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Use of non-conventional yeasts (Candida oleophila, Starmerella lactis-condensi, Hanseniaspora uvarum and Lachancea thermotolerans) for enhancing the sensory quality of craft beer

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Abstract:	In recent years, craft beer production has grown significantly, sparking interest in using non-conventional yeasts to produce beers with distinctive flavors. This work investigated the impact of unconventional yeast strains, including Hanseniaspora uvarum YGA34 (EP1), Lachanchea thermotolerans MNF105 (EP2), Candida oleophila YS209 (EP3) and Starmerella lactis-condensi MN412 (EP4), as co-starter cultures alongside Saccharomyces cerevisiae US-05. The control trial was inoculated with S. cerevisiae US-05 (TC) alone. For the first time, C. oleophila and St. lactis-condensi were used for beer production and the results were also compared with H. uvarum and L. thermotolerans. These strains were previously selected from high-sugar matrices like manna and fermented honey by-products, exhibited logarithmic growth cycles ranging from 5 to 8 during experimental fermentations. Interestingly, St. lactis-condensi MN412 and L. thermotolerans MNF105 strains efficiently consumed fructose, glucose, and sucrose in the beer must before the commercial strain S. cerevisiae US-05 was introduced. Lachancea thermotolerans MNF105 consumed more maltose than the other strains,

	albeit slightly less than the control strain. Among the beer samples, esters were the most prevalent compounds, ranging from 91.2 to 237.3 mg/L. Notably, the EP2 trial exhibited the highest ester content (237.3 mg/L). Specifically, ethyl octanoate was the dominant compound, identified at 125.5 mg/L in the EP2 trial. These unconventional yeast strains exhibited significant differences compared to beers brewed with S. cerevisiae alone. Additionally, their application led to an increase in volatile organic compounds. In conclusion, novel yeast strains isolated from high-sugar matrices showed excellent technological properties, making them promising co-starters and starter in innovative craft beer production.

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DIPARTIMENTO SCIENZE AGRARIE, ALIMENTARI e FORESTALI
SAAF DIPARTIMENTO SCIENZE AGRARIE ALIMENTARI FORESTALI

Palermo, 25/07/2024

Dear Editor,

I am pleased to submit our paper titled: "*Use of non-conventional yeasts (Candida oleophila, Starmerella lactis-condensi, Hanseniaspora uvarum and Lachancea thermotolerans) for enhancing the sensory quality of craft beer*" to your attention. The present work investigated the impact of unconventional yeast strains, including *Hanseniaspora uvarum* YGA34 (EP1), *Lachanchea thermotolerans* MNF105 (EP2), *Candida oleophila* YS209 (EP3) and *Starmerella lactis condensi* MN412 (EP4), as co-starter cultures in craft beer production. Additionally, the commercial yeast strain *S. cerevisiae* US-05 was used for sequential inoculation after 72 h and as part of the control trial.

These strains were previously selected from high-sugar matrices like manna and fermented honey by-products. Very limited information has been published to date on yeast biodiversity characterizing the mentioned sugary extracts and no data have been reported on *Candida oleophila* and *Starmerella lactis condensi* in craft beer production.

The microbiological, physico-chemical and sensory parameters as well as the composition of volatile organic compounds of the beers were carried out. Interestingly, the strains efficiently consumed fructose, glucose and sucrose in the wort before the introduction of the commercial strain *S. cerevisiae* US-05. In addition, *L. thermotolerans* MNF105 consumed maltose more than the other strains, although slightly less than the control strain. The selected strains exhibited superior performance across various aspects, including VOCs production and subsequent sensory enhancements, as validated by sensory analysis. Notably, distinct non-*Saccharomyces* yeast strains contribute to this enhanced complexity through their ability to generate diverse and perceptible VOCs. Esters were the most abundant compounds in the beer samples, with ethyl octanoate being the dominant compound.

To our knowledge, no scientific researches have been carried out on these topics. From recent issues, we believe that our research article may be of interest for Food Research International readers.

The manuscript has been prepared following FRI authors' guidelines.

I hope the paper could be revised by FRI reviewers.

With my best personal regards,
Nicola Francesca

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- Yeast strains from high sugar matrices present interesting brewing characteristics
- EP4 trial inoculated with MN412 produced highest amount of glycerol
- Non-*Saccharomyces* showed the ability to generate diverse and perceptible VOCs
- EP2 trial exhibited the highest ester content
- The overall organoleptic investigation showed a preference for MNF105

Use of non-conventional yeasts (*Candida oleophila, Starmerella lactis-condensi, Hanseniaspora uvarum* and *Lachancea thermotolerans*) for enhancing the sensory quality of craft beer

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ABSTRACT

In recent years, craft beer production has grown significantly, sparking interest in using non-conventional yeasts to produce beers with distinctive flavors. This work investigated the impact of unconventional yeast strains, including *Hanseniaspora uvarum* YGA34 (EP1), *Lachanchea thermotolerans* MNF105 (EP2), *Candida oleophila* YS209 (EP3) and *Starmerella lactis-condensi* MN412 (EP4), as co-starter cultures alongside *Saccharomyces cerevisiae* US-05. The control trial was inoculated with *S. cerevisiae* US-05 (TC) alone. For the first time, *C. oleophila* and *St. lactis-condensi* were used for beer production and the results were also compared with *H. uvarum* and *L. thermotolerans*.

These strains were previously selected from high-sugar matrices like manna and fermented honey by-products, exhibited logarithmic growth cycles ranging from 5 to 8 during experimental fermentations. Interestingly, *St. lactis-condensi* MN412 and *L. thermotolerans* MNF105 strains efficiently consumed fructose, glucose, and sucrose in the beer must before the commercial strain *S. cerevisiae* US-05 was introduced. *Lachancea thermotolerans* MNF105 consumed more maltose than the other strains, albeit slightly less than the control strain. Among the beer samples, esters were the most prevalent compounds, ranging from 91.2 to 237.3 mg/L. Notably, the EP2 trial exhibited the highest ester content (237.3 mg/L). Specifically, ethyl octanoate was the dominant compound, identified at 125.5 mg/L in the EP2 trial. These unconventional yeast strains exhibited significant differences compared to beers brewed with *S. cerevisiae* alone. Additionally, their application led to an increase in volatile organic compounds. In conclusion, novel yeast strains isolated from high-sugar matrices showed excellent technological properties, making them promising co-starters and starter in innovative craft beer production.

Keywords: *Candida oleophila*; *Starmerella lactis-condensi; Hanseniaspora uvarum*; *Lachanchea thermotolerans*; Non-*Saccharomyces*; Beer fermentation; volatile organic compounds; Beer aroma.

1. Introduction

In brewing history, non-*Saccharomyces* yeast species were often viewed negatively due to potential issues related to beer safety, turbidity, filterability, off-flavours, acidity, and flavour profile alterations (Miguel et al., 2022; Varela, 2016). However, when correctly selected and utilized, non-*Saccharomyces* yeasts can impart favourable properties. Extensive research has revealed their significant role in shaping beer aroma and enhancing sensory characteristics (Van Rijswijck, Wolkers–Rooijackers, Abee, & Smid, 2017). Notably, yeast selection impacts metabolites like acetate, ethyl esters, and higher alcohols (Pires, Teixeira, Bràanyik, & Vicente, 2014) prompting investigations into the use of non-*Saccharomyces* strains in beer production.

Thanks to their unique characteristics, some non-*Saccharomyces* yeasts have proven suitable for producing low-alcohol beers, functional brews, sour beers, and enhancing beer flavours (Basso, Alcarde, & Portugal, 2016; Holt, Mukherjee, Lievens, Verstrepen, & Thevelein, 2018, Francesca et al., 2023; Toh, Chua, Lu, & Liu, 2020). While research on their positive attributes is relatively recent, their potential in the fermented beverage industry, especially brewing, is becoming increasingly evident. In recent studies, researchers have explored yeasts isolated from unconventional brewing sources to discover novel strains that enhance beer aromas (Colomer, Funch, & Forster, 2019; Larroque et al., 2021). Notably, some investigations focused on isolations from highly sugary matrices, such as manna ash (Guarcello et al., 2019), and from alcoholic beverages derived from fermented honey by-products (Matraxia et al., 2021). So far, non-*Saccharomyces* yeasts isolated from unconventional sources and investigated for their potential applications in brewing included *Zygosaccharomyces*, *Pichia*, *Saccharomycodes*, *Torulaspora*, *Lachanchea* and *Hanseniaspora* (Sannino, Mezzasoma, Buzzini, & Turchetti, 2019). In a study by Canonico, Agarbati, Comitini and Ciani (2016), the presence of *Torulaspora delbrueckii* led to low-alcohol beers with distinctive analytical and aromatic profiles. Similarly, Callejo et al. (2019) explored yeast applications in beer production and found that *Torulospora delbrueckii* and *Saccharomycodes ludwigii*, followed by *Lachanchea thermotolerans*, can produce beers with

reduced ethanol content and characteristic aromas. Additionally, *Schizosaccharomyces pombe* was identified as capable of increasing both ethanol and acetaldehyde contents. Matraxia et al. (2021) found that *Hanseniaspora uvarum* effectively improved glycerol and acetic acid concentrations. This strain also exhibited heightened sensory complexity and intensity, thriving in the presence of ethanol and hops. Furthermore, Pirrone et al. (2022) demonstrated that beers produced thorugh sequential inoculation of *H. uvarum* and *S. cerevisiae* featured elevated ester content and reduced alcohol concentration. In another study, Francesca et al. (2023) found that the yeast strain *L. thermotolerans* MNF105 produced lower lactic acid, contributing to beer flavour balance. Additionally, it synthesized ethyl lactate, a compound commonly associated with this species.

The ability of specific yeast species and strains to selectively ferment sugars enables their sequential use alongside *S. cerevisiae*, leading to enhanced beer flavour and reduced sweetness due to residual sugars. Non-*Saccharomyces* yeasts, however, have limited ethanol tolerance and may not fully consume the primary sugars in beer wort. Consequently, they are often co-fermented with *Saccharomyces* species to complete the fermentation process (Cubillos, Gibson, Grijalva-Vallejos, Krogerus, & Nikulin, 2019). This mixed or sequential inoculation approach holds promise for producing innovative beer flavours (Holt et al., 2018). Notably, there is no existing literature on the use of *Candida oleophila* and *Starmerella lactis-condensi* in brewing until this study, which reports their results as co-starters in brewing. Having previously been applied only in oenological research (Francesca et al., 2024).

Based on the above considerations, the present study aimed to: (i) enhance understanding of non-*Saccharomyces* yeasts in beer production; (ii) evaluate for the first time the effect of *C. oleophila* and *St. lactis-condensi* on beer production; (iii) improve the sensory quality of craft beer; and (iv) enhance understanding about yeast ecology in high-sugar environments.

2. Materials and methods

2.1.Yeast strains and media

The yeast strains used in this research included *H. uvarum* YGA34, *L. thermotolerans* MNF105, *C. oleophila* YS209, and *St. lactis-condensi* MN412. These strains belong to the collection of the Department of Agricultural, Food and Forest Sciences (SAAF; University of Palermo, Italy). Isolated from manna (Guarcello et al., 2019) and honey by-products (Gaglio et al., 2017), they were specifically selected based on their high-performance during beer wort fermentation. Additionally, the commercial yeast strain *S. cerevisiae* US-05 (from Lallemand Inc., Montreal, Canada) was used for sequential inoculation after 72 h and as part of the control trial. Yeast reactivation from cryogenic storage followed the procedure described by Pirrone et al. (2022). All media and the supplements were obtained from Oxoid (Thermofisher, Milan, Italy).

2.2.Experimental plan

Experimental beer productions were conducted at a laboratory scale (5 L batch) in accordance with experimental plan reported in Figure 1.

The wort for the fermentation trials was prepared in a double batch using a 40-L all-in-one microbrewing plant Klarstein mod. 10031629 (Chal-Tec GmbH Berlin, Germany).

