A novel microbiological approach to impact the aromatic composition of sour loquat beer

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ABSTRACT

The growing interest in novel beer development determined the exploitation of unconventional yeasts isolated from novel ecological niches to generate unexplored sensory profiles. In recent years, there is an increasing interest in generating beers brewed with the addition of fruits. For the first time, *Lachancea thermotolerans* MNF105 and *Saccharomyces cerevisiae* MN113 isolated from manna, were tested as starter cultures to process loquat beer to improve the sensory profile. Innovatively, the yeast species *L. thermotolerans* was investigated for the production of sour fruit beer. Sour fruit beers produced with *L. thermotolerans* MNF105 were more balanced than the respective control, especially in terms of perceived acidity during sensory analysis. This could be due to the lower lactic acid production (0.49 g/L) compared to the respective control (1.74 g/L). The overall organoleptic investigation showed a preference for *S. cerevisiae* MN113 (TF1) isolated from manna. Experimental trials conducted with the selected strains demonstrated the absence of off-odour and off-flavour and improved aroma perception. Aldehydes and alcohols were the most abundant compounds emitted from the beers. *S. cerevisiae* MN113 and *L. thermotolerans* MNF105, manna related yeasts, showed great technological properties, representing promising starters for the production of fruit beer and sour fruit beer.

1. Introduction

Beer is the oldest and most popular alcoholic beverage in the world. Precisely, it ranks third among beverages, after tea and water (Anderson et al., 2019; Callejo et al., 2020). Recent developments have focused on the selection of *Saccharomyces* and non-*Saccharomyces* yeasts from sugar-rich sources, in order to find new yeast strains capable of producing innovative fermented alcoholic beverages, e.g. Guarcello et al. (2019) provided a survey on the ecological niches associated with the highly sugary source represented by manna. Instead, Matraxia et al. (2021) investigated yeast composition of a highly alcoholic beverages (Spiritu re fascitrari) obtained from the fermentation of honey by-products. On the other hand, Sinacori et al. (2014) deepened the knowledge of the microbial community of southern Italian honeys. In

particular, manna is a sugary substance obtained from the solidification of processed sap of different *Fraxinus* sp. (Schicchi et al., 2007; Yücedag & Sen, 2008). As a source with a high sugar content, manna hosts osmophilic microorganisms, in particular those are able to survive in a viable form under the extremely stressful conditions generated by the osmotic pressure (Guarcello et al., 2019). The study conducted by Guarcello et al. (2019) resulted in the isolation of several yeast species and *Lachancea thermotolerans* showed characteristics useful to act as starters or co-starters in food applications such as sour beer production. *Lachancea* and other non-*Saccharomyces* yeasts including *Pichia*, *Saccharomycodes*, *Zygosaccharomyces*, *Hanseniaspora*, and *Torulaspora* are being evaluated for their potential use as starter cultures in brewing (Sannino et al., 2019). Domizio et al. (2016) registered a lactic acid production by *L. thermotolerans* allowing the production of sour beer
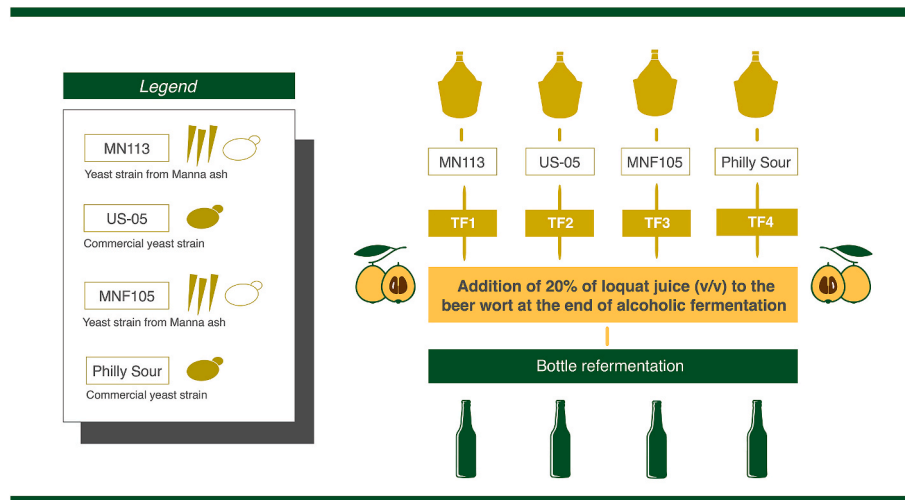

Fig. 1. Experimental plan of loquat beer production.

without the deliberate addition of bacteria with a consistent shortening of the transformation process and positively affecting the taste and aroma. Canonico et al. (2019) confirmed the significant decrease in the pH of the medium inoculated with pure cultures of *L. thermotolerans* due to the production of large amounts of lactic acid and also registered a defined production of ethyl butyrate and ethyl acetate. On the other hand, Zdaniewicz et al. (2020) showed that some strains of *L. thermotolerans* possess a limited lactic acid production capacity with a marginal influence on pH drop, but observed a higher production of ethyl lactate compared to *S. cerevisiae*.

Beer market is worldwide dominated by traditional beer types, but there is an increasing interest in the production of beers brewed with the addition of fruit (Patraşcu et al., 2018). Several traditional beer processes are being implemented with the addition of fruit to produce novel sour fruit beers, e.g. the typical Belgian lambic beer, brewed with a blend of barley malt and unmalted wheat is added with "Kriek" cherries or "Framboise" raspberries, and subjected to spontaneous fermentation (De Keersmaecker, 1996; Glover, 2001; Protz, 1995; Spitaels et al., 2014). This type of beverage became popular due to its rich fruity flavor and refreshing properties due also to a pleasant acidity (Gorzelany et al., 2022; Martìnez et al., 2017; Zapata et al., 2019). At the same time, the consumption of tropical fruits is also becoming popular worldwide due to their nutritional and health properties (Aquilani, Laureti, Poponi, & Secondi, 2015). Many of these fruits, such as banana, passion fruit, annona, mango, and loquat, are being studied for their use in brewing, with the aim of increasing the amount of ethanol by adding sugars, enriching in terms of volatile organic compounds and, in some cases, improving the final acidity (Carvalho et al., 2009; De Melo et al., 2017; Gasiński et al., 2020; Pirrone et al., 2022; Santos et al., 2021).

Many tropical fruits such as mango, avocado and papaya have recently spread from their origin areas to Mediterranean countries (Adiletta et al., 2020; Farina et al., 2020; Migliore et al., 2017) while others, such as loquat have been around for a long time. Loquat (*Eriobotrya japonica* Lindl.) is an evergreen tree native to southeastern China. Today, loquat trees are cultivated in many countries around the world (Badenes et al., 2013). In particular, the species *E. japonica* is well adapted throughout Mediterranean countries (Reig et al., 2014) when Spain is the first country for fruit production (Reig et al., 2011). Italian production of loquat fruit is almost entirely concentrated on the northern coast of Sicily, mainly within Palermo province (Farina et al., 2011). Sicilian loquat is characterised by orange-fleshed and white-fleshed fruits that are rich in nutrients, highly aromatic and with high acidity (Gentile et al., 2016); for this reason, these fruits were considered for brewing purposes (Farina et al., 2016; Pirrone et al., 2022). Furthermore, their acidity makes them of great interest for the production of

sour fruit beers. To our knowledge, however, no previous research has assessed the effect of *L. thermotolerans* isolated from a novel ecological niche such as manna ash or other high sugar matrices to produce sour fruit beer. Based on the above considerations, the present research aimed to: (i) evaluate for the first time the effect of *L. thermotolerans* strain (MNF105) isolated from manna for fruit sour beer production; (ii) improve the knowledge on a *S. cerevisiae* strain (MN113) isolated from manna as a possible starter culture in fruit beer production; (iii) study loquat fruit addition to produce craft beer; (iv) to deepen our knowledge on microbial ecology of manna ash as novel source of yeast starter.

