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Microbial interaction dynamics of *Candida oleophila* and *Starmerella lactis-condensi* with *Saccharomyces cerevisiae* in winter-melon beer fermentation

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ABSTRACT

Candida oleophila YS209 and *Starmerella lactis-condensi* MN412 are non-*Saccharomyces* yeasts isolated from sugar-rich environments. The ecological behaviour of these yeasts in brewing systems remains poorly understood. This study investigated their interaction dynamics with *Saccharomyces cerevisiae* US-05 in a winter-melon beer fermentation using sequential and co-inoculation strategies. Yeast population dynamics, physicochemical parameters, volatile organic compounds, antioxidant properties, and sensory attributes were evaluated. Initial yeast concentrations ranged from 5.74 to 6.53 Log CFU/mL, with *S. cerevisiae* reaching 7.16 Log CFU/mL in monoculture. Non-*Saccharomyces* populations decreased after day 6 but increased following fruit juice addition. In sequential fermentations, *C. oleophila* reached 6.10 Log CFU/mL. Sequential inoculation with *C. oleophila* YS209 resulted in higher glycerol production (3.15 g/L) and enhanced ester synthesis (182.32 mg/L), with ethyl octanoate as the predominant compound. Finished beers showed ethanol contents ranging from 6.07 to 6.28% (v/v). Winter melon beers exhibited higher total phenolic content (681–827 mg GAE/L) than raw juice (475.8 mg GAE/L) and increased antioxidant capacity (+109%). Sensory analysis showed more fruity and floral attributes, particularly in sequential fermentations. Overall, *C. oleophila* YS209 and *St. lactis-condensi* MN412 influenced yeast succession and fermentation outcomes, supporting their potential application as functional bioflavouring yeasts in fruit-based beer fermentation.

1. Introduction

Beer is one of the most widely consumed alcoholic beverages globally. Beer is brewed through the fermentation of water, malt, hops and yeast, this beverage boasts a centuries-old tradition and deep cultural significance (Baiano, 2021; Meussdoerffer, 2009). In recent years, the craft beer industry has shown growing interest in fruit beers, a distinctive category characterized by the addition of whole fruits or fruit derivatives (juices, purees) to the traditional brewing matrix. Winter-melon is a widely cultivated Cucurbitaceae fruit, appreciated for its diverse shapes, flavors, and textures, and suitable for juice preparation (Lija & Beevy, 2021; Sun et al., 2018). Owing to its high consumption, it generates substantial waste along the supply chain, including discarded fruits and processing by-products such as peels, pulp, and seeds (Silva et al., 2025). Global production reached approximately 29 million tons in 2023 (FAOSTAT, 2025), and these underutilized materials represent a promising resource for functional product development (Rico et al., 2020; Silva et al., 2024).

The incorporation of fruit aims to enhance the sensory complexity of beer and to meet consumer demand for innovative, flavour-rich, and

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potentially health-promoting products (Croonenberghs et al., 2024; Gorzelany et al., 2022; Nardini & Garaguso, 2020; Pereira et al., 2020; Villacreces et al., 2022; Zhao et al., 2023). Accordingly, numerous studies have investigated the use of specific fruits in beer production to assess their effects on fermentation performance, volatile and phenolic profiles, and sensory attributes. These include persimmon (Cho et al., 2018; Martínez et al., 2017), cherry (Kawa-Rygielska et al., 2019), strawberry (Rodman et al., 2018), grape (Leni et al., 2023), peaches (Jung et al., 2017), and various tropical fruits (Carvalho et al., 2009; De Melo et al., 2017; Francesca et al., 2023; Gasinski et al., 2020; Santos et al., 2021). Despite the wide range of fruits explored, winter-melon (*Cucumis melo* L. var. *inodorus*) remains largely uninvestigated as a substrate for beer production. Its high sugar content, pleasant aroma, and valuable nutritional profile, including amino acids, organic acids, and phenolic compounds (Petkova & Antova, 2015; Rodríguez-Pérez et al., 2013; Silva et al., 2025), make it a promising candidate for innovative fruit-based beers. In addition, *Cucumis melo* fruits and seeds contain significant levels of bioactive compounds, such as antioxidants and polyphenols, contributing to their potential health-promoting properties (Bouaziz et al., 2020; Mallek-Ayadi et al., 2022).

Over the past decade, the brewing sector has increasingly explored the use of non-*Saccharomyces* yeasts, recognized for their ability to broaden diversity and contribute to the creation of novel beer profiles (Michel et al., 2016). Strains such as *Lachancea thermotolerans*, *Torulaspora delbrueckii*, *Hanseniaspora uvarum*, and *Metschnikowia pulcherrima* have shown potential in modulating acidity, enhancing aromatic expression, and producing specific volatile metabolites (e.g., esters and higher alcohols) (Basso et al., 2016; Canonico et al., 2016; Fernández -Cruz et al., 2020; Larroque et al., 2021; Varela et al., 2016). Within the range of non-*Saccharomyces* yeasts being studied for brewing applications, *Candida oleophila* and *Starmerella lactis-condensi* emerge as promising candidates, though their potential remains relatively underexplored. These strains, originally isolated from sugar-rich matrices such as manna and honey, have shown potential in enhancing aromatic complexity (Gaglio et al., 2017; Guarcello et al., 2019). Pirrone et al. (2025) recently investigated the application of *C. oleophila* and *S. lactis-condensi* in beer fermentations, reporting improved aromatic complexity, higher concentrations of acetate esters and terpenes, and a notable difference in sensory characteristics compared with fermentations relying solely on *Saccharomyces cerevisiae*. Non-*Saccharomyces* yeasts are often employed in co-inoculation or sequential fermentation strategies to take advantage of their specific metabolic contributions while relying on the competitive dominance of *S. cerevisiae* in the later stages to ensure complete fermentation and microbial stability (Holt et al., 2018; Matraxia et al., 2021). Recent studies have demonstrated that the combination of non-*Saccharomyces* yeasts with fruit additions can enhance fruity and floral aromas, while promoting a more diverse and expressive fermentation profile (Francesca et al., 2023; Pirrone et al., 2022). Their compatibility with fruit-based substrates represents a promising strategy to enhance the aromatic complexity and the bioactive profile of beer. This synergistic interaction is particularly relevant when applied to underutilized fruits such as winter-melon, which provide not only a fermentable substrate but also distinctive aromatic characteristics and valuable nutritional components. Despite increasing interest in fruit-based fermentations, the ecological behaviour, competitive dynamics and their influence on metabolite formation and fermentation outcomes of non-*Saccharomyces* yeasts in mixed beer fermentation systems remain insufficiently characterised. In particular, their persistence, interactions, and overall impact on fermentation performance in sugar-rich matrices such as winter-melon juice are still poorly understood. Based on these considerations, the present study was designed to provide a comprehensive evaluation of the ecological behaviour and technological potential of selected non-*Saccharomyces* yeasts in a fruit-enriched brewing system. Particular attention was given to their interaction with *S. cerevisiae* under different inoculation strategies, in order to better understand how microbial succession influences fermentation performance and final beer quality. Accordingly, the objectives of the present study were to: (i) evaluate the fermentation performance of *C. oleophila* and *St. lactis-condensi* in melon-based beer production; (ii) compare co-inoculation and sequential inoculation strategies with *S. cerevisiae* to identify the most effective fermentation protocol; (iii) assess the impact of these yeast combinations on the volatile profile and sensory properties of the final product; (iv) explore the potential of winter-melon juice as a functional adjunct in brewing.

2. Materials and methods

2.1. Yeast strains and media

In this work, the yeast strains *C. oleophila* YS209 and *St. lactis-condensi* MN412 were selected for experimental trials. Both microorganisms are preserved within the microbial collection of the Department of Agricultural, Food, and Forest Sciences (SAAF), University of Palermo (Italy), were originally isolated from sugar-rich substrates, manna (Guarcello et al., 2019) and honey-derived by-products (Gaglio et al., 2017). Their inclusion in the experimental design was motivated by previous evidence highlighting their outstanding aptitude for beer wort fermentation. For comparison, the commercial brewing strain *S. cerevisiae* US-05 (Fermentis, Marcq-en-Baroeul Cedex, France) was employed either as a monoculture or in co-inoculation and sequential fermentation strategies, with the latter involving the addition of *S. cerevisiae* 48 h after non-*Saccharomyces* inoculation. Yeast revival from cryopreserved stocks was carried out following the protocol reported by Pirrone et al. (2022). All microbiological media and chemical reagents were obtained from Oxoid (ThermoFisher, Milan, Italy).

2.2. Experimental plan and production

Fermentation assays were carried out at laboratory scale using 5-L batch, following the experimental plan illustrated in Fig. 1. The wort preparation process followed the protocol described by Pirrone et al. (2025), with some modifications to the recipe. Specifically, the grain bill included 2.5 kg of wheat malt (BestMalz, Heidelberg, Germany) and 3.5 kg of Maris Otter pale ale malt (Simpson Malts, Northumberland, UK). The milled malts were then combined with 30.0 L of water supplemented with 10 g each of $CaSO_4$ and $CaCl_2$ to adjust the pH, following the procedure outlined by Marconi et al. (2016).

The mashing procedure was conducted under controlled temperature steps. The mash was first maintained at 65 °C for 60 min to allow the full conversion of starches, and the end of saccharification was verified using the iodine test, following the approach described by Mayer et al. (2016). The temperature was then increased to 72 °C for 10 min. Afterwards, the mixture was brought to 78 °C for another 10 min to inactivate enzymatic activity. During lautering phase, the wort was clarified through recirculation over the grain bed and subsequently sparged with 19 L of water at 78 °C, reaching a final volume of 46.5 L. At the beginning of the boiling stage, 20 g of Mandarina Bavaria hop pellets (α-acid content: 9.7% w/w) were added to the wort, followed by a second addition of 10 g of the same hop cultivar during the final 10 min of the boil. A total volume of 80 L was produced through a double-batch brewing process executed on the same day.

The beer wort had the standard quality parameters: pH 5.3 and 11.4 °Bx. Sampling was carried out both on the uninoculated wort and throughout fermentation at different time intervals: days 1, 2, 4, 6, 9, 13, and 16.

2.2.1. Preparation and inoculation of yeast strains

The experimentation included five fermentation trials, each inoculated as follows: ME1, *St. lactis-condensi* MN412 initially, followed by *S. cerevisiae* US-05 strain after 48 h; ME2, *C. oleophila* YS209 initially, followed by *S. cerevisiae* US-05 strain after 48 h; ME3, *St. lactis-condensi* MN412 co-inoculated with *S. cerevisiae* US-05 strain; ME4, *C. oleophila*

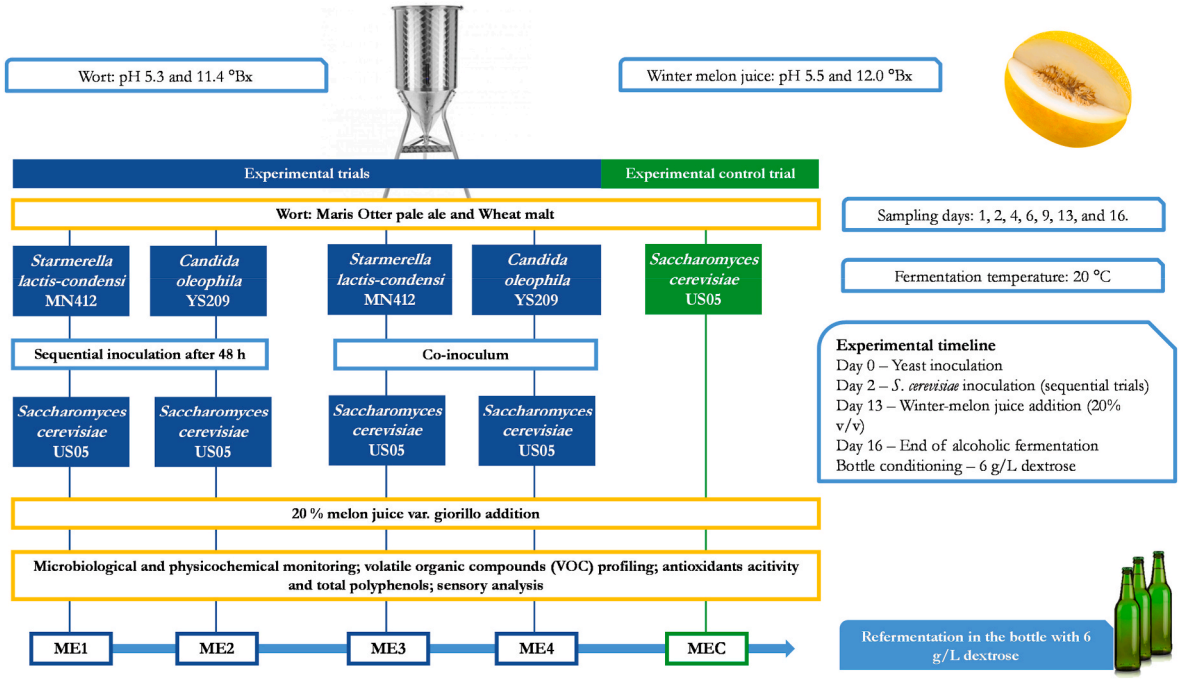

Fig. 1. Schematic representation of the experimental design for beer production.

YS209 co-inoculated with *S. cerevisiae* US-05 strain; MEC (control), *S. cerevisiae* US-05 strain as control. Yeast inoculation was performed at an initial density of approximately 2.0 × 10^6 cells/mL, following the procedure outlined by Holt et al. (2018). Fermentations were conducted at 20 °C using 5-L glass fermenters equipped with airlock valves to maintain anaerobic conditions. At the end of alcoholic fermentation, the beers were transferred into 0.5-L bottles and primed with 6 g/L dextrose in accordance with the protocol described by Francesca et al. (2023). Bottle conditioning proceeded at 20 °C for 25 days, as recommended by Callejo et al. (2019). Upon completion of maturation, the samples were submitted to sensory evaluation. All fermentation trials were carried out in triplicate.

