance is temperature-dependent, as refrigeration limits microbial turnover and enhances the ecological dominance and bioprotective activity of the inoculated strains. This also explains why inoculation alone cannot compensate for the absence of cold storage under fully ambient conditions. From a safety-energy perspective, the combined AT/8 regime represents a practical compromise, in which an initial ambient phase is followed by sufficient refrigeration to restore microbial stability while substantially reducing overall energy demand. From an industrial standpoint, bioprotective yeasts should therefore be considered a complementary tool rather than a replacement for refrigeration, enabling process optimisation without compromising product safety or quality. Practical implementation may differ across production settings, requiring adaptation to plant-specific conditions and process configurations.

From a sustainability perspective, a key outcome is the demonstrated potential to reduce refrigeration time from 180 to 90 days without compromising safety or quality. Although specific LCA data for table olives remain limited, evidence from the broader food cold chain and the olive oil sector identifies refrigeration as a major energy hotspot. In line with these benchmarks, halving the refrigeration phase yields estimated savings consistent with reported ranges, suggesting industrial relevance (Espadas-Espadas-Aldana et al., 2019; Heard & Miller, 2016). Given that refrigeration is a major contributor to energy consumption and greenhouse gas emissions in table olive processing, reducing the cold storage period represents a meaningful step toward lowering the environmental footprint of the Castelvetrano method. However, these energy and emission estimates should be considered indicative, as precise quantification (e.g., kWh per batch or CO_2-eq reduction) requires dedicated modelling (Rus-Fernández & Fuentes, 2024).

The energy and emission estimates presented here are intended as indicative only and do not account for plant-specific parameters, such as thermal load, product thermal mass, cold-room efficiency, refrigerant type, or system configuration.

The main limitations of this study should be acknowledged. Sensory testing could not be performed on ambient-only batches due to safety concerns, resulting in an unbalanced comparison across treatments. Additionally, presumptive pathogen detection on HEA was not confirmed by biochemical or molecular analyses, and therefore microbial safety under ambient storage requires further investigation. Energy savings estimate are based on indicative assumptions and do not account for operational parameters specific to the plant, which may influence the actual magnitude of the observed effects. Future research should focus on optimising the nutritional ecology of *Candida* in brine to enhance resilience and dominance, exploring co-inoculation strategies with selected LAB as low-impact barriers, and extending validation to other cultivars and processing styles. The integration of metagenomics and volatilomics would also strengthen the causal links between microbial ecology and sensory outcomes. Finally, quantifying energy performance and environmental indicators across different production scales is essential to fully assess sustainability benefits.

Overall, *C. boidinii* LC1 and *C. norvegica* OC10 show strong potential as bioprotective starters, supporting non-fermentative stability, preserving colour and texture, and enabling substantial reductions in refrigeration time. Their use aligns with both quality objectives and cleaner production principles, offering a viable pathway toward more sustainable Castelvetrano olive processing, provided further validation across diverse production contexts.

Beyond the demonstrated microbiological stability and sensory performance, reducing refrigeration time from 180 to 90 days provides a relevant environmental co-benefit that strengthens the cleaner-production value of the intervention. Halving cold storage duration enables a reduction in electricity demand and associated Scope 2 emissions, with avoided impacts scaling with refrigerated volume and system efficiency. These savings are particularly significant in table-olive processing, where long-term refrigeration is a major energy hotspot. The integration of bioprotective *Candida* cultures, therefore enhances not only product quality and safety but also resource efficiency, aligning with waste-prevention principles and SDG 12. Although the calculations rely on conservative assumptions regarding cold-room volume and emission factors, the results indicate that microbial bioprotection can function as a dual-purpose strategy, supporting both technological performance and environmental sustainability.