The beer recipe consisted of 5.5 kg of pilsner malt (BestMalz, Heidelberg, Germany), 3 kg of pale ale malt (BestMalz, Heidelberg, Germany), 0.5 kg of wheat malt (BestMalz, Heidelberg, Germany), and 0.1 kg of acidified malt (Mich. Weyermann® GmbH & Co. KG Brennerstraße 17-19, 96052 Bamber) to adjust the mash pH. The malts were ground using a double roller mill (Brouwland, Beverlo, Belgium) with a roller distance of 1.20 mm. They were then mixed with 35.5 L of water containing 10 g each of $CaSO_4$ and $CaCl_2$ for pH correction following the method described by Marconi, Rossi, Galgano, Sileoni, & Perretti, et al. (2016). The mashing process occurred at 65°C for one hour, ensuring complete saccharification, which was confirmed using an iodine solution, as described by Mayer et al. (2016). The temperature was raised to 72°C and maintained for 10 min. To deactivate enzymes, the mixture was heated to 78°C for 10 min. Next, during the lautering phase, the wort was recirculated over the spent and rinsed grains with 19 L of water heated to 78°C,

resulting in a wort volume of 46.5 L. The wort was then boiled for 60 min. At the start of the boil, 10 g of Styrian wolf pellets with 12% w/w α-acids were added, followed by another 30 g of the same hops 5 min before the end of the boil. The resulting volume was 40 L. After clarifying the wort using a whirlpool with a 10-min recirculation and a 10-min rest (Marconi et al., 2016) it was cooled for 20 min using a stainless-steel wort chiller. The beer wort met standard quality parameter: pH 5.2 and 14°P (Plato degree). This experiment utilised 80 l of wort produced in a double-batch process on the same day.

The study involved 5 experimental trials, inoculated with different strains as follows: EP1, *H. uvarum* YGA34 initially, followed by *S. cerevisiae* US-05 strain after 72 h; EP2, *L. thermotolerans* MNF105 initially, followed by *S. cerevisiae* US-05 strain after 72 h; EP3, *C. oleophila* YS209 initially, followed by *S. cerevisiae* US-05 strain after 72 h; EP4, *St. lactis-condensi* MN412 initially, followed by *S. cerevisiae* US-05 strain after 72 h; TC (control), *S. cerevisiae* US-05 strain as control. The yeast strains were inoculated at a density of approximately 2.0×10^6 cells/mL, as reported by Holt et al. (2018). The fermentation process was carried out at 20°C in hermetically sealed glass fermenters (5 L) equipped with an airlock valve. Samples were collected from the uninoculated wort and daily until the inoculation of *S. cerevisiae*, at which point the sampling was reduced to every three days (day 1, 2, 3, 6, 9 and 12). After completing the fermentation process, the beer was transferred into 0.5 L bottles, and 6 g/L of dextrose was added, following the method described by Francesca et al. (2023). Subsequently, bottle conditioning occurred at 20°C for 25 days (Callejo et al., 2019). Finally, sensory analysis was conducted on the experimental beers. All fermentation experiments were performed in triplicate.

2.3. Microbiological analyses

The samples were subjected to microbiological analysis with plate counts immediately after sampling. The following media were used: Wallerstein Laboratory nutrient agar (WL) for *Saccharomyces* populations (Di Maio, Polizzotto, Planeta, & Oliva, 2011) and Lysine agar (LA) for

non-*Saccharomyces* populations (Iris, Antonio, Antonia, & Antonio, 2020). The colonies from the two agar media were presumptively identified based on their morphological characteristics. Identification was confirmed only after determining cell morphology through microscopic inspection (Cavazza, Grando & Zini, 1992). Genetic characterisation was then performed on three isolates with the same morphology from a given sample.

Genomic DNA for PCR assays was using the InstaGene Matrix kit (Bio-Rad, Hercules, CA, USA) according to the manufacturer's protocol. Differentiation of yeasts was performed by RFLP analysis using the region spanning the internal transcribed spacers (ITS1 and ITS2) and the 5.8 S rRNA gene (Esteve-Zarzoso, Belloch, Uruburu, & Querol, 1999). One isolate per group was further analysed by sequencing the D1/D2 region of the 26 S rRNA gene to confirm the preliminary identification obtained by RFLP analysis as indicated by Alfonzo et al. (2020). DNA sequencing reactions were performed at BMR Genomics (Padova, Veneto, Italy). Sequence identity was determined by BlastN search against the NCBI non-redundant sequence database (http://www.ncbi.nlm.nih.gov). Sequences were manually corrected using Chromas 2.6.2. (Technelysium Pty Ltd., Australia).

The strain typing of *H. uvarum*, *L. thermotolerans*, *C. oleophila* and *St. lactis-condensi* strains was carried out by DNA (RAPD)-PCR analysis using the primer M13 (Binati et al., 2019), while for *S. cerevisiae*, interdelta analysis (Legras and Karst, 2003) was used. Amplification, visualisation of bands and result analysis were conducted using Gelcompar II software, version 6.5 (Applied-Maths, Sint-Martens-Latem, Belgium), in accordance with the methodology described by Alfonzo et al. (2021). The comparison of the polymorphic and interdelta profiles of the isolates from the different trials and the inoculated strains (*H. uvarum* YGA34, *L. thermotolerans* MNF105, *C. oleophila* YS209, *St. lactis-condensi* MN412 and *S. cerevisiae* US-05) enabled the percentage of dominance to be determined. All analyses were performed in triplicates.

2.4. Determination of physicochemical parameters

The study analyzed various parameters, including pH, Brix (°Bx), different acids (acetic, lactic, tartaric, and malic), sugars (fructose, glucose, maltose, and sucrose), glycerol, alcohol content (% vol), density (FG), real extract (°P), energy (kcal/100g), apparent extract (°P), original extract (°P), and real attenuation (%). The methodology followed that described by Francesca et al. (2023).

2.5. Determination of volatile organic compounds of beer samples

2.5.1. Standard solutions

Ethyl octanoate (standard for the ester fraction), 2-heptanol (standard for the alcoholic fraction), and limonene (standard for terpenes) (Fisher Scientific S.L.C, 28108 – Alcobendas-Madrid) were used as standard for calibration line. Standard solutions were prepared at five different concentrations (38 ppm, 82 ppm, 138 ppm, 170 ppm, 250 ppm for ethyl octanoate, 14 ppm, 62 ppm, 100 ppm, 159 ppm, 222 ppm for 2 heptanol, and 31 ppm, 63 ppm, 125 ppm, 169 ppm and 250 ppm for limonene).

2.5.2 SPME-GC/MS analysis

Beer samples (10 mL) were placed in a 20 mL SPME glass vial (Gerstel, 75.5 x 22.5mm) together with 1 g of sodium chloride. Gas chromatographic analyses were performed with Agilent 7000C GC system, fitted with a fused silica Agilent DB-5MS capillary column (30 m × 0.25 mm i.d.; 0.25 μm film thickness), coupled to an Agilent triple quadrupole Mass Selective Detector MSD 5973; ionization voltage 70 eV; electron multiplier energy 2000 V; transfer line temperature, 295 ◦C. Solvent Delay: 3.5 min. Helium was the carrier gas (1 mL/min).

The column temperature was initially kept constant at 40°C for 2 min (during splitless injection), subsequently, increasing the temperature by 4 °C/min was set to 60°C, at which it was kept constant for 2 min. Increasing the temperature by 2°C/min., it was raised to 90°C, from 190°C to 230°C, increasing by 5°C/min and finally left at 230°C for 15 min. The analytes in the fiber were automatically injected at 250°C with the splitless mode. The mass spectrometer was set in MS mode in order to acquire all mass-to-charge ratios from 35 to 450 amu (0.1 amu). Identification of

compounds was carried out using Adams, NIST 11, Wiley 9 and FFNSC 2 mass spectral database. These identifications were also confirmed by other published mass spectra.

2.6. Sensory evaluation

Sixteen judges (9 men and 7 women) aged between 28 and 48, selected from the University of Palermo students and personnel, assessed the beers. The sensory analysis in this research followed ethical standards for sensory studies. All participants gave their voluntary consent and were informed about the study. Participant information and privacy were protected by anonymization and appropriate measures. Informed consent was obtained from all participants prior to publication of experimental data. In addition, no institutional and/or national ethical approval is required for this study. The beer evaluation study followed the methodology described by Francesca et al. (2023), with some modifications to the descriptors. These descriptors covered aspects such as odour (intensity, complexity, fruity, floral, hoppy, honey/caramel, wheat/cereal, acetic, spicy, sulfury, alcohol and DMS), taste (intensity, complexity, sweet, bitter, acid, astringent, fruity, spicy, hoppy, sapidity, wheat/cereal, balsamic, alcohol, body, DMS, oxidized/aged), and overall acceptance. The final scores were based on the average of the three assessments.

2.7. Statistical and explorative multivariate analyses

An analysis of variance (ANOVA) was conducted to detect significant differences among physico-chemical parameters during the brewing process, VOCs, and sensory analysis of the final beer. The post-hoc Tukey's method facilitated pairwise comparisons across all data points. Statistical significance was assigned to $P \leq 0.05$ (Mazzei, Francesca, Moschetti, & Piccolo, 2010). Additionally, a Principal Component Analysis (PCA) was performed using XLstat software version 2019.2.2 (Addinsoft, New York, NY, USA) for Excel to explore the correlation between aromas identified in VOCs and sensory attributes. Only aromas with an odour activity value > 1 were considered in the analysis.

3. Results

3.1. *Evaluation of population dynamics*

The growth kinetics during fermentation were investigated, and the results are depicted in Figure 2. In uninoculated wort, microbial levels were below the detection limit for both WL and LA media. However, the inoculum cell densities varied between 5.95 and 6.35 Log CFU/mL. After 3 d, all experimental trials exhibited an increase of approximately 1 Log cycle. Similar trends were observed in the control trial TC. Moreover, on the third day, sequential inoculation of the commercial yeast strain *S. cerevisiae* US-05 resulted in a range of cell densities for the *Saccharomyces* genus between 6.10 and 6.65 Log CFU/mL. Subsequently, the number of *Saccharomyces* spp. increased in the following sampling day, except for the control trial TC, which showed a decrease. Additionally, the experimental trials demonstrated a decrease in all non-*Saccharomyces* species

Starting from the 9th day of sampling, both *Saccharomyces* and non-*Saccharomyces* cell counts decreased and continued to decline until the end of the fermentation process. The highest cell counts were registered observed on the 3rd d for *C. oleophila* YS209 in trial EP3 (7.54 Log CFU/mL), followed by *L. thermotolerans* MNF105 in trial EP2 (7.36 Log CFU/mL). Meanwhile, *S. cerevisiae* US-05 in EP4 (7.16 Log CFU/mL) showed more consistent growth compared to the control trial TC, which reached values of 7.10 Log. Moreover, 60 yeast isolates were collected from each trial, resulting in a total of 300 yeast isolates (EP1, EP2, EP3, EP4 and TC). DNA analysis (RAPD-PCR) demonstrated that all non-*Saccharomyces* yeast strains showed an identical RAPD profile to the inoculated strains (*H. uvarum* YGA34, *L. thermotolerans* MNF105, *C. oleophila* YS209, *St. lactis-condensi* MN412 *and S. cerevisiae* US-05) in the respective trials (EP1, EP2, EP3 and EP4). In the TC treatment, the interdelta profiles of the *S. cerevisiae* were comparable to those of the inoculated *S. cerevisiae* strain US-05.

