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Enhancing aroma and stability in Sicilian Maiorca wheat beer: Impact of *Torulaspora delbrueckii* via inoculation methods

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ABSTRACT

In response to the rising demand for alternative yeasts in brewing, this study explored the potential of *Torulaspora delbrueckii*, a yeast known for its floral notes in winemaking, to enhance the aromatic profile of wheat beer. We developed a recipe using a 1:1 blend of Maiorca wheat malt and grain and employed different inoculation methods to compare the effects of *T. delbrueckii* with a traditional *Saccharomyces cerevisiae* fermentation. Extensive physicochemical analyses were conducted on both the wort and beer, including volatile aroma compounds, by using solid-phase microextraction (SPME) coupled with gas chromatography-mass spectrometry (GC-MS). Identification was based on spectral library matching and retention index comparison. Results were compared to descriptive sensory analysis of the experimental beers. Eighteen key volatile compounds were detected, including phenethyl alcohol, ethyl acetate, and isoamyl acetate, with *T. delbrueckii* enhancing fruity and ester notes, especially in co-inoculated and sequentially inoculated beer samples. The introduction of *T. delbrueckii* during primary fermentation increased desirable aromatic compounds such as esters (fruity, floral), phenolics, and malty notes while reducing undesirable compounds like diacetyl, octanoic acid, oxidized aromas, and aldehydes. These findings indicate that *T. delbrueckii* could play a beneficial role in enhancing beer stability and sensory quality.

1. Introduction

Beer, one of the most ancient and cherished alcoholic beverages worldwide, has undergone significant transformations throughout its history. It has evolved from a simple fermented grain drink to a complex and varied product (Meussdoerffer, 2009). Brewers continuously seek innovative ways to enhance the flavor, aroma, and quality of their brews. Specific yeast strains have played a pivotal role in achieving this objective (Baiano, 2021; Pirrone et al., 2022, 2025; Pirrone, Naselli, et al., 2025; Matraxia et al., 2021; Francesca et al., 2023). Among the various strains investigated, *Torulaspora delbrueckii* has gained prominence as a noteworthy and promising microorganism in the field of beer production (Canonico et al., 2016; Michel et al., 2016). This non-*Saccharomyces* yeast species has gained considerable attention lately for its ability to influence various aspects of beer fermentation and sensory characteristics. Its effects include improving aromatic complexity, mouthfeel, and overall beer character, and also imparting favorable organoleptic features to the final product (Canonico et al., 2017).

Traditionally, brewer's yeast, scientifically known as *Saccharomyces cerevisiae*, has been the primary yeast for manufacturing beer. Its resilient fermentation abilities, high tolerance to alcohol, and ability to generate a smooth and reliable flavor profile have established it as the preferred yeast. Currently, *S. cerevisiae* remains an indispensable ingredient in the brewing sector, and it is still the top-performing yeast strain used in numerous beer genres. However, the rise in craft brewing and

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the demand for unique and varied beer profiles have created an opportunity for alternative yeast strains to gain popularity. *Torulaspora* species have been researched for their potential in winemaking for many years, but their potential in beer production has become more evident recently (Tataridis et al., 2013).

T. delbrueckii exhibits several traits that make it an interesting prospect for the brewing industry, such as its capacity to generate esters like ethyl acetate, isoamyl acetate, and phenylethyl acetate, which produce fruity and floral aromas (Gallone et al., 2016; Li et al., 2022). These compounds can enhance the aromatic complexity of beer, rendering it an intriguing selection for brewers who desire to produce unique and flavorful brews with a distinct aroma profile. Furthermore, *T. delbrueckii* is known for low production of undesirable compounds (off-flavors) (Azzolini et al., 2015; Michel et al., 2016; Belda et al., 2017; Toh et al., 2018; Canonico et al., 2018). Eliminating undesirable compounds could lead to an overall improvement in the sensory characteristics of beer, rendering *T. delbrueckii* a valuable addition to the quest for high-quality brewing. Another interesting feature of *T. delbrueckii* is its capacity for co-fermentation with *S. cerevisiae*. This method involves utilizing different yeast strains simultaneously throughout the fermentation process. Several studies have explored the dynamics of co-fermentation between *T. delbrueckii* and *Saccharomyces* species, uncovering the potential for synergy in achieving specific flavor and aroma profiles (Canonico et al., 2017; Canonico et al., 2020). Apart from aroma and flavor, another important aspect of beer appreciation is mouthfeel. *Torulaspora delbrueckii* has been noted for its potential to enhance mouthfeel, contributing to a fuller and smoother beer texture. This characteristic is of particular interest to brewers crafting styles like wheat beers, where a soft and creamy mouthfeel is desired. In a study by Canonico et al. (2017), it was demonstrated that *T. delbrueckii* strains co-fermenting with *S. cerevisiae* produced beers with higher levels of glycerol that can contribute to the beer body and mouthfeel. This finding suggests that *T. delbrueckii* has the potential to influence the physical attributes of beer, providing brewers with a versatile tool to shape their brews according to desired style and characteristics. In this context, this study aims to delves into the present scientific comprehension of *T. delbrueckii*'s role in beer production and its potential to open up innovative avenues for craft brewers striving to craft beers with unique and captivating sensory characteristics. We investigated the impact of *T. delbrueckii* yeast on the aroma, quality and co-fermentation interactions with *S. cerevisiae* during the production of wheat beer. A recipe was formulated using a 1:1 mixture of Maiorca wheat malt and grain, and multiple inoculation techniques were employed to investigate the effects of *T. delbrueckii*. Comprehensive physicochemical analyses were performed on both the wort and the resulting beer, including the assessment of volatile aroma compounds through solid-phase microextraction (SPME) combined with gas chromatography-mass spectrometry (GC-MS). The findings were then compared to the outcomes of a descriptive sensory evaluation of the experimental beers. The results of this study have the potential to be of significant benefit to breweries that are seeking to innovate while maintaining the highest standards of quality. *T. delbrueckii* represents a promising yeast strain with the potential to drive advances in brewing techniques, particularly in the context of craft and speciality beers, where differentiation is of paramount importance.