5. Conclusions

Targeted inoculation with *C. boidinii* LC1 and *C. norvegica* OC10 within the Castelvetrano process was shown to effectively support product quality and control microbial proliferation under refrigerated and combined storage conditions, while also contributing to a potential reduction in the environmental impact of production. By halving the refrigeration period, the proposed strategy may lower electricity consumption and the associated emissions. Nevertheless, these environmental benefits are derived from indicative energy estimates and should be validated through more detailed quantitative assessments. Although the approach appears technically feasible and economically attractive at an industrial scale, microbiological safety was not confirmed through targeted pathogen identification. Future studies should therefore include confirmatory safety analyses and comprehensive life-cycle assessments to robustly quantify system-wide impacts and to explore potential synergies with complementary sustainable technologies. Overall, *Candida*-based bioprotection emerges as a promising strategy to enhance technological performance while supporting sustainability-driven optimisation of table olive processing. The proposed approach is technically

feasible under real industrial processing conditions, demonstrating potential for scalability. It offers a practical solution for improving product quality and supporting energy-efficient processing. However, further validation across diverse industrial settings is necessary.

Statement for studies in humans/animals

1. Human Studies Statement

This research included a non-invasive sensory evaluation involving only adult trained assessors. According to the internal regulations of the Comitato Etico di Ateneo, University of Palermo, this type of study: involves no clinical procedures, collects no personal or sensitive data, poses no physical or psychological risk to participants, and is therefore exempt from formal ethical approval under the institution's policy. All assessors were fully informed about the purpose and procedures of the study and voluntarily provided written informed consent prior to participation. The study complied with the ethical principles of the Declaration of Helsinki and followed ISO 13,299:2016 guidelines for sensory analysis.

2. Animal Studies Statement

This research did not involve experiments on animals.

3. Data Protection and Privacy

No identifiable or sensitive personal information was collected. All sensory data were handled anonymously and in compliance with EU GDPR regulations.

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CRediT authorship contribution statement

Davide Alongi: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Giulio Perricone:** Data curation, Formal analysis, Methodology. **Matteo Pollon:** Data curation, Methodology, Software, Writing – original draft. **Antonino Pirrone:** Data curation, Formal analysis. **Enrico Viola:** Software, Visualization. **Clara Vitaggio:** Data curation, Formal analysis. **Vincenzo Naselli:** Methodology, Validation. **Francesco La Croce:** Conceptualization, Investigation. **Raimondo Gaglio:** Visualization, Writing – original draft. **Onofrio Corona:** Supervision, Validation, Writing – original draft, Writing – review & editing. **Luca Settanni:** Supervision, Writing – original draft, Writing – review & editing. **Giancarlo Moschetti:** Conceptualization, Writing – original draft, Writing – review & editing. **Nicola Francesca:** Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. **Antonio Alfonzo:** Conceptualization, Investigation, Methodology, Validation, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2026.102267.

Data availability

Data will be made available on request.

References

Abriouel, H., Benomar, N., Lucas, R., & Gálvez, A. (2011). Culture-independent study of the diversity of microbial populations in brines during fermentation of naturally fermented Aloreña green table olives. *International Journal of Food Microbiology, 144*, 487–496. https://doi.org/10.1016/j.ijfoodmicro.2010.11.006

Alfonzo, A., Francesca, N., Naselli, V., Gaglio, R., Corona, O., Seminerio, V., Settanni, L., La Croce, F., & Moschetti, G. (2023). Effect of glucose and inactivated yeast additions on the fermentation performances of *lactiplantibacillus pentosus* OM13 during the production of Nocellara del Belice table olives. *Fermentation, 9*, Article 634. https://doi.org/10.3390/fermentation9070634. Article.

Alfonzo, A., Alongi, D., Prestianni, R., Pirrone, A., Naselli, V., Viola, E., De Pasquale, C., La Croce, F., Gaglio, R., Settanni, L., Francesca, N., & Moschetti, G. (2024). Enhancing the quality and safety of Nocellara del Belice green table olives produced using the Castelvetrano method. *Food Microbiology, 120*, Article 104477. https://doi.org/10.1016/j.fm.2024.104477. Article.