3.2 *Physicochemical changes during fermentation*

The wort's chemical composition included 42.66 g/L maltose, 13.65 g/L sucrose, 12.64 g/L glucose and 1.56 g/L fructose. At the end of alcoholic fermentation, pH values for different trials ranged from 3.58 to 4.02. Notably, strains-specific differences were observed in sugar, glycerol, acetic acid, and lactic acid concentrations (Table 1). Instead, the trend of the consumption of the different sugars during the first three days of alcoholic fermentation by the different non-*Saccharomyces* is shown in Figure 3. In the EP2 and EP4 trials, inoculated with *L. thermotolerans* MNF105 and *St. lactis-condensi* MN412, respectively, glucose, and sucrose were nearly depleted after three days of fermentation before *S. cerevisiae* US-05 inoculation. In addition, with regard to fructose, strain MNF105 showed superior performance, consuming the entirety of the sugar in three days, followed by *C. oleophila* YS209 (EP3 trial), which showed similar behaviour. It is noteworthy that *H. uvarum* YGA34 and *C. oleophila* YS209, in EP1 and EP3, respectively, demonstrated the capacity to metabolise fructose, glucose, and sucrose, albeit at a relatively slower rate. With regard to maltose, the *L. thermotolerans* strain MNF105 also exhibited a higher consumption rate, albeit to a slightly lesser extent than the control strain. After introducing the commercial strain *S. cerevisiae* US-05, all sugars were consumed, resulting in comparable levels across all trials. In fact, after 12 days of fermentation, all trials yielded a final sugar concentration below 1.46 g/L. Interestingly, in the EP2 trial inoculated with *L. thermotolerans* MNF105, the lactic acid concentration reached 0.70 g/L at the end of alcoholic fermentation, whereas other trials had values between 0.15 and 0.18 g/L. Conversely, the EP2 trial showed slightly higher acetic acid levels (0.24 g/L) compared to (ranging from 0.02 to 0.15 g/L). Glycerol content peaked at 3.36 g/L in the trial inoculated with *St. lactis-condensi*, with no significant differences observed among the remaining trials. Prior to the inoculation of *S. cerevisiae*, the *L. thermotolerans* strain MNF105 (EP2) produced a greater quantity of glycerol (0.71 g/L), although this was only a slight increase in comparison to the other non-*Saccharomyces* strains (between 0.39 and 0.62 g/L). Regarding ethanol, values ranged from 5.80 to 6.32. The lowest value occurred in EP2, while the other tests showed similar results. Density

values did not differ significantly between the trials. However, real attenuation values were highest for EP3 (67.70%), EP4 (69.20%), and TC (67.70%), intermediate for EP1 (66.20%), and lowest for EP2 (63.20%).

3.3. Volatile organic compound composition

In Table 3, the experimental beers exhibited a diverse array of volatile organic compounds (VOCs) falling into six identified classes: alcohols, ketones, carboxylic acids, esters, terpenes, and others. Notably, all novel strains used in beer production showed significant differences in detected compounds. Among these, the strain *L. thermotolerans* MNF105 (EP2) produced the highest VOC concentration, particularly with an abundance of esters.

Esters dominated as the most abundant class, reaching a remarkable concentration of 237.3 mg/L in the EP2 trial. Following closely was TC trial inoculated with *S. cerevisiae* US-05, which had 198.5 mg/L of esters. The primary ester compound identified was ethyl octanoate., with the highest value (157.1 mg/L) observed in the control trial (TC), followed by 125.5 mg/L in the EP2 trial with *L. thermotolerans* MNF105. Among the other most abundant esters, ethyl-trans-4-decenoate had concentrations of 31.4 mg/L in TC and 25.9 in EP2. Ethyl decanoate (caprate) reached 38.1 mg/L in EP2 and 2.7 mg/L in EP3). Isoamyl acetate was present at 27.0 mg/L in EP2 and 2.0 mg/L in EP1 and EP3. Overall, the ester class comprised 18 different compounds, but only seven of them had olfactory activity values greater than one. These active esters include ethyl butanoate, isoamyl acetate, ethyl hexanoate, ethyl octanoate, isoamyl hexanoate, ethyl nonanoate, and ethyl decanoate.

In the EP2 trials, twelve compounds showed higher values, with six surpassing the odour threshold. In comparison, the control trial TC had five such compounds. Notably, the EP3 trial (inoculated with *C. oleophila* YS209) and in the EP1 trial (inoculated with *H. uvarum* YGA34) detected and perceptibly enhanced seven ester compounds, contributing to the sensory complexity and variability of the beers.

In terms of alcohols, the trial results were comparable, except for the EP4 trial (inoculated with *St. lactis-condensi* MN412), which showed a higher of 128.90 mg/L. Across all trials, both 1-octanol and 2-undecanol were detected with an odour activity value >1, as detailed in Table 3. Interestingly, the EP2 trial identified more active compounds than the other trials. Notably, it detected 4-vinylguaiacol (2.30 mg/L), a compound absents in the remaining trials.

Among the detected terpenoid compounds, only three had an odour activity value greater than one: limonene, β-myrcene, and linalool. Remarkably, the trial inoculated with the commercial *S. cerevisiae* strain US-05 exhibited higher values for active compounds. Specifically, β-myrcene was present at 6.1 mg/L (only in TC), limonene reached 14.0 mg/L in EP2 (followed by 12.3 mg/L in EP1), and linalool was at 24.4 mg/L in EP4 (followed by 21.6 mg/L in EP3). Additionally, styrene exhibited an odour activity value greater than one. Notably, the EP3 trial inoculated with *C. oleophila* YS209 exhibited the highest styrene value at 73.7 mg/L.

3.4. Sensory evaluation

Quantitative sensory analysis results, depicted in Fig. 4, revealed significant differences among the experimental beers. These variations suggest that the non-*Saccharomyces* yeast strains positively influenced the organoleptic quality of beers. The panellists expressed appreciation for all trials, with the EP2 trial (inoculated with *L. thermotolerans* MNF10 yeast) emerging as the most preferred. Notably, both EP2 and EP3 trials, respectively inoculated with *L. thermotolerans* and *C. oleophila*, stood out for their overall acceptability (scoring 6.6 and 6.2). Specifically, the EP2 trial received the highest scores for aroma intensity and fruity notes (6.6 and 6.2, respectively), while the EP3 trial excelled in complexity (scoring 6.3 points).

The sensory profile of EP2 beer stood out due to its highest overall acceptability scores and superior ratings across 10 attributes. These attributes included aroma (intensity, spiciness, and fruitiness) and taste (intensity, acidity, astringency, fruity, spicy, body, and overall acceptability). The panellists particularly favored the EP3 trial, which exhibited higher values for five attributes: aroma

complexity, taste complexity, bitterness, wheat/cereal notes, and oxidised/aged flavours. EP2 achieved the highest aroma score for the parameter "fruity" (6.6 points), while EP3 excelled in flavour complexity (6.8 points). Notably, trials EP1 and EP4 also revealed significant parameters. EP1 was remarkable for its complexity, while EP4 highlighted hop aroma. The participants observed that beers brewed with different non-*Saccharomyces* yeast strains displayed greater complexity, likely due to their ability to produce distinct aroma compounds. These experimental beers were easily distinguishable from those of the control trial, where the commercial *S. cerevisiae* US-05 strain scored higher in "hoppy" attribute, but had overall lower complexity and body.

3.5 Statistical and explorative multivariate analyses

The graphical representation of VOCs analysis is presented in Fig. 5. The hierarchical dendrogram combined with heat map plot showed that the different non-*Saccharomyces* yeast strains significantly affected VOCs emitted from beers. The VOC concentrations between the trials resulted in the grouping of the trials into five different clusters.

3.6 Correlation between VOCs and sensory profiles

A principal component analysis (PCA) was conducted to assess the correlation between VOCs above the perception threshold and sensory attributes related to aroma. The findings, illustrated in Figure 6, reveal that the F1 factor accounted for 67.74% of the total variance, while the F2 factor explained 37.89%. The biplot graph highlights significant diversity among experimental trials, with VOCs and sensory profiles differing significantly from the control beer.

EP2 beer exhibited associations with several esters, including ethyl hexanoate, ethyl decanoate, isoamyl acetate, and octanol, contributing to fruity aromas (Verstrepen et al., 2003). Additionally, this trial was linked to the production of 4-vinylguaiacol, which imparts spicy notes (Vanbeneden, Gils, Delvaux, & Delvaux, 2008). The panellists noted that beers brewed with the *St. lactis*-

condensi MN412 yeasts strain exhibited greater complexity, attributed to the strain's ability to produce and diversify various VOCs.

The aroma of hops, more pronounced in EP4 beer, is attributed to Linalool (Jiang et al., 2023). Similarly, the control trial (TC) is associated with limonene and β-myrcene, both attributable to hops. Additionally, esters such as ethyl octanoate and isoamyl hexanoate contribute to the fruity aroma (Verstrepen et al., 2003). In contrast, EP1 and EP3 beers were statistically linked to three descriptors: styrene, honey/caramel, and ethyl nonanoate. Ethyl nonanoate and styrene are responsible for the honey/caramel aroma, producing sweet and balsamic notes, respectively (Meilgaard, 1975).

4. Discussion

The quality of beer depends on several ingredients, such as malt, hop, water. Moreover, the yeasts used as starters and co-starter are of paramount relevance to improve shelf life and sensory characteristics of beer. In this research, the results of microbiological analyses are in line with typical yeast growth dynamics observed during sequential inoculation into fermenting beer wort (Bourbon-Melo et al., 2020; Matraxia et al., 2021). To assess strain persistence, colony shape and cellular morphology were phenotypically investigated. This investigation identified characteristic members of the *Hanseniaspora*, *Candida*, *Starmerella*, *Lachanchea*, and *Saccharomyces* genera (Cadez, Pagnocca, Raspor, & Rosa, 2014; Jindamorakot et al., 2009; Chand-Goyal, Eckert, Droby, & Atkinson, 1998; Csoma, Kállai, Czentye, & Sipiczki, 2023; Fell, Statzell-Tallman, & Kurtzman, 2004; Kurtzman, Fell, Boekhout, & Robert, 2011). Following inoculation, the cell density of all non-*Saccharomyces* strains rapidly exceeded 7 Log CFU/mL, with minor variations across trials. Prior research has highlighted the impact of these cell densities on the sensory quality of the final beer product (Du Plessis et al., 2017). However, after day 6, non-*Saccharomyces* levels declined, likely due to reduced available nutrients (such as sugars) and increase stress factors (including ethanol) (Domizio, House, Joseph, Bisson, & Bamforth, 2016; Matraxia et al., 2021; Francesca et

al., 2024). Simultaneously, this reduction in non-*Saccharomyces* population can be attributed to interactions with *S. cerevisiae* (Tristezza et al., 2016). Possible mechanisms include nutrient competition or the presence of metabolites produced by *S. cerevisiae* that inhibit non-*Saccharomyces* growth (Domizio et al., 2011; Wang, Mas, & Esteve-Zarzoso 2015). These fermentation dynamics are consistent with previous observations by Pirrone et al. (2022), Matraxia et al. (2021), and Wu et al. (2024).

In the physicochemical analysis, the presence of different non-*Saccharomyces* strains in trials EP1, EP2, EP3, and EP4 did not impact the attenuation capacity of the commercial *S. cerevisiae* strain US-05. These trials exhibited similar values to the control trial (TC). Among the tested non-*Saccharomyces* yeast strains, only *L. thermotolerans* MNF105 in the EP2 trial displayed a similar trend to the control strain by metabolizing maltose. Notably, Domizio et al. (2016) confirmed that specific strains of *L. thermotolerans* and *S. cerevisiae* share comparable maltose utilization capabilities. In contrast, Callejo et al. (2019) found that *Lachancea* strains had lower maltose fermentation capacity compared to *S. cerevisiae*. Additionally, our results align with those reported by Francesca et al. (2023) regarding the yeast strain *L. thermotolerans* MNF105. The sugar consumption kinetics of *S. cerevisiae* US-05 in the TC trial followed the general pattern of the species, with glucose and fructose being consumed before maltose (Pirrone et al., 2022; Tan et al., 2021).

The *St. lactis-condensi* yeast strain MN412, used in the EP4 trial, exhibited robust glucose and fructose consumption during the initial fermentation phase. Notably, this strain does not metabolize maltose. Similarly, other species within this genus, such as *Starmerella bombicola*, also lack the ability to utilize maltose (García, Esteve-Zarzoso, Cabellos, & Arroyo, 2018). In the EP3 trial, inoculated with *C. oleophila* YS209, a similar trend was observed: consumption of glucose, fructose, and sucrose during initial fermentations prior to *S. cerevisiae* inoculation, but an inability to consume maltose. Furthermore, Francesca et al. (2024) already registered this yeast strain's preference for fructose over other sugars.

In trial EP1, *H. uvarum* YGA 34 exhibited limited fermentation capabilities for the primary sugars in beer wort. This is consistent with the findings of Matraxia et al. (2021), which indicated that this species is unable to metabolize maltose, a result also supported by Pirrone et al. (2022).