2. Materials and methods

2.1. Yeast strains and media

Yeast strains applied in this research were *S. cerevisiae* MN113 and *L. thermotolerans* MNF105. Yeast strains belongs to the collection of the Department of Agricultural, Food and Forest Sciences (SAAF; University of Palermo, Italy); they were isolated from manna (Guarcello et al., 2019) and selected for their high performances during beer wort fermentation. Commercial yeast strains *L. thermotolerans* Philly Sour and *S. cerevisiae* US-05 (both from Allemand Inc., Montreal, Canada) were employed for the control trials. Yeast reactivation from cryogenic storage was performed as reported by Pirrone et al. (2022). Yeast propagation was then carried out in broth cultures with YPD medium, incubated overnight at 28 °C and then re-inoculated in sterile flasks containing YPD, where the cells were left to grow. Media component were procured from Oxoid (Rodano, Italy).

2.2. Experimental plan

Experimental high fermentation beers were conducted on a medium scale (5 L batch) employing four different inocula to better understand the impact of inoculum during fermentation. The wort for the fermentation trials was produced with a 40-L all-in-one microbrewing plant Klarstein mod. 10031629 (Chal-Tec GmbH Berlin, Germany). Pilsner malt (4.5 kg) and wheat malt (4.5 kg; BestMalz, Heidelberg, Germany), pre-ground by a double roller mill (Brouwland, Beverlo, Belgium) with roller distance at 1.20 mm, were added to 34 L of water containing $CaSO_4$ (10 g) and $CaCl_2$ (10 g) for pH correction (Marconi et al., 2016). The mash was performed at different temperature/time combinations: 45 °C for 15 min (acid rest); 52 °C for 15 min (protease step); 62 °C for 30 min (β-amylase); 72 °C for 20 min (α-amylase); and 78 °C for 10 min (mash-out); until the sugars are completely converted (Mayer et al., 2016).

The lautering phase was performed by rinsing the grains with 18 L of H₂O heated at 78 °C; the resulting in a total wort volume of 41 L. The wort was then boiled for 60 min. At the beginning of boiling, 45 g of hops (Mandarina Bavaria - pellets, 9.7% w/w α-acids) were added. After that, the resulting volume was 37 L. Clarification of the wort was carried out using a whirlpool that included recirculation for 10 min and resting for 10 min (Marconi et al., 2016). The wort was cooled for 20 min in a stainless-steel wort chiller until 21 °C and then prepared for yeast inoculation. The quality parameters of beer wort were: 5.60 pH and 12 °Bx (Brix degree). Loquat juice, used to prepare the fruit beers, was extracted from the fruits of the cultivar 'Claudia' of *Eriobotrya japonica* Lindl reaped from a local orchard (37°5′39.54 "N, 13°25′25.85 "E). The fruits were harvested when fully ripe as determined by colorimeter (Minolta, Osaka, Japan). After cutting and pre-washing, subsequent washings were performed as reported by Alfonzo et al. (2018). Subsequently, fruit juice was obtained as reported by Pirrone et., (2022). At the conclusion of the alcoholic fermentation (day 10th), 20% (v/v) of loquat juice was added in all experiments according to Gasiski et al. (2020). The values of pH and the sugar content of the juice were measured before addition to beer. Four experimental trials were inoculated as reported in Fig. 1, as follows: TF1 with *S. cerevisiae* MN113; TF2 with *S. cerevisiae* US05 (control trial for *Saccharomyces*); TF3 with *L. thermotolerans* MNF105; TF4 with *L. thermotolerans* Philly sour (control trial for *Lachancea*). Trials were inoculated with each yeast strain at approximately 2.0×10^6 cells/mL (Holt et al., 2018). Fermentation took place at 20 °C in glass fermenters (5 L) with hermetic closure equipped with an airlock valve. Samples were collected from uninoculated wort, after the inoculum of each yeast strain, at 3 and 6 days of fermentation, upon completion of primary alcoholic fermentation (day 10), the following day after adding the loquat juice (day 11) and at the conclusion of secondary alcoholic fermentation (day 16). At the end of the fermentation process, the beer was transferred into 0.33 L bottles by adding 6 g/L of dextrose. The bottles were conditioned at 20 °C for 25 days (Callejo et al., 2019). After this period, sensory analysis was conducted on the beers. All fermentation experiments were carried out in triplicate.

2.3. Microbiological analyses

Each sample was subjected to microbiological analysis by plate count. Two different media were used: Wallerstein Laboratory nutrient agar (WL) and Lysine Agar medium (LA) for *Saccharomyces* (Di Maio et al., 2011) and non-*Saccharomyces* (Iris et al., 2020) populations, respectively. Based on their morphological characteristics, the colonies from the two agar media were presumptively identified as *Saccharomyces* and *Lachancea* only after cell morphology determination by microscopic inspection (Cavazza et al., 1992). All analyses were performed in triplicate.

2.4. Physicochemical analysis

The pH values were measured with a pH meter, model number Mod.70 XS/50010162 (Cheimika, Pellezzano, Italy) and the °Bx were estimated with a refractometer, model number DBR Salt (Zetalab srl, Padova, Italy). The determination of acetic acid, lactic acid, tartaric acid, fructose, glucose, glycerol, malic acid, maltose, and sucrose, was carried out as reported by Matraxia et al. (2021). BeerFoss™ FT Go (FOSS Italia srl, Padova, Italy) was used to measure alcohol (% vol), density (FG), real extract (°P), energy (kcal/100 g), apparent extract (°P), original extract (°P), specific gravity (°P) and real attenuation (%) of the final beers.

2.5. Analysis of volatile organic compounds in beer samples

The analysis of volatile organic compounds (VOC) in beer samples was performed as reported by Alfonzo et al. (2021). Quantification was carried out using three calibration lines. For compounds belonging to classes other than the standards, similarity was used for quantification. A dilution factor was applied to the reported data.

2.6. Sensory evaluation

Thirteen judges (aged between 27 and 45, 8 men and 5 women) were selected from the University of Palermo to evaluate fruit beer. All panellists had experience in beer production and acted as beer judges in several beer tasting sessions. The judges received preliminary training to target the sensory characteristics that depict the attributes of the fruit beers. To eliminate the effect of beer colour perception on taste perception, samples were offered to panellists in private tasting cabins with uniform illumination. The samples were appropriately labelled with randomly generated number codes and delivered in standard ISO tasting glasses with a watch glass stopper (100 mL at 16 °C). Sensory evaluations were carried out under blind tasting conditions at the sensory analysis laboratory of SAAF Department – University of Palermo, Italy.

Beer sensory evaluations were conducted in accordance with the methodology described by Marconi et al. (2016) and the ISO standards. The attributes evaluated were: visual perception (appearance), odour-based olfactory sensations (through the nostril, orthonasally) and flavour (through the back of the throat, retronasally), oral sensations that are based on taste, mouthfeel and overall quality. All panellists identified 32 sensory descriptive attributes in terms of appearance, odour, flavour, taste and overall quality. The scores ranged from 0 to 9 (on an unstructured 9 cm scale). A score of 0 meant that the attribute was low, while a score of 9 indicated that the attribute was extremely strong. In addition, panellists also visually judged the intensity of the colour using the same scale with the terms "straw yellow" and "amber orange" anchored to the left and right limits (Barry et al., 2018; Jackson, 2016). For evaluating the fruit beer attributes, the following descriptors were used: appearance (colour), odour (intensity, complexity, fruity, loquat, floral, hoppy, wheat/cereal, honey/caramel, acetic, oxidized/aged, sulphury, alcohol and DMS) and taste (intensity, complexity, sweet, bitter, acid, astringent, fruity, loquat, spicy, hoppy, sapidity, wheat/cereal, burnt/cooked, alcohol, body, DMS and oxidized/aged). The average of the three assessments was used to obtain the final scores.