2. Materials and methods

2.1. Wheat grain

The common wheat variety, Maiorca (*Triticum vulgare* Host. var. albidum Koern), was obtained from a commercial cereal farm called 'MolinOro 100 % grano siciliano' located in Valledolmo, Palermo, Sicily. The wheat was cultivated using conventional agronomic practices. The farm is situated at Latitude 37°43', Longitude 13°45', and an altitude of 450 m above sea level. The soil in the area is sandy clay. Samples were harvested in June 2022 and stored at temperatures between 4 and 6 °C until the malting process, which took place in October 2022. After malting, the resulting malt samples were vacuum-sealed and stored at 12 °C for 3 weeks before undergoing analytical procedures.

2.2. Malting conditions

Maiorca wheat malting tests were conducted in triplicate using an automated malting system (Phoenix Biosystems, Adelaide, Australia) at the Department of Agricultural, Food and Forest Sciences (SAAF) at the University of Palermo (Italy). The Maiorca wheat samples underwent a cleaning process to eliminate glumes and husks. The malting procedures followed the conditions outlined by Alfeo et al. (2018). In each malting basket, 800 g of grains were steeped in water at 15 °C for 5 h, followed by an 8-h air rest, and an additional 4 h in water, achieving a steeping-out moisture level of 41 %. Germination took place over 120 h at 15 °C and 95 % relative humidity. Subsequently, the samples were dried and kilned for a total of 34.5 h, with the following temperature regimen: 3 h at 55 °C, 12 h at 60 °C, 10 h at 65 °C, 5 h at 70 °C, and 4.5 h at 75 °C.

2.3. Malts and hops

The malted Maiorca malt (MM) was employed in a 1:1 ratio with unmalted Maiorca wheat (UM); the blended sample of Maiorca grains (BM) was used in the grist of experimental beer. Rice husk was introduced to the mash, constituting 5 % of the wheat malt content in the recipe. For hops, Slovenian Styrian Golding hops with 4,6 % w/w alpha acids were utilized.

2.4. Yeast strains

The yeast strains used in this study belong to the species *T. delbrueckii* (LEVEL^2 BIODIVA™, Lallemand Oenology) and *S. cerevisiae* (SafAle™ US-05, Fermentis Lessafre). As indicated by the Supplier, *T. delbrueckii* strain has been selected for its ability to significantly increase the aromatic and taste complexity of wines by producing polyols in high quantities during the first steps of alcoholic fermentation. Polyols, or polyalcohols, are carbohydrates containing several hydroxyl groups (-OH) produced by the fermenting yeast: their main functions are protection against osmotic stress, redox balancing and inhibition of acetic acid production pathways. The *S. cerevisiae* commercial strain US-05, a neutral ale yeast, was used as the control.

2.5. Congress trial conditions

The blended sample of Maiorca grains (BM) obtained with 1:1 ratio of MM and UM was mashed with I-CUBE MASH BATH - R8 in accordance with Analytica EBC method 4.4 (1997). A portion of 50.0 g of the sample was weighed in the mash beakers and the mashing was performed. The filtration was performed using fluted filter paper (Whatman Schleicher & Schuell Qualitative Folded Filter Paper Grade 597 ½; 320 mm diameter) in a 200 mm diameter funnel with a stem that reaches the bottom of the conical flask. After filtration, the wort was measured in terms of color, pH, extract, and specific gravity according to the EBC method [respectively EBC method 8.3 (1997); EBC method 4.5.1 (2004); EBC method 4.4 (1997) and EBC method 8.2.2 (2004)].

2.6. Micro-brewing conditions

The beers for the experiment were brewed using the Klarstein mod. 10031629 microbrewery plant (Chal-Tec GmbH, Berlin, Germany) and fermented in a stainless-steel fermenter with a hermetic closure. A combination of malted and unmalted Maiorca wheat in a 1:1 ratio, totaling seven kilograms, was milled using a double roller mill (Mattmill Kompakt, Germany), with the roller distance set at 1.20 mm. This

mixture was then added to 25 L of water.