Regarding lactic acid levels, *L. thermotolerans* MNF105 in trial EP2 displayed significantly higher concentrations of this compound compared to other trials. This is attributed to the species' ability to produce lactic acid during beer wort fermentation (Domizio et al., 2016; Francesca et al., 2023). Remarkably, Zdaniewicz, Satora, Pater, and Bogacz, (2020) found that this species lacks the capacity for beer acidification, emphasizing the strain-dependent nature of this characteristic.

The acetic acid levels at the end of fermentation are comparable across the different trials, aligning with findings from various studies on beer wort fermentation (Matraxia et al., 2021; Pirrone et al., 2022; Viana et al., 2021). Furthermore, prior to the inoculation of *S. cerevisiae*, the *L. thermotolerans* strain MNF105 produced a greater quantity of glycerol, although this was only a slight increase in comparison to the other non-*Saccharomyces* strains. At the end of alcoholic fermentation, glycerol contents in the trials were also similar, with the EP4 trial (inoculated with *St. lactis-condensi* MN412) exhibiting the highest value. These glycerol values correspond to those reported by Matraxia et al., (2021) and Viana et al. (2021) in their beer studies. Notably, the alcohol and final attenuation values obtained were higher than those reported by Cirlincione et al. (2023), who produced beers fermented with the commercial *S. cerevisiae* strain US-05. This difference may be attributed to a higher sugar content in the initial wort.

In fermented beverages, esters play a crucial role in creating the sweet and fruity aroma. Their concentration depends on yeast enzymatic activity (Pires et al., 2014). These compounds significantly influence the final flavour profile of beer (Holt, Miks, de Carvalho, Foulquie-Moreno, & Thevelein, 2019). Ethyl octanoate, known for its apple-like flavour, is abundant in fruity and sweet properties (Verstrepen et al., 2003). This compound has been detected in wheat beers made with Maiorca wheat malt (Gugino et al., 2024). While there are more than 90 esters produced in beer, the most notable ones include ethyl octanoate (sour apple), isoamyl acetate (banana-like),

ethyl acetate (sweet fruit), and ethyl decanoate (apple-like) (Verstrepen et al., 2003; Viejo, Fuentes, Torrico, Godbole, & Dunshea 2019). These yeast-derived secondary metabolites significantly contribute to defining beer aroma. Even minor changes in ester concentrations can profoundly impact the overall beer scent (Thompson-Witrick et al., 2015).

Ethyl trans-4-decenoate was detected in beers produced with *S. cerevisiae* var. *diastaticus*, *S. cerevisiae* var. *bayanus*, and *Brettanomyces claussenii* (Matukas et al., 2022). Moreover, Ocvirk, Mlinarič, and Košir (2018) found ethyl decanoate in wheat beer produced with a condenser placed on top of the fermenter to reduce losses and Viejo et al. (2019) in different beers produced from top, bottom, and spontaneous fermentation. Isoamyl acetate was observed in samples taken at different stages of the brewing process of lager beer and in traditional sorghum beer (Alves et al., 2020; Attchelouwa et al., 2020). All these compounds contribute to the diverse aroma profiles of beer.

Alcohols in beer contribute to various aromas, including alcoholic, floral, or solvent notes (Esslinger, 2009). In particular, octanol was detected in beers during a study on temperature impact during storage (Ferreira et al., 2022). Undecanol appeared in sour beers during a study evaluating the probiotic strain *Lacticaseibacillus paracasei* subsp. *paracasei* F19 with yeast *S. cerevisiae* US-05 (Praia, Herkenhoff, Broedel, Frohme, & Saad, 2022). 4-vinylguaiacol was found in beer brewed using synthetic wort and fermented with *Pichia anomala* BCMO15_2 and *S. cerevisiae* 00/30. Historically considered an off-flavour, this compound is now recognized as an essential aroma in some white beers (Larroque et al., 2021; Vanbeneden et al., 2008).

Terpenes detected in beer are attributed to hops (Brendel, Hofmann & Granvogl, 2020; Dietz, Cook, Huismann, Wilson, & Ford, 2020; Ramírez & Viveros, 2021). Brendel et al. (2020) detected β-myrcene in alcohol-free beer made with various hop varieties. Additionally, Limonene, the second most abundant terpenoid in nature, contributes to beer's citrus or pine flavour (Ramírez and Viveros, 2021; Kemp et al., 2021). It was identified in beers hopped with Citra and Simcoe varieties (Sharp, Qian, Clawson, & Shellhammer, 2016) and in beers brewed with Amarillo, Cascade, and Centellian hops (Van Holle et al., 2021). Linalool, a significant component of beer

aroma, is one of the most desired hop oil compounds (Rutnik, Knez Hrnčič, & Jože Košir, 2022; Dietz et al., 2020). It imparts a pleasant floral aroma. Rutnik et al. (2023) found it more prevalent in beer brewed with Styrian Wolf hops rather than other hop varieties.

Furthermore, styrene is a compound commonly found in wheat beers, as evidenced by multiple studies in the scientific literature (Schwarz, Stübner, & Methner, 2012; Gugino et al., 2024). It originates from the decarboxylation of cinnamic acid during boiling or through enzymatic processes during fermentation. Small amounts of styrene can also be found in grains, coffee, and dried fruits. Notably, Gugino et al., (2024) demonstrated its presence in beers processed from wheat Sicilian grains, such as Maiorca, at varying percentages.

Numerous studies suggest that utilizing various non-*Saccharomyces* yeasts can enhance and modify the flavour profile of fermented beverages, particularly beer (Gutiérrez, Boekhout, Gojkovic, Katz, 2018; Larroque et al., 2021; Pirrone et al., 2022). An overall sensory evaluation favoured EP2 beer inoculated with *L. thermotolerans* MNF105, which exhibited pronounced intensity and fruity notes. These results are consistent with those of Francesca et al. (2023), where the MNF105 strain enhanced aromatic characteristics in fruit beer production. Our findings corroborated those of Postigo, Esteban, and Arroyo (2023), indicating that the strain *L. thermotolerans* CLI 1232 exhibits a harmonious acidity and a fruity aroma profile. Additionally, other trials were well appreciated by panellists. Remarkably, several studies have explored *H. uvarum* yeast. Matraxia et al. (2021) showed its ability to enhance fruity aromas. Pirrone et al. (2022) further showed that beers produced through sequential inoculation of *H. uvarum* YGA34 and *S. cerevisiae* MN113 or US-05 (T3 and T4) exhibit elevated ester levels and reduced alcohol concentration.

Furthermore, a groundbreaking study investigated the use of *C. oleophila* and *St. lactis-condensi* as co-starters in brewing. Francesca et al. (2024) revealed that employing *St. lactis-condensi* MN412 and *C. oleophila* YS209 positively impacted the final wines, enhancing fruity and floral intensity, as confirmed by sensory analysis.

5. Conclusion

In this work, we explored the impact of unconventional yeast strains, specifically *H. uvarum* YGA34, *L. thermotolerans* MNF105, *C. oleophila* YS209, and *St. lactis-condensi* MN412, as co-starter cultures on the physico-chemical and sensory properties of beer. These strains were sourced from matrices rich in sugar content, such as manna and honey by-products. In particular, the previously unstudied strains *C. oleophila* YS209 and *St. lactis-condensi* MN412 were introduced to the brewing process. After 72 hours of fermentation, the commercial yeast strain *S. cerevisiae* US-05 was inoculated. The selected strains exhibited superior performance across various aspects, including VOCs production and subsequent sensory enhancements, as validated by sensory analysis. Experimental trials with these strains revealed no off-odour or off-flavour and greater aromatic complexity. Notably, distinct non-*Saccharomyces* yeast strains contribute to this enhanced complexity through their ability to generate diverse, perceptible VOCs.

In particular, the beers produced exhibit higher ester concentrations and lower alcohol content. Consequently, the beers brewed with *L. thermotolerans* MNF105 demonstrated the greatest abundance of active VOCs compared to other trials, a conclusion supported by sensory analysis. This study contributes to the limited scientific understanding of non-*Saccharomyces* yeasts' role as co-starters for beer production. Further research is required to assess the impact on wort fermentation in industrial contexts and across varying wort compositions.

Declarations 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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745 **Table 1.** Conventional chemical parameters monitored in samples beer during the alcoholic
746 fermentation of beer.

Parameters	Trials					S.S.
	EP1	EP2	EP3	EP4	TC	
D-fructose (g/L)						
wort	1.56 ± 0.04	1.56 ± 0.04	1.56 ± 0.04	1.56 ± 0.04	1.56 ± 0.04	
1d	1.15 ± 0.04^{a}	0.81 ± 0.02^{c}	1.02 ± 0 .01^{b}	1.04 ± 0.02^{b}	0.84 ± 0.01^{c}	***
2d	0.74 ± 0.03^{a}	0.31 ± 0.01^{c}	0.62 ± 0 .01^{b}	0.72 ± 0.02^{a}	0.24 ± 0.01^{d}	***
3d	0.31 ± 0.02^{a}	0.01 ± 0.00^{c}	0.22 ± 0 .01^{b}	0.32 ± 0.02^{a}	0.04 ± 0.01^{c}	***
6d	0.21 ± 0.03^{a}	0.00 ± 0.00^{c}	0.07 ± 0.01^{b}	0.03 ± 0.01^{bc}	0.01 ± 0.01^{c}	***
9d	0.02 ± 0.00^{a}	0.00 ± 0.00^{b}	0.02 ± 0.00^{a}	0.00 ± 0.00^{b}	0.03 ± 0.01^{a}	***
12d	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.02 ± 0.00^{a}	0.01 ± 0.01^{a}	0.04 ± 0.01^{a}	N.S.
D-glucose (g/L)						
wort	12.64 ± 0.25	12.64 ± 0.25	12.64 ± 0.25	12.64 ± 0.25	12.64 ± 0.25	
1d	10.45 ± 0.18^{a}	7.44 ± 0.17^{c}	10.03 ± 0.21^{a}	8.43 ± 0.19^{b}	7.98 ± 0.18^{b}	***
2d	8.03 ± 0.20^{a}	2.05 ± 0.09^{d}	7.42 ± 0.11^{b}	3.57 ± 0.12^{c}	2.21 ± 0.07^{d}	***
3d	6.13 ± 0.12^{a}	0.16 ± 0.02^{d}	4.81 ± 0.15^{b}	0.57 ± 0.04^{c}	0.15 ± 0.02^{d}	***
6d	2.34 ± 0.11^{a}	0.08 ± 0.02^{b}	0.20 ± 0.01^{b}	0.15 ± 0.01^{b}	0.07 ± 0.00^{b}	***
9d	0.09 ± 0.01^{b}	0.06 ± 0.00^{c}	0.10 ± 0.01^{ab}	0.06 ± 0.01^{c}	0.12 ± 0.01^{a}	***
12d	0.07 ± 0.00^{bc}	0.05 ± 0.00^{c}	0.10 ± 0.01^{ab}	0.06 ± 0.01^{c}	0.11 ± 0.02^{a}	***
Maltose (g/L)						
wort	42.66 ± 0.75	42.66 ± 0.75	42.66 ± 0.75	42.66 ± 0.75	42.66 ± 0.75	
1d	41.55 ± 0.41^{a}	39.12 ± 0.34^{b}	41.51 ± 0.51^{a}	42.06 ± 0.71^{a}	39.45 ± 0.34^{b}	***
2d	40.23 ± 0.45^{a}	34.05 ± 0.31^{b}	40.61 ± 0.48^{a}	41.67 ± 0.62^{a}	33.21 ± 0.32^{b}	***
3d	39.25 ± 0.42^{b}	28.50 ± 0.28^{c}	39.71 ± 0.21^{b}	41.26 ± 0.34^{a}	26.27 ± 0.19^{d}	***
6d	25.54 ± 0.18^{a}	17.50 ± 0.24^{d}	24.05 ± 0.17^{b}	23.01 ± 0.31^{c}	12.54 ± 0.16^{e}	***
9d	9.20 ± 0.15^{b}	7.24 ± 0.10^{c}	10.24 ± 0.21^{a}	9.24 ± 0.12^{b}	2.25 ± 0.05^{d}	***
12d	1.30 ± 0.08^{a}	0.71 ± 0.04^{c}	0.92 ± 0.03^{b}	0.75 ± 0.08^{c}	0.53 ± 0.03^{d}	***
D-sucrose (g/L)						
wort	13.65 ± 0.35	13.65 ± 0.35	13.65 ± 0.35	13.65 ± 0.35	13.65 ± 0.35	
1d	11.29 ± 0.18^{a}	9.75 ± 0.27^{b}	10.93 ± 0.24^{a}	9.23 ± 0.21^{bc}	8.84 ± 0.16^{c}	***
2d	8.91 ± 0.24^{a}	2.95 ± 0.11^{d}	7.42 ± 0.12^{b}	3.44 ± 0.15^{c}	2.42 ± 0.09^{e}	***
3d	6.48 ± 0.15^{a}	0.13 ± 0.02^{c}	3.70 ± 0.14^{b}	0.26 ± 0.07^{c}	0.18 ± 0.06^{c}	***
6d	4.38 ± 0.21^{a}	0.13 ± 0.03^{b}	0.20 ± 0.07^{b}	0.17 ± 0.04^{b}	0.13 ± 0.03^{b}	***
9d	0.11 ± 0.03^{a}	0.10 ± 0.02^{a}	0.13 ± 0.02^{a}	0.09 ± 0.01^{a}	0.14 ± 0.03^{a}	N.S.
12d	0.09 ± 0.02^{a}	0.10 ± 0.03^{a}	0.11 ± 0.01^{a}	0.09 ± 0.04^{a}	0.10 ± 0.01^{a}	N.S.
Lactic acid (g/L)						
wort	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
1d	0.01 ± 0.00^{b}	0.13 ± 0.01^{a}	0.01 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	***
2d	0.05 ± 0.02^{b}	0.39 ± 0.03^{a}	0.01 ± 0.00^{b}	0.02 ± 0.00^{b}	0.02 ± 0.01^{b}	***
3d	0.04 ± 0.02^{b}	0.69 ± 0.05^{a}	0.03 ± 0.01^{b}	0.02 ± 0.01^{b}	0.02 ± 0.01^{b}	***
6d	0.05 ± 0.02^{b}	0.70 ± 0.05^{a}	0.04 ± 0.01^{b}	0.02 ± 0.00^{b}	0.02 ± 0.01^{b}	***
9d	0.05 ± 0.03^{b}	0.72 ± 0.04^{a}	0.04 ± 0.02^{b}	0.03 ± 0.01^{b}	0.04 ± 0.01^{b}	***
12d	0.04 ± 0.03^{b}	0.72 ± 0.04^{a}	0.05 ± 0.02^{b}	0.03 ± 0.01^{b}	0.04 ± 0.01^{b}	***
Acetic acid (g/L)						
wort	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
1d	0.01 ± 0.00^{b}	0.03 ± 0.00^{a}	0.01 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	
2d	0.05 ± 0.02^{ab}	0.08 ± 0.01^{a}	0.02 ± 0.00^{bc}	0.00 ± 0.00^{c}	0.01 ± 0.00^{c}	***
3d	0.12 ± 0.02^{b}	0.18 ± 0.03^{a}	0.05 ± 0.01^{c}	0.01 ± 0.00^{c}	0.03 ± 0.01^{c}	***
6d	0.15 ± 0.01^{b}	0.23 ± 0.02^{a}	0.05 ± 0.00^{c}	0.02 ± 0.01^{c}	0.03 ± 0.01^{c}	***
9d	0.15 ± 0.03^{b}	0.25 ± 0.02^{a}	0.06 ± 0.01^{c}	0.01 ± 0.00^{c}	0.04 ± 0.01^{c}	***