2.2.2. Extraction and processing of winter-melon juice

The winter-melon juice used to prepare the fruit beers was extracted from the fruits of the *Cucumis melo* var. inodorus cv. Giorillo harvested in an orchard belonging to the "Altamore Franco" company (Roccamena, Italy). The selection of fruits for juice extraction was restricted to those classified as processing by-products, defined as fruits that were undersized or exhibited minor morphological defects. After cutting and preliminary rinsing, the fruits underwent successive washing steps following the procedure described by Alfonzo et al. (2018). The extraction of winter-melon juice was then performed according to the method reported by Pirrone et al. (2022). Prior to use, the winter-melon juice was pasteurized (75 ± 1 °C for 30 s), to reduce microbial load and ensure microbiological stability (Lee et al., 2025; Wurlitzer et al., 2019). At the end of primary alcoholic fermentation (day 13), winter-melon juice was added to all fermentations at 20% (v/v), in accordance with the protocol of Gasinski et al. (2020).

2.3. Monitoring of strain density

The collected samples were processed immediately for microbiological assessment through plate-count analysis. Serial dilutions were prepared in Ringer's solution (Sigma-Aldrich, Milan, Italy), and 0.1-mL aliquots from each dilution step were spread onto Wallerstein Laboratory (WL) nutrient agar and Lysine Agar (LA). These media enabled the selective enumeration of *Saccharomyces* spp. (Di Maio et al., 2011) and non-*Saccharomyces* yeasts (Iris et al., 2020). Plates were incubated at 28 °C for 48–72 h. Preliminary colony identification was performed on the basis of macro- and micro-morphological characteristics, which were subsequently validated through microscopic examination of cellular traits (Cavazza et al., 1992; Pallmann et al., 2001).

For genotypic characterization, three isolates exhibiting comparable colony morphologies were selected from each sampling point. Genomic DNA was extracted for PCR-based analyses using the InstaGene Matrix kit (Bio-Rad, Hercules, CA, USA), following the manufacturer's protocol. Species-level discrimination was subsequently performed through restriction fragment length polymorphism (RFLP) analysis of the ribosomal DNA region spanning ITS1, ITS2, and the 5.8S rRNA gene, according to the method established by Esteve-Zarzoso et al. (1999).

To corroborate the preliminary identifications, a single representative isolate from each group was subjected to sequencing of the D1/D2 domain of the 26S rRNA gene, following the protocol described by Alfonzo et al. (2020). Sequencing was performed by BMR Genomics (Padova, Italy), and taxonomic affiliation was determined through BlastN comparison against the NCBI non-redundant database. The accuracy of the resulting sequences was further confirmed through manual inspection and editing using Chromas 2.6.2 (Technelysium Pty Ltd., Australia).

Strain-level differentiation was performed using species-specific molecular approaches. *C. oleophila* and *St. lactis-condensi* isolates were genotyped through RAPD-PCR using the M13 primer, following the methodology of Binati et al. (2019), whereas *S. cerevisiae* strains were distinguished by interdelta fingerprinting according to Legras and Karst (2003). Amplification products were visualized and analysed using GelCompar II software (version 6.5; Applied-Maths, Sint-Martens-Latem, Belgium), applying the analytical workflow described by Alfonzo et al. (2021). Comparative assessment of the resulting polymorphic profiles with those of the inoculated reference strains (*C. oleophila* YS209, *St. lactis-condensi* MN412, and *S. cerevisiae* US-05) enabled evaluation of strain dominance across the fermentation trials. All genotyping analyses were conducted in triplicate to ensure methodological robustness and reproducibility.

2.4. Analysis of physicochemical parameters

The pH levels were assessed using a Mod.70 XS/50010162 pH meter (Cheimika, Pellezzano, Italy). The quantification of organic acids (acetic and lactic), sugars (fructose, glucose, maltose, and sucrose), and glycerol in both wort and beer samples was carried out using an iCubio iMagic M9 enzymatic analyser (Shenzhen iCubio Biomedical Technology Co., Shenzhen, China). The analytical procedure followed the protocol described by Matraxia et al. (2021). The system dispensed the required reagents and sample aliquots into the cuvette, carried out the incubation at a controlled temperature, measured absorbance at the appropriate wavelength, and automatically calculated analyte concentrations based on the corresponding calibration curves. All standards and enzymatic reagents employed in the analyses were supplied by R-Biopharm AG (Darmstadt, Germany). In addition, the final ethanol concentration was determined using the procedure outlined by Chawafambira (2021). Each measurement was performed in triplicate.

2.5. Determination of antioxidants and total polyphenols

2.5.1. Extraction of antioxidants

The extraction of extractable antioxidants was carried out using an aqueous-organic system, following the protocols described by Durazzo et al. (2017, 2019). In detail, about 3–5.5 g of each sample was weighed and placed into 50 mL test tubes. These tubes were then mixed with 20 mL of a methanol: water solution (50:50, v/v) which had been previously acidified to a pH of 2. The test tubes were vortexed for approximately 3 min and then gently shaken for 1 h at 100 rpm in a thermostatic bath at room temperature. Thereafter, the test tubes were placed in a centrifuge (2500 rpm for 10 min). Following centrifugation, the upper layer was extracted using a glass Pasteur pipette and transferred into a new 50 mL test tube. The residue was then subjected to a second extraction by adding 20 mL of an acetone: water mixture (70:30, v/v), repeating the same shaking and centrifugation operations. The two extracts (methanolic and acetone) were subsequently combined and subjected to a second round of centrifugation at 2800 rpm for 15 min. The resulting supernatant was transferred into tubes and directly used for the determination of antioxidant properties and total polyphenol content.

2.5.2. Total polyphenols

The Total Polyphenol Content (TPC) was determined using the Folin-Ciocalteu colorimetric method (Singleton & Rossi, 1965). The extractable polyphenol samples were centrifuged for a period of 10 min at a speed of 3000 g, with the objective of removing suspended particles that had been formed during the freezing process. Subsequently, 100 μL of the samples were mixed with 3 mL of a 2% (w/v) sodium carbonate solution and 100 μL of a 50% (w/v) Folin-Ciocalteau reagent. To prepare the calibration curve, 100 μL aliquots of aqueous gallic acid solutions in the range 50-700 mg/L were placed in a test tube, then 3 mL of 2% sodium carbonate and 100 μL of Folin-Ciocalteu reagent were added. The reaction was kept in the dark for 60 min. Following the incubation period, spectroscopic analysis was conducted on a PerkinElmer Lambda 365 UV-VIS spectrometer (PerkinElmer; Waltham, MA, USA), utilising a 765-nm wavelength setting. The concentration of TPC content was determined by comparison with values obtained with a standard gallic acid solution. The TPC values were expressed in milligrams of gallic acid equivalent per litre (mg GAE/L) in fresh weight (FW). Each sample was analysed in triplicate.

2.5.3. Trolox equivalent antioxidant capacity

The antioxidant activity was determined using Trolox equivalent antioxidant capacity (TEAC) method as described by Re et al. (1999), which measures the ability to trap free radicals present in the medium. The green-blue cationic radical ABTS + stock solution was prepared from a solution of 7 mM 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 140 mM potassium persulphate (K2S2O8) for 12-16 h in the dark at room temperature, resulting in stability for up to 48 h. Subsequently, ABTS was diluted approximately 1:100 with ethanol and adjusted to an extinction coefficient of 0.70 ± 0.02 at 734 nm at 30 °C in the thermostatic spectrophotometer (PerkinElmer UV-VIS Lambda 365 – PerkinElmer; Waltham, MA, USA). For the calibration curve, a 5 mM Trolox stock solution was prepared with concentrations ranging from 0 to 400 μM Trolox in volumetric flasks. Initially, 2 mL of the ABTS radical was added to the cuvette to measure its extinction coefficient, after which 100 μL of the samples or standard solutions were added and the extinction coefficient was measured again after 3 min. The analysis was performed in triplicate, and the results were expressed as Trolox equivalents (TE) μM/L. A suitable dilution had previously been optimised to obtain an inhibition percentage between 20 and 80%. It was determined that none of the samples required dilution.

2.5.4. Oxygen radical absorbance capacity

The Oxygen Radical Absorbance Capacity (ORAC) method is based on the capacity of antioxidant compounds to sequester oxygen radicals (Ou et al., 2001). The capacity is measured by evaluating the decrease in fluorescein fluorescence induced by oxidative damage caused by free radicals generated by the thermal decomposition of 2,2′-azinobis (2-amidinopropane) dihydrochloride (AAPH). The antioxidant capacity of the sample was determined based on the time and percentage of protection against fluorescence loss and was quantified using a standard curve obtained using Trolox. The reaction was performed with a VICTOR 1420 multilabel plate counter (PerkinElmer; Waltham, MA, USA) using a fluorescence filter with excitation and emission wavelengths of 485 and 535 nm, respectively. The experiments were conducted at a temperature of 37 °C, employing fluorescein sodium and AAPH at concentrations of 0.015 and 120 mg/mL, respectively. The samples were then diluted to a ratio of 1:50 with a 75 mM phosphate buffer, with a pH of approximately 7.4. To this end, 80 μL of fluorescein, 40 μL of AAPH and 80 μL of diluted sample, Trolox (standard) or phosphate buffer (blank) were added to a 96 white multi-well plate. Fluorescence was recorded at 5-min intervals for a period of 90 min, until the fluorescence of the test sample was less than five per cent of the initial value. The results were expressed in TE μM/L. The analysis was performed in triplicate.

2.6. Analysis of VOCs in beer samples

Volatile organic compounds were analysed by SPME extraction followed by GC-MS. For SPME, 10 mL of sample were transferred into 20 mL vials (Gerstel, 75.5 × 22.5 mm) containing 1 g of sodium chloride. Reagents, calibration standards, and the GC-MS analytical procedure were applied as reported in Pirrone et al. (2025). Compound identification was performed by comparison with validated mass-spectral libraries (Adams, NIST 11, Wiley 9, FFNSC 2) and confirmed using published reference spectra.

2.7. Sensory evaluation

A sensory evaluation was performed to characterise the organoleptic properties of the experimental beers obtained. The panel consisted of seventeen trained assessors (10 males and 7 females), aged 26-47 years, all affiliated with the University of Palermo. All participants had prior experience in brewing processes and in the sensory assessment of fruit beers, including those derived from experimental fermentations. Before the evaluation, the judge took part in training sessions designed to standardise their perception and scoring of the sensory descriptors relevant to fruit-based beers. Sensory evaluations were conducted under blind tasting conditions in the sensory analysis laboratory of the SAAF Department, University of Palermo, Italy. Samples were coded with random three-digit numbers and presented in randomized order to avoid potential positional bias. All evaluations were conducted under

controlled conditions, with samples served at standardized temperature (12 ± 1 °C) and volume (30 mL). Between samples, panellists were provided with water to cleanse the palate.

The methodology for sample preparation and sensory analysis followed the approach described by Francesca et al. (2023), with some modifications to the evaluated attributes. To assess the sensory characteristics of the fruit beers, the panel considered a set of descriptors covering both odour and taste. For odour evaluation, the following descriptors were included: intensity, complexity, fruity, melon, floral, hoppy, wheat/cereal, honey/caramel, spicy, acetic, sulphury, and dimethyl sulphide (DMS). For taste, the assessed attributes were: intensity, complexity, sweetness, bitterness, acidity, astringency, fruity, melon, spicy, hoppy, wheat/cereal, burnt/cooked, alcoholic perception, body, DMS, oxidized/aged notes, and overall acceptance. Each sample was evaluated in triplicate by the panel, and the final score for each descriptor was calculated as the average of the three assessments. Participants provided written informed consent after receiving a clear and comprehensive description of the sensory evaluation protocol. All participants confirmed that they fully understood the procedures and that the assessment involved no anticipated risks associated with the odour or taste attributes of the sample. Informed consent forms, countersigned by both panellists and the panel supervisor, was collected before the evaluation began. All procedures were conducted in full compliance with relevant laws, institutional policies, and ethical principles.

2.8. Statistical analyses

Analysis of variance (ANOVA) was applied to determine significant differences among the physicochemical parameters monitored during brewing, the total polyphenol content (TPC), the antioxidant capacity assays, the VOC concentration, and the sensory attributes of the final beers. Post-hoc comparisons were performed using Tukey's test, with statistical significance set at $P \leq 0.05$ (Mazzei et al., 2010).

To further examine the structure of the volatile dataset, a Heat Map Clustered Analysis (HMCA) was performed, integrating hierarchical clustering with heat-map visualization to illustrate the distribution of compounds across samples (Martorana et al., 2017). Data were autoscaled prior to analysis (Gaglio et al., 2017), and clustering was conducted according to Ward's method. VOC abundance was represented using a color gradient ranging from yellow (lowest concentration) to red (highest). Subsequently, Principal Component Analysis (PCA) was performed to explore the relationships between volatile compounds and sensory attributes. Only volatile compounds with an odour activity value (OAV) greater than 1 were included in the analysis, in order to focus exclusively on those with potential sensory relevance. The graphical representation of TPC and total antioxidant capacity was generated using GraphPad Prism version 8 (GraphPad Software, LLC, USA). However, all statistical analyses, including those related to TPC and antioxidant capacity, as well as all other graphical outputs were performed using XLStat software (version 2019.2.2, Addinsoft, New York, USA) within Microsoft Excel.

3. Results and discussion

3.1. Growth kinetics during fermentation

Growth kinetics were monitored during fermentation, and the results are shown in Fig. 2. In uninoculated wort and winter-melon juice, microbial levels remained below the detection limit for both WL and LA media. In contrast, the initial inoculum exhibited cell densities ranging from 5.74 to 6.53 Log CFU/mL. After four days of fermentation, all experimental trials showed an increase of approximately 0.5 Log units, with the control trial (MEC) showing the most pronounced rise. On day 2, sequential inoculation of the commercial yeast strain *S. cerevisiae* US-05 in ME1 and ME2 resulted in *Saccharomyces* cell densities ranging from 6.18 to 6.25 Log CFU/mL. Previous studies demonstrated that

Fig. 2. Monitoring yeast concentrations during alcoholic fermentation. Data are expressed as mean ± standard deviation (n = 3). Error bars represent standard deviation.

variations in yeast cell density can significantly influence the sensory properties of the final product (Du Plessis et al., 2017).