The process of mashing took place at 65 °C for 60 min, ensuring complete saccharification, as confirmed by testing with an iodine solution following the method outlined by Mayer et al. (2016). To deactivate the enzymes, the mixture was heated to 78 °C for 10 min. The lautering phase involved a primary recirculation of wort over the spent grain, followed by rinsing with 19 L of water heated to 78 °C.

The resulting wort, which had a volume of 38 L, underwent a 60-min boil. Hops (48 g) were added at the beginning of the boiling process, with a quantity of 1.5 g/L in relation to the final volume of the beer wort. After boiling, the wort was efficiently cooled using a stainless-steel chiller until it reached 20 °C. The wort was then transferred to a stainless-steel fermenter and inoculated with the selected yeast strain following the experiment protocol. The standard quality parameters of the beer wort included a pH of 5.6 and 12 °P (Plato degree).

2.7. Fermentation trials

The beer wort was divided into four 8-liter batches and subjected to various *T. delbrueckii* strain inoculation methods, and compared with beer produced with the commercial *S. cerevisiae* US-05 strain fermentation used as a Control. To determine the optimal use of *T. delbrueckii* for improving the final product, four experimental conditions were tested: TC (control beer with US-05 strain), T1 (beer with co-inoculation of US-05 and *T. delbrueckii*), T2 (beer with *T. delbrueckii* inoculation and subsequent addition of US-05 after 48 h), and T3 (beer with *T. delbrueckii* inoculation during refermentation). From preliminary fermentation trials, three wort fermentations were carried out as follows: (TC) inoculated at 5.00×10^9 CFU/L *S. cerevisiae* SafAle™ US-05; (T1) co-inoculated at 5.00×10^9 CFU/L *S. cerevisiae* SafAle™ US-05, and 5×10^9 CFU/L *T. delbrueckii* LEVEL² BIODIVA™; (T2) inoculated at 5×10^9 CFU/L *T. delbrueckii* LEVEL² BIODIVA™, followed by sequential inoculation of 5.00×10^9 CFU/L *S. cerevisiae* SafAle™ US-05 after 48 h; (T3) the worth was inoculated at 5.00×10^9 CFU/L *S. cerevisiae* SafAle™ US-05, fermented for 15 days until 1009 SG and refermented for 20 days in bottle with 5×10^9 CFU/L *T. delbrueckii* LEVEL² BIODIVA™. Each trial was done in triplicate at 18 °C under static conditions. The fermentation kinetics were monitored three times a week, until the end of the fermentation. The yeast viability was monitored using plate viable cell counts on WL Nutrient Agar (Oxoid, Hampshire, UK), useful to quantify and discriminate between *T. delbrueckii* yeast from the *S. cerevisiae* starter strain by colony morphology and color (Pallmann et al., 2001). During fermentation, a temperature of 18 °C was maintained for 15 days. The fermentation was considered complete when the specific gravity was constant for two consecutive days. The beer samples were stored at a temperature of 2 °C for 8 days to induce precipitation of suspended yeasts and trubs. The beers were bottled into brown glass bottles and sucrose (9 g/L) was added to perform fermentation in the bottle and to ensure the production of 6 g of CO_2 per liter of beer.

2.8. Malt and wort analysis

The analyses were performed in triplicate according to the Analytica European Brewery Convention (EBC) (Analytica-EBC, 2007). In detail, the moisture (%) of malts was determined by EBC method 4.2 (2000). Proteins and soluble proteins were calculated as total nitrogen (TN, dry basis %, db %) and soluble nitrogen (SN, db %), respectively, according to the EBC method 4.3.1 (2004) and 4.9.1 (1997) and then multiplied by 6.25. The Kolbach Index (%) was calculated in accordance with EBC methods 4.9.1(1997). The malt extract (db %), extract difference and pH were calculated respectively according to EBC method 4.4 (1997), EBC method 4.5 (1997) and EBC method 4.6 (1997). The saccharification rate, fermentability (%), free amino nitrogen (FAN, mg/100 g db), and wort color were determined by EBC methods 4.4.1 (1997), 4.11.1 (1999), 4.10 (1997), and 8.3 (International Method, 1997), respectively. The speed of filtration was measured using EBC methods 4.4.3 (1997). The total volume of filtration was 350 mL for all samples. The viscosity of the congress wort was measured using an Ostwald viscometer with a capillary diameter of 0.5 mm and constant K (Richtwert) 0.03, following EBC method 8.2 (International Method, 1997). The Megazyme assay kit (Megazyme International, Ireland) was used to determine total starch content (db %) following the AOAC Method 996.11 (2005) supplied with the assay kit, and a malt amylase assay kit (Megazyme International) was employed to quantify α and β amylases in malt flours. The enzyme activities were measured by reading the assay absorbance using a Beckmann DU650 spectrophotometer (Pasadena, California, US) and reported as units per gram of dry matter (U/g). One unit of activity is defined as the amount of enzyme required to release 1 μmol of reducing sugar equivalents per min under the defined assay conditions. The β-glucan and β-glucanase content were determined using Megazyme assay kit (respectively K-BGLU and K-MBGL- Megazyme International) following EBC Methods 3.10.1 and 8.13.1 for malt and wort β-glucan content and Azo-barley glucan method for β-glucanase content.