12d	0.15 ± 0.04^{b}	0.24 ± 0.03^{a}	0.07 ± 0.02^{bc}	0.02 ± 0.01^{c}	0.05 ± 0.02^{c}	***
Glycerol (g/L)						
wort	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
1d	0.10 ± 0.01^{bc}	0.13 ± 0.01^{b}	0.08 ± 0.00^{c}	0.08 ± 0.01^{c}	0.45 ± 0.03^{a}	***
2d	0.38 ± 0.02^{b}	0.38 ± 0.02^{b}	0.22 ± 0.01^{c}	0.20 ± 0.01^{c}	1.11 ± 0.08^{a}	***
3d	0.62 ± 0.02^{b}	0.71 ± 0.05^{b}	0.42 ± 0.03^{c}	0.39 ± 0.02^{c}	1.93 ± 0.12^{a}	***
6d	1.83 ± 0.09^{ab}	2.04 ± 0.13^{a}	1.67 ± 0.08^{b}	1.05 ± 0.09^{c}	1.95 ± 0.11^{ab}	***
9d	2.30 ± 0.05^{b}	2.62 ± 0.04^{a}	2.15 ± 0.06^{b}	1.96 ± 0.04^{c}	2.52 ± 0.09^{a}	***
12d	2.91 ± 0.11^{b}	3.04 ± 0.10^{b}	2.78 ± 0.08^{bc}	3.36 ± 0.09^{a}	2.55 ± 0.10^{c}	***

Values are expressed as average of three measurements.

Beer fermented by: *H. uvarum* YGA34 and after 72 h *S. cerevisiae* US-05 strain (EP1); *L. thermotolerans* MNF105 and after 72 h *S. cerevisiae* US-05 strain (EP2); *C. oleophila* YS209 and after 72 h *S. cerevisiae* US-05 strain (EP3); *St. lactis-condensi* MN412 and after 72 h *S. cerevisiae* US-05 strain (EP4); *S. cerevisiae* US-05 strain as control (TC).

Abbreviations: S.S., statistical significance; N.S., not significant.

Data in the same line followed by the same letter are not significantly different according to Tukey's test. Symbols: ***, $P < 0.001$.

Table 2. Conventional chemical parameters monitored in samples beer during the alcoholic fermentation of beer.

	EP1	EP2	EP3	EP4	TC	S.S.
Alcohol (v/v)	6.06 ± 0.12^{a}	5.80 ± 0.11^{b}	6.19 ± 0.15^{a}	6.32 ± 0.21^{a}	6.19 ± 0.14^{a}	***
Density (FG)	1.010 ± 0.09^{a}	1.012 ± 0.08^{a}	1.009 ± 0.12^{a}	1.008 ± 0.09^{a}	1.009 ± 0.10^{a}	N.S.
Real extract (°P)	4.59 ± 0.12^{b}	5.01 ± 0.13^{a}	4.39 ± 0.08^{bc}	4.18 ± 0.07^{c}	4.39 ± 0.10^{bc}	***
Energy (kcal/100g)	36.00 ± 0.12^{b}	41.30 ± 0.13^{a}	33.35 ± 0.08^{c}	30.71 ± 0.08^{d}	33.35 ± 0.09^{c}	***
Apparent extract (°P)	2.56 ± 0.10^{b}	3.07 ± 0.11^{a}	2.31 ± 0.08^{bc}	2.05 ± 0.13^{c}	2.31 ± 0.07^{bc}	***
Original extract (°P)	13.80 ± 0.08^{a}	13.78 ± 0.06^{a}	13.82 ± 0.10^{a}	13.81 ± 0.08^{a}	13.80 ± 0.07^{a}	N.S.
Real attenuation (%)	66.20 ± 1.15^{ab}	63.20 ± 1.82^{b}	67.70 ± 1.35^{a}	69.20 ± 1.38^{a}	67.70 ± 1.25^{a}	**
pH	3.92 ± 0.10^{ab}	3.58 ± 0.08^{b}	3.85 ± 0.12^{ab}	4.02 ± 0.06^{a}	3.95 ± 0.15^{a}	**

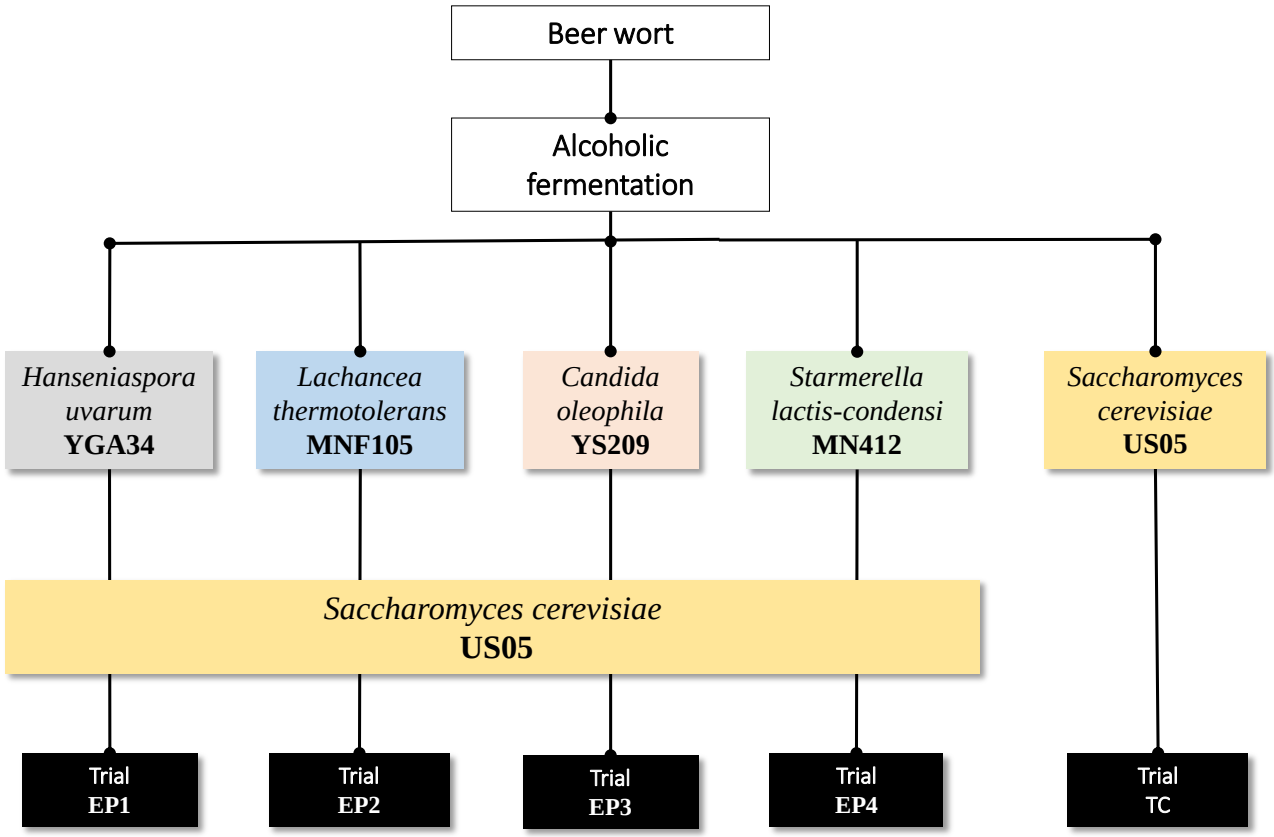
Values are expressed as average of three measurements.
Beer fermented by: *H. uvarum* YGA34 and after 72 h *S. cerevisiae* US-05 strain (EP1); *L. thermotolerans* MNF105 and after 72 h *S. cerevisiae* US-05 strain (EP2); *C. oleophila* YS209 and after 72 h *S. cerevisiae* US-05 strain (EP3); *St. lactis-condensi* MN412 and after 72 h *S. cerevisiae* US-05 strain (EP4); *S. cerevisiae* US-05 strain as control (TC).
Abbreviations: S.S., statistical significance; N.S., not significant.
Data in the same line followed by the same letter are not significantly different according to Tukey's test. Symbols: ***, $P < 0.001$; **, $P < 0.01$.

767 **Table 3.** VOCs detected in beer samples. Compounds detected by SPME/GC-MS (all values in mg/L).