2.7. Statistical and explorative multivariate analyses

ANOVA test was performed to identify significant differences between the chemical parameters determined during the brewing process (lactic acid, acetic acid, tartaric acid, glucose, fructose, sucrose, maltose, glycerol, and malic acid), microbiological analysis (yeast counts), VOCs and sensory analysis (descriptive quantitative analysis). The post-hoc Tukey's method was used to pairwise compare all the data. Statistical significance was attributed to $P \leq 0.05$ (Mazzei et al., 2010). Heat Map Clustered Analysis (HMCA) was used to visualize VOC concentrations, based on a hierarchical dendrogram with a heat map graph, displaying individual content values in the data matrix as colours (Martorana et al., 2017). Colour intensity was used to represent the relative VOC concentration values, ranging from yellow (lowest quantity) to red (highest quantity). A heat map analysis of VOC concentration was carried out using the autoscaled data (Gaglio et al., 2017). The heat map was created using ascending hierarchical clustering based on Ward's method, while statistical data analysis and graph construction were performed using XLStat software version 2019.2.2 (Addinsoft, New York, USA) for Excel. The data collected during the alcoholic fermentation (VOC's, sensory and chemical parameters) from the several trials were compared to investigate relationships using an exploratory multivariate technique. Agglomerative Hierarchical Clustering (AHC) was performed to explore the relationships among the trials particularly between sensory and chemical parameter data.
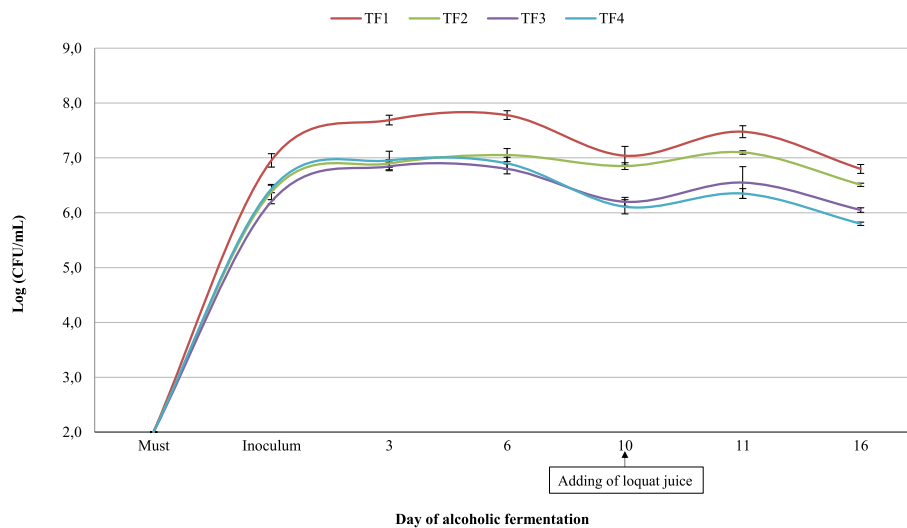
Fig. 2. Monitoring of yeast concentrations during alcoholic fermentation. Beer fermented by: *S. cerevisiae* MN113 (TF1); *S. cerevisiae* US05 (TF2); *L. thermotolerans* MNF105 (TF3); *L. thermotolerans* PHILLY SOUR (TF4).

Table 1
Conventional chemical parameters identified in beer wort and *Eriobotrya japonica* juice.

Parameters (g/L)	Wort	Loquat juice
D-fructose	4.52 ± 0.22	38.25 ± 0.64
D-glucose	7.56 ± 0.28	31.56 ± 0.45
Maltose	36.24 ± 0.05	0.00 ± 0.00
D-sucrose	18.05 ± 0.21	35.15 ± 0.30
Acetic acid	0.00 ± 0.00	0.04 ± 0.04
Lactic acid	0.00 ± 0.00	0.04 ± 0.04
L-Malic Acid	0.10 ± 0.01	12.51 ± 0.15
Glycerol	0.00 ± 0.00	0.00 ± 0.00
Tartaric acid	0.09 ± 0.01	0.95 ± 0.13

Table 2
Physicochemical parameters identified in the final fruit beers.

	TF1	TF2	TF3	TF4	S.S.
Alcohol (% vol)	4.35 ± 0.12[b]	5.04 ± 0.11[a]	4.28 ± 0.15[b]	4.21 ± 0.21[b]	***
Density (FG)	1012.6 ± 1.1[a]	1007.4 ± 1.2[b]	1013.4 ± 0.8[a]	1013.2 ± 0.5[a]	***
Real extract (°P)	5.19 ± 0.12[a]	4.13 ± 0.13[b]	5.33 ± 0.08[a]	5.28 ± 0.07[a]	***
Energy (kcal/ 100g)	44 ± 0.12[a]	43 ± 0.13[a]	44 ± 0.08[a]	43 ± 0.08[a]	N.S.
Apparent extract (°P)	3.69 ± 0.12[a]	2.35 ± 0.12[b]	3.87 ± 0.12[a]	3.83 ± 0.12[a]	***
Original extract (°P)	11.78 ± 0.08[a]	11.8 ± 0.06[b]	11.8 ± 0.10[a]	11.65 ± 0.08[a]	***
Specific gravity (°P)	1014.5 ± 1.10[a]	1009.2 ± 0.90[b]	1015.2 ± 1.20[a]	1015 ± 1.23[a]	***
Real attenuation (%)	57.5 ± 1.15[b]	66.4 ± 1.82[a]	56.4 ± 1.35[b]	56.2 ± 1.38[b]	***
pH	3.80 ± 0.10[a]	3.81 ± 0.08[a]	3.65 ± 0.12[ab]	3.49 ± 0.06[b]	*

Values are expressed as average of three measurements.
Abbreviations: S.S., statistical significance.
Beer fermented by: *S. cerevisiae* MN113 (TF1); *S. cerevisiae* US05 (TF2); *L. thermotolerans* MNF105 (TF3); *L. thermotolerans* PHILLY SOUR (TF4). 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; *P < 0.05; N.S., not significant.

3. Results and discussion

3.1. Evaluation of population dynamics

Growth kinetics during fermentation are shown in Fig. 2. Microbial levels in uninoculated wort and loquat juice were below the detection limit on both WL and LA media (data not shown). On the contrary, all inoculated trials showed yeast cell densities varying between 6.2 and 7.0 Log CFU/mL; these values increased just after 1 d. Starter yeasts increased about 0.5 Log cycles their levels after 3 d for all trials. Similar trends were observed for *L. thermotolerans* (Fig. 2), which increased significantly over the first 3 d until 6.9 and 7.0 log (CFU/mL) for trials TF3 and TF4, respectively. From the 6th day of fermentation onward the inoculated trials began to decrease the numbers of *Lachancea*. Similarly, the levels of *S. cerevisiae* in trials TF1 and TF2 showed an increase in the first few days and displayed cell densities of 7.8 and 7.1 (CFU/mL), respectively, and then began to decrease. Interestingly, trial TF1 trial inoculated with *S. cerevisiae* MN113 showed a more consistent growth than trial TF2 inoculated with the control strain US05. The results are comparable to the dynamics of yeast growth during fermentation in wort beer (Matraxia et al., 2021; Toh et al., 2020). The decrease of *Lachancea* levels after day 4 can be due to various factors including the decrease in nutrients available as sugar (Domizio et al., 2016; Michel et al., 2016). On day 11th of fermentation, with the addition of loquat juice, yeast cell density increased in all trials. *S. cerevisiae* MN113 in trial TF1 had the greatest cell counts at the conclusion of AF (6.8 Log CFU/mL), while *L. thermotolerans* MNF105 in trial TF3 (6.1 Log CFU/mL) demonstrated higher results than control TF4 (5.8 Log CFU/mL). Yeast growth dynamics occurred in fruit beer, confirming those observed by De Melo et al. (2017) and Pirrone et al. (2022).