2.9. Beer analysis

The measurement of pH of the experimental beer before and after fermentation was conducted with a pH meter Mod.70 XS/50010162 (Cheimika, Pellezzano, Italy). BeerFoss™ FT Go (FOSS Italia srl, Padova, Italy) was used to determine the following parameters of the experimental beer: alcohol (% vol), Original Gravity (SG), Final gravity (SG), Apparent attenuation (%), Real attenuation (%), Original extract (°P), Apparent extract (°P), Real extract (°P), and Alcohol (% Vol).

2.10. Volatile aroma compounds analysis

The aroma volatiles of beers were extracted and analyzed by Headspace-Solid-Phase Microextraction (HS-SPME) coupled with Gas Chromatography-Mass Spectrometry (GC-MS) following a previously optimized method (Cincotta et al., 2022; Verzera et al., 2021). In detail, a 40 mL vial equipped with a "mininert" valve (Supelco, Bellefonte, PA, USA) was filled with 15 mL of each wort and beer sample by adding 5 g of sodium chloride and stirred at 40 °C for 30 min. Afterwards, a DVB/CAR/PDMS (Divinylbenzene/Carboxen/Polydimethylsiloxane) fiber with film thickness of 50/30 μm (Supelco, Bellefonte, PA, USA) was used to perform the extraction of the volatiles in the headspace of the vial for 30 min. After sampling, the SPME fiber was kept for 3 min in the splitless injector at 260 °C of a Shimadzu GC 2010 Plus gas chromatograph directly interfaced with a TQMS 8040 triple quadrupole mass spectrometer (Shimadzu, Milan, Italy) by using the following conditions: polar capillary column, VF-WAXms, 60 m, 0.25 mm i.d., 0.25 μm film thickness (Agilent Technologies Italia S.p.A., Milan, Italy); oven temperature, 45 °C held for 5 min, increased to 80 °C at 10 °C/min and up to 240 °C at 2 °C/min; carrier gas, helium at a constant flow of 1 mL/min; transfer line temperature, 250 °C; acquisition range, 30–400 *m/z*; scan speed, 1428 amu/s. Each compound was successively identified using mass spectral data, the NIST '18 (NIST/EPA/NIH Mass Spectra Library, version 2.0, USA) and FFNSC 3.0 databases, linear retention indices (LRIs) calculated according to the Van Den Dool & Kratz equation (Van Den Dool and Kratz, 1963), literature data, and the injection of standards. 2-Octanol was used as an internal standard and for quantitative purposes as reported by Medina et al. (2023). Each sample was analyzed in triplicate.

2.11. Sensory analysis

A Quantitative Descriptive Analysis (QDA) was conducted to evaluate the sensory attributes of beer samples, focusing on color, odor, taste, and overall quality. Following the 13.4 EBC methods (1997), 10 trained panelists (5 males, 5 females, aged 23–30) were selected and assessed under ISO-8589: 2007 (2007) controlled laboratory conditions at the Sensory Analysis Laboratory of the University of Palermo (Italy).

The evaluation took place in individual booths under white light at room temperature, where panelists assessed 80 mL beer samples served at 8°C, with randomized presentation order and identification codes using Smart Sensory Box software (Smart Sensory Solutions S.r.l., Sassari, Italy). Water was provided for palate cleansing between samples. The descriptive test aimed to provide a comprehensive sensory profile, considering visual, olfactory, and gustatory attributes, based on 33 descriptors from the Beer Flavour Wheel (Meilgaard et al., 1979) and EBC methods 10.12 (1979). Four attributes were chosen to evaluate visual characteristics including color, turbidity, foam persistency, and foam structure; fifteen were selected related to odor including odor intensity, estery, fruity, floral, hoppy, grainy, honey, malty, caramel, phenolic, solvent-like, diacetyl, sulfury, yeasty, and oxidized; and thirteen gustatory traits included taste intensity, acid, sweet, salty, bitter, estery, fruity, spicy, oxidized, astringent, carbonation, alcoholic, and body. The panelists were also asked to rate their overall acceptance. The panelists were said to gradually sip the sample and describe it using the tablet connected to a Smart Sensory Box. The sensory attributes were assessed using an unstructured nine-point scale anchored at the left end with "absent" and at the right end with "high". Informed consent forms, signed by both the panelists and the panel leader, were collected prior to the evaluation. All procedures complied with relevant laws, institutional guidelines, and ethical principles.