Alvanoudi, P., Ordoudi, S. A., Nakas, A., Assimopoulou, A. N., & Mantzouridou, F. T. (2024). Brine volatilome changes along the spontaneous fermentation of Spanish-style cv. Chalkidiki green olives under high and low NaCl conditions. *Food and Bioprocess Technology, 17*, 1462–1478. https://doi.org/10.1007/s11947-023-03211-0

Ambra, R., Lucchetti, S., Moneta, E., Peparaio, M., Nardo, N., Baiamonte, I., Di Costanzo, M. G., Saggia Civitelli, E., & Pastore, G. (2017). Effect of partial substitution of sodium with potassium chloride in the fermenting brine on organoleptic characteristics and bioactive molecules occurrence in table olives debittered using spanish and Castelvetrano methods. *International Journal of Food Science and Technology, 52*, 662–670. https://doi.org/10.1111/ijfs.13319

Aponte, M., Blaiotta, G., La Croce, F., Mazzaglia, A., Farina, V., Settanni, L., & Moschetti, G. (2012). Use of selected autochthonous lactic acid bacteria for Spanish-style table olive fermentation. *Food Microbiology, 30*, 8–16. https://doi.org/10.1016/j.fm.2011.10.005

Arroyo-López, F. N., Romero-Gil, V., Bautista-Gallego, J., Rodríguez-Gómez, F., Jiménez-Díaz, R., García-García, P., Querol, A., & Garrido-Fernández, A. (2012). Yeasts in table olive processing: Desirable or spoilage microorganisms? *International Journal of Food Microbiology, 160*, 42–49. https://doi.org/10.1016/j.ijfoodmicro.2012.08.003

Bacceli, M., Simone, N., Lanza, B., & Cichelli, A. (2023). A novel approach for the characterization of the textural properties of table olives: Acoustic compression related to sensory analysis. *Foods (Basel, Switzerland), 12*, Article 241. https://doi.org/10.3390/foods12020241. Article.

Bautista-Gallego, J., Rodríguez-Gómez, F., Barrio, E., Querol, A., Garrido-Fernández, A., & Arroyo-López, F. N. (2011). Exploring the yeast biodiversity of green table olive industrial fermentations for technological applications. *International Journal of Food Microbiology, 147*, 89–96. https://doi.org/10.1016/j.ijfoodmicro.2011.03.013

Ben Said, L., Gaudreau, H., Dallaire, L., Tessier, M., & Fliss, I. (2019). Bioprotective culture: A new generation of food additives for the preservation of food quality and safety. *Industrial Biotechnology, 15*, 138–147. https://doi.org/10.1089/ind.2019.29175.lbs

Bencresciuto, G. F., Mandalà, C., Migliori, C. A., Cortellino, G., Vanoli, M., & Bardi, L. (2023). Assessment of starters of lactic acid bacteria and killer yeasts: Selected strains in lab-scale fermentations of table olives (*Olea europaea* L.) cv. Leccino. *Fermentation, 9*, Article 182. https://doi.org/10.3390/fermentation9020182. Article.

Beuchat, L. R. (1993). Selective media for detecting and enumerating foodborne yeasts. *International Journal of Food Microbiology, 19*, 1–14. https://doi.org/10.1016/0168-1605(93)90119-2

Bevilacqua, A., Perricone, M., Cannarsi, M., Corbo, M., & Sinigaglia, M. (2009). Technological and spoiling characteristics of the yeast microflora isolated from Bella di Cerignola table olives. *International Journal of Food Science and Technology, 44*, 2198–2207. https://doi.org/10.1111/j.1365-2621.2009.02060.x

Busetta, G., Garofalo, G., Barbera, M., Di Trana, A., Claps, S., Lovallo, C., Franciosi, E., Gaglio, R., & Settanni, L. (2023). Metagenomic, microbiological, chemical and sensory profiling of Caciocavallo Podolico Lucano cheese. *Food Research International, 169*, Article 112926. https://doi.org/10.1016/j.foodres.2023.112926. Article.

Campus, M., Değirmencioğlu, N., & Comunian, R. (2018). Technologies and trends to improve table olive quality and safety. *Frontiers in Microbiology, 9*, Article 617. https://doi.org/10.3389/fmicb.2018.00617. Article.

Caridi, A., Panebianco, F., Scibetta, S., & Schena, L. (2019). Selection of yeasts for their anti-mold activity and prospective use in table olive fermentation. *Journal of Food Processing and Preservation, 43*, Article e14259. https://doi.org/10.1111/jfpp.14259. Article.