RT (min)	Compounds	Aroma Description	Odour Threshold	EP1 (OAV)	EP2 (OAV)	EP3 (OAV)	EP4 (OAV)	TC (OAV)	S.S.
	∑Alcohols			95.4 ± 2.7^{b}	89.8 ± 3.0^{bc}	85.2 ± 2.4^{c}	128.9 ± 3.3^{a}	66.7 ± 2.4^{d}	***
3.453	1-Pentanol	Alcoholic, iodoform-like	80[1]	47.8 ± 1.6^{ab} (<1)	46.7 ± 1.2^{b} (<1)	45.0 ± 1.1^{b} (<1)	51.2 ± 0.8^{a} (<1)	32.8 ± 0.8^{c} (<1)	***
14.000	1-Octanol	coconut, walnut, oily	0.04[1]	1.7 ± 0.1^{a} (42.5)	1.7 ± 0.1^{a} (42.5)	1.5 ± 0.2^{ab} (37.5)	1.2 ± 0.0^{b} (30.0)	1.6 ± 0.1^{a}(40.0)	**
15.000	Phenethyl alcohol	Rose, perfumy	125[1]	42.2 ± 0.8^{b}(<1)	38.1 ± 1.6^{bc} (<1)	35.7 ± 0.9^{c} (<1)	71.8 ± 2.2^{a} (<1)	28.1 ± 1.1^{d} (<1)	***
20.649	2-Undecanol	fatty acids, coconut	0.5[2]	3.7 ± 0.2^{bc} (7.4)	1.0 ± 0.0^{d} (2.0)	3.0 ± 0.2^{c} (6.0)	4.7 ± 0.3^{a} (9.4)	4.2 ± 0.4^{ab} (8.4)	***
20.799	4-Vinylguaiacol	spicy, cloves, phenolic	0.12[2]	0.0 ± 0.0^{b} (<1)	2.3 ± 0.1^{a} (19.2)	0.0 ± 0.0^{b} (<1)	0.0 ± 0.0^{b} (<1)	0.0 ± 0.0^{b} (<1)	***
	∑Ketones			0.2 ± 0.0^{ab}	0.2 ± 0.0^{ab}	0.1 ± 0.0^{b}	0.3 ± 0.1^{a}	0.1 ± 0.0^{b}	**
17.150	3-Hydroxy-2-methyl-1-phenyl-1-propanone	unknown	unknown	0.2 ± 0.0^{ab}(n.d.)	0.2 ± 0.0^{ab} (n.d.)	0.1 ± 0.0^{b} (n.d.)	0.3 ± 0.1^{a} (n.d.)	0.1 ± 0.0^{b} (n.d.)	**
	∑Carboxylic acids			0.7 ± 0.1^{a}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.3 ± 0.0^{b}	***
2.354	Acetic acid	acid, acetic, pungent	71[2]	0.7 ± 0.1^{a} (<1)	0.0 ± 0.0^{c} (<1)	0.0 ± 0.0^{c} (<1)	0.0 ± 0.0^{c} (<1)	0.0 ± 0.0^{b} (<1)	***
19.949	β-hydroxydodecanoic acid	unknown	unknown	0.0 ± 0.0^{a} (<1)	0.0 ± 0.0^{a} (<1)	0.0 ± 0.0^{a} (<1)	0.0 ± 0.0^{a} (<1)	0.3 ± 0.0^{a} (<1)	N.S.
	∑Esters			127.4 ± 5.0^{c}	237.3 ± 8.2^{a}	105.5 ± 2.6^{d}	91.2 ± 2.6^{d}	198.5 ± 5.7^{b}	***
1.904	Ethyl acetate	Fruity, sweet	30[1]	1.0 ± 0.1^{b} (<1)	3.2 ± 0.2^{a} (<1)	0.7 ± 0.0^{bc} (<1)	0.5 ± 0.0^{c} (<1)	0.7 ± 0.1^{bc} (<1)	***
4.303	Isobutyl acetate	Banana, sweet, fruity	1.6[2]	0.0 ± 0.0^{a} (<1)	0.5 ± 0.0^{a} (<1)	0.0 ± 0.0^{a} (<1)	0.0 ± 0.0^{a} (<1)	0.0 ± 0.0^{a} (<1)	N.S.
5.203	Ethyl butanoate	Papaya, apple, perfumed	0.40[1]	0.6 ± 0.0^{a} (1.5)	0.5 ± 0.1^{ab} (1.2)	0.5 ± 0.0^{ab} (1.2)	0.4 ± 0.0^{b} (1.0)	0.4 ± 0.0^{b} (1.0)	N.S.
8.002	Isoamyl acetate	Fruity, banana, pear	0.50[2]	2.0 ± 0.2^{b} (4.0)	27.0 ± 1.0^{a} (54.0)	2.0 ± 0.1^{b} (4.0)	0.0 ± 0.0^{c} (<1)	1.8 ± 0.1^{b} (3.6)	***
12.151	Ethyl hexanoate (caproate)	Apple, fruity, aniseed	0.17[1]	4.2 ± 0.4^{b} (24.7)	5.6 ± 0.5^{a} (32.9)	1.9 ± 0.2^{c} (11.2)	5.5 ± 0.3^{a} (32.3)	1.8 ± 0.1^{c} (10.6)	***
12.551	Hexyl acetate	aromatic, perfumed	3.5[2]	0.2 ± 0.0^{a} (<1)	1.2 ± 0.0^{a} (<1)	0.3 ± 0.0^{a} (<1)	0.4 ± 0.0^{a} (<1)	0.3 ± 0.0^{a} (<1)	N.S.
12.651	2-Methylbutyl isobutanoate	unknown	unknown	0.2 ± 0.0^{a} (n.d.)	0.0 ± 0.0^{a} (n.d.)	0.0 ± 0.0^{a} (n.d.)	0.0 ± 0.0^{a} (n.d.)	0.4 ± 0.0^{a} (n.d.)	N.S.
18.199	Ethyl octanoate	Apple, sweet, fruity	0.37[1]	103.2 ± 3.4^{c} (278.9)	125.5 ± 2.5^{b} (339.1)	91.8 ± 1.8^{d} (248.1)	77.3 ± 2.1^{e} (208.9)	157.1 ± 3.2^{a} (424.2)	***
19.299	Isoamyl hexanoate (caproate)	Apple, fruity, aniseed	0.17[1]	0.2 ± 0.0^{a} (1.2)	0.4 ± 0.0^{a} (2.3)	0.2 ± 0.0^{a} (1.2)	0.4 ± 0.0^{a} (2.3)	0.5 ± 0.0^{a} (2.9)	N.S.
19.499	2-Phenylethyl hexanoate (caproate)	unknown	unknown	0.9 ± 0.1^{b} (n.d.)	3.9 ± 0.2^{a} (n.d.)	0.8 ± 0.1^{b} (n.d.)	1.1 ± 0.0^{b} (n.d.)	0.5 ± 0.0^{c} (n.d.)	***
20.199	Propyl octanoate	unknown	unknown	0.0 ± 0.0^{a} (n.d.)	0.3 ± 0.0^{a} (n.d.)	0.0 ± 0.0^{a}(n.d.)	0.0 ± 0.0^{a} (n.d.)	0.0 ± 0.0^{a} (n.d.)	N.S.
20.449	Ethyl nonanoate	Fruity, fatty acids, sweet	1.20[2]	1.5 ± 0.2^{a} (1.3)	1.1 ± 0.1^{bc} (<1)	1.4 ± 0.1^{ab} (1.2)	0.9 ± 0.0^{c} (<1)	1.1 ± 0.0^{bc} (<1)	***
21.698	Butyl octanoate (caprylate)	unknown	unknown	0.4 ± 0.0^{a} (n.d.)	0.7 ± 0.0^{a} (n.d.)	0.3 ± 0.0^{a} (n.d.)	0.3 ± 0.0^{a} (n.d.)	0.6 ± 0.0^{a} (n.d.)	N.S.
22.698	Ethyl-trans-4-decenoate	Green, pineapple, pear	unknown	10.6 ± 0.6^{c} (n.d.)	25.9 ± 1.1^{b} (n.d.)	1.6 ± 0.1^{d} (n.d.)	1.7 ± 0.1^{d} (n.d.)	31.4 ± 2.1^{a} (n.d.)	***

22.748	Ethyl decanoate (caprate)	Caprylic, fruity, apple	0.57[2]	0.8 ± 0.0[b] (1.4)	38.6 ± 2.3[a] (67.7)	2.7 ± 0.2[b] (4.7)	1.2 ± 0.1[b] (2.1)	0.0 ± 0.0[b] (<1)	***
23.998	Isoamyl caprylate	spicy, orange, pear	2[2]	1.1 ± 0.0[b] (<1)	1.5 ± 0.1[a] (<1)	0.9 ± 0.0[c] (<1)	1.0 ± 0.0[c] (<1)	1.3 ± 0.1[b] (<1)	***
27.197	Ethyl dodecanoate (laurate)	caprylic, soapy, estery	2[2]	0.5 ± 0.0[bc] (<1)	1.2 ± 0.1[a] (<1)	0.4 ± 0.0[c] (<1)	0.5 ± 0.0[bc] (<1)	0.6 ± 0.0[b] (<1)	***
34.645	Ethyl palmitate	Fatty acids, fruity, sweet	1.50[2]	0.0 ± 0.0[a] (<1)	0.2 ± 0.0[a] (<1)	0.0 ± 0.0[a] (<1)	0.0 ± 0.0[a] (<1)	0.0 ± 0.0[a] (<1)	N.S.
	∑Terpenes			37.5 ± 2.7[b]	34.1 ± 3.8[b]	26.9 ± 1.4[b]	33.9 ± 2.8[b]	162.2 ± 6.7[a]	
10.351	Camphene	unknown	unknown	0.0 ± 0.0[b] (n.d.)	0.0 ± 0.0[b] (n.d.)	0.0 ± 0.0[b] (n.d.)	0.0 ± 0.0[b] (n.d.)	2.1 ± 0.2[a] (n.d.)	***
11.301	β-Myrcene	herbs, resinous, spicy	0.20[2]	0.0 ± 0.0[b] (<1)	0.0 ± 0.0[b] (<1)	0.0 ± 0.0[b] (<1)	0.0 ± 0.0[b] (<1)	6.1 ± 0.3[a] (15.5)	***
11.801	β-Pinene	pine, mint, eucalyptus	unknown	0.0 ± 0.0[b] (n.d.)	0.0 ± 0.0[b] (n.d.)	0.0 ± 0.0[b] (n.d.)	0.0 ± 0.0[b] (n.d.)	8.7 ± 0.5[a] (n.d.)	***
12.851	o-Cymene	unknown	unknown	1.7 ± 0.1[b] (n.d.)	1.3 ± 0.1[bc] (n.d.)	1.0 ± 0.0[c] (n.d.)	1.6 ± 0.1[b] (n.d.)	5.6 ± 0.4[a] (n.d.)	***
12.951	Limonene	Citrus, fruity	0.1[1]	12.3 ± 1.2[a] (123.0)	14.0 ± 2.1[a] (140.0)	7.3 ± 0.5[b] (73.0)	5.2 ± 0.4[b] (52.0)	10.8 ± 0.31[a] (108.0)	***
14.850	p-Cymenene	unknown	unknown	3.1 ± 0.2[a] (n.d.)	2.5 ± 0.2[a] (n.d.)	2.4 ± 0.2[a] (n.d.)	2.7 ± 0.2[a] (n.d.)	8.3 ± 0.6[a] (n.d.)	***
15.200	Linalool	citrus, rosewood-like, aniseed	0.008[2]	20.4 ± 1.2[ab](2550.0)	16.3 ± 1.4[b] (2037.5)	16.2 ± 0.7[b] (2025.0)	24.4 ± 2.1[a] (3050.0)	21.6 ± 1.6[a](2700.0)	***
	∑Other			42.1 ± 2.1[b]	33.0 ± 1.8[c]	73.7 ± 2.4[a]	18.1 ± 0.9[d]	29.7 ± 1.7[c]	***
8.402	Styrene	balsamic	20[2]	42.1 ± 2.1[b] (2.1)	33.0 ± 1.8[c] (1.6)	73.7 ± 2.4[a] (3.7)	18.1 ± 0.9[d] (<1)	29.7 ± 1.7[c] (1.5)	***