A decline in non-*Saccharomyces* populations was observed from day 6 onward, persisting until the addition of melon juice. Concurrently, *Saccharomyces* spp. density also decreased from day 9. Strain persistence was evaluated through phenotypic analysis of colony morphology and cellular traits, enabling identification of representative species from the *Candida*, *Starmerella*, and *Saccharomyces* genera (Chand-Goyal et al., 1998; Csoma et al., 2023; Kurtzman et al., 2011). The reduction in *Candida* and *Starmerella* populations after day 4 may be linked to nutrient depletion (e.g. sugars), competitive interaction with dominant *Saccharomyces* strains (Michel et al., 2016; Tristezza et al., 2016), and the accumulation of stress-inducing metabolites, particularly ethanol (Domizio et al., 2016; Matraxia et al., 2021).

Peak cell counts were recorded on day 4 for *S. cerevisiae* US-05 in trial MEC (7.16 Log CFU/mL), followed by *S. lactis-condensi* MN412 in trial ME1 (6.98 Log CFU/mL). The lowest value was observed in ME3, where *S. lactis-condensi* MN412 reached 4.95 Log CFU/mL at day 16. Matraxia et al. (2021) reported that co-inoculation of *S. cerevisiae* and *H. uvarum* in beer wort enhanced *Saccharomyces* growth compared to sequential inoculation. In contrast, the present study did not reveal significant differences between inoculation strategies. Following the addition of winter-melon juice (day 13), yeast levels increased across all trials, likely due to the renewed availability of fermentable sugars. Notably, ME2 showed the strongest response among non-*Saccharomyces* strains, with *C. oleophila* YS209 reaching 6.10 Log CFU/mL. The higher persistence of *C. oleophila* under sequential inoculation suggests that delayed introduction of *S. cerevisiae* allows this strain to establish temporarily and exert a measurable impact on fermentation dynamics. These findings are consistent with previously reported yeast dynamics in wort- and fruit-based beers, where fruit supplementation provides additional fermentable sugars that stimulate yeast proliferation and elevate cell density (Francesca et al., 2023; Pirrone et al., 2022; Wu et al., 2024). This confirms that fruit-derived sugars act as ecological drivers modulating yeast population structure during mixed fermentations.

In total, 70 yeast isolates were recovered from each trial, yielding 350 isolates across ME1, ME2, ME3, ME4, and MEC. RAPD-PCR molecular characterisation confirmed that all non-*Saccharomyces* isolates shared RAPD profiles identical to those of the inoculated strains (*C. oleophila* YS209, *St. lactis-condensi* MN412, and *S. cerevisiae* US-05) in trials ME1 – ME4. In the TC treatment, interdelta fingerprinting patterns of *S. cerevisiae* showed high similarity to the inoculated reference strain *S. cerevisiae* US-05.

3.2. Physicochemical changes during fermentation

The physicochemical parameters of winter-melon juice and wort are summarized in Table 1. The initial wort showed a pH value of 5.3 and 11.4 °Bx, whereas the melon juice had a pH of 5.5 and 12.0 °Bx. The pH values of the juice are consistent with those reported by Chambi et al. (2016), ranging from 5.46 to 6.07. However, the sugar content in our samples was slightly higher than their reported range (7.2 - 10.6 °Bx) and notably above the mean value of 6.00 °Bx reported by Yıkmış (2020). Such differences may reflect variations in cultivation area, varietal area, agronomic practices, and seasonal factors, all of which are affect sugar accumulation in melons. This variability highlights the influence of raw material composition on the fermentation matrix and subsequent yeast metabolic activity.

Table 1
Sugars composition identified in beer wort and *Cucumis melo* var. inodorus juice.

Parameters (g/L)	Wort	Melon juice
D-fructose	1.42 ± 0.08	28.25 ± 0.63
D-glucose	10.89 ± 0.25	23.42 ± 0.41
Maltose	38.67 ± 0.31	0.00 ± 0.00
D-sucrose	14.20 ± 0.15	34.15 ± 0.35

Analyses of fermentation dynamics (Table 2) revealed differences among inoculation strategies in the evolution of glucose, fructose, sucrose, maltose, glycerol, acetic acid, and lactic acid. During the first two days, co-inoculation trials (ME3 and ME4) showed significantly higher sugar consumption compared to sequential inoculation (ME1 and ME2). These patterns reflect differences in early fermentation kinetics driven by yeast interactions and inoculation timing.

Trials with *C. oleophila* (ME2 and ME4) demonstrated more pronounced metabolism relative to *St. lactis-condensi* (ME1 and ME3), with rapid depletion of glucose and fructose prior to *S. cerevisiae* inoculation. As previously reported by Pirrone et al. (2025), both strains actively consumed glucose, fructose, and sucrose before the addition of *S. cerevisiae*; however, neither strain metabolizes maltose, and the reduction observed is attributable to *S. cerevisiae*. The faster fructose consumption observed in *St. lactis-condensi* indicates species-specific sugar preferences (Francesca et al., 2024).

Overall, ME4 trial (co-inoculation of *C. oleophila* and *S. cerevisiae* US-05) produced values most comparable to the MEC control (*S. cerevisiae* US-05). The enhanced sugar consumption observed in co-inoculation trials is attributable to *S. cerevisiae*, which achieved rates exceeding those of the control. These results suggest that co-inoculation can accelerate substrate utilisation without substantially altering final residual sugar levels. Notably, *C. oleophila* YS209 consistently showed the highest sugar consumption within the first two days in both ME2 and ME4 trials. Following juice addition, glucose, sucrose, and fructose concentrations increased, but were fully depleted by the end of alcoholic fermentation. These findings corroborate those of Matraxia et al. (2021), who reported no significant differences in final sugar consumption across inoculation strategies. Organic acids and glycerol production also varied among trials. Acetic acid concentrations ranged from 0.02 to 0.07 g/L, while lactic acid concentrations varied between 0.06 and 0.16 g/L, consistent with values reported by Pirrone et al. (2025). Glycerol reached its highest concentration in ME2 (3.15 g/L, sequential inoculation with *C. oleophila* YS209) surpassing the MEC control (*S. cerevisiae* US-05 in MEC, 2.96 g/L). This increase may be related to strain-dependent metabolic behaviour under sequential inoculation, as reported in previous studies. These values exceeded those reported in fruit beer studies by Gasinski et al. (2020) and Kawa-Rygielska et al. (2019), highlighting the role of selected yeast strains in enhancing glycerol production. Ethanol concentrations ranged from 6.07 to 6.28% v/v. The lowest value was observed in ME2, while the remaining treatments showed comparable levels, indicating slight variability among trials. Overall, ethanol production remained within a narrow range, indicating that inoculation strategy primarily influenced secondary metabolite formation rather than final alcohol yield.

3.3. Determination of antioxidants and total polyphenols

The results for TPC and TAC (ORAC and TEAC) in melon juice and melon beer are illustrated in Fig. 3. The extractable TPC in melon beers (Fig. 3a) ranged from 681.4 to 827.4 mg GAE/L, significantly higher than the 475.8 mg GAE/L measured in melon juice. The highest TPC was observed in sample ME3, produced through co-inoculation with *St. lactis-condensi* (MN412) and *S. cerevisiae* (US-05), representing a 73.9% increase compared to melon juice. In the TEAC assay (Fig. 3b), samples collected at the end of alcoholic fermentation showed a 75.3-109.3% increase in antioxidant capacity compared to the reference melon juice. The highest TEAC values were recorded in samples ME1 and ME2 (887.7–897 μM Trolox/L), achieved through sequential inoculation with *St. lactis-condensi* and *C. oleophila*, followed by *S. cerevisiae*. These findings align with Nardini and Garaguso (2020), who demonstrated that fruit addition to beer elevates phenolic and antioxidant content. In this study, the higher values in melon beers can be attributed both to the inherent phenolic compounds in beer, primarily derived from hops (Oladokun et al., 2017), and to the additional contribution of melon

Table 2
Conventional physico-chemical parameters monitored in samples during the alcoholic fermentation.

	ME1	ME2	ME3	ME4	MEC	S.S.
D-fructose (g/L)						
1d	1.08 ± 0.02^{b}	1.26 ± 0.02^{a}	1.04 ± 0.01^{b}	1.08 ± 0.02^{b}	1.03 ± 0.01^{c}	***
2d	0.98 ± 0.03^{b}	1.17 ± 0.01^{a}	0.82 ± 0.01^{c}	0.95 ± 0.02^{b}	0.85 ± 0.01^{c}	***
4d	0.41 ± 0.02^{b}	0.62 ± 0.00^{a}	0.21 ± 0.01^{d}	0.32 ± 0.02^{c}	0.15 ± 0.01^{e}	***
6d	0.11 ± 0.01^{b}	0.15 ± 0.00^{a}	0.03 ± 0.01^{d}	0.07 ± 0.01^{c}	0.01 ± 0.01^{d}	***
9d	0.02 ± 0.00^{b}	0.04 ± 0.01^{a}	0.00 ± 0.00^{c}	0.02 ± 0.00^{b}	0.00 ± 0.01^{c}	***
13d (+Fr)	4.25 ± 0.05^{b}	4.80 ± 0.04^{a}	3.95 ± 0.06^{cd}	4.10 ± 0.12^{bc}	3.80 ± 0.10^{d}	***
16 d (End AF)	0.02 ± 0.01^{a}	0.03 ± 0.00^{a}	0.01 ± 0.01^{a}	0.02 ± 0.02^{a}	0.04 ± 0.02^{a}	N.S.
D-glucose (g/L)						
1d	10.45 ± 0.07^{a}	10.1 ± 0.11^{b}	9.24 ± 0.12^{d}	9.06 ± 0.14^{d}	9.65 ± 0.09^{c}	***
2d	9.55 ± 0.10^{b}	9.88 ± 0.09^{b}	8.15 ± 0.11^{d}	8.23 ± 0.12^{d}	8.65 ± 0.07^{c}	***
4d	6.84 ± 0.08^{b}	7.54 ± 0.05^{a}	6.14 ± 0.05^{c}	6.02 ± 0.04^{cd}	5.91 ± 0.05^{d}	***
6d	4.61 ± 0.07^{b}	4.95 ± 0.06^{a}	4.21 ± 0.03^{c}	3.84 ± 0.01^{d}	3.25 ± 0.04^{e}	***
9d	1.01 ± 0.04^{b}	1.25 ± 0.04^{a}	0.98 ± 0.04^{b}	0.88 ± 0.01^{c}	0.65 ± 0.03^{d}	***
13d (+Fr)	3.50 ± 0.02^{e}	3.80 ± 0.02^{c}	3.95 ± 0.02^{b}	3.74 ± 0.01^{d}	4.10 ± 0.02^{a}	***
16 d (End AF)	0.02 ± 0.01^{bc}	0.05 ± 0.01^{a}	0.03 ± 0.01^{ab}	0.01 ± 0.00^{bc}	0.00 ± 0.00^{c}	***
Maltose (g/L)						
1d	37.50 ± 0.42^{a}	36.70 ± 0.33^{a}	34.95 ± 0.25^{b}	34.25 ± 0.24^{b}	37.12 ± 0.23^{a}	***
2d	36.94 ± 0.45^{a}	35.98 ± 0.32^{a}	31.35 ± 0.38^{c}	30.20 ± 0.42^{d}	33.21 ± 0.32^{b}	***
4d	21.14 ± 0.31^{a}	23.55 ± 0.25^{c}	26.42 ± 0.22^{b}	24.05 ± 0.33^{c}	26.34 ± 0.19^{b}	***
6d	22.15 ± 0.19^{a}	19.45 ± 0.14^{b}	15.47 ± 0.17^{c}	14.05 ± 0.27^{d}	12.47 ± 0.17^{e}	***
9d	10.55 ± 0.16^{a}	9.80 ± 0.11^{b}	8.80 ± 0.22^{c}	7.55 ± 0.13^{d}	3.25 ± 0.09^{e}	***
13d (+Fr)	1.55 ± 0.08^{a}	0.95 ± 0.05^{b}	0.55 ± 0.03^{c}	0.31 ± 0.08^{d}	0.53 ± 0.03^{c}	***
16 d (End AF)	0.05 ± 0.01^{b}	0.22 ± 0.05^{a}	0.02 ± 0.01^{b}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	***
D-sucrose (g/L)						
1d	13.98 ± 0.22^{a}	13.29 ± 0.27^{ab}	12.65 ± 0.35^{b}	11.55 ± 0.32^{c}	12.78 ± 0.41^{b}	***
2d	10.95 ± 0.15^{a}	10.54 ± 0.12^{b}	8.95 ± 0.12^{c}	8.50 ± 0.11d	8.20 ± 0.09d	***
4d	6.85 ± 0.21^{b}	7.44 ± 0.05^{a}	5.24 ± 0.15^{c}	4.22 ± 0.08d	4.15 ± 0.11d	***
6d	4.88 ± 0.11^{b}	5.55 ± 0.04^{a}	2.98 ± 0.07^{c}	2.20 ± 0.10d	0.13 ± 0.08e	***
9d	0.28 ± 0.04^{b}	0.42 ± 0.02^{a}	0.25 ± 0.02^{b}	0.13 ± 0.03^{c}	0.14 ± 0.07^{c}	***
13d (+Fr)	7.38 ± 0.14^{a}	6.45 ± 0.18^{c}	7.12 ± 0.21^{a}	6.58 ± 0.18^{bc}	6.98 ± 0.12^{ab}	***
16 d (End AF)	0.15 ± 0.01^{a}	0.02 ± 0.01^{c}	0.02 ± 0.01^{c}	0.05 ± 0.00^{b}	0.03 ± 0.00^{bc}	***
Lactic acid (g/L)						
1d	0.01 ± 0.01^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	N.S.
2d	0.03 ± 0.01^{a}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	0.02 ± 0.00^{ab}	0.02 ± 0.01^{ab}	N.S.
4d	0.08 ± 0.02^{a}	0.03 ± 0.01^{b}	0.02 ± 0.01^{b}	0.08 ± 0.01^{a}	0.10 ± 0.02^{a}	***
6d	0.10 ± 0.02^{a}	0.05 ± 0.02^{b}	0.05 ± 0.01^{b}	0.08 ± 0.02^{ab}	0.11 ± 0.01^{a}	**
9d	0.11 ± 0.03^{a}	0.05 ± 0.02^{b}	0.05 ± 0.01^{b}	0.10 ± 0.01^{ab}	0.15 ± 0.02^{a}	***
13d (+Fr)	0.11 ± 0.03^{ab}	0.06 ± 0.01^{b}	0.06 ± 0.02^{b}	0.11 ± 0.02^{ab}	0.15 ± 0.01^{a}	**
16 d (End AF)	0.12 ± 0.02^{b}	0.06 ± 0.01^{c}	0.06 ± 0.01^{c}	0.12 ± 0.01^{b}	0.16 ± 0.00^{a}	***
Acetic acid (g/L)						
1d	0.00 ± 0.00^{a}	0.01 ± 0.01^{a}	0.01 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	N.S:
2d	0.02 ± 0.02^{a}	0.03 ± 0.03^{a}	0.02 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.01^{a}	N.S.
4d	0.04 ± 0.02^{a}	0.03 ± 0.03^{a}	0.05 ± 0.01^{a}	0.01 ± 0.00^{a}	0.03 ± 0.01^{a}	N.S.
6d	0.06 ± 0.01^{a}	0.04 ± 0.02^{ab}	0.05 ± 0.00^{ab}	0.02 ± 0.01^{b}	0.03 ± 0.01^{ab}	N.S.
9d	0.06 ± 0.03^{a}	0.04 ± 0.02^{ab}	0.06 ± 0.01^{a}	0.01 ± 0.00^{b}	0.04 ± 0.01^{ab}	N.S.
13d (+Fr)	0.06 ± 0.04^{a}	0.05 ± 0.03^{a}	0.07 ± 0.02^{a}	0.02 ± 0.01^{a}	0.05 ± 0.02^{a}	N.S.
16 d (End AF)	0.06 ± 0.01^{a}	0.05 ± 0.02^{ab}	0.07 ± 0.01^{a}	0.02 ± 0.00^{b}	0.05 ± 0.01^{ab}	*
Glycerol (g/L)						
1d	0.10 ± 0.01d	0.15 ± 0.01^{c}	0.18 ± 0.02^{bc}	0.20 ± 0.01^{b}	0.25 ± 0.03^{a}	***
2d	0.28 ± 0.02^{c}	0.34 ± 0.02^{bc}	0.40 ± 0.02^{b}	0.38 ± 0.01^{b}	0.48 ± 0.05^{a}	***
4d	0.58 ± 0.03^{c}	0.51 ± 0.05^{c}	1.15 ± 0.04^{b}	1.30 ± 0.02^{a}	1.18 ± 0.05^{b}	***
6d	1.45 ± 0.08^{ab}	1.35 ± 0.10^{b}	1.55 ± 0.09^{ab}	1.64 ± 0.09^{a}	1.53 ± 0.08^{ab}	N.S.
9d	2.15 ± 0.07^{ab}	2.05 ± 0.09^{b}	2.10 ± 0.06^{ab}	2.25 ± 0.05^{a}	2.09 ± 0.09^{b}	N.S.
13d (+Fr)	2.77 ± 0.11^{a}	2.61 ± 0.09^{ab}	2.61 ± 0.08^{ab}	2.44 ± 0.09^{b}	2.78 ± 0.11^{a}	N.S.
16 d (End AF)	2.91 ± 0.10^{b}	3.15 ± 0.08^{a}	2.86 ± 0.11^{b}	2.75 ± 0.07^{b}	2.96 ± 0.08^{ab}	*
Ethanol (%, (v/v))						
16 d (End AF)	6.13 ± 0.07^{a}	6.07 ± 0.11^{a}	6.28 ± 0.09^{a}	6.21 ± 0.08^{a}	6.16 ± 0.08^{a}	N.S.