2.12. Statistical analysis

All the data were evaluated by Matlab software (MathWorks Inc., Nutick, Massachusetts, United States). Sensory data were analyzed using Smart Sensory Box. One-way Analysis of Variance (ANOVA) with Tukey's post hoc test was used for multiple comparisons and a p-value < 0.05 was considered significant. In order to individuate samples with similar characteristics, hierarchical cluster analysis was performed using Matlab software. All experiments were conducted in triplicate and results were reported as mean ± standard deviation.

3. Result and discussion

3.1. Wheat and malt quality parameters

Table 1 reports the quality characteristics of wheat and malt. The moisture content of the samples ranged from 6.06 % to 12.77 % w/w. The malt samples MM and BM had the lowest values, which are consistent with those found in other barley and wheat malts (Mascia et al., 2014; Alfeo et al., 2021; Gugino et al., 2024). The sample of unmalted wheat exhibited a moisture content of 12.77 ± 0.06 % w/w, which falls within the optimal range for preventing fungal growth and ensuring proper grain storage.

Table 1
Wheat and malts quality parameters.

	Unmalted Maiorca (UM)	Malted Maiorca (MM)	Blended Maiorca (BM)
Moisture (% w/w)	12.77 ± 0.06^{c}	6.06 ± 0.05^{a}	8.55 ± 0.06^{b}
TCW (g db)	36.32 ± 0.10^{c}	32.5 ± 0.21^{a}	33.95 ± 0.55^{b}
Proteins (% db)	12.81 ± 0.17^{c}	12.12 ± 0.40^{a}	12.43 ± 0.12^{ab}
Sol. Proteins (% db)	-	4.66 ± 0.06	3.82 ± 0.11
Kolbach Index (%)	-	38.5 ± 1.29	30.75 ± 1.09
Starch (% db)	69.51 ± 2.98^{a}	62.39 ± 0.26^{a}	64.85 ± 0.44^{a}
GE (%)	97.83 ± 0.29	-	-
β-glucan (g/100 g db)	3.01 ± 0.17^{b}	0.36 ± 0.03^{a}	0.97 ± 0.02^{a}
α-amylase (CU/g db)	0.55 ± 0.15^{a}	176.14 ± 3.27^{c}	86.73 ± 1.39^{b}
β-amylase (BU/g db)	36.9 ± 0.01^{a}	41.28 ± 1.50^{a}	43.03 ± 1.16^{a}
Endo- β-glucanases (U/kg db)	-	16.14 ± 0.15	10.53 ± 1.38
Endo−1,4-β-D-xylanase (U/g db)	-	1.2 ± 0.02	0.88 ± 0.11

GE= germination energy; TCW= thousand corn weight; db = dry basis; FAN = free amino nitrogen; BU = Betamyl Units; CU = Cerendo Units; U=Units of enzyme; Values in the same line followed by different letter are statistically different ($p < 0.05$)

The thousand kernel weight was highest in the unmalted wheat sample, with a value of 36.32 ± 0.10 g db. In contrast, the Maiorca malt sample had the lowest weight, precisely 32.5 g db, while the Maiorca blend sample weighed 33.95 g db. The results indicate that unmalted wheat has a higher kernel weight than MM and BM.

The protein content of the samples ranged from 12.12 % db to 12.81 % db. The highest protein content was found in unmalted wheat, while the lowest was found in Maiorca malt. Majorca malt and unmalted wheat had the lowest and highest values, respectively, with the BM sample falling in between at 12.43 % db. These values are consistent with typical protein content values for wheat malt found in the literature (Mascia et al., 2014; Alfeo et al., 2021; Gugino et al., 2024; Gugino et al., 2025; Benanti et al., 2023). The degree of malting modification can be determined by the soluble protein content calculated on congress worts. In this study, the two samples, MM and BM, showed different values, with MM having a higher value of 4.66 % db and BM having a lower value of 3.82 % db. The Kolbach Index value also showed the same trend, with BM having a lower value of 30.75 % and MM having a higher value of 38.5 %. The difference in the samples can be attributed to the fact that the BM sample consisted of half of unmalted Maiorca wheat.

The total starch content in the samples did not show any significant differences, with values ranging between 62.39 % db and 69.51 % db.

The unmalted Maiorca wheat had the highest value, and there were no significant differences between this sample and the MM and BM samples, indicating that there was no loss of extract due to malting. The good degree of modification shown by the Kolbach index attests to the adequacy of the malting process in the samples studied.

Table 1 shows that the β-glucan content varied significantly among the samples. Unmalted cereals have variable β-glucan content depending on the type of cereal. Barley and oats have abundant β-glucans, while rice, rye, and wheat have β-glucan content ranging from 0.04 % to 2.9 % (Lazaridou et al., 2007). The malting process degrades these compounds through the action of the enzyme β-glucanase, resulting in generally low β-glucan content after malting. The low degradation of β-glucans during malting has a negative impact on the viscosity of wort and the filtration stage during brewing. The malt samples studied had a β-glucan content in line with the values found in the literature (Wang et al., 2004; Alfeo et al., 2021). In particular, the content of β-glucans was higher in the unmalted wheat (3.01 g 100 g) and very similar between the MM and BM samples, 0.36 ± 0.03 g/100 g and 0.97 g/100 g, respectively.