Cirlincione, F., Francesca, N., Settanni, L., Donnini, D., Venturella, G., & Gargano, M. L. (2021). Microbial safety of black summer truffle collected from Sicily and Umbria regions, Italy. *Journal of Food Quality and Hazards Control, 8*, 13–20. https://doi.org/10.18502/jfqhc.8.1.5458

Consonni, R., & Cagliani, L. R. (2019). NMR studies on Italian PDO olive oils and their potential in olive-tree-derived products characterization. *European Journal of Lipid Science and Technology, 121*, Article 1800174. https://doi.org/10.1002/ejlt.201800174. Article.

Espadas-Aldana, G., Vialle, C., Belaud, J. P., Vaca-Garcia, C., & Sablayrolles, C. (2019). Analysis and trends for life cycle assessment of olive oil production. *Sustainable Production and Consumption, 19*, 216–230. https://doi.org/10.1016/j.spc.2019.04.003

European Environment Agency (EEA). (2025). Greenhouse gas emission intensity indicators. Retrieved from https://www.eea.europa.eu Accessed January 7, 2026.

Fasolato, L., Carraro, L., Facco, P., Cardazzo, B., Balzan, S., Taticchi, A., Andreani, N. A., Montemurro, F., Martino, M. E., Di Lecce, G., Toschi, T. G., & Novelli, E. (2016). Agricultural by-products with bioactive effects: A multivariate approach to evaluate microbial and physicochemical changes in a fresh pork sausage enriched with phenolic compounds from olive vegetation water. *International Journal of Food Microbiology, 228*, 34–43. https://doi.org/10.1016/j.ijfoodmicro.2016.04.003

Galanakis, C. M. (2020). *The interaction of food industry and environment.* London: Academic Press.

Giavalisco, M., Lavanga, E., Ricciardi, A., & Zotta, T. (2024). Starter cultures for the production of fermented table olives: Current status and future perspectives. *Fermentation, 10*, Article 351. https://doi.org/10.3390/fermentation10070351. Article.

Golomb, B. L., Morales, V., Jung, A., Yau, B., Boundy-Mills, K. L., & Marco, M. L. (2013). Effects of pectinolytic yeast on the microbial composition and spoilage of olive fermentations. *Food Microbiology, 33*, 97–106. https://doi.org/10.1016/j.fm.2012.09.004

Guarcello, R., Gaglio, R., Todaro, A., Alfonzo, A., Schicchi, R., Cirlincione, F., Moschetti, G., & Francesca, N. (2019). Insights into the cultivable microbial ecology of "manna" ash products extracted from *Fraxinus angustifolia* (Oleaceae) trees in Sicily, Italy. *Frontiers in Microbiology, 10*, Article 984. https://doi.org/10.3389/fmicb.2019.00984. Article.

Heard, B. R., & Miller, S. A. (2016). Critical research needed to examine the environmental impacts of expanded refrigeration on the food system. *Environmental Science & Technology, 50*, 12060–12071. https://doi.org/10.1021/acs.est.6b02740

Hernández, A., Martín, A., Aranda, E., Pérez-Nevado, F., & Córdoba, M. G. (2007). Identification and characterization of yeast isolated from the elaboration of seasoned green table olives. *Food Microbiology, 24*, 346–351. https://doi.org/10.1016/j.fm.2006.07.022

International Energy Agency (IEA). (2024). Emission factors. https://www.iea.org/data-and-statistics Accessed January 7, 2026.

International Olive Council. (2025). IOC statistics dashboard. Retrieved from https://www.internationaloliveoil.org/wp-content/uploads/2025/07/statistics.xlsx Accessed September 18, 2025.

ISPRA. (2024). Italian National Inventory Report. Retrieved from https://www.isprambiente.gov.it Accessed January 7, 2026.

Kailis, S., & Harris, D. J. (2007). *Producing table olives.* Collingwood: Landlinks Press.

Karacan, M. A., Yilmaz, I. C., & Yilmaz, D. (2023). Key implications on food storages in cold chain by energy management perspectives. *Frontiers in Sustainable Food Systems, 7*, Article 1250646. https://doi.org/10.3389/fsufs.2023.1250646. Article.