Values are expressed as mean ± standard deviation (n = 3).
Abbreviations: S.S., statistical significance.
Beer fermented by: ME1, *St. lactis-condensi* MN412 initially, followed by *S. cerevisiae* US-05 strain after 48 h; ME2, *C. oleophila* YS209 initially, followed by *S. cerevisiae* US-05 strain after 48 h; ME3, *St. lactis-condensi* MN412 co-inoculated with *S. cerevisiae* US-05 strain; ME4, *C. oleophila* YS209 co-inoculated with *S. cerevisiae* US-05 strain; MEC (control), *S. cerevisiae* US-05 strain as control. Different letters within the same row indicate significant differences according to Tukey's test. Symbols: ***, $P < 0.001$; **, $P < 0.01$; *$P < 0.05$; N.S., not significant.

juice.

The inoculation strategies likely facilitated greater release of phenolics bound to the melon matrix. Furthermore, interactions among yeasts during mixed fermentations may have altered the redox potential of the medium, thereby reducing oxidative losses and improving phenolics stability. Both inoculation type (co-inoculation or sequential) and fermentation duration proved critical, influencing extraction efficiency and enzymatic conversion of precursors into more soluble, bioactive forms. Similar outcomes have been reported in fruit beers and mixed fermentations, where non-*Saccharomyces* yeasts enhanced polyphenol content and antioxidant activity (Piazzon et al., 2010; Zhao et al., 2013). Regarding to ORAC (Fig. 3c), the highest value was recorded in ME2 (148,706 μM Trolox/L). These values reflect the strong influence of winter-melon sugars and mixed-yeast interactions on redox-related

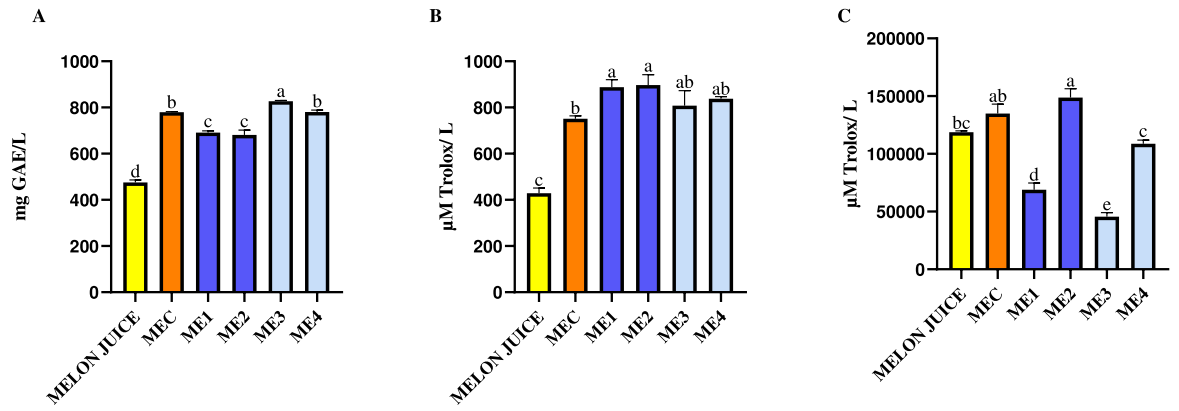

Fig. 3. Total polyphenols (A) and total antioxidant capacity: TEAC and ORAC (B, C) in melon juice and melon beers. Beer fermented by: ME1, *St. lactis-condensi* MN412 initially, followed by *S. cerevisiae* US-05 strain after 48 h; ME2, *C. oleophila* YS209 initially, followed by *S. cerevisiae* US-05 strain after 48 h; ME3, *St. lactis-condensi* MN412 co-inoculated with *S. cerevisiae* US-05 strain; ME4, *C. oleophila* YS209 co-inoculated with *S. cerevisiae* US-05 strain; MEC (control), *S. cerevisiae* US-05 strain as control. Data are expressed as mean ± standard deviation (n = 3). Error bars represent standard deviation. Different letters (a-d) in the same graph indicate significant differences between melon juice and melon beers. Results are expressed as mean ± standard deviation performed in three technical replicates. One-way ANOVA followed by Tukey's test ($p < 0.05$). GAE: Gallic acid equivalent; ORAC: Oxygen radical absorbance capacity; TEAC: Trolox equivalent antioxidant capacity.

metabolite formation. The ME2 value was approximately 7.5-fold higher, thereby underscoring the remarkable antioxidant potential imparted by melon enrichment. This increase highlights the synergistic effect of white melon juice and the microbial strains employed. To date, no published studies have examined melon beer. Lower ORAC values were observed in ME1 and ME3 (45,625–68,919 µM Trolox/L), indicating reduced antioxidant capacity compared to melon juice. In ME4, co-inoculation of *C. oleophila* YS209 with *S. cerevisiae* US-05 yielded 108, 700 µM Trolox/L, a value comparable to melon juice (118,701 µM Trolox/L). Overall, the ME2 biotechnological approach was most relevant under the tested conditions, yielding the highest antioxidant values in both TEAC and ORAC assays. Recent findings corroborate these results. Chen et al. (2023) reported that simultaneous inoculation of *S. cerevisiae* with *L. plantarum* increased antioxidant capacity in apple cider compared to single-yeast fermentation. Nardini (2023) demonstrated that "special beers" enriched with fruits, vegetables, or aromatic plants exhibit higher phenolic content and antioxidant activity than conventional beers. Similarly, Grieco et al. (2024) showed that wheat beers fermented sequentially with non-*Saccharomyces* and *S. cerevisiae* achieved higher TPC values (~566 mg GAE/L) than those fermented with *S. cerevisiae* alone.

It can therefore be hypothesised that the presence of non-*Saccharomyces* yeasts or sequential strategies promote greater hydrolysis of bound phenolic precursors (via yeast-produced β-glucosidase/esterase), improved utilisation of residual sugars (reducing oxidative stress and stabilizing polyphenols), and activation of yeast-specific metabolic pathways that generate more active free or transformed phenolic compounds. This literature supports the observation that sample ME2, inoculated sequentially with *St. lactis-condensi* MN412 and *S. cerevisiae* US-05, showed elevated TPC values, highlighting the positive effect of yeast selection and sequential inoculation.

3.4. Volatile organic compound composition

The volatile organic compounds (VOCs) in winter-melon juice were primarily composed of alcohols (12.4 mg/L), esters (6.24 mg/L), and aldehydes (2.24 mg/L) (Table 3). Within each class, the compounds were 3-nonen-1-ol (12.4 mg/L), ethyl acetate (3.03 mg/L), and trans-2-nonenal (2.24 mg/L). These results differ from those reported by Sun et al. (2022), who investigated "Large Round" winter-melon juices under various storage conditions and identified terpenes such as limonene and pinene, likely reflecting varietal differences in the melons studied.

In the experimental beers, VOC profiles varied considerably across strains and inoculation strategies, as illustrated in the heat map (Fig. 4). Table 4 summarizes the wide range of VOCs detected, grouped into four categories: alcohols, esters, terpenes, and others. Such variability indicates that yeast metabolism and microbial succession play a central role in modulating aroma development during fruit-beer fermentation.

Table 3
Concentrations of volatile organic compounds in winter melon juice determined by SPME–GC/MS (mg/L).

RT	Compounds	
	∑Alcohols	**12.4**
20.499	3-Nonen-1-ol	12.4
	∑Esters	**6.24**
4.353	Methyl acetate	0.44
5.353	Ethyl acetate	3.03
7.602	Ethyl propanoate	0.40
9.002	Ethyl isobutanoate	0.35
9.552	Methyl-2-methyl butanoate	0.40
10.351	Ethyl butanoate	0.68
11.851	Ethyl-2-methyl butanoate	0.31
21.598	Ethyl octanoate	0.63
	∑Aldehydes	**2.24**
20.898	*trans*-2-Nonenal	2.24

Esters were the most abundant class of VOCs across all trials, reaching the highest concentration in the ME2 trial (182.32 mg/L), which employed sequential inoculation with *C. oleophila* YS209 followed by *S. cerevisiae* US-05 after 48 h.

In contrast, the control trial MEC, inoculated only with *S. cerevisiae* US-05, revealed a lower but still substantial ester concentration of 137.45 mg/L, the second highest overall. A total of 18 ester compounds were identified, 10 of which exhibited olfactory activity values greater than one, indicating a significant sensory contribution. These included ethyl butanoate, isoamyl acetate, ethyl hexanoate, ethyl heptanoate, ethyl octanoate, isoamyl hexanoate, 2-phenylethyl acetate, ethyl nonanoate, ethyl decanoate, and ethyl dodecanoate. Esters are among the most important volatile compounds in fermented beverages, contributing sweet and fruity aroma notes. Their formation depends largely on yeast metabolism, involving both enzymatic activity and non-enzymatic esterification reactions between alcohols and organic acids (Pires et al., 2014; Zhang et al., 2023). Ester production is highly strain-dependent (Pires et al., 2014) and plays a crucial role in shaping beer aroma and overall sensory perception (Holt et al., 2019).