3.2. Physico-chemical analysis

Physicochemical composition of loquat juice and wort is presented in Table 1. Loquat juice was characterised by pH 3.70 and 11.20 °Bx (data not shown), whereas the original wort by pH 5.60 and 12 °Bx (data not shown). Instead, Table 2 shows data registered after FOSS analysis. Ethanol production was not significantly different between the trials, and therefore no differences were shown between *L. thermotolerans* and *S. cerevisiae* for this parameter. Instead, pH values at the end of alcoholic fermentation ranged from 3.44 to 3.81. According to Domizio et al. (2016), *L. thermotolerans* strain 101 was able to reduce pH from 5.60 to 3.77 during wort fermentation. Our isolate L. *thermotolerans* MNF105,

Table 3
Conventional chemical parameters monitored in samples beer during the alcoholic fermentation.

	TF1	TF2	TF3	TF4	S.S.
D-fructose (g/L)					
3d	0.21 ± 0.04^a	0.12 ± 0.03^a	0.20 ± 0.05^a	0.10 ± 0.02^a	*
6d	0.04 ± 0.03^b	0.15 ± 0.03^a	0.00 ± 0.00^b	0.00 ± 0.00^b	***
10d	0.04 ± 0.03^b	0.19 ± 0.06^a	0.00 ± 0.00^b	0.00 ± 0.00^b	**
11d (+Fr)	5.54 ± 0.25^{ab}	5.26 ± 0.12^b	6.10 ± 0.23^a	5.62 ± 0.18^{ab}	*
End AF	0.01 ± 0.00^b	0.01 ± 0.00^b	0.02 ± 0.01^{ab}	0.04 ± 0.01^a	**
D-glucose (g/L)					
3d	0.27 ± 0.09^{bc}	0.12 ± 0.05^c	0.65 ± 0.10^a	0.44 ± 0.08^{ab}	***
6d	0.04 ± 0.02^a	0.05 ± 0.02^a	0.10 ± 0.02^a	0.08 ± 0.05^a	N.S.
10d	0.03 ± 0.01^a	0.02 ± 0.01^a	0.03 ± 0.00^a	0.04 ± 0.00^a	N.S.
11d (+Fr)	7.38 ± 0.15^a	7.41 ± 0.49^a	7.31 ± 0.38^a	7.22 ± 0.32^a	N.S.
End AF	0.03 ± 0.00^b	0.04 ± 0.01^b	0.05 ± 0.05^a	0.04 ± 0.02^b	**
Maltose (g/L)					
3d	7.40 ± 0.40^b	3.70 ± 0.31^b	26.17 ± 1.75^a	24.00 ± 2.20^a	***
6d	6.95 ± 0.12^b	1.40 ± 0.19^c	17.16 ± 1.15^a	16.02 ± 1.11^a	***
10d	6.84 ± 0.15^a	1.37 ± 0.14^b	8.01 ± 0.89^a	7.66 ± 1.20^a	***
11d (+Fr)	5.84 ± 0.21^a	0.82 ± 0.10^b	5.92 ± 0.19^a	5.79 ± 0.18^a	***
End AF	5.42 ± 0.22^a	0.45 ± 0.05^b	5.82 ± 0.61^a	5.53 ± 0.83^a	***
D-sucrose (g/L)					
3d	0.34 ± 0.09^c	0.24 ± 0.05^c	2.30 ± 0.06^a	2.11 ± 0.04^b	***
6d	0.19 ± 0.03^{ab}	0.22 ± 0.03^a	0.12 ± 0.04^b	0.13 ± 0.02^b	*
10d	0.14 ± 0.10^a	0.20 ± 0.04^a	0.11 ± 0.01^a	0.10 ± 0.05^a	N.S.
11d (+Fr)	5.95 ± 0.40^{ab}	5.46 ± 0.32^{ab}	6.34 ± 0.12^a	5.21 ± 0.41^b	*
End AF	0.16 ± 0.05^a	0.07 ± 0.02^a	0.18 ± 0.09^a	0.13 ± 0.08^a	N.S.
Lactic acid (g/L)					
3d	0.07 ± 0.05^a	0.08 ± 0.02^a	0.07 ± 0.02^a	0.07 ± 0.02^a	N.S.
6d	0.06 ± 0.03^c	0.07 ± 0.01^c	0.51 ± 0.05^b	1.59 ± 0.26^a	***
10d	0.09 ± 0.02^{bc}	0.08 ± 0.04^c	0.61 ± 0.08^b	2.25 ± 0.34^a	***.
11d (+Fr)	0.07 ± 0.04^b	0.09 ± 0.05^b	0.51 ± 0.12^b	2.20 ± 0.43^a	***
End AF	0.04 ± 0.02^b	0.05 ± 0.02^b	0.49 ± 0.08^b	1.74 ± 0.36^a	***
Acetic acid (g/L)					
3d	0.13 ± 0.02^a	0.01 ± 0.00^b	0.02 ± 0.01^b	0.16 ± 0.05^a	**
6d	0.13 ± 0.03^{ab}	0.02 ± 0.00^c	0.05 ± 0.02^{bc}	0.16 ± 0.04^a	**
10d	0.15 ± 0.05^a	0.04 ± 0.01^b	0.14 ± 0.04^{ab}	0.18 ± 0.06^a	*
11d (+Fr)	0.08 ± 0.02^a	0.04 ± 0.01^a	0.09 ± 0.02^a	0.12 ± 0.07^a	N.S.
End AF	0.14 ± 0.02^a	0.03 ± 0.01^b	0.09 ± 0.02^{ab}	0.13 ± 0.05^a	N.S.
L-Malic Acid (g/L)					
3d	0.16 ± 0.08^a	0.17 ± 0.05^a	0.13 ± 0.05^a	0.10 ± 0.02^a	N.S.
6d	0.16 ± 0.05^a	0.18 ± 0.03^a	0.15 ± 0.03^a	0.12 ± 0.03^a	N.S.
10d	0.18 ± 0.05^a	0.20 ± 0.05^a	0.17 ± 0.04^a	0.14 ± 0.04^a	N.S.
11d (+Fr)	2.23 ± 0.19^a	2.41 ± 0.19^a	2.25 ± 0.15^a	2.13 ± 0.14^a	N.S.

Table 3 (*continued*)

	TF1	TF2	TF3	TF4	S.S.
End AF	1.56 ± 0.12^a	1.81 ± 0.11^a	1.83 ± 0.15^a	1.80 ± 0.21^a	N.S.
Glicerol (g/L)					
3d	3.18 ± 0.19^a	2.95 ± 0.18^{ab}	2.54 ± 0.20^b	3.23 ± 0.10^a	*
6d	3.20 ± 0.20^a	3.02 ± 0.20^a	3.15 ± 0.12^a	3.34 ± 0.22^a	N.S.
10d	3.38 ± 0.18^a	3.08 ± 0.14^a	3.38 ± 0.15^a	3.52 ± 0.28^a	N.S.
11d (+Fr)	3.28 ± 0.20^a	3.15 ± 0.11^a	3.50 ± 0.10^a	3.48 ± 0.19^a	N.S.
End AF	3.32 ± 0.22^a	3.17 ± 0.09^a	3.54 ± 0.16^a	3.51 ± 0.21^a	N.S.
Tartaric acid (g/L)					
3d	0.15 ± 0.02^a	0.18 ± 0.02^a	0.14 ± 0.01^a	0.16 ± 0.03^a	N.S.
6d	0.15 ± 0.02^a	0.21 ± 0.02^a	0.14 ± 0.03^a	0.16 ± 0.04^a	N.S.
10d	0.13 ± 0.03^a	0.22 ± 0.03^a	0.15 ± 0.04^a	0.17 ± 0.04^a	N.S.
11d (+Fr)	0.32 ± 0.05^a	0.37 ± 0.04^a	0.38 ± 0.09^a	0.34 ± 0.05^a	N.S.
End AF	0.32 ± 0.04^a	0.38 ± 0.09^a	0.38 ± 0.08^a	0.34 ± 0.08^a	N.S.