Kaya, Ü., Öztürk Güngör, F., Çamoğlu, G., Akkuzu, E., Aşik, Ş., & Köseoğlu, O. (2017). Effect of deficit irrigation regimes on yield and fruit quality of olive trees (cv. Memecik) on the Aegean coast of Turkey. *Irrigation and Drainage, 66*, 820–827. https://doi.org/10.1002/ird.2156

Lamendola, M., Mirabile, G., Muratore, J., & Torta, L. (2024). Fungi associated with olive tree (cv. Nocellara del Belice) decay in Trapani Province (Sicily, Italy). *Pathogens (Basel, Switzerland), 13*, Article 932. https://doi.org/10.3390/pathogens13110932. Article.

Leventdurur, S., Sert-Aydın, S., Boyaci-Gunduz, C. P., Agirman, B., Ben Ghorbal, A., Francesca, N., Martorana, A., & Erten, H. (2016). Yeast biota of naturally fermented black olives in different brines made from cv. Gemlik grown in various districts of the Cukurova region of Turkey. *Yeast (Chichester, England), 33*, 289–301. https://doi.org/10.1002/yea.3170

Lawrence, A., Thollander, P., Andrei, M., & Karlsson, M. (2019). Specific energy consumption/use (SEC) in energy management for improving energy efficiency in industry: Meaning, usage and differences. *Energies, 12*, Article 247. https://doi.org/10.3390/en12020247. Article.

Malheiro, R., Casal, S., Baptista, P., & Pereira, J. A. (2015). A review of *Bactrocera oleae* (Rossi) impact in olive products: From the tree to the table. *Trends in Food Science & Technology, 44*, 226–242. https://doi.org/10.1016/j.tifs.2015.04.009

Marchi, B., & Zanoni, S. (2022). Cold chain energy analysis for sustainable food and beverage supply. *Sustainability, 14*, Article 11137. https://doi.org/10.3390/su141811137. Article.

Marques, C., Güneş, S., Vilela, A., & Gomes, R. (2025). Life-cycle assessment in agri-food systems and the wine industry: A circular economy perspective. *Foods (Basel, Switzerland), 14*, Article 1553. https://doi.org/10.3390/foods14091553. Article.

Martín-Vertedor, D., Schaide, T., Boselli, E., Martínez, M., Arias-Calderón, R., & Pérez-Nevado, F. (2021). Effects of different controlled temperatures on Spanish-style fermentation processes of olives. *Foods (Basel, Switzerland), 10*, Article 666. https://doi.org/10.3390/foods10030666. Article.

Martorana, A., Alfonzo, A., Settanni, L., Corona, O., La Croce, F., Caruso, T., Moschetti, G., & Francesca, N. (2015). An innovative method to produce green table olives based on "pied de cuve" technology. *Food Microbiology, 50*, 126–140. https://doi.org/10.1016/j.fm.2015.03.008

Nikolaivits, E., Termentzi, A., Skaltsounis, A. L., Fokialakis, N., & Topakas, E. (2017). Enzymatic tailoring of oleuropein from *Olea europaea* leaves and product identification by HRMS/MS spectrometry. *Journal of Biotechnology, 253*, 48–54. https://doi.org/10.1016/j.jbiotec.2017.05.020

Ramírez, E., Gandul-Rojas, B., Romero, C., Brenes, M., & Gallardo-Guerrero, L. (2015). Composition of pigments and colour changes in green table olives related to processing type. *Food Chemistry, 166*, 115–124. https://doi.org/10.1016/j.foodchem.2014.05.154

Rodríguez-Gómez, F., Romero-Gil, V., Arroyo-López, F. N., Roldán-Reyes, J. C., & Torres-Gallardo, R. (2017). Assessing the challenges in the application of potential probiotic lactic acid bacteria in the large-scale fermentation of Spanish-style table olives. *Frontiers in Microbiology, 8*, Article 915. https://doi.org/10.3389/fmicb.2017.00915. Article.

Romeo, F. V., Piscopo, A., Mincione, A., & Poiana, M. (2012). Quality evaluation of different typical table olive preparations (cv. Nocellara del Belice). *Grasas y Aceites, 63*, 19–25. https://doi.org/10.3989/gya.058511

Ruiz-Barba, J. L., Cortés-Delgado, A., Sánchez, A. H., López-López, A., & Montaño, A. (2024). Naturally fermented Gordal and Manzanilla green table olives: Effect of single yeast starters on fermentation and final characteristics of the products. *Fermentation, 10*, 439. https://doi.org/10.3390/fermentation10090439. Article.