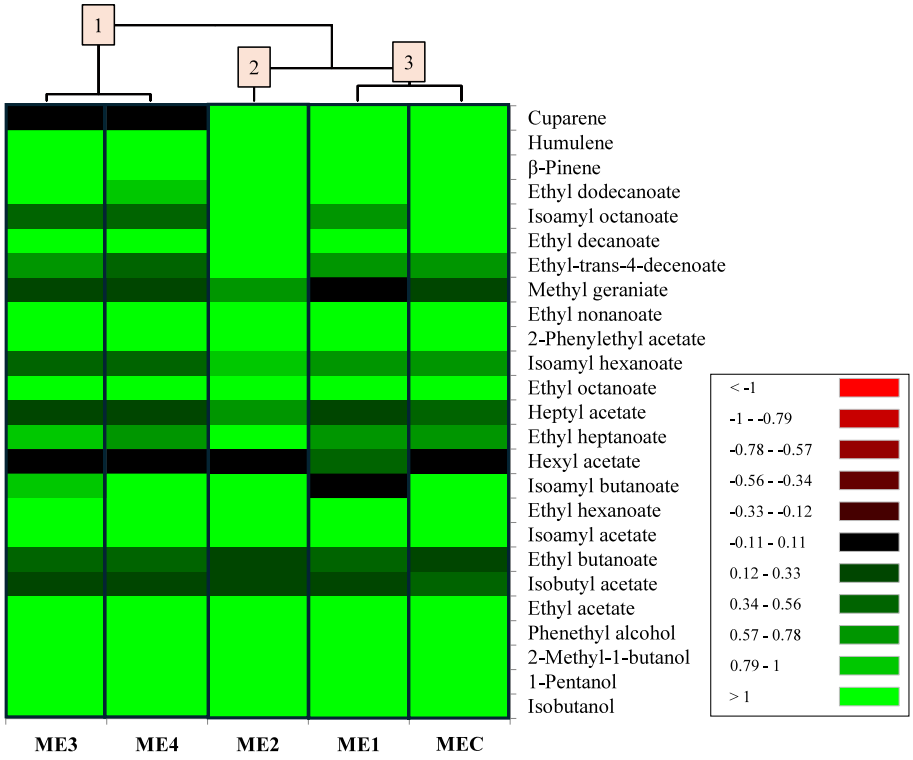

Fig. 4. Distribution of VOCs in fruit beers. The heat map plot illustrates the relative concentrations of various VOCs, providing a visual comparison of their presence across different samples. Beer fermented by: ME1, *St. lactis-condensi* MN412 initially, followed by *S. cerevisiae* US-05 strain after 48 h; ME2, *C. oleophila* YS209 initially, followed by *S. cerevisiae* US-05 strain after 48 h; ME3, *St. lactis-condensi* MN412 co-inoculated with *S. cerevisiae* US-05 strain; ME4, *C. oleophila* YS209 co-inoculated with *S. cerevisiae* US-05 strain; MEC (control), *S. cerevisiae* US-05 strain as control. Data were autoscaled prior to analysis and hierarchical clustering was performed using Ward's method.

Sequential inoculation consistently yielded higher ester levels than co-inoculation. The trials ME1 and ME2 recorded 116.57 mg/L and 182.32 mg/L, respectively, while ME3 and ME4 showed lower concentrations (94.56 mg/L and 84.48 mg/L, respectively). Ethyl octanoate was the most abundant ester, peaking in trial ME2 (97.07 mg/L), followed by MEC (74.99 mg/L). Ethyl decanoate reached 43.09 mg/L in ME2 compared to 18.45 mg/L recorded in MEC, while isoamyl acetate was present at 18.63 mg/L in MEC and 18.09 mg/L in ME3. Pirrone et al. (2025) reported lower concentrations of these compounds under sequential inoculation with *S. cerevisiae* US-05 and *C. oleophila* YS209, although similar trends were observed for *St. lactis-condensi* MN412. For ethyl octanoate, Pirrone et al. (2025) reported slightly higher levels (77.30 mg/L) than the 73.94 mg/L measured in the present study.

Among the esters produced during beer fermentation, ethyl octanoate (sour apple), isoamyl acetate (banana-like), ethyl acetate (sweet fruit), and ethyl decanoate (apple-like) are particularly influential (Verstrepen et al., 2003; Viejo et al., 2019). Even minor variations in their concentrations can significantly alter sensory perception (Thompson Witrick et al., 2017). Ethyl octanoate, with its apple-like aroma, plays a major role in imparting fruity sweetness (Verstrepen et al., 2003) and has been detected in wheat beers brewed with Maiorca wheat malt (Gugino et al., 2024). Isoamyl acetate has been identified throughout lager brewing and in traditional sorghum beers (Alves et al., 2020; Attchelouwa et al., 2020). Ethyl decanoate has been reported in wheat beers brewed with condensers to reduce volatilisation losses (Ocvirk et al., 2018), as well as in beers produced via top, bottom, and spontaneous fermentations (Viejo et al., 2019), including stout, lager, and wheat beers (De Flaviis et al., 2022). Collectively, these esters are central to the diverse aroma profiles of different beer styles.

Alcohol concentrations across trials ranged between 83.04 and 118.20 mg/L, with all compounds showing odour activity values < 1. Terpenes varied between 8.29 and 49.54 mg/L, with ME1 recording the highest and ME3 the lowest values. Among the terpenoids detected, only humulene had an odour activity value > 1 (33.22 mg/L in ME1, 17.61 mg/L in ME2). These concentrations were higher than those in MEC (14.93 mg/L) and in co-inoculation trials ME3 and ME4. Terpenoids can originate from yeast biosynthesis (Wang et al., 2018) or hops (Brendel et al., 2020; Dietz et al., 2020; Ramírez & Viveros, 2021). Rodolfi et al. (2023) identified humulene in beers produced via dry-hopping with different cultivars under varied fertilisation practices. Overall, sequential inoculation produced higher concentrations of aroma active VOCs, particularly esters and terpenes, than co-inoculation trials. This suggests that sequential inoculation influenced the formation of key volatile molecules, thereby contributing to differences in aromatic profile and complexity of fruit beers.

3.5. Sensory evaluation

The results of the quantitative sensory analysis are presented in Fig. 5. These results revealed significant differences among the trials. Non-*Saccharomyces* yeasts significantly influenced microbial dynamics and metabolite formation, with the impact varying across inoculation strategies; sensory differences were consistent with these metabolic shifts. These sensory differences are consistent with the strain-dependent metabolic and aromatic profiles observed during fermentation. Importantly, none of beers had defects and all trials were positively received by the tasting panel. ME2 displayed the sensory attributes most strongly associated with its specific metabolic profile. Panellists noted that beers brewed with sequential inoculation showed greater complexity, likely related to the ability of non-*Saccharomyces* yeasts to generate distinctive aroma compounds. This enhanced complexity is in line with the higher concentrations of aroma-active esters and volatile compounds observed under sequential inoculation. The presence of melon juice was consistently perceived and positively evaluated, with

Table 4
Concentrations of volatile organic compounds in fruit beer determined by SPME–GC/MS (mg/L).

RT	Compounds	Aroma Description	Odour Threshold	ME1 (OAV)	ME2 (OAV)	ME3 (OAV)	ME4 (OAV)	MEC (OAV)	S. S.
	∑Alcohols			**45.60 ± 0.62^{b}**	**47.20 ± 0.75^{b}**	**55.58 ± 0.59^{a}**	**37.23 ± 0.84^{b}**	**38.69 ± 0.40^{c}**	***
5.703	2-Methylpropanol	alcoholic, malty, solvent	80[a]	3.46 ± 0.05^{c} (<1)	3.16 ± 0.09^{d} (<1)	4.99 ± 0.11^{a} (<1)	4.02 ± 0.15^{b} (<1)	3.82 ± 0.08^{b} (<1)	***
8.452	1-Pentanol	Alcoholic, iodoform-like	80[b]	13.77 ± 0.24^{c} (<1)	15.4 ± 0.21^{b} (<1)	26.07 ± 0.24^{a} (<1)	12.56 ± 0.31^{d} (<1)	15.54 ± 0.10^{b} (<1)	***
8.552	2-Methyl-1-butanol	Alcoholic, banana, vinous	65[b]	12.09 ± 0.12^{a} (<1)	10.57 ± 0.10^{b} (<1)	7.75 ± 0.09^{d} (<1)	12.09 ± 0.21^{a} (<1)	8.68 ± 0.08^{c} (<1)	***
19.949	Phenethyl alcohol	Rose, perfumy	125[b]	16.28 ± 0.21^{b} (<1)	18.07 ± 0.35^{a} (<1)	16.77 ± 0.15^{b} (<1)	8.56 ± 0.17^{d} (<1)	10.65 ± 0.14^{c} (<1)	***
	∑Esters			**116.75 ± 1.56^{c}**	**182.32 ± 2.01^{a}**	**94.56 ± 1.80^{d}**	**84.48 ± 1.90^{e}**	**137.45 ± 1.71^{b}**	***
5.353	Ethyl acetate	Fruity, sweet	30[b]	3.96 ± 0.10^{b} (<1)	3.5 ± 0.12^{c} (<1)	2.18 ± 0.14^{d} (<1)	1.73 ± 0.08^{e} (<1)	4.71 ± 0.11^{a} (<1)	***
9.453	Isobutyl acetate	Banana, sweet, fruity	1.60[a]	0.29 ± 0.03^{b} (<1)	0.31 ± 0.02^{b} (<1)	0.31 ± 0.02^{b} (<1)	0.26 ± 0.01^{b} (<1)	0.44 ± 0.02^{a} (<1)	***
10.351	Ethyl butanoate	Papaya, apple, perfumed	0.40[a]	0.35 ± 0.00^{b} (<1)	0.32 ± 0.02^{bc} (<1)	0.44 ± 0.01^{a} (1.10)	0.40 ± 0.03^{a} (1.00)	0.28 ± 0.01^{c} (<1)	***
12.751	Isoamyl acetate	Fruity, banana, pear	0.50[a]	11.61 ± 0.08^{c} (23.22)	9.70 ± 0.09^{d} (19.4)	18.09 ± 0.21^{b} (36.18)	11.46 ± 0.15^{c} (22.92)	18.63 ± 0.30^{a} (37.26)	***
16.350	Ethyl hexanoate	Apple, fruity, aniseed	0.17[a]	2.59 ± 0.07^{e} (15.23)	8.32 ± 0.15^{b} (48.94)	3.45 ± 0.10^{d} (20.29)	9.01 ± 0.24^{a} (53.00)	4.32 ± 0.14^{c} (25.41)	***
16.650	Isoamyl butanoate	Fruity, melon, berry	unknown	0.00 ± 0.00^{c} (n. d.)	1.45 ± 0.05^{a} (n. d.)	0.99 ± 0.08^{b} (n. d.)	1.10 ± 0.07^{b} (n.d.)	1.14 ± 0.06^{b} (n. d.)	***
16.700	Hexyl acetate	aromatic, perfumed	3.50[a]	0.43 ± 0.02^{a} (<1)	0.00 ± 0.00^{b} (<1)	0.00 ± 0.00^{b} (<1)	0.00 ± 0.00^{b} (<1)	0.00 ± 0.00^{b} (<1)	***
18.949	Ethyl heptanoate	fruity, perfumed, fatty	0.40[a]	0.60 ± 0.04^{c} (1.50)	1.15 ± 0.05^{a} (2.87)	0.88 ± 0.04^{b} (0.22)	0.57 ± 0.04^{c} (1.42)	0.66 ± 0.03^{c} (1.65)	***
19.349	Heptyl acetate	pear, fruity, aromatic	1.40[a]	0.21 ± 0.00^{d} (<1)	0.60 ± 0.03^{a} (<1)	0.31 ± 0.02^{c} (<1)	0.33 ± 0.01^{c} (<1)	0.42 ± 0.02^{b} (<1)	***
21.598	Ethyl octanoate	Apple, sweet, fruity	0.37[b]	73.94 ± 0.45^{b} (199.83)	97.07 ± 0.42^{a} (262.35)	49.87 ± 0.35^{c} (134.78)	47.5 ± 0.52^{d} (128.38)	74.99 ± 0.28^{b} (202.67)	***
22.748	Isoamyl hexanoate	Apple, fruity, aniseed	0.17[b]	0.66 ± 0.05^{b} (3.88)	0.78 ± 0.04^{a} (4.58)	0.45 ± 0.02^{c} (2.65)	0.47 ± 0.04^{c} (2.76)	0.59 ± 0.03^{b} (3.47)	***
23.048	2-Phenylethyl acetate	roses, honey, apple, flowery	0.20[a]	1.74 ± 0.10^{d} (8.70)	2.30 ± 0.12^{c} (11.50)	2.82 ± 0.15^{b} (14.10)	1.73 ± 0.16^{d} (8.65)	3.59 ± 0.09^{a} (17.95)	***
23.748	Ethyl nonanoate	Fruity, fatty acids, sweet	1.20[a]	3.48 ± 0.21^{b} (2.90)	5.14 ± 0.24^{a} (4.28)	4.60 ± 0.31^{a} (3.83)	2.77 ± 0.18^{c} (2.31)	5.17 ± 0.07^{a} (4.31)	***
24.447	Methyl geraniate	Floral, rose-fatty	unknown	0.00 ± 0.00^{c} (n. d.)	0.63 ± 0.05^{a} (n. d.)	0.27 ± 0.02^{b} (n. d.)	0.26 ± 0.01^{b} (n.d.)	0.28 ± 0.02^{b} (n. d.)	***
25.647	Ethyl-trans-4-decenoate	Green, pineapple, pear	unknown	0.70 ± 0.02^{bc} (n.d.)	1.11 ± 0.04^{a} (n. d.)	0.59 ± 0.06^{c} (n. d.)	0.39 ± 0.05^{d} (n.d.)	0.77 ± 0.05^{b} (n. d.)	***
25.997	Ethyl decanoate	Caprylic, fruity, apple	0.57[a]	11.74 ± 0.24^{c} (20.60)	43.09 ± 0.31^{a} (75.60)	7.57 ± 0.15^{d} (13.28)	5.08 ± 0.21^{e} (8.91)	18.45 ± 0.28^{b} (32.37)	***
27.097	3-Methylbutyl octanoate	fruity, orange, pear, melon	2[a]	0.77 ± 0.05^{c} (<1)	1.27 ± 0.11^{b} (<1)	0.39 ± 0.04^{d} (<1)	0.49 ± 0.03^{d} (<1)	1.49 ± 0.08^{a} (<1)	***
30.096	Ethyl dodecanoate	caprylic, soapy, estery	2[a]	3.68 ± 0.10^{b} (1.84)	5.58 ± 0.15^{a} (2.79)	1.35 ± 0.08^{c} (<1)	0.93 ± 0.07^{d} (<1)	1.52 ± 0.12^{c} (<1)	***
	∑Terpenes			**49.54 ± 0.61^{a}**	**28.67 ± 0.38^{b}**	**8.29 ± 0.30^{d}**	**4.93 ± 0.28^{e}**	**14.93 ± 0.50^{c}**	***
16.050	β-Pinene	pine, mint, eucalyptus	unknown	2.12 ± 0.12^{b} (n. d.)	2.79 ± 0.08^{a} (n. d.)	2.17 ± 0.09^{b} (n. d.)	2.01 ± 0.10^{bc} (n.d.)	1.78 ± 0.07^{c} (n. d.)	***
27.597	Humulene	spicy, herbaceous	0.81	33.22 ± 0.24^{a} (41.52)	17.61 ± 0.15^{b} (22.01)	6.12 ± 0.21^{d} (7.65)	2.92 ± 0.18^{e} (3.65)	8.75 ± 0.15^{c} (10.94)	***
27.997	Cuparene	unknown	unknown	14.20 ± 0.25^{a} (n.d.)	8.27 ± 0.15^{b} (n. d.)	0.00 ± 0.00^{d} (n. d.)	0.00 ± 0.00^{d} (n.d.)	4.40 ± 0.28^{c} (n. d.)	***