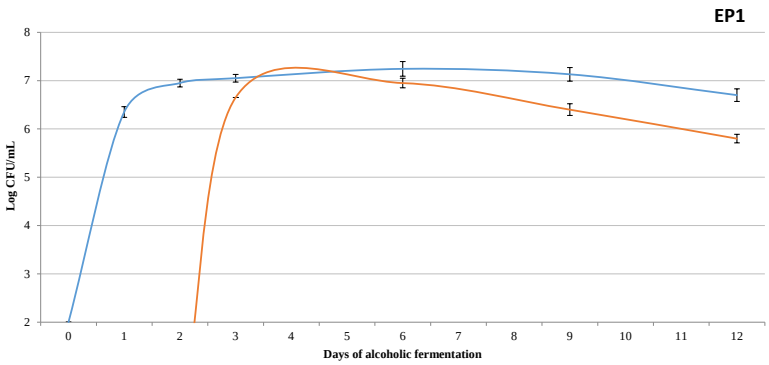
768 Values are expressed as average of three measurements ± standard deviation.
769 Concentrations are calculated using ethyl octanoate, 2-heptanol, and limonene as the standards for the calibration lines
770 Concentrations are calculated using limonene as the standard for the calibration line.
771 Compounds in each class are ordered according to their retention time.
772 Odour threshold as reported in literature.
773 Abbreviations: S.S., statistical significance; RT, retention time using a non-polar DB-5MS column; OAV, odour activity value; n.d., not determinable;
774 N.S., not significant.
775 Beer fermented by: *H. uvarum* YGA34 and after 72 h *S. cerevisiae* US-05 strain (EP1); *L. thermotolerans* MNF105 and after 72 h *S. cerevisiae*
776 US-05 strain (EP2); *C. oleophila* YS209 and after 72 h *S. cerevisiae* US-05 strain (EP3); *St. lactis-condensi* MN412 and after 72 h *S. cerevisiae*
777 US-05 strain (EP4); *S. cerevisiae* US-05 strain as control (TC). Data in the same line followed by the same letter are not significantly different
778 according to Tukey's test. Symbols: ***, P < 0.001; **, P < 0.01; * P < 0.05.
779 [1]Maarse, H. (2017). Volatile compounds in foods and beverages. Routledge.
780 [2]Zunkel, M., Gastl, M., Schoenberger, C., Sedin, D., Becker, T. (2011). Beer flavor database. In ASBC 74th Annual Meeting. Ft. Myers.
781

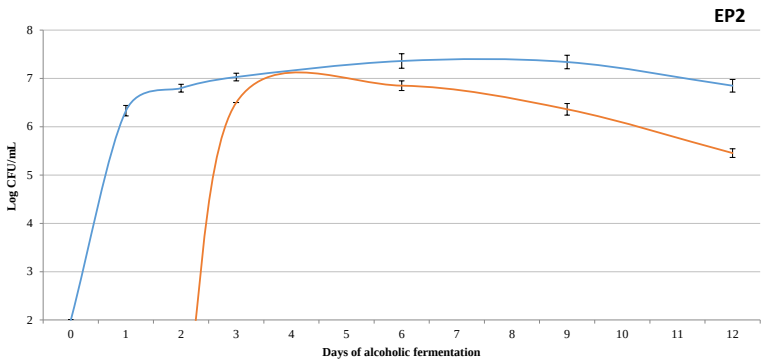


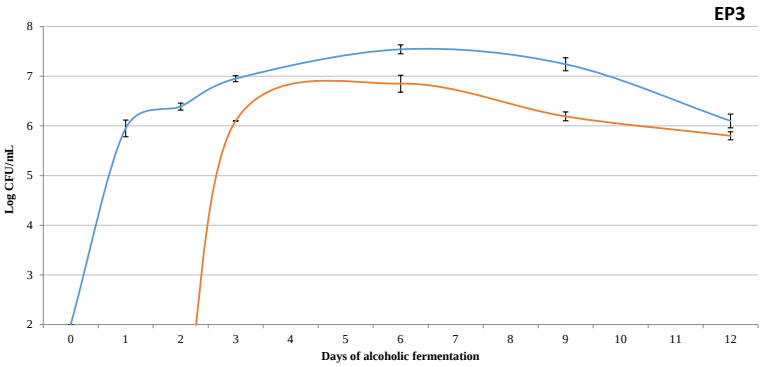
782

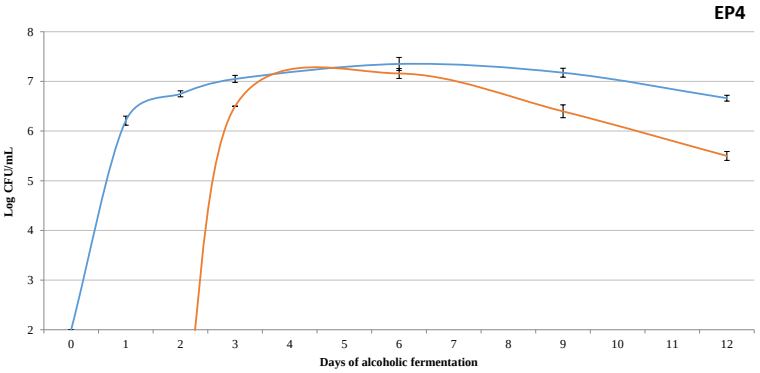
783 **Fig. 1**. Experimental plan of beer production

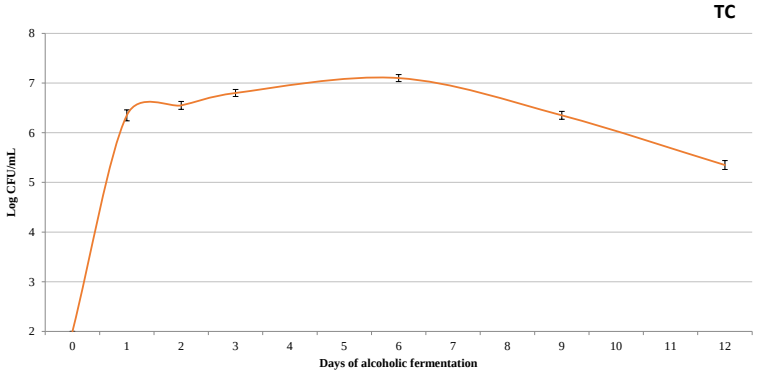
784


EP1
Log CFU/mL
Days of alcoholic fermentation


EP2
Log CFU/mL
Days of alcoholic fermentation


EP3
Log CFU/mL
Days of alcoholic fermentation


EP4
Log CFU/mL
Days of alcoholic fermentation


TC
Log CFU/mL
Days of alcoholic fermentation

785

Fig. 2. Trend of microbial count monitored during alcoholic fermentation. Beer fermented by: *H. uvarum* YGA34 and after 72 h *S. cerevisiae* US-05 strain (EP1); *L. thermotolerans* MNF105 and after 72 h *S. cerevisiae* US-05 strain (EP2); *C. oleophila* YS209 and after 72 h *S. cerevisiae* US-05 strain (EP3); *St. lactis-condensi* MN412 and after 72 h *S. cerevisiae* US-05 strain (EP4); *S. cerevisiae* US-05 strain as control (TC). Symbols: —, *Saccharomyces;* —, non-*Saccharomyces.*

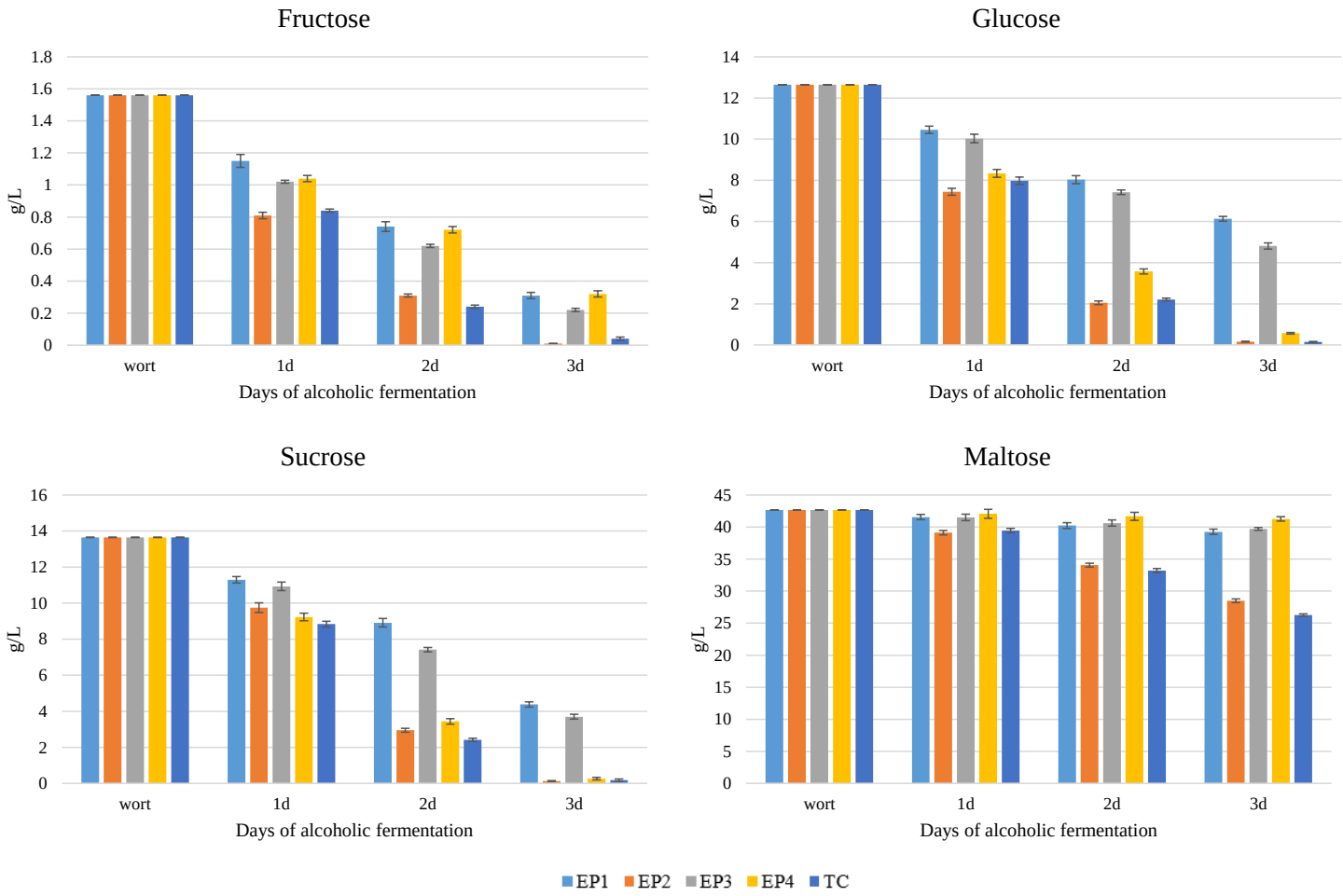
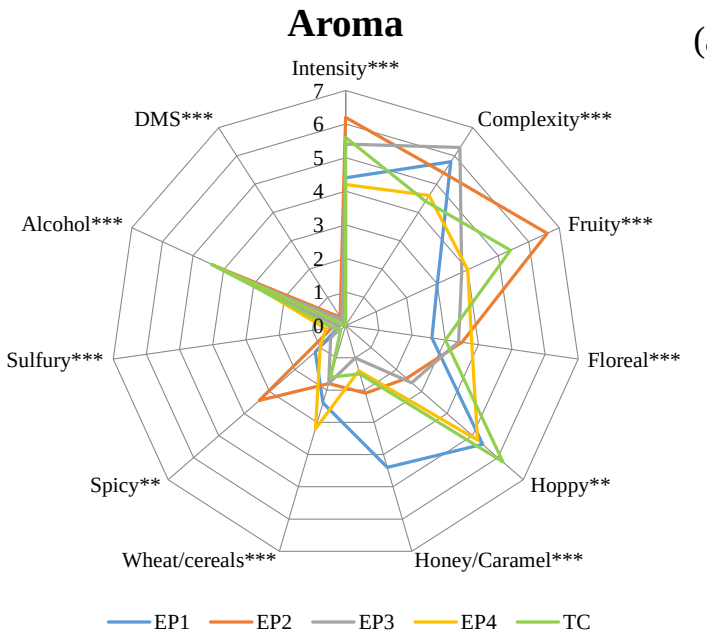


Fig. 3. Trend of the consumption of the different sugars during the first three days of alcoholic fermentation (before inoculation of *S. cerevisiae* US-05 yeast strain). Yeast strains applied: *H. uvarum* YGA34 (EP1); *L. thermotolerans* MNF105 (EP2); *C. oleophila* YS209 (EP3); *St. lactis-condensi* MN412 (EP4); *S. cerevisiae* US-05 strain as control (TC).