3.2. Starter yeast dynamics during the fermentation

Fig. 1 shows the cell growth dynamic of pure, co-inoculated, and sequential *S. cerevisiae* US-05 fermentations with *T. delbrueckii*. Pure *S. cerevisiae* US-05 fermentation used as a control (TC) reached a cell concentration $> 10^{7}$ CFU/mL after two days of fermentation and maintained it until the end of the process. In the T1 fermentation trial, where *S. cerevisiae* was co-inoculated with *T. delbrueckii* (Fig. 1A), US-05 reached a population of 2.1×10^{6} CFU/mL on day 2 and maintained this level of viable cells until day 7 before gradually decreasing to 3.0×10^{5} CFU/mL at the end of the fermentation process. Here, the growth kinetics of *T. delbrueckii* showed a similar trend to that of pure *S. cerevisiae* (TC) fermentation, gradually decreasing after 7 days up to a concentration of 2.1×10^{6} CFU/mL. These data indicate that at this inoculation ratio of 1:1 with US-05, *T. delbrueckii* dominated the process from the beginning of fermentation positively affecting the characteristics of the beer. These fermentation kinetics are in line with those reported by Canonico et al. (2016, 2017, 2020) and Toh et al. (2018), showing how

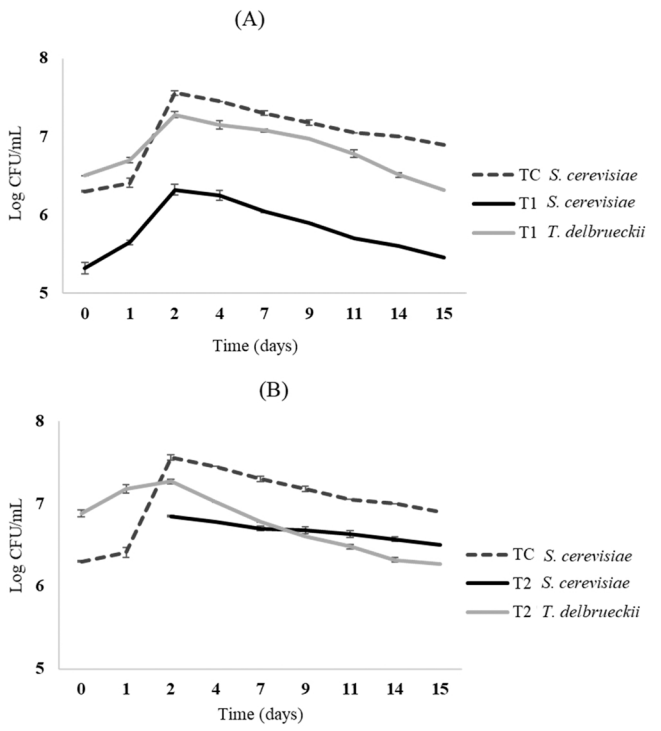

Fig. 1. Growth dynamics of co-inoculated (A) and sequentially inoculated (B) *S. cerevisiae* US-05 and *T. delbrueckii* in comparison to dynamic of *S. cerevisiae* US-05 used as pure starter culture. TC: inoculation at 5.00×10^9 CFU/L *S. cerevisiae* SafAle™ US-05; T1: co-inoculation at 5.00×10^9 CFU/L *S. cerevisiae* SafAle™ US-05 and 5×10^9 CFU/L *T. delbrueckii* LEVEL² BIODIVA™; T2: inoculation at 5×10^9 CFU/L *T. delbrueckii* LEVEL² BIODIVA™, followed by sequential inoculation of 5.00×10^9 CFU/L *S. cerevisiae* SafAle™ US-05 after 48 h.

T. *delbrueckii* used in co-inoculation with *S. cerevisiae* improves the aroma profile of the beer, opening new opportunities to modify the taste and aroma of beers in the future.

Similar growth dynamics were observed in the case of sequential fermentation (T2) of *S. cerevisiae* added 48 h after *T. delbrueckii* (Fig. 1B). However, in the latter case, although *S. cerevisiae* had a slightly decreasing growth trend since inoculation, the growth of *T. delbrueckii* was adversely influenced by the US-05 strain (Fig. 1B). This is clearly visible after day 7, when *S. cerevisiae* has a slightly higher cell concentration than *T. delbrueckii*, and at the end of fermentation, when both the two species had a cell concentration around 10^6 CFU/mL; this finding is indicative of progressive competition by *S. cerevisiae*, which however did not prevent *T. delbrueckii* from influencing the analytical and aromatic characteristics of the fermenting must in the early stages of fermentation. The reduced viability of *S. cerevisiae* during the initial phase after inoculation, due to the constant presence of the *T. delbrueckii* strain inoculated 2 days earlier, was previously observed by Canonico et al. (2015, 2020) in two different studies carried out on sequentially inoculated grape must.