Values are expressed as average of three measurements.
Abbreviations: S.S., statistical significance.
Beer fermented by: *S. cerevisiae* MN113 (TF1); *S. cerevisiae* US05 (TF2); *L. thermotolerans* MNF105 (TF3); *L. thermotolerans* PHILLY SOUR (TF4). 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; *P < 0.05; N.S., not significant.

Table 4
Volatile compounds identified in *Eriobotrya japonica* juice by GC-MS, after a liquid-liquid extraction of the sample with dichloromethane.

KI[a]	KI[b]	Ident.[c]	Compound	Loquat juice (ppm)
			∑Esters	**1.37**
612	610	2	Ethyl acetate	0.60
743	769	2	Methyl 2-methylbutanoate	0.02
1394	1382	2	Methyl cinnamate	0.04
-	1423	2	N-Acetyl-L-proline methyl ester	0.07
1966	1997	2	Methyl 3,4,5 trimethoxycinnamate	0.64
			∑Alcohols	**0.55**
809	807	2	2,3-Butanediol	0.02
857	866	2	2-Hexen-1-ol	0.19
874	874	1,2	3-Hexen-1-ol	0.10
-	896	2	3-Phenyl-2-butanol	0.02
1513	1498	2	2,4-Di-tert-butylphenol	0.22
			∑Aldehydes	**0.13**
810	818	2	Hexanal	0.07
1224	1215	2	3,4-Dimethylbenzaldehyde	0.06
			∑Ketones	**0.17**
743	769	2	Acetoin	0.08
1278	1278	1,2	2-Hydroxy-2-methyl-1-phenyl-1-propanone	0.09
			∑Terpenes	**0.59**
1026	1024	2	Carvomenthene	0.16
1031	1031	1,2	Limonene	0.19
2835	2789	2	Squalene	0.24
			∑Other	**0.10**

[a] Kovats indices based on literature (https://webbook.nist.gov/).
[b] Kovats indices on a DB-5MS apolar column[c]; Ident.: 1 = kovats index identical to bibliography; 2 = identification based on comparison of MS.

that produced noticeably sour beers, reached final pH values from 5,60 to 3.65, while the control strain reached a lower value of 3.49. In this study *L. thermotolerans* determined a pH dropping similar to that observed with *S. cerevisiae*. Trials TF1 and TF3 showed similar alcohol, density and attenuation values, while trial TF2 showed lower density
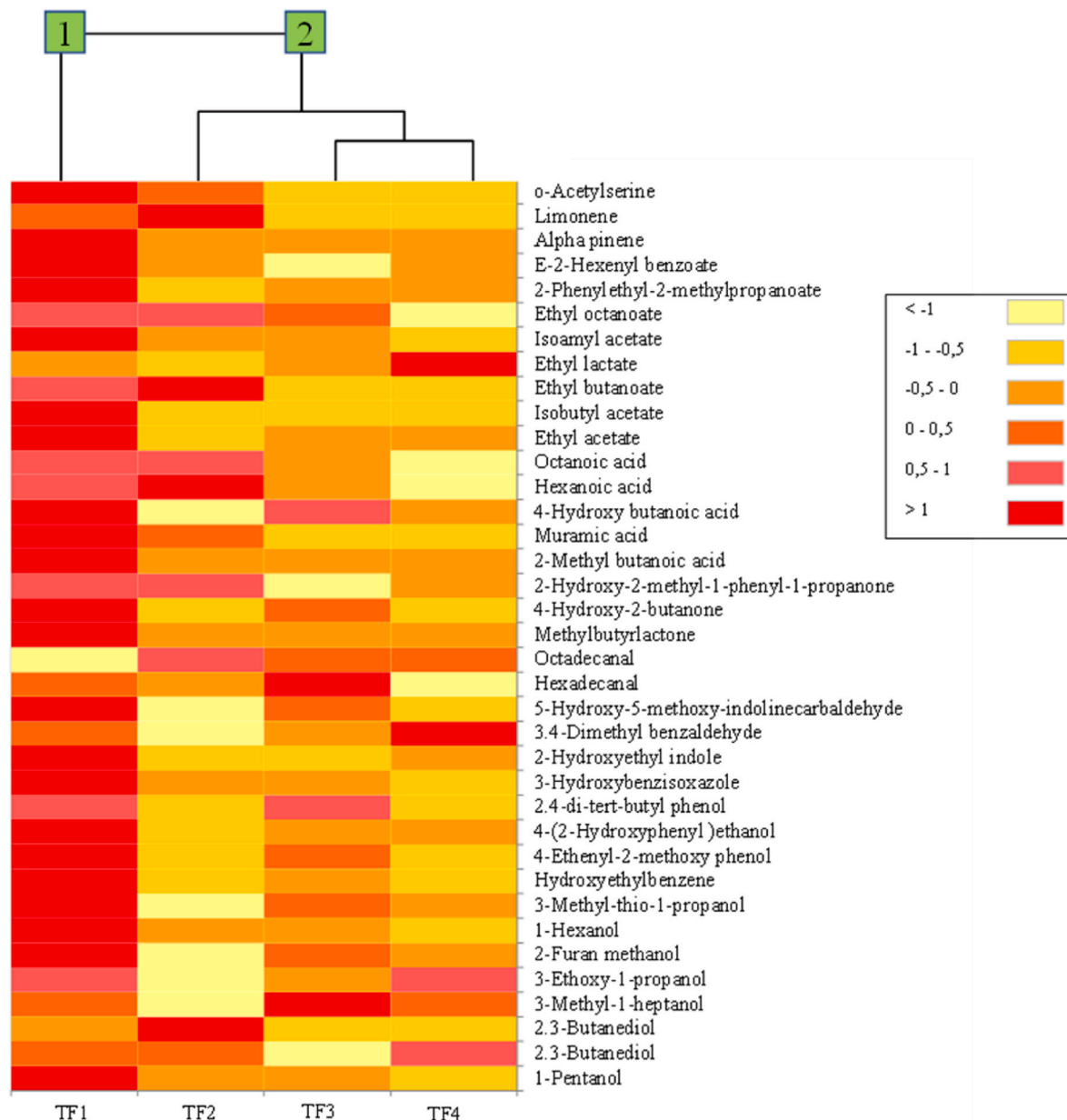
Fig. 3. Distribution of volatile organic compounds among fruit beers. The heat map plot depicts the relative concentration of each VOCs. Beer fermented by: *S. cerevisiae* MN113 (TF1); *S. cerevisiae* US05 (TF2); *L. thermotolerans* MNF105 (TF3); *L. thermotolerans* PHILLY SOUR (TF4).

values and consequently higher alcohol concentration and attenuation. However, some differences among strains were registered for physico-chemical parameters and for the main sugars, acids and glicerol (Table 3). Regarding sugar content, except trial TF2, which had a final sugar content of 0.57 g/L, the other trials reached higher values between 5.62 and 6.07 g/L after 16 d of fermentation (Table 3). The sugar consumption kinetics showed that the selected control strain US05 (TF2) was characterised by the best performances, although the behaviour of strain MN113 in trial TF1 was almost comparable. After 3 d of alcoholic fermentation, both strains of *S. cerevisiae* entirely consumed glucose and fructose, but only partially the other sugars. In case of maltose, trial TF1 showed a slower consumption than what observed for trial TF2. Similar observations were made with *Lachancea* strains and the residual maltose content in all trials were comparable. These results confirm those of Domizio et al. (2016) who stated that *L. thermotolerans* and *S. cerevisiae* strains possess similar maltose utilisation capacities. In contrast, Callejo et al. (2019) registered a lower maltose fermentation capacity in *Lachancea* strains when compared to *S. cerevisiae* strain. The kinetics of

sugar consumption by *S. cerevisiae* registered in our study followed the general trend of this species, with glucose and fructose used before maltose (Pirrone et al., 2022; Tan et al., 2021). Glucose, fructose and sucrose concentrations increased when loquat juice was added to the wort, and were totally fermented during the alcoholic fermentation.