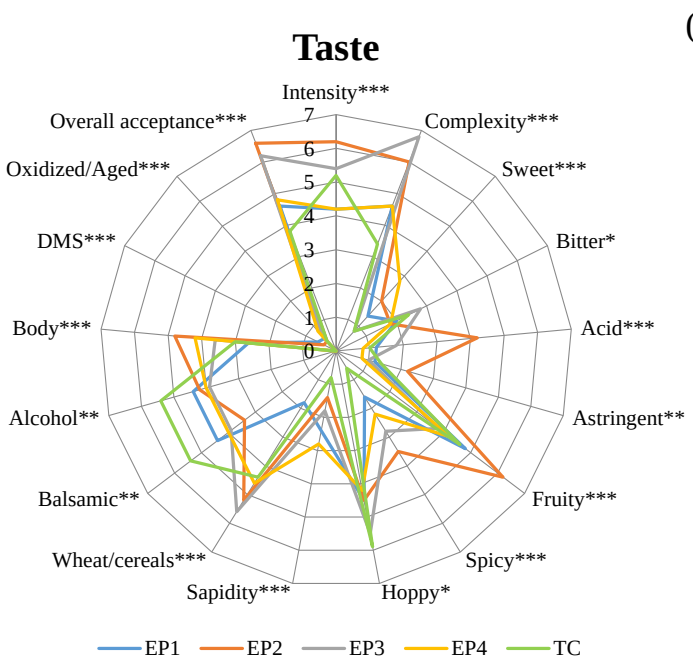


Fig. 4. Sensory analysis performed on beers: spider plots of average scores for aroma (a), taste attributes and overall quality of bottled beers (b), determined by judges during tasting sessions. Beer fermented by: *H. uvarum* YGA34 and after 72 h *S. cerevisiae* US-05 strain (EP1); *L. thermotolerans* MNF105 and after 72 h *S. cerevisiae* US-05 strain (EP2); *C. S. cerevisiae lactis-condensi* MN412 and after 72 h *S. cerevisiae* US-05 strain (EP4); *S. cerevisiae* US-05 strain as control (TC). Symbols: ***, $P < 0.001$; **, $P < 0.01$; * $P < 0.05$.

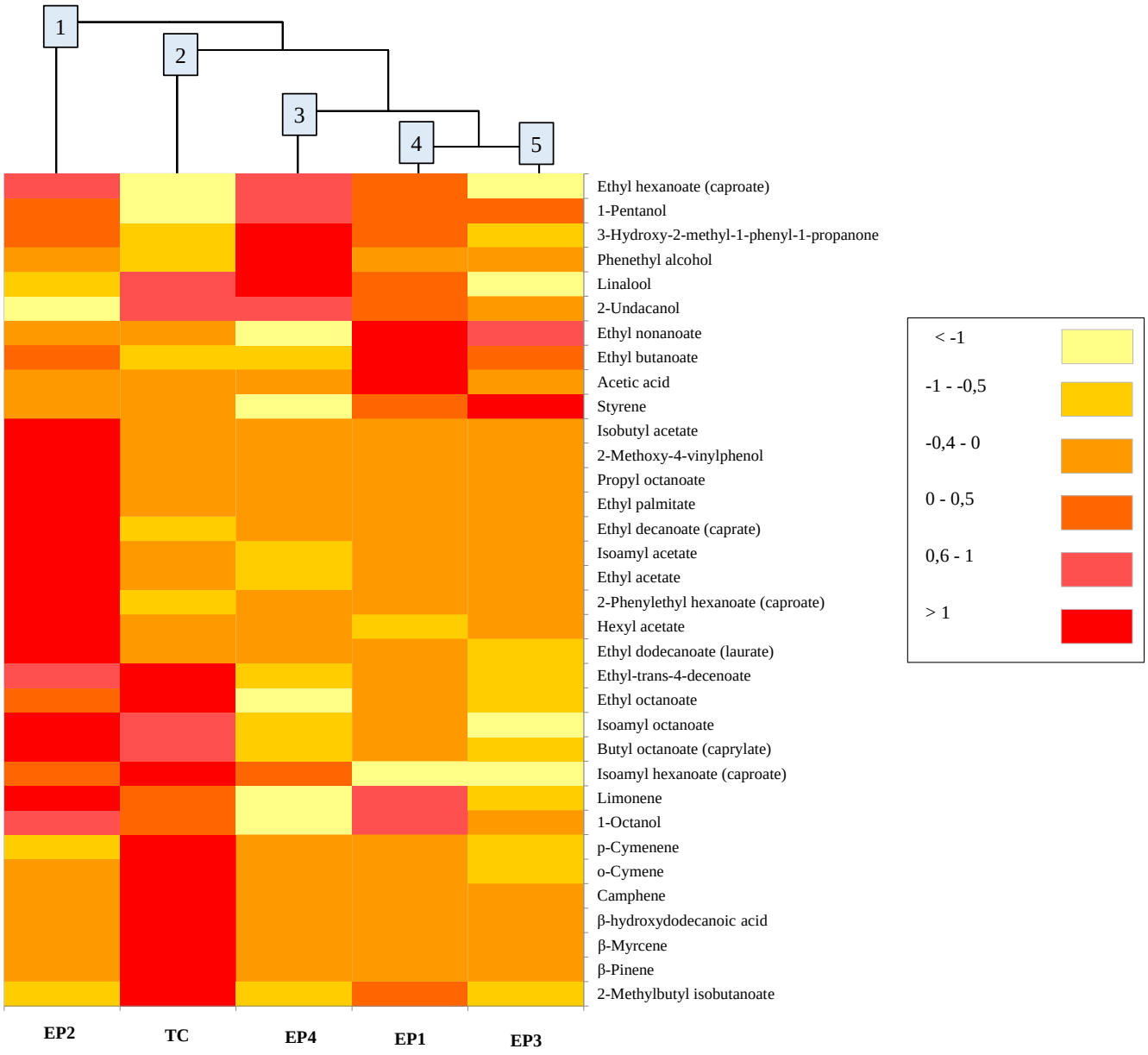


Fig. 5. Distribution of volatile organic compounds among beers. The heat map plot depicts the relative concentration of each VOCs. The number of clusters (from 1 to 5) are reported on the top of the figure. Beer fermented by: *H. uvarum* YGA34 and after 72 h *S. cerevisiae* US-05 strain (EP1); *L. thermotolerans* MNF105 and after 72 h *S. cerevisiae* US-05 strain (EP2); *C. oleophila* YS209 and after 72 h *S. cerevisiae* US-05 strain (EP3); *St. lactis-condensi* MN412 and after 72 h *S. cerevisiae* US-05 strain (EP4); *S. cerevisiae* US-05 strain as control (TC).

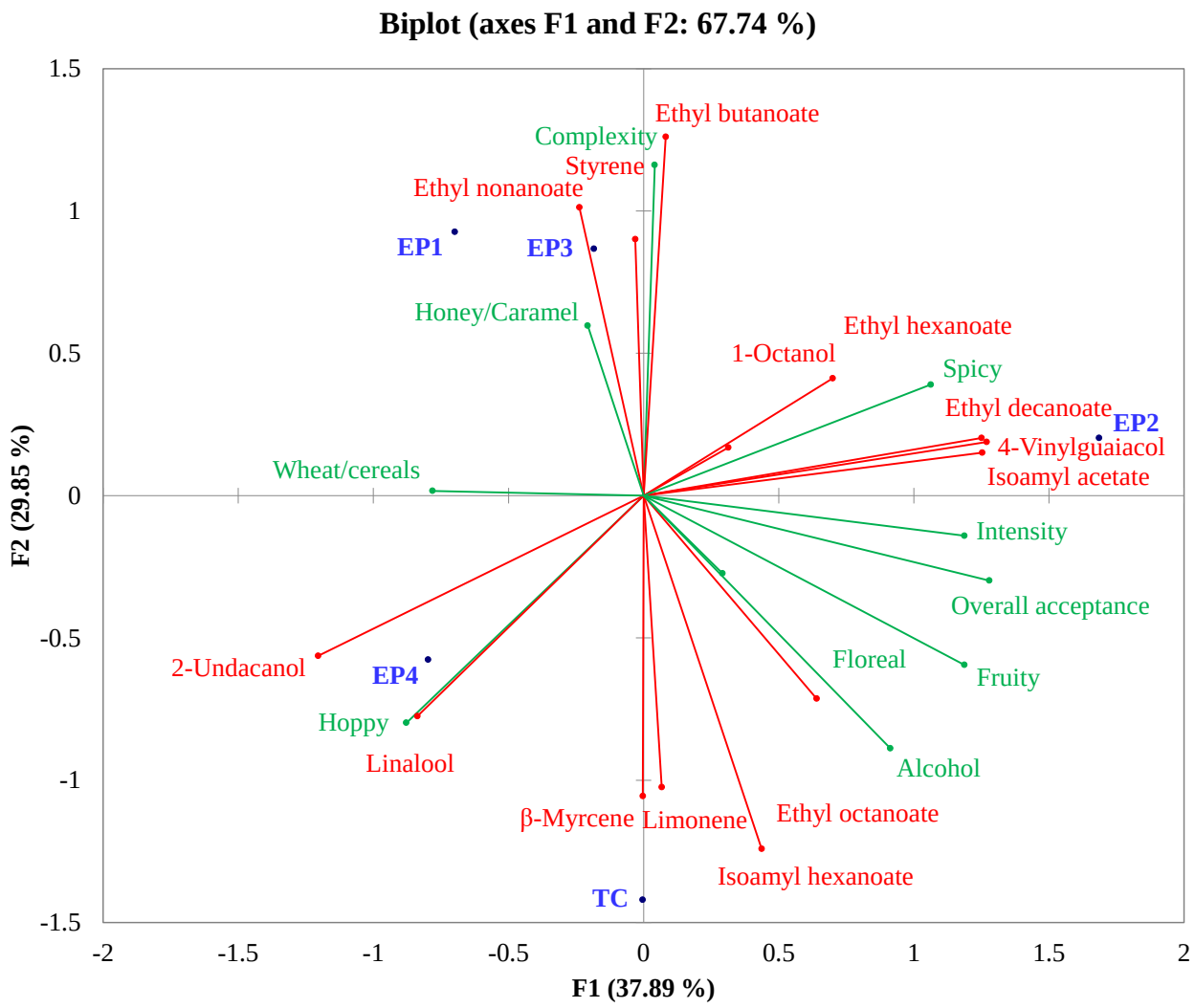
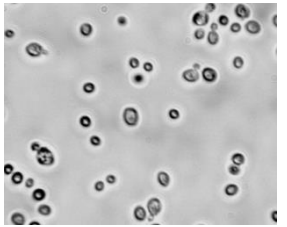


Fig. 6. Principal component analysis (PCA) biplot for VOCs above the perception threshold and sensory attributes (only olfactory parameters). Beer fermented by: *H. uvarum* YGA34 and after 72 h *S. cerevisiae* US-05 strain (EP1); *L. thermotolerans* MNF105 and after 72 h *S. cerevisiae* US-05 strain (EP2); *C. oleophila* YS209 and after 72 h *S. cerevisiae* US-05 strain (EP3); *St. lactis-condensi* MN412 and after 72 h *S. cerevisiae* US-05 strain (EP4); *S. cerevisiae* US-05 strain as control (TC).

Graphical Abstract

Use of yeasts previously isolated and selected from manna and honey by-products



Fermentation

Production of experimental top-fermented beers

Beer analysis

- Physico-chemical analysis
- Analysis of volatile organic compounds
- Sensory evaluation

CONFLICT OF INTEREST

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