Concentrations are calculated using ethyl octanoate, 2-heptanol, and limonene as the standards for the calibration lines.
Compounds in each class are ordered according to their retention time.
RT, retention time using a non-polar DB-5MS column.
Values are expressed as mean ± standard deviation (n = 3).
Abbreviations: S.S., statistical significance.
Beer fermented by: ME1, *St. lactis-condensi* MN412 initially, followed by *S. cerevisiae* US-05 strain after 48 h; ME2, *C. oleophila* YS209 initially, followed by *S. cerevisiae* US-05 strain after 48 h; ME3, *St. lactis-condensi* MN412 co-inoculated with *S. cerevisiae* US-05 strain; ME4, *C. oleophila* YS209 co-inoculated with *S. cerevisiae* US-05 strain; MEC (control), *S. cerevisiae* US-05 strain as control. Different letters within the same row indicate significant differences according to Tukey's test. Symbols: ***, $P < 0.001$; **, $P < 0.01$; *$P < 0.05$; N.S., not significant.
[a] Zunkel et al. (2011)
[b] Maarse (1991)

particular emphasis on the sensory attributes derived from the specific fermentation profile. ME1 and ME2 trials were especially noteworthy, and both achieved high overall acceptability scores (5.80 and 6.40, respectively).

The sensory profile of the ME2 beer was distinguished by the highest overall acceptability scores and superior ratings across eight attributes, while ME1 followed with seven. Specifically, ME2 trial achieved top scores for aroma intensity (7.2), aromatic complexity (6.8), and fruity

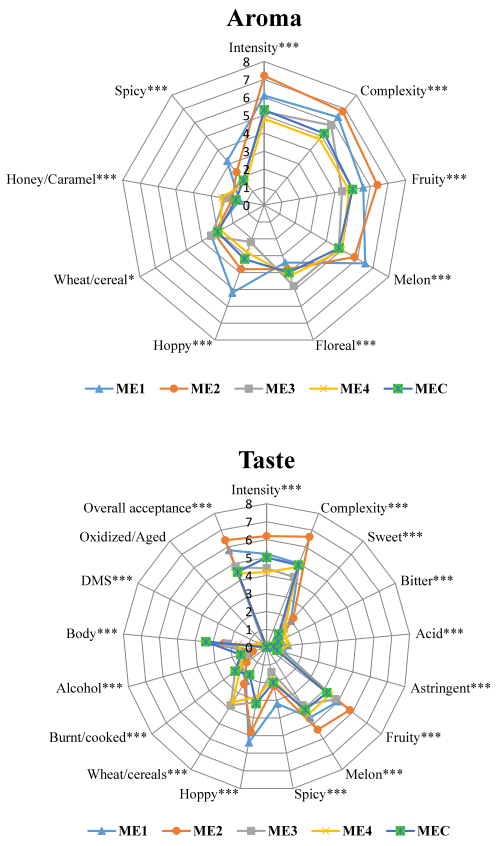

Fig. 5. Sensory analysis of the beers included spider plots illustrating the average scores for aroma, taste attributes, and overall quality of bottled fruit beers, as assessed by judges during tasting sessions. Beer fermented by: ME1, *St. lactis-condensi* MN412 initially, followed by *S. cerevisiae* US-05 strain after 48 h; ME2, *C. oleophila* YS209 initially, followed by *S. cerevisiae* US-05 strain after 48 h; ME3, *St. lactis-condensi* MN412 co-inoculated with *S. cerevisiae* US-05 strain; ME4, *C. oleophila* YS209 co-inoculated with *S. cerevisiae* US-05 strain; MEC (control), *S. cerevisiae* US-05 strain as control. Symbols: ***, $P < 0.001$; **, $P < 0.01$; *$P < 0.05$.

notes (6.4). ME1, in contrast, excelled in melon (6.5), hoppy (5.2), wheat/cereal (3.4), and spicy notes (3.2). In terms of flavour, ME2 outperformed in intensity, complexity, sweetness, fruity notes, and melon attributes, whereas ME1 scored higher in sourness, astringency, spiciness, and hoppiness. These differences suggest that sequential inoculation favoured a more balanced and complex sensory profile, while trials characterised by stronger cereal- and hop-derived attributes showed reduced overall aromatic complexity. Comparable findings have been reported in other studies. Pirrone et al. (2025) observed similar increases in complexity with sequential inoculation of *C. oleophila* YS209 and *S. cerevisiae* US-05, likely due to the strong capacity of strain YS209 to produce aromatic compounds. Francesca et al. (2024) reported analogous results in Frappato wine, where both YS209 and MN412 positively influenced fruity and floral intensity with YS209 contributing most to overall complexity. By contrast, ME3 and ME4 trials, in which *C. oleophila* YS209 and *St. lactis condensi* were co-inoculated with *S. cerevisiae* US-05, exhibited lower values across several parameters, even though these beers were still well detected by the panel. These experimental beers were clearly distinct from the control MEC trial, brewed solely with commercial *S. cerevisiae* US-05 strain, which scored higher for "Burnt/Cooked", "Alcohol", and "Body" attributes, but exhibited lower overall complexity and intensity. Overall, the sensory evaluation indicates that sequential inoculation with non-*Saccharomyces* yeasts proved to be the most effective strategy for enhancing aromatic complexity and consumer acceptability in fruit-based beer production.

3.6. Multivariate analysis and correlation between volatile organic compounds and sensory profiles

Fig. 4 illustrates the results of the VOC analysis. The hierarchical dendrogram, together with the heat map, showed that the inoculation strategies of *C. oleophila* YS209 and *St. lactis-condensi* MN412 had a marked impact on the VOC profiles of the fruit beers. Differences in VOC concentrations among the samples led to their separation into three distinct clusters. Specifically, the melon beer trials separated into two groups: ME3 and ME4 clustered together, whereas ME1, ME2, and MEC grouped within another main cluster. These findings emphasize that co-inoculation and sequential inoculation represent distinct approaches, each producing specific effects on the VOC profile, as reflected in the dendrogram and heat map.

PCA was then applied to investigate the relationship between VOCs (above their odour thresholds) and sensory attributes, with a focus on aroma descriptors. As shown in Fig. 6, the first factor F1 accounted for 56.03% of the overall variance, while the factor F2 explained 18.71%. The resulting biplot clearly distinguished the beer samples, highlighting their unique aromatic profiles.

ME2 was strongly associated with several esters, ethyl nonanoate, ethyl heptanoate, ethyl decanoate, ethyl octanoate, isoamyl hexanoate, and ethyl dodecanoate, all known to provide fruity and sweet notes (Verstrepen et al., 2003). This correlation was confirmed by sensory evaluation, as ME2 received the highest scores for fruitiness, aromatic complexity, and overall intensity. By contrast, ME1 was more closely linked to hop-derived attributes, largely driven by humulene, a compound typical of hop aroma (Rodolfi et al., 2023). Meanwhile, ME3 and MEC were associated with floral and honey/caramel descriptors, with the floral notes particularly attributed to phenylethyl acetate, an ester widely recognized for its contribution to such aromas (Verstrepen et al., 2003). Overall, the results clarify how non-*Saccharomyces* yeasts modulate microbial succession, sugar utilisation and stress-response pathways within a mixed fermentation ecosystem. These ecological dynamics explain the observed differences in metabolite formation and highlight strain-dependent metabolic adaptation in a fruit-enriched fermentation matrix.

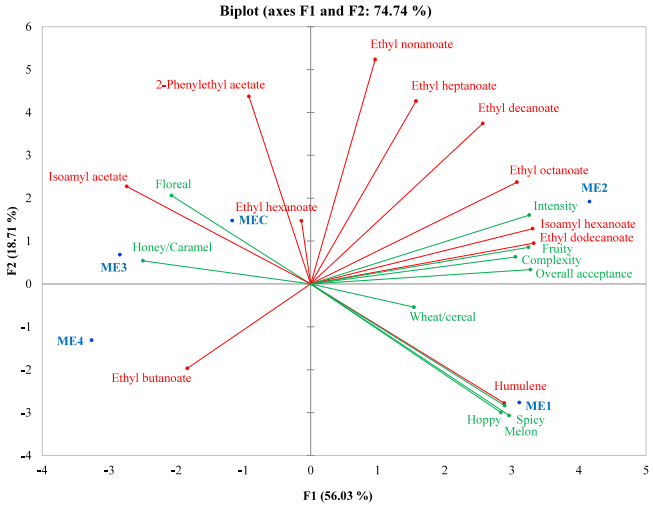

Fig. 6. Principal component analysis (PCA) biplot for VOCs above the perception threshold and sensory attributes (only olfactory parameters). Beer fermented by: ME1, *St. lactis-condensi* MN412 initially, followed by *S. cerevisiae* US-05 strain after 48 h; ME2, *C. oleophila* YS209 initially, followed by *S. cerevisiae* US-05 strain after 48 h; ME3, *St. lactis-condensi* MN412 co-inoculated with *S. cerevisiae* US-05 strain; ME4, *C. oleophila* YS209 co-inoculated with *S. cerevisiae* US-05 strain; MEC (control), *S. cerevisiae* US-05 strain as control. PCA was performed on autoscaled data, only compounds with OAV >1 were included.

4. Conclusions

This study advances understanding of the ecological roles of *C. oleophila* YS209 and *St. lactis-condensi* MN412 in mixed fermentation applied to fruit-based beer production. It emphasises their influence on microbial succession, metabolic pathways, and the formation of volatile compounds as well as on physicochemical, antioxidant, and sensory properties of the final beers. By comparing sequential and co-inoculation approaches in a fruit-enriched fermentation matrix, the study demonstrates how these non-*Saccharomyces* yeasts modulate sugar utilisation patterns, stress-response behaviours, and strain-dependent fermentation outputs. In particular, sequential inoculation was associated with higher glycerol production, increased levels of aroma-active esters, and greater sensory complexity. The integration of population dynamics, volatile profiling, antioxidant properties, and multivariate analyses provides a comprehensive view of the ecological interactions occurring within mixed-yeast systems and their impact on beer quality attributes. These findings contribute to a broader understanding of yeast behaviour in complex fermentation ecosystems, highlighting the potential of non-*Saccharomyces* species to modulate microbial and biochemical trajectories in food fermentations and to contribute to the development of differentiated sensory profiles in craft brewing. Overall, the combined use of winter-melon juice and selected non-*Saccharomyces* yeasts represents a promising biotechnological strategy for the production of innovative fruit beers with enhanced functional and sensory value. Further studies should investigate the mechanistic basis of strain resilience and metabolic adaptation under different environmental conditions and explore the scalability and applicability of this approach to other fruit matrices.

CRediT authorship contribution statement

Antonino Pirrone: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Valentina Craparo:** Methodology, Formal analysis, Data curation. **Giulio Perricone:** Investigation, Data curation. **Ignazio Maria Gugino:** Methodology, Investigation. **Enrico Viola:** Visualization, Software. **Vincenzo Naselli:** Methodology, Data curation. **Azzurra Vella:** Formal analysis, Data curation. **Irene Dolce:** Methodology, Formal analysis. **Marta Cuenca-Ortolá:** Writing – original draft, Visualization, Software, Formal analysis. **Mónica Gandía:** Writing – review & editing, Software, Formal analysis. **Antonio Cilla:** Writing – review & editing, Writing – original draft, Validation, Supervision, Conceptualization. **Antonella Maggio:** Writing – review & editing, Writing – original draft, Validation, Supervision, Formal analysis, Data curation. **Raimondo Gaglio:** Investigation, Conceptualization. **Luca Settanni:** Writing – review & editing, Writing – original draft, Supervision. **Giancarlo Moschetti:** Writing – review & editing, Writing – original draft, Validation. **Nicola Francesca:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Funding acquisition. **Antonio Alfonzo:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Conceptualization.

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Declaration of competing interest

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

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Data availability

Data will be made available on request.