3.3. Enzyme activities

Table 1 reports the enzyme activity of MM and BM samples. Malt enzymes are crucial quality indicators, as the quality of the wort and the course of fermentation depend on their activity during the brewing stage. The α- and β-amylase enzymes are primarily responsible for the degradation of starch, thereby contributing to the production of simple sugars such as maltose and glucose (Kunze, 2005). Endo-β-glucanases and endo-1,4-β-D-xylanase are responsible for degrading non-starch polysaccharides such as β-glucans and arabinoxylans, which affect must viscosity (Psota et al., 2018). The α-amylase content varied significantly in the analyzed samples, being double in the MM sample (176.14 CU/g db) compared to the BM sample (86.73 CU/g db). The difference in α-amylase values between the two samples is due to the addition of half-unmalted wheat in the BM sample. It is worth noting that the unmalted Maiorca wheat had very low α-amylase values (0.55 CU/g db). The β-amylase content was similar in all three samples studied. Enzymes such as α-amylase, β-glucanase, and Dxylanase are activated during germination. The β-amylases are already active from the beginning of germination and are present in unmalted grain (Briggs, 1998). In the studied samples, the β-amylase content did not show any statistically significant differences and ranged from 36.9 BU/g db to 43.03 BU/g db in UM and BM samples, respectively. The malt samples were analyzed for endo-β-glucanases and endo-1,4-β-D-xylanase enzymes. Both enzymes had slightly higher values in the MM sample than in BM, which is consistent with previous studies on Maiorca malt (Gugino et al., 2024).

3.4. Congress wort analysis

Table 2 displays the characteristics of Maiorca malt and Maiorca blend worts, which were studied to comprehend their brewing behaviour. The main differences between the two samples were observed in extract, colour, free amino nitrogen content, and saccharification. The dry extract was analysed as a parameter for assessing brewing yield. It provides a measure of the soluble compounds obtained from the malt. Typically, about 90 % of these compounds are carbohydrates, while the remaining portion consists of amino acids, peptides of various sizes, phenolic compounds, and lipids (Burger and LaBerge, 1985; Rani and Bhardwaj, 2021). The samples analysed in this study exhibited varying values of extract on dry matter. The highest value was observed in sample MM (84.27 % db), while the lowest was found in sample BM (79.18 % db). No significant differences were observed in the fine-coarse extract, despite the difference in grinding. The pH of the two samples was similar, with sample MM having a value of 6.18 and sample BM having a slightly lower value of 6.12.

Regarding colour, the two samples appeared visibly different, a difference that was confirmed by the colour analysis. The MM sample had a more intense colour with a value of 5.27 EBC units, while the BM sample had a lighter colour with a value of 3.87 EBC units. The viscosity of the two samples did not vary greatly, ranging from 1.48 to 1.51 mPa.s for the MM and BM samples, respectively. These values are consistent with those reported in the literature for wheat malt (Alfeo et al., 2018; Faltermaier et al., 2014). The viscosity and spent values were found to be correlated with the β-glucan content, which also showed a similar correlation trend between the two samples. The β-glucan content varied slightly between 241.59 and 264.3 mg/L in the MM and BM samples, respectively. Free Amino Nitrogen (FAN) refers to the soluble protein fraction consisting of peptides and amino acids that are available to yeast and used for their cell growth (Hill and Stewart, 2019). The FAN content in the malt samples studied ranged from 117.61 to 80.74 mg/L

Table 2
Quality parameters of congress worts.

	MM	BM
Extract (% db)	84.27 ± 0.93	79.18 ± 0.57
Extract fine-course difference (% db)	1.45 ± 0.05	1.43 ± 0.04
pH	6.18 ± 0.04	6.12 ± 0.07
Colour (EBC unit)	5.27 ± 0.22	3.87 ± 1.53
Viscosity (mPa.s)	1.48 ± 0.01	1.51 ± 0.04
β-glucan (mg/L)	241.59 ± 5.19	264.3 ± 25.38
FAN (mg/L)	117.61 ± 1.23	80.74 ± 1.46
Fermentability (%)	81.48 ± 0.43	79.01 ± 3.65
Saccharification (min)	< 10	10 <s < 15

s = saccarification; FAN = free amino nitrogen; Congress wort of Maiorca malt (MM) and Blended Maiorca grains (BM).

in MM and BM, respectively. The reduction in FAN content in the BM sample may be attributed to the presence of half of unmalted Maiorca wheat in the mixture.

The fermentability values were higher in the Maiorca Malt (MM) sample at 81.48 % compared to the BM sample at 79.01 %. The MM sample also had a lower saccharification time, indicating a faster conversion of starch into fermentable sugars during mash. These differences may be attributed to the addition of unmalted wheat in the BM sample, which resulted in lowered enzyme activity.