The concentrations of lactic acid, acetic acid, malic acid and tartaric acid, produced during fermentation, are reported in Table 3. Comparing *L. thermotolerans* MNF105 (TF3 test) with the commercial strain, it produced moderate amounts of lactic acid and had a slight influence on pH (TF4) (0.52 and 2.25 g/L, respectively), but although limited, both *L. thermotolerans* showed an ability not possessed by *S. cerevisiae*. In the present study, in accordance with the work of Domizio et al. (2016), the ability of *L. thermotolerans* to produce lactic acid by acidifying beer wort was highlighted. In contrast, the study conducted by Zdaniewicz et al. (2020) showed that this species does not have the necessary capacity for beer acidification. However, the limited lactic acid production by *L. thermotolerans* MNF105 strain can be considered a positive trait for the production of fruit beers, as these which are characterized by a high

Table 5

Volatile compound concentrations in beer samples. Compounds detected by GC-MS, after a liquid-liquid extraction of the sample with dichloromethane.

KI[a]	KI[b]	Ident.[c]	Compounds	TF1[1]	TF2[1]	TF3[1]	TF4[1]	S.S.
			∑Alcohols	**100.63 ± 1.89[a]**	**35.09 ± 1.03[c]**	**40.66 ± 0.95[b]**	**30.99 ± 0.83[d]**	***
764	785	2	1-Pentanol	34.99 ± 0.26[a]	14.98 ± 0.24[b]	14.21 ± 0.12[c]	10.06 ± 0.31[d]	***
809	807	2	2,3-Butanediol	0.81 ± 0.04[a]	0.82 ± 0.06[a]	0.48 ± 0.04[b]	0.88 ± 0.03[a]	***
816	816	2	2,3-Butanediol	0.30 ± 0.02[b]	0.58 ± 0.03[a]	0.20 ± 0.01[c]	0.23 ± 0.01[c]	***
-	818	2	3-Methyl-1-heptanol	0.13 ± 0.01[b]	0.00 ± 0.00[c]	0.20 ± 0.02[a]	0.12 ± 0.01[b]	***
848	854	2	3-Ethoxy-1-propanol	0.14 ± 0.01[a]	0.08 ± 0.01[b]	0.10 ± 0.01[b]	0.13 ± 0.00[a]	***
865	867	2	2-Furan methanol	0.11 ± 0.01[a]	0.00 ± 0.00[c]	0.07 ± 0.01[b]	0.06 ± 0.01[b]	***
878	878	1,2	1-Hexanol	0.17 ± 0.00[a]	0.11 ± 0.00[b]	0.12 ± 0.02[b]	0.09 ± 0.01[b]	***
983	984	2	3-Methyl-thio-1-propanol	0.24 ± 0.02[a]	0.11 ± 0.01[c]	0.18 ± 0.02[b]	0.16 ± 0.01[b]	***
1134	1125	2	Hydroxyethylbenzene	46.30 ± 0.88[a]	16.77 ± 0.55[c]	21.70 ± 0.46[b]	13.91 ± 0.15[d]	***
1311	1314	2	4-Ethenyl-2-methoxy phenol	0.36 ± 0.04[a]	0.11 ± 0.01[c]	0.26 ± 0.02[b]	0.12 ± 0.01[c]	***
1432	1441	2	4-(2-Hydroxyphenyl) ethanol	0.19 ± 0.01[a]	0.11 ± 0.01[c]	0.13 ± 0.01[bc]	0.14 ± 0.01[b]	***
1505	1507	2	2,4-di-tert-butyl phenol	0.70 ± 0.04[a]	0.53 ± 0.03[b]	0.71 ± 0.06[a]	0.52 ± 0.02[b]	**
1750	1766	2	2-Hydroxyethyl indole	15.19 ± 0.49[a]	0.49 ± 0.06[d]	1.92 ± 0.12[c]	4.44 ± 0.24[b]	***
			∑Aldehydes	**3.40 ± 0.13[a]**	**6.34 ± 0.23[a]**	**3.88 ± 0.17[a]**	**3.44 ± 0.19[a]**	N.S.
1224	1215	2	3,4-Dimethyl benzaldehyde	0.24 ± 0.01[b]	0.14 ± 0.01[d]	0.20 ± 0.00[c]	0.30 ± 0.02[a]	***
-	1796	2	5-Hydroxy-5-methoxy-indolinecarbaldehyde	0.29 ± 0.01[a]	0.14 ± 0.00[c]	0.24 ± 0.01[b]	0.17 ± 0.02[c]	***
1817	1820	2	Hexadecanal	1.42 ± 0.04[ab]	1.37 ± 0.12[ab]	1.58 ± 0.11[a]	1.12 ± 0.08[b]	*
2020	2027	2	Octadecanal	1.45 ± 0.07[b]	1.99 ± 0.11[a]	1.86 ± 0.05[a]	1.85 ± 0.07[a]	***
			∑Ketones	**0.78 ± 0.04[a]**	**0.41 ± 0.03[b]**	**0.41 ± 0.01[b]**	**0.41 ± 0.02[b]**	***
941	954	2	Methylbutyrlactone	0.04 ± 0.01[a]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	***
964	962	2	4-Hydroxy-2-butanone	0.39 ± 0.02[a]	0.06 ± 0.01[d]	0.23 ± 0.00[b]	0.12 ± 0.00[c]	***
1278	1278	1,2	2-Hydroxy-2-methyl-1-phenyl-1-propanone	0.35 ± 0.01[a]	0.35 ± 0.02[a]	0.18 ± 0.01[c]	0.29 ± 0.02[b]	***
			∑Carboxylic acids	**1.91 ± 0.12[a]**	**1.76 ± 0.07[a]**	**1.05 ± 0.06[b]**	**0.47 ± 0.03[c]**	***
876	869	2	2-Methyl butanoic acid	0.11 ± 0.01[a]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	***
916	917	2	4-Hydroxy butanoic acid	0.09 ± 0.01[a]	0.00 ± 0.00[d]	0.07 ± 0.00[b]	0.03 ± 0.00[c]	***
1015	1014	2	Hexanoic acid	0.50 ± 0.04[a]	0.57 ± 0.03[a]	0.31 ± 0.02[b]	0.20 ± 0.01[c]	***
1195	1187	2	Octanoic acid	1.16 ± 0.06[a]	1.15 ± 0.04[a]	0.64 ± 0.03[b]	0.21 ± 0.02[c]	***
			∑Esters	**2.71 ± 0.18[a]**	**1.20 ± 0.08[c]**	**1.69 ± 0.09[b]**	**2.89 ± 0.17[a]**	***
612	610	2	Ethyl acetate	1.27 ± 0.08[a]	0.32 ± 0.02[c]	0.53 ± 0.04[b]	0.49 ± 0.03[b]	***
801	799	2	Isobutyl acetate	0.05 ± 0.01[a]	0.04 ± 0.01[a]	0.04 ± 0.00[a]	0.04 ± 0.00[a]	N.S.
818	821	2	Ethyl butanoate	0.08 ± 0.01[a]	0.11 ± 0.02[a]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	***
836	831	2	Ethyl lactate	0.47 ± 0.03[c]	0.22 ± 0.01[d]	0.69 ± 0.03[b]	2.06 ± 0.12[a]	***
881	884	2	Isoamyl acetate	0.19 ± 0.02[a]	0.09 ± 0.01[b]	0.09 ± 0.00[b]	0.07 ± 0.00[b]	***
1206	1198	2	Ethyl octanoate	0.24 ± 0.01[a]	0.22 ± 0.00[a]	0.16 ± 0.01[b]	0.00 ± 0.00[c]	***
1256	1259	2	Phenyl ethyl acetate	0.20 ± 0.01[a]	0.06 ± 0.00[c]	0.08 ± 0.01[bc]	0.09 ± 0.01[b]	***
			∑Terpenes	**0.14 ± 0.02[a]**	**0.16 ± 0.01[a]**	**0.08 ± 0.01[b]**	**0.06 ± 0.00[b]**	***
939	939	1,2	α-pinene	0.03 ± 0.01[a]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	***
1031	1031	1,2	Limonene	0.11 ± 0.01[b]	0.16 ± 0.01[a]	0.08 ± 0.01[c]	0.06 ± 0.00[c]	***

[1]Values are expressed in ppm, averaged over three samples each analysed in triplicate. Data in the same line followed by the same letter are not significantly different according to Tukey's test. Symbols: ***, $P < 0.001$; **, $P < 0.01$; *$P < 0.05$.