References

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

Alfonzo, A., Gaglio, R., Miceli, A., Francesca, N., Di Gerlando, R., Moschetti, G., & Settanni, L. (2018). Shelf life evaluation of fresh-cut red chicory subjected to different minimal processes. *Food Microbiology, 73*, 298–304. https://doi.org/10.1016/j.fm.2018.02.008

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

Alves, V., Gonçalves, J., Figueira, J. A., Ornelas, L. P., Branco, R. N., Câmara, J. S., & Pereira, J. A. (2020). Beer volatile fingerprinting at different brewing steps. *Food Chemistry, 326*, Article 126856. https://doi.org/10.1016/j.foodchem.2020.126856

Attchelouwa, C. K., N'guessan, F. K., Marcotte, S., Amoikon, T. L., Charmel, M., & Djè, M. K. (2020). Characterisation of volatile compounds associated to sensory changes during the storage of traditional sorghum beer by HS-GC/FID and SPME-GC/MS. *Journal of Agriculture and Food Research, 2*, Article 100088. https://doi.org/10.1016/j.jafr.2020.100088

Baiano, A. (2021). Craft beer: An overview. *Comprehensive Reviews in Food Science and Food Safety, 20*, 1829–1856. https://doi.org/10.1111/1541-4337.12693

Basso, R. F., Alcarde, A. R., & Portugal, C. B. (2016). Could non-*Saccharomyces* yeasts contribute on innovative brewing fermentations? *Food Research International, 86*, 112–120. https://doi.org/10.1016/j.foodres.2016.06.002

Binati, R. L., Innocente, G., Gatto, V., Celebrin, A., Polo, M., Felis, G. E., & Torriani, S. (2019). Exploring the diversity of a collection of native non-*Saccharomyces* yeasts to develop co-starter cultures for winemaking. *Food Research International, 122*, 432–442. https://doi.org/10.1016/j.foodres.2019.04.043

Bouaziz, A., Djidel, S., Bentaher, A., & Khennouf, S. (2020). Polyphenolic content, antioxidant and anti-inflammatory activities of melon (*Cucumis melo* L. var. inodorus) seeds. *Journal of Drug Delivery and Therapeutics, 10*, 22–26. https://doi.org/10.22270/jddt.v10i2-s.4022

Brendel, R., Schwolow, S., Rohn, S., & Weller, P. (2020). Gas-phase volatilomic approaches for quality control of brewing hops based on simultaneous GC-MS-IMS and machine learning. *Analytical and Bioanalytical Chemistry, 412*, 7085–7097. https://doi.org/10.1007/s00216-020-02842-y

Callejo, M. J., García Navas, J. J., Alba, R., Escott, C., Loira, I., González, M. C., & Morata, A. (2019). Wort fermentation and beer conditioning with selected non-*Saccharomyces* yeasts in craft beers. *European Food Research and Technology, 245*, 1229–1238. https://doi.org/10.1007/s00217-019-03244-w

Canonico, L., Agarbati, A., Comitini, F., & Ciani, M. (2016). *Torulaspora delbrueckii* in the brewing process: A new approach to enhance bioflavour and to reduce ethanol content. *Food Microbiology, 56*, 45–51. https://doi.org/10.1016/j.fm.2015.12.005

Carvalho, G. B., Silva, D. P., Bento, C. V., Vicente, A. A., Teixeira, J. A., Felipe, M. D. G. A., & Almeida e Silva, J. B. (2009). Banana as adjunct in beer production: Applicability and performance of fermentative parameters. *Applied Biochemistry and Biotechnology, 155*, 53–62. https://doi.org/10.1007/s12010-008-8458-y

Cavazza, A., Grando, M. S., & Zini, C. (1992). Rilevazione della flora microbica di mosti e vini. *Vignevini, 9*, 17–20.

Chambi, H. N. M., Lima, W. C. V., & Schmidt, F. L. (2016). Osmotic dehydration of yellow melon using red grape juice concentrate. *Food Science and Technology, 36*, 468–475. https://doi.org/10.1590/1678-457X.01416

Chand-Goyal, T., Eckert, J. W., Droby, S., & Atkinson, K. (1998). A method for studying the population dynamics of Candida oleophila on oranges in the grove, using a selective isolation medium and PCR technique. *Microbiology Research, 153*, 265–270.

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

Chen, X., Lin, M., Hu, L., Xu, T., Xiong, D., Li, L., & Zhao, Z. (2023). Research on the effect of simultaneous and sequential fermentation with *Saccharomyces cerevisiae* and *Lactobacillus plantarum* on antioxidant activity and flavor of apple cider. *Fermentation, 9*, 102. https://doi.org/10.3390/fermentation9020102

Cho, J. H., Kim, I. D., Dhungana, S. K., Do, H. M., & Shin, D. H. (2018). Persimmon fruit enhanced quality characteristics and antioxidant potential of beer. *Food Science and Biotechnology, 27*, 1067–1073. https://doi.org/10.1007/s10068-018-0340-2

Croonenberghs, A. P., Bongaerts, D., Bouchez, A., De Roos, J., & De Vuyst, L. (2024). Fruit beers, beers with or without a co-fermentation step with fruits. *Current Opinion in Biotechnology, 86*, Article 103081. https://doi.org/10.1016/j.copbio.2024.103081

Csoma, H., Kállai, Z., Czentye, K., & Sipiczki, M. (2023). *Starmerella lactis-condensi*, a yeast that has adapted to the conditions in the oenological environment. *International Journal of Food Microbiology*. , Article 110282. https://doi.org/10.1016/j.ijfoodmicro.2023.110282

De Flaviis, R., Santarelli, V., Mutarutwa, D., Giuliani, M., & Sacchetti, G. (2022). Volatiles profile of 'Blanche' wheat craft beer as affected by wheat origin: A chemometric study. *Food Chemistry, 385*, Article 132696. https://doi.org/10.1016/j.foodchem.2022.132696

De Melo, V. F., Araújo, G. S., Bispo, J. A. C., Del Bianchi, V. L., & De Carvalho, G. B. M. (2017). Effect of different concentrations of bush passion fruit pulp and temperature in the production of beer. *African Journal of Biotechnology, 16*, 1150–1158. https://doi.org/10.5897/AJB2016.15613

Di Maio, S., Polizzotto, G., Planeta, D., & Oliva, D. (2011). A method to discriminate between the *Candida stellata* and *Saccharomyces cerevisiae* in mixed fermentation on WLD and lysine agar media. *South African Journal for Enology and Viticulture, 32*, 35–41. https://doi.org/10.21548/32-1-1364

Dietz, C., Cook, D., Huismann, M., Wilson, C., & Ford, R. (2020). The multisensory perception of hop essential oil: A review. *Journal of the Institute of Brewing, 126*, 320–342. https://doi.org/10.1002/jib.622

Domizio, P., House, J. F., Joseph, C. M. L., Bisson, L. F., & Bamforth, C. W. (2016). *Lachancea thermotolerans* as an alternative yeast for the production of beer. *Journal of the Institute of Brewing, 122*, 599–604. https://doi.org/10.1002/jib.362

Du Plessis, H., Du Toit, M., Nieuwoudt, H., Van der Rijst, M., Kidd, M., & Jolly, N. (2017). Effect of *Saccharomyces*, non-*Saccharomyces* yeasts and malolactic fermentation strategies on fermentation kinetics and flavor of Shiraz wines. *Fermentation, 3*, 64. https://doi.org/10.3390/fermentation3040064

Durazzo, A., Lisciani, S., Camilli, E., Gabrielli, P., Marconi, S., Gambelli, L., Aguzzi, A., Lucarini, M., Maiani, G., Casale, G., & Marletta, L. (2017). Nutritional composition and antioxidant properties of traditional Italian dishes. *Food Chemistry, 218*, 70–77. https://doi.org/10.1016/j.foodchem.2016.08.120

Durazzo, A., Lucarini, M., Santini, A., Camilli, E., Gabrielli, P., Marconi, S., Lisciani, S., Aguzzi, A., Gambelli, L., & Novellino, E. (2019). Antioxidant properties of four commonly consumed popular Italian dishes. *Molecules, 24*, 1543. https://doi.org/10.3390/molecules24081543

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

FAOSTAT. (2025). *Food and Agriculture Organization of the United Nations*. https://www.fao.org/faostat/en/#data/QCL. (Accessed 6 February 2025).

Fernández-Cruz, E., Carrasco-Galán, F., Cerezo-López, A. B., Valero, E., Morcillo-Parra, M.Á., Beltrán, G., Torija, M. J., Troncoso, A. M., & García-Parrilla, M. C. (2020). Occurrence of melatonin and indolic compounds derived from L-tryptophan yeast metabolism in fermented wort and commercial beers. *Food Chemistry, 331*, Article 127192. https://doi.org/10.1016/j.foodchem.2020.127192

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

Francesca, N., Pirrone, A., Gugino, I., Prestianni, R., Naselli, V., Settanni, L., Todaro, A., Guzzon, R., Maggio, A., Porrello, A., Bruno, M., Farina, V., Passafiume, R., Alfonzo, A., Moschetti, G., & Gaglio, R. (2023). A novel microbiological approach to impact the aromatic composition of sour loquat beer. *Food Bioscience, 55*, Article 103011. https://doi.org/10.1016/j.fbio.2023.103011

Gaglio, R., Alfonzo, A., Francesca, N., Corona, O., Di Gerlando, R., Columba, P., & Moschetti, G. (2017). Production of the Sicilian distillate "Spiritu re fascitrari" from honey by-products: An interesting source of yeast diversity. *International Journal of Food Microbiology, 261*, 62–72. https://doi.org/10.1016/j.ijfoodmicro.2017.09.004

Gasinski, A., Kawa-Rygielska, J., Szumny, A., Czubaszek, A., Gąsior, J., & Pietrzak, W. (2020). Volatile compounds content, physicochemical parameters, and antioxidant activity of beers with addition of mango fruit (*Mangifera indica*). *Molecules, 25*, 3033. https://doi.org/10.3390/molecules25133033

Gorzelany, J., Patyna, M., Pluta, S., Kapusta, I., Balawejder, M., & Belcar, J. (2022). The effect of the addition of ozonated and non-ozonated fruits of the Saskatoon berry (*Amelanchier alnifolia* Nutt.) on the quality and pro-healthy profile of craft wheat beers. *Molecules, 27*, 4544. https://doi.org/10.3390/molecules27144544

Grieco, F., Fiore, A., Gerardi, C., Tufariello, M., Romano, G., & Baiano, A. (2024). Optimisation of quality features of new wheat beers fermented through sequential inoculation of non-*Saccharomyces* and *Saccharomyces* yeasts. *Heliyon, 10*, Article e37598. https://doi.org/10.1016/j.heliyon.2024.e3759

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*, 984. https://doi.org/10.3389/fmicb.2019.0098

Gugino, I. M., Alfeo, V., Ashkezary, M. R., Marconi, O., Pirrone, A., Francesca, N., Cincotta, F., Verzera, A., & Todaro, A. (2024). Maiorca wheat malt: A comprehensive analysis of physicochemical properties, volatile compounds, and sensory evaluation in brewing process and final product quality. *Food Chemistry, 435*, Article 137517. https://doi.org/10.1016/j.foodchem.2023.137517

Holt, S., Miks, M. H., de Carvalho, B. T., Foulquié-Moreno, M. R., & Thevelein, J. M. (2019). The molecular biology of fruity and floral aromas in beer and other alcoholic beverages. *FEMS Microbiology Reviews, 43*, 193–222. https://doi.org/10.1093/femsre/fuy041

Holt, S., Mukherjee, V., Lievens, B., Verstrepen, K. J., & Thevelein, J. M. (2018). Bioflavoring by non-conventional yeasts in sequential beer fermentations. *Food Microbiology, 72*, 55–66. https://doi.org/10.1016/j.fm.2017.11.008

Iris, L., Antonio, M., Antonia, B. M., & Antonio, S. L. J. (2020). Isolation, selection, and identification techniques for non-*Saccharomyces* yeasts of oenological interest. In *Biotechnological progress and beverage consumption* (pp. 467–508). Academic Press. https://doi.org/10.1016/B978-0-12-816678-9.00015-1.

Jung, K. M., Kim, S. Y., Seo, E. C., Lee, H. I., Kwon, O. H., Kim, H. R., Dhungana, S. K., Park, Y. S., & Kim, I. D. (2017). Influence of peach (*Prunus persica* L. Batsch) fruit addition on quality characteristics and antioxidant activities of beer. *International journal of sciences, 6*(08), 186–191. https://doi.org/10.18483/ijSci.1411

Kawa-Rygielska, J., Adamenko, K., Kucharska, A. Z., Prorok, P., & Piórecki, N. (2019). Physicochemical and antioxidative properties of Cornelian cherry beer. *Food Chemistry, 281*, 147–153. https://doi.org/10.1016/j.foodchem.2018.12.093

Kurtzman, C. P., Fell, J. W., Boekhout, T., & Robert, V. (2011). Methods for isolation, phenotypic characterization and maintenance of yeasts. In C. P. Kurtzman, J. W. Fell, & T. Boekhout (Eds.), *The yeasts: A taxonomic Study* (5th ed., pp. 87–110). USA: Elsevier. https://doi.org/10.1016/B978-0-444-52149-1.00007-0.

Larroque, M. N., Carrau, F., Fariña, L., Boido, E., Dellacassa, E., & Medina, K. (2021). Effect of *Saccharomyces* and non-*Saccharomyces* native yeasts on beer aroma compounds. *International Journal of Food Microbiology, 337*, Article 108953. https://doi.org/10.1016/j.ijfoodmicro.2020.108953

Lee, S. H., Nordin, S., & Mahror, N. (2025). Effect of different pasteurization temperatures on the physicochemical and microbiological quality of rock melon juice during storage at 4° C. *Pertanika Journal of Tropical Agricultural Science, 48*(5). https://doi.org/10.47836/pjtas.48.5.12

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

Leni, G., Romanini, E., Bertuzzi, T., Abate, A., Bresciani, L., Lambri, M., Dell'Asta, M., & Gabrielli, M. (2023). Italian grape ale beers obtained with Malvasia di Candia aromatica grape variety: Evolution of phenolic compounds during fermentation. *Foods, 12*, 1196. https://doi.org/10.3390/foods12061196

Lija, M., & Beevy, S. S. (2021). A review on the diversity of melon. *Plant Science Today, 8*, 995–1003. https://doi.org/10.14719/pst.1300

Maarse, H. (1991). *Volatile compounds in foods and beverages*. Routledge.