Rus-Fernández, P., & Fuentes, A. (2025). Fermentation starters and bacteriocins as biocontrol strategies for table olives preservation: A mini-review. *Journal of the Science of Food and Agriculture, 105*, 3550–3556. https://doi.org/10.1002/jsfa.13874

Sabatini, N., & Marsilio, V. (2008). Volatile compounds in table olives (Olea europaea L., Nocellara del Belice cultivar). *Food Chemistry, 107*, 1522–1528. https://doi.org/10.1016/j.foodchem.2007.10.008

Sabatini, N. (2010). A comparison of the volatile compounds in Spanish-style, Greek-style and Castelvetrano-style green olives of the Nocellara del Belice cultivar: Alcohols, aldehydes, ketones, esters and acids. In V. R. Preedy, & R. R. Watson (Eds.), *Olives and olive oil in health and disease prevention* (pp. 219–231). London: Academic Press. https://doi.org/10.1016/B978-0-12-374420-3.00024-3.

Sánchez, A. H., López-López, A., Beato, V. M., de Castro, A., & Montaño, A. (2017). Stability of color in spanish-style green table olives pasteurized and stored in plastic containers. *Journal of the Science of Food and Agriculture, 97*, 3631–3641. https://doi.org/10.1002/jsfa.8222

Sgroi, F., Modica, F., Baviera-Puig, A., Giamporcaro, G., & Sciortino, C. (2024). Consumer response to PDO table olives: An analysis of consumer behavior using ordered logistic regression. *Research in World Agriculture Economics, 5*, Article 1233. https://doi.org/10.36956/rwae.v5i4.1233. Article.

Sidari, R., Martorana, A., & De Bruno, A. (2019). Effect of brine composition on yeast biota associated with naturally fermented Nocellara Messinese table olives. *LWT – Food Science and Technology, 109*, 163–170. https://doi.org/10.1016/j.lwt.2019.04.010

Sinacori, M., Francesca, N., Alfonzo, A., Cruciata, M., Sannino, C., Settanni, L., & Moschetti, G. (2014). Cultivable microorganisms associated with honeys of different geographical and botanical origin. *Food Microbiology, 38*, 284–294. https://doi.org/10.1016/j.fm.2013.07.013

Soler-Rivas, C., Espín, J. C., & Wichers, H. J. (2000). Oleuropein and related compounds. *Journal of the Science of Food and Agriculture, 80*, 1013–1023. https://doi.org/10.1002/(SICI)1097-0010(20000515)80:7<1013::AID-JSFA571>3.0.CO;2-C

Tarantini, A., Crupi, P., Ramires, F. A., D'Amico, L., Romano, G., Blando, F., Branco, P., Clodoveo, M. L., Corbo, F., Cardinali, A., & Bleve, G. (2024). Study of the effects of pasteurization and selected microbial starters on functional traits of fermented table olives. *Food Microbiology, 122*, Article 104537. https://doi.org/10.1016/j.fm.2024.104537. Article.

Tofalo, R., Perpetuini, G., Schirone, M., Suzzi, G., & Corsetti, A. (2013). Yeast biota associated with naturally fermented table olives from different Italian cultivars. *International Journal of Food Microbiology, 161*, 203–208. https://doi.org/10.1016/j.ijfoodmicro.2012.12.011

Wang, Y., Fan, Y., Li, X., Yang, J., & Zhang, X. (2025). Experimental validation of a novel CO_2 refrigeration system for cold storage: Achieving energy efficiency and carbon emission reductions. *Energies, 18*, Article 1129. https://doi.org/10.3390/en18051129. Article.

Zinno, P., Guantario, B., Perozzi, G., Pastore, G., & Devirgiliis, C. (2017). Impact of NaCl reduction on lactic acid bacteria during fermentation of Nocellara del Belice table olives. *Food Microbiology, 63*, 239–247. https://doi.org/10.1016/j.fm.2016.12.001