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

[a] Kovats indices based on literature (https://webbook.nist.gov/).

[b] Kovats indices on a DB-5MS apolar column[c]; Ident.: 1 = kovats index identical to bibliography; 2 = identification based on comparison of MS.

basal acidic taste. At the end of the secondary alcoholic fermentation, the amounts of acids other than lactic acid, were comparable to those previously reported by Pirrone et al. (2022) and Viana et al. (2021), who analysed beers and fruit beers. Regarding glycerol, the concentrations registered in our study (3.17 – 3.54 g/L) are higher than those reported by Gazinski et al. (2020) and Kawa-Rygielska et al. (2019), where they studied fruit beers. With the addition of loquat juice, the levels of malic and tartaric acid, particularly high in loquat fruits (Toker et al., 2013), increased in all trials. Moreover, all strains consumed malic acid, with *S. cerevisiae* strain MN113 consuming the largest amount.

3.3. Volatile organic compound composition

The VOC of loquat juice is characterised by esters (1.42 ppm), terpenes (0.65 ppm), alcohols (0.55 ppm), ketones (0.17 ppm) and aldehydes (0.13 ppm) (Table 4). The analyses performed on the juice are similar to those of Pirrone et al. (2022), but in this case ketones were detected. In contrast to Planeta et al. (2021), who analysed several loquat fruits, no compounds belonging to the acid class were detected, probably due to a decrease in oxidative phenomena. The final beers showed a higher VOC complexity, characterised by seven classes: alcohols, aldehydes, ketones, carboxylic acids, esters, terpenes and others. The experimental beers differed for a variety of aroma compounds, as shown in the heat map (Fig. 3), where the relationships among the beers

are based on the concentration of each compound detected. Beers fermented with *S. cerevisiae* MN113 (TF1) showed the highest content of aroma compounds, particularly alcohols (Table 5). Alcohols were quantitatively and numerically the most abundant class in all beers, with the highest concentration in TF1 (100.63 ppm) followed by TF3 (40.66 ppm). This class is known for floral, solvent or alcoholic flavors (Eβlinger, 2009). The most abundant alcohol in both fruit beers was hydroxyethylbenzene (46.30 and 21.70 ppm in TF1 and TF, respectively) followed by pentanol (34.99 ppm and 14.98 ppm in TF1 and TF2, respectively). Eight different ester-class compounds are present in the samples; however, their levels are strain-dependent (Pires et al., 2014). The main ester active in aroma is ethyl acetate, a secondary metabolite of alcoholic fermentation, which is responsible for the fruity aroma (Canonico et al., 2016). Moreover, ethyl lactate was also present, especially in beers from trials TF3 and TF4 (0.69 ppm and 2.06 ppm, respectively), as this compound is generally produced by the species *L. thermotolerans*. Some strains within this yeast species have been shown to produce ethyl lactate and have been found in sour beers (Witrick et al., 2017). In addition, this compound is also detected during wine fermentation (Gobbi et al., 2013). Among the compounds detected, 2,3-butanediol, 2,4-di-tert-butyl phenol, 3,4-dimethyl benzaldehyde, 2-hydroxy-2-methyl-1-phenyl-1-propanone, ethyl acetate and limonene were present in both loquat juice and fruit beer. Specifically, 2,3-butanediol is a molecule produced by *S. cerevisiae* (Song et al., 2019), while 2,
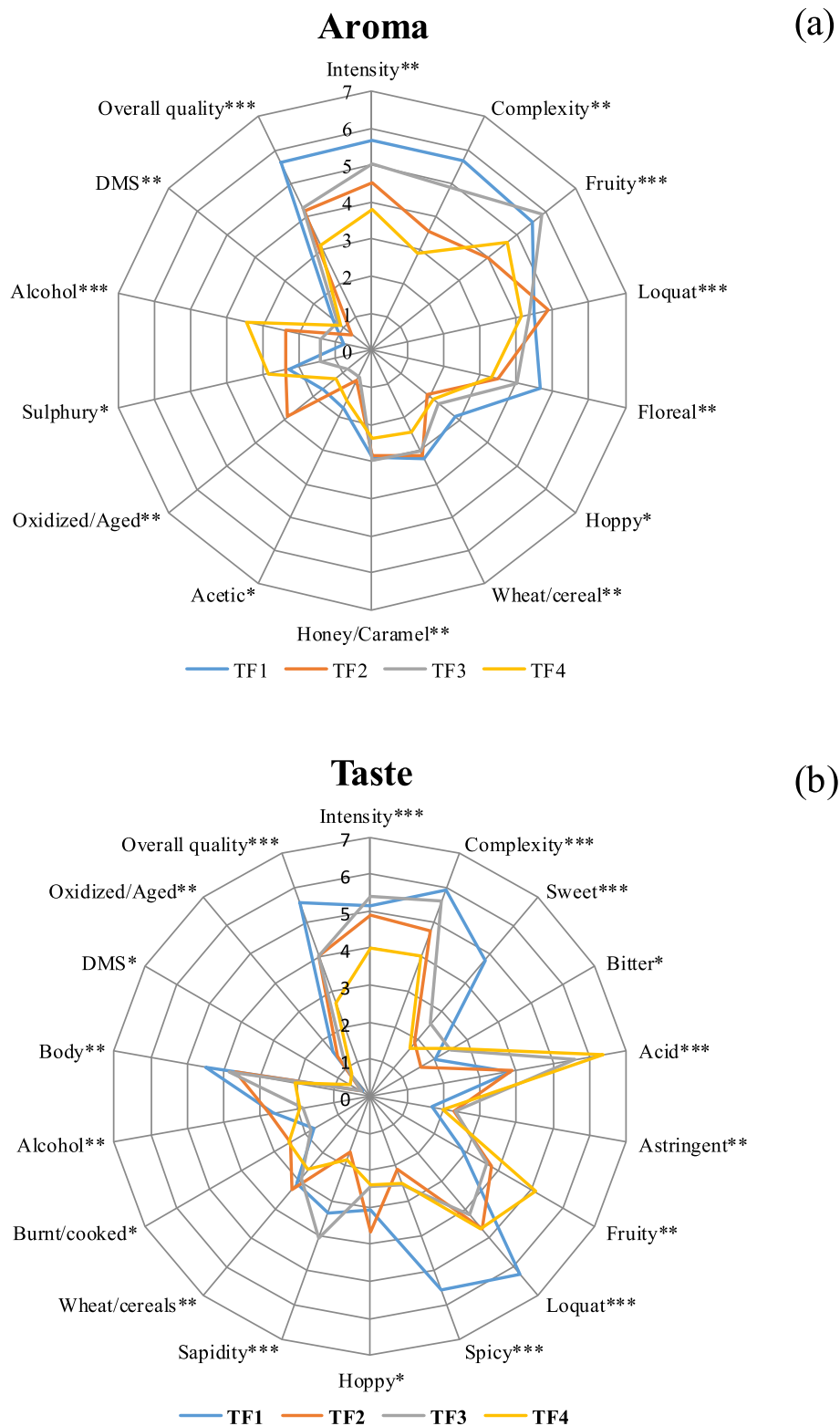
Fig. 4. Sensory analysis performed on beers: spider plots of average scores for aroma (a), taste attributes and overall quality of bottled fruit beers (b), determined by judges during tasting sessions. Beer fermented by: *S. cerevisiae* MN113 (TF1); *S. cerevisiae* US05 (TF2); *L. thermotolerans* MNF105 (TF3); *L. thermotolerans* PHILLY SOUR (TF4). Symbols: ***, P < 0.001; **, P < 0.01; *P < 0.05.