Mallek-Ayadi, S., Bahloul, N., Baklouti, S., & Kechaou, N. (2022). Bioactive compounds from *Cucumis melo* L. fruits as potential nutraceutical food ingredients and juice processing using membrane technology. *Food Sciences and Nutrition, 10*, 2922–2934. https://doi.org/10.1002/fsn3.2888

Marconi, O., Rossi, S., Galgano, F., Sileoni, V., & Perretti, G. (2016). Influence of yeast strain, priming solution and temperature on beer bottle conditioning. *Journal of the Science of Food and Agriculture, 96*(12), 4106–4115. https://doi.org/10.1002/jsfa.7611

Martínez, A., Vegara, S., Martí, N., Valero, M., & Saura, D. (2017). Physicochemical characterization of special persimmon fruit beers using Bohemian Pilsner malt as a base. *Journal of the Institute of Brewing, 123*, 319–327. https://doi.org/10.1002/jib.434

Martorana, A., Alfonzo, A., Gaglio, R., Settanni, L., Corona, O., La Croce, F., Vagnoli, P., Caruso, T., Moschetti, G., & Francesca, N. (2017). Evaluation of different conditions to enhance the performances of *Lactobacillus pentosus* OM13 during industrial production of spanish-style table olives. *Food Microbiology, 61*, 150–158. https://doi.org/10.1016/j.fm.2016.08.007

Matraxia, M., Alfonzo, A., Prestianni, R., Francesca, N., Gaglio, R., Todaro, A., Alfeo, V., Perretti, G., Columba, P., Settanni, L., & Moschetti, G. (2021). Non-conventional yeasts from fermented honey by-products: Focus on *Hanseniaspora uvarum* strains for craft beer production. *Food Microbiology, 99*, Article 103806. https://doi.org/10.1016/j.fm.2021.103806

Mayer, H., Ceccaroni, D., Marconi, O., Sileoni, V., Perretti, G., & Fantozzi, P. (2016). Development of an all rice malt beer: A gluten free alternative. *LWT-Food Science and Technology, 67*, 67–73. https://doi.org/10.1016/j.lwt.2015.11.037

Mazzei, P., Francesca, N., Moschetti, G., & Piccolo, A. (2010). NMR spectroscopy evaluation of direct relationship between soils and molecular composition of red wines from Aglianico grapes. *Analytica Chimica Acta, 673*, 167–172. https://doi.org/10.1016/j.aca.2010.06.003

Meussdoerffer, F. G. (2009). A comprehensive history of beer brewing. In H. M. Eßlinger (Ed.), *Handbook of brewing* (pp. 1–42). Wiley-VCH. https://doi.org/10.1002/9783527623488.ch1.

Michel, M., Meier-Dörnberg, T., Jacob, F., Methner, F., Wagner, R. S., & Hutzler, M. (2016). Pure non-*saccharomyces* starter cultures for beer fermentation with a focus on secondary metabolites and practical applications. *Journal of the Institute of Brewing, 122*, 569–587. https://doi.org/10.1002/jib.381

Nardini, M. (2023). An overview of bioactive phenolic molecules and antioxidant properties of beer: Emerging trends. *Molecules, 28*, 3221. https://doi.org/10.3390/molecules28073221

Nardini, M., & Garaguso, I. (2020). Characterization of bioactive compounds and antioxidant activity of fruit beers. *Food Chemistry, 305*, Article 125437. https://doi.org/10.1016/j.foodchem.2019.125437

Ocvirk, M., Mlinarič, N. K., & Košir, I. J. (2018). Comparison of sensory and chemical evaluation of lager beer aroma by gas chromatography and gas chromatography/mass spectrometry. *Journal of the Science of Food and Agriculture, 98*, 3627–3635. https://doi.org/10.1002/jsfa.8840

Oladokun, O., James, S., Cowley, T., Dehrmann, F., Smart, K., Hort, J., & Cook, D. (2017). Perceived bitterness character of beer in relation to hop variety and the impact of hop aroma. *Food Chemistry, 230*, 215–224. https://doi.org/10.1016/j.foodchem.2017.03.031

Ou, B., Hampsch-Woodill, M., & Prior, R. L. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry, 49*, 4619–4626. https://doi.org/10.1021/jf010586o

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

Pereira, I. M. C., Matos, J. D., Figueiredo, R. W., Carvalho, J. D. G., Figueiredo, E. A. T., Menezes, N. V. S., & Gaban, S. V. F. (2020). Physicochemical characterization, antioxidant activity, and sensory analysis of beers brewed with cashew peduncle (*Anacardium occidentale*) and orange peel (*Citrus sinensis*). *Food Science and Technology, 40*, 749–755. https://doi.org/10.1590/fst.17319

Petkova, Z., & Antova, G. (2015). Proximate composition of seeds and seed oils from melon (*Cucumis melo* L.) cultivated in Bulgaria. *Cogent Food & Agriculture, 1*, Article 1018779. https://doi.org/10.1080/23311932.2015.1018779

Piazzon, A., Forte, M., & Nardini, M. (2010). Characterization of phenolics content and antioxidant activity of different beer types. *Journal of Agricultural and Food Chemistry, 58*, 10677–10683. https://doi.org/10.1021/jf101975

Pires, E. J., Teixeira, J. A., Brányik, T., & Vicente, A. A. (2014). Yeast: The soul of beer's aroma – A review of flavour-active esters and higher alcohols produced by the brewing yeast. *Applied Microbiology and Biotechnology, 98*, 1937–1949. https://doi.org/10.1007/s00253-013-5470-0

Pirrone, A., Naselli, V., Gugino, I. M., Porrello, A., Viola, E., Craparo, V., Vella, A., Alongi, D., Seminerio, V., Carusi, M., Radici, C., Amato, F., Guzzon, R., Todaro, A., Gaglio, R., Settanni, L., Maggio, A., Moschetti, G., Francesca, N., & Alfonzo, A. (2025). Use of non-conventional yeasts for enhancing the sensory quality of craft beer. *Food Research International, 208*, Article 116164. https://doi.org/10.1016/j.foodres.2025.116164

Pirrone, A., Prestianni, R., Naselli, V., Todaro, A., Farina, V., Tinebra, I., Guzzon, R., Badalamenti, N., Maggio, A., Gaglio, R., Settanni, L., Bruno, M., Moschetti, G., Alfonzo, A., & Francesca, N. (2022). Influence of indigenous *Hanseniaspora uvarum* and *Saccharomyces cerevisiae* from sugar-rich substrates on the aromatic composition of loquat beer. *International Journal of Food Microbiology, 379*, Article 109868. https://doi.org/10.1016/j.ijfoodmicro.2022.109868

Ramírez, A., & Viveros, J. M. (2021). Brewing with *Cannabis sativa* vs. *Humulus lupulus*: A review. *Journal of the Institute of Brewing, 127*(3), 201–209. https://doi.org/10.1002/jib.654

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*, 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Rico, X., Gullón, B., Alonso, J. L., & Yáñez, R. (2020). Recovery of high value-added compounds from pineapple, melon, watermelon and pumpkin processing by-products: An overview. *Food Research International, 132*, Article 109086. https://doi.org/10.1016/j.foodres.2020.109086

Rodman, A. D., Fraga, E. S., & Gerogiorgis, D. (2018). On the application of a nature-inspired stochastic evolutionary algorithm to constrained multi-objective beer fermentation optimisation. *Computers & Chemical Engineering, 108*, 448–459. https://doi.org/10.1016/j.compchemeng.2017.10.019

Rodolfi, M., Valentoni, A., Pretti, L., Sanna, M., Guidotti, S., Marchioni, I., & Ganino, T. (2023). From hop to beer: Influence of different organic foliar fertilisation treatments on hop oil profile and derived beers' flavour. *Plants, 12*, 1861. https://doi.org/10.3390/plants12091861

Rodríguez-Pérez, C., Quirantes-Piné, R., Fernández-Gutiérrez, A., & Segura-Carretero, A. (2013). Comparative characterization of phenolic and other polar compounds in Spanish melon cultivars by using high-performance liquid chromatography coupled to electrospray ionization quadrupole-time of flight mass spectrometry. *Food Research International, 54*, 1519–1527. https://doi.org/10.1016/j.foodres.2013.09.011

Santos, M. A. S., Ribeiro, P. V. L., Andrade, C. P., Machado, A. R. G., Souza, P. G., & Kirsch, L. D. S. (2021). Physicochemical and sensory analysis of craft beer made with soursop (*Annona muricata* L.). *Acta Science Policy Technologie Aliment, 20*, 103–112. https://doi.org/10.17306/J.AFS.2021.0845

Silva, M. A., Albuquerque, T. G., Alves, R. C., Oliveira, M. B. P., & Costa, H. S. (2024). Melon peel flour: Utilization as a functional ingredient in bakery products. *Food & Function, 15*, 1899–1908. https://doi.org/10.1039/D3FO05268K

Silva, M. A., Albuquerque, T. G., Ferreira, D. M., Alves, R. C., Oliveira, M. B. P., & Costa, H. S. (2025). Nutritional and bioactive profiling of *Cucumis melo* L. by-products: Towards a circular food economy. *Molecules, 30*, 1287. https://doi.org/10.3390/molecules30061287

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–158. https://doi.org/10.5344/ajev.1965.16.3.144

Sun, X., Baldwin, E. A., Manthey, J., Dorado, C., Rivera, T., & Bai, J. (2022). Effect of preprocessing storage temperature and time on the physicochemical properties of winter melon juice. *Journal of Food Quality, 2022*, Article 3237639. https://doi.org/10.1155/2022/3237639

Sun, X., Baldwin, E., Plotto, A., Cameron, R., Manthey, J., Dorado, C., & Bai, J. (2018). The effect of cultivar and processing method on the stability, flavor, and nutritional properties of winter melon juice. *Lebensmittel-Wissenschaft & Technologie, 97*, 223–230. https://doi.org/10.1016/j.lwt.2018.06.059

Thompson Witrick, K., Duncan, S. E., Hurley, K. E., & O'Keefe, S. F. (2017). Acid and volatiles of commercially available Lambic beers. *Beverages, 3*, 51. https://doi.org/10.3390/beverages3040051

Tristezza, M., Tufariello, M., Capozzi, V., Spano, G., Mita, G., & Grieco, F. (2016). The oenological potential of *Hanseniaspora uvarum* in simultaneous and sequential co-fermentation with *Saccharomyces cerevisiae* for industrial wine production. *Frontiers in Microbiology, 7*, 670. https://doi.org/10.3389/fmicb.2016.00670

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

Verstrepen, K. J., Derdelinckx, G., Dufour, J. P., Winderickx, J., Thevelein, J. M., Pretorius, I. S., & Delvaux, F. R. (2003). Flavor-active esters: Adding fruitiness to beer. *Journal of Bioscience and Bioengineering, 96*, 110–118. https://doi.org/10.1016/S1389-1723(03)90112-5

Viejo, C. G., Fuentes, S., Torrico, D. D., Godbole, A., & Dunshea, F. R. (2019). Chemical characterization of aromas in beer and their effect on consumers liking. *Food Chemistry, 293*, 479–485. https://doi.org/10.1016/j.foodchem.2019.04.114

Villacreces, S., Blanco, C. A., & Caballero, I. (2022). Developments and characteristics of craft beer production processes. *Food Bioscience, 45*, Article 101495. https://doi.org/10.1016/j.fbio.2021.101495

Wang, C., Liwei, M., Park, J. B., Jeong, S. H., Wei, G., Wang, Y., & Kim, S. W. (2018). Microbial platform for terpenoid production: *Escherichia coli* and yeast. *Frontiers in microbiology, 9*, Article 2460. https://doi.org/10.3389/fmicb.2018.02460

Wu, D., Chen, Z., Lv, Y., Yang, L., Liu, H., Qiu, R., Xu, H., Li, J., Lu, J., & Cai, G. (2024). Coculturing of non-*Saccharomyces cerevisiae* and *Saccharomyces cerevisiae* for improving the aroma quality and antioxidant characteristics of beer with Yuan'an yellow tea. *Food Bioscience*. , Article 103725. https://doi.org/10.1016/j.fbio.2024.103725

Wurlitzer, N. J., Dionísio, A. P., Lima, J. R., Garruti, D. D. S., Silva Araújo, I. M. D., da Rocha, R. F. J., & Maia, J. L. (2019). Tropical fruit juice: Effect of thermal treatment and storage time on sensory and functional properties. *Journal of Food Science & Technology, 56*(12), 5184–5193. https://doi.org/10.1007/s13197-019-03987-0

Yıkmış, S. (2020). Sensory, physicochemical, microbiological and bioactive properties of red watermelon juice and yellow watermelon juice after ultrasound treatment. *Journal of Food Measurement and Characterization, 14*, 1417–1426. https://doi.org/10.1007/s11694-020-00391-7

Zhang, K., Zhang, T. T., Guo, R. R., Ye, Q., Zhao, H. L., & Huang, X. H. (2023). The regulation of key flavor of traditional fermented food by microbial metabolism: A review. *Food Chemistry X*. , Article 100871. https://doi.org/10.1016/j.fochx.2023.100871

Zhao, H., Li, H., Sun, G., Yang, B., & Zhao, M. (2013). Assessment of endogenous antioxidative compounds and antioxidant activities of lager beers. *Journal of the Science of Food and Agriculture, 93*, 910–917. https://doi.org/10.1002/jsfa.5824

Zhao, X., Yin, Y., Fang, W., & Yang, Z. (2023). What happens when fruit married with beer? *International Journal of Gastronomy and Food Science, 32*, Article 100716. https://doi.org/10.1016/j.ijgfs.2023.100716

Zunkel, M., Gastl, M., Schoenberger, C., Sedin, D., & Becker, T. (2011, June). In *Beer flavor database. Conference presentation at the 74th Annual Meeting of the American Society of Brewing Chemists*. Fort Myers, FL.