4-di-tert-butyl-phenol is produced by yeasts and was found in *Luzhou-*-Flavor Liquor (Ding et al., 2015). Instead, 3,4-dimethylbenzaldehyde was found in dry-cured hams, where several yeasts were found, in particular *Debaryomyces* was the dominant yeast species (Gong et al., 2023). Furthermore, 2-hydroxy-2-methyl-1-phenyl-1-propanone was

found in a traditional Chinese liquor (Huangjiu) obtained by fermenting a pool of different species of bacteria, yeasts and fungi (Wang et al., 2022). Instead, ethyl acetate has been found in sorghum beers fermented with *S. cerevisiae* yeast (Tokpohozin et al., 2019). But it has also been found in fruit lambic beers, mainly associated with activity by
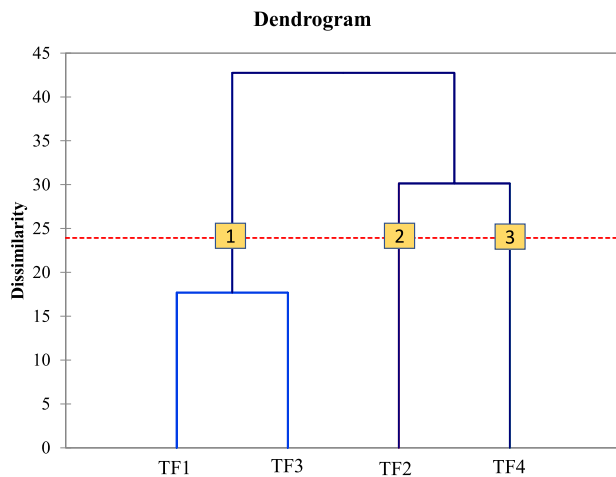
Dendrogram

Fig. 5. Dendrogram of strains resulting from AHC based on values of chemical and sensory aspect of beer experimental productions Dissimilarity is calculated by Euclidean distance. Agglomeration is calculated by Ward's method. Beer fermented by: *S. cerevisiae* MN113 (TF1); *S. cerevisiae* US05 (TF2); *L. thermotolerans* MNF105 (TF3); *L. thermotolerans* PHILLY SOUR (TF4).

Brettanomyces (Bongaerts et al., 2021). Limonene is found in lemon and other citrus fruits, but in general in more than 300 plants (Jongedijk et al., 2016). Moreover, this compound is the second most distributed terpenoid in nature and can also be associated with hops (Ramírez & Viveros, 2021). Finally, most of these compounds are produced by the metabolism of microorganisms, while only limonene is attributed to fruits and hops.

3.4. Sensory evaluation

The data from the sensory evaluation are reported in Fig. 4. The differences among loquat experimental beers were significant, thus, yeasts isolated from manna impacted differently from control yeasts beer aroma. All panellists were able to recognise loquat addition and the use of wheat malt in all beers and they did not reveal any defects. In particular, trial TF1 beers showed the highest scores for fourteen attributes [aroma (Fig. 4a): intensity, complexity, floreal, hoppy, wheat/cereal, acetic and overall impression; taste (Fig. 4b): complexity, sweet, loquat, spicy, body, oxidized/aged and overall impression], while those from trial TF3 only for five (aroma: fruity, honey/caramel; taste: intensity, astringent and sapidity). The beer from trial TF2 were easily recognised from the other beers and they reached a general low acceptance, but the commercial *L. thermotolerans* strain used in trial TF4 determined an excessive acidity and the beers received the worst appreciation. On the other hand, the beers from TF1 and TF3 received fair overall quality scores (5.56 and 4.01, respectively). Several studies reported that the use of *L. thermotolerans* increased the perceived acidity due to an increase in the total acidity of beers (Osburn et al., 2018; Peces-Pérez et al., 2022; Romero-Rodríguez et al., 2022). The overall organoleptic investigation showed a preference for trial TF1 beers, which showed a higher residual sugar content at the end of fermentation with pronounced notes of spice and loquat flavour, followed by those from trial TF3.

3.5. Statistical and explorative multivariate analyses

The AHC categorised the evidence according to their mutual dissimilarity and relationship (Fig. 5). This evaluation categorised the trials using forty-two variables chosen based on the outcomes from sensory characteristics and chemical parameters. The different trials of loquat beers were visibly divided into three clusters, considering a

dissimilarity of 35%. In one cluster were grouped the trials using manna-isolated strains, namely MN113 (TF1) and MNF105 (TF3), while in two other different clusters were grouped the trials using controls, namely US-05 (TF2) and MNF105 (TF4). The variables that greatly influenced the clustering were fruity, intensity of colour and acid. The graphical representation of the VOC analysis is reported in Fig. 3. The hierarchical dendrogram combined with heat map graph revealed that different strains significantly influence the VOCs released from the trials. The concentrations of the VOCs among loquat beers resulted in a cluster with TF1 trials and a main cluster with a grouping of TF2, TF3 and TF4 trials.

4. Conclusion

In this work, yeast strains belonging to the species *L. thermotolerans* and *S. cerevisiae* isolated from sugar-rich matrices, i.e., manna, were tested to assess their effect as starter cultures on the physicochemical and organoleptic properties of loquat beer. In particular, the selected strain *L. thermotolerans* MNF105 derived from these matrices was applied for the first time in brewing. From different point of view, fermentation with the chosen strains produced better results compared to that with the corresponding commercial controls. Experimental trials conducted with the selected strains demonstrated the absence of off-odours and off-flavours and improved aroma perception. Instead, these strains have been shown to be able to conduct beer fermentation, producing a good amount of alcohol and also the ability to produce particular flavors that can modify and enhance the aromatic complexity of fruit beers. Moreover, the modest lactic acid production of the *L. thermotolerans* MNF105 strain is a positive ability for the production of sour fruit beers, as these already have excessive acidity as a base due to the low pH of the fruit. The overall organoleptic investigation showed a preference for *S. cerevisiae* MN113 (TF1), which showed higher residual sugar content at the end of AF, followed by TF3, in which *L. thermotolerans* also isolated from manna was used, which produced a more balanced beer than the commercial control. Aldehydes and alcohols were the most prevalent VOCs in beers. Beers brewed with *S. cerevisiae* MN113 (TF1) are characterised by higher concentrations of alcohols, ketones and carboxylic acids. In particular, ethyl acetate was also higher, a secondary metabolite of alcoholic fermentation responsible for the fruity aroma. Interestingly, the samples inoculated with *Lachancea* strains have a greater content of ethyl lactate, a compound produced by this species. The manna-related yeasts *S. cerevisiae* MN113 and *L. thermotolerans* MNF105 showed great technological properties and represent promising starters for the production of fruit beer and sour fruit beer. The use of unconventional yeasts represents a potential to be exploited for the production of aromatically diversified beers (Cubillos et al., 2019). Furthermore, *L. thermotolerans* represents a viable alternative to bacteria for the production of sour beers (Postigo et al., 2023).

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Declaration of competing interest

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

Data availability

Data will be made available on request.

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