3.5. Beer analysis

Physicochemical analyses were conducted on the experimental beer samples to understand the effects of different fermentation protocols. The results are reported in Table 3. The wort produced in the pilot production had a density of 5.61 and a pH of 11.83 °P. Following fermentation, the produced beers exhibited notable variations in pH, colour, real and apparent attenuation, and alcoholic strength. The latter is typically associated with the attenuation value attained by the yeast strains.

Specifically, T1 and T2 beers had a lower pH value (4.33 and 4.29, respectively) than T3 and TC beers, which had slightly higher values of 4.50 and 4.55, respectively. The diversity in colour was recorded between 3.21 EBC units to 6.9 EBC units, with the lowest value in T2 beer and the highest in TC beer. T1 and T2 beers had a lighter yellow colour than T3 and TC beers, which had a deeper yellow colour tending towards golden.

The yeast strains studied exhibited varying trends in real and apparent attenuation, and consequently, overall alcohol content. Notably, the beers fermented with the *T. delbruekii* yeast strain (T1, T2 and T3, respectively) showed higher values for both real and apparent attenuation compared to the TC control beer.

3.6. Volatile organic compounds of beer

Table 4 reports the volatile composition of the experimental beers. Over 100 volatile aroma compounds were identified and quantified in the analyzed beers. Their identification was based on spectral library matching and retention index comparison. The compounds were classified into seven categories: organic acids, alcohols, aldehydes, esters, ketones, terpenes, and other aromatic compounds. Several studies in the literature demonstrate that many of the identified compounds contribute to the characteristic flavors of wheat beer (De Flaviis et al., 2021; De Flaviis et al., 2022b; Francesca et al., 2023; Gugino et al., 2024; Li et al., 2012; Langos et al., 2013).

Table 3
Beer and wort quality parameters.

	TC	T1	T2	T3
Wort pH	5.61 ± 0.00^{a}	5.61 ± 0.00^{a}	5.61 ± 0.00^{a}	5.61 ± 0.00^{a}
Beer pH	4.55 ± 0.01^{b}	4.33 ± 0.05^{a}	4.29 ± 0.04^{a}	4.5 ± 0.02^{b}
Beer color (EBC)	6.65 ± 0.02^{b}	3.38 ± 0.08^{a}	3.21 ± 0.03^{a}	6.9 ± 0.01^{c}
density OG	1.048 ± 0.00^{a}	1.048 ± 0.00^{a}	1.048 ± 0.00^{a}	1.048 ± 0.00^{a}
density FG	1.009 ± 0.00^{b}	1.007 ± 0.00^{a}	1.005 ± 0.00^{a}	1.006 ± 0.00^{a}
Apparent attenuation (%)	81 ± 0.00^{a}	86 ± 1.41^{b}	90 ± 1.41^{b}	87 ± 0.00^{b}
Real attenuation (%)	65 ± 0.00^{a}	70 ± 1.41^{b}	73 ± 1.41^{b}	71 ± 0.00^{b}
Original extract (°P)	11.83 ± 0.00^{b}	11.83 ± 0.00^{a}	11.83 ± 0.00^{a}	11.83 ± 0.00^{a}
Apparent extract (°P)	2.31 ± 0.00^{b}	1.67 ± 0.18^{a}	1.16 ± 0.18^{a}	1.54 ± 0.00^{a}
Real extract (°P)	4.03 ± 0.45^{b}	3.51 ± 0.00^{a}	3.09 ± 0.15^{a}	3.4 ± 0.15^{a}
Alcohol (% vol)	5.07 ± 0.00^{a}	5.4 ± 0.09^{b}	5.66 ± 0.10^{b}	5.46 ± 0.00^{b}

OG =original gravity; FG= final gravity; °P = Plato;
Worts and beers produced by micro-brewing trials with fermentation protocols: TC (control beer with US-05 strain), T1 (beer with co-inoculation of US-05 and *T. delbrueckii* strain), T2 (beer with *T. delbrueckii* strain inoculation and subsequent addition of US-05 strians after 48 h), and T3 (beer with *T. delbrueckii* strain inoculation during refermentation). Values in the same line followed by different letter are statistically different ($p < 0.05$)

Eleven organic acids were identified, differently contributing to the total acid content; octanoic acid was the most abundant in all samples, followed by decanoic, hexanoic, and acetic, which was generally more abundant in T1 beer. The concentration of octanoic acid ranged from 0.477 to 0.806 mg/L, with the highest concentration observed in the T1 sample and the lowest in the T2 sample. According to Müller et al. (2021), octanoic acid contributes to the off-flavour cheesy aroma.

Fifteen volatile alcohols have been identified and statistically significant differences resulted among the studied samples in terms of total amount, ranging from 121.655 to 166.085 mg/L. The beers T2 and T1 had the highest values, with values of 136.950 and 166.085 mg/L, respectively; the lowest values of 123.925 and 124.655 mg/L, were found in beers TC and T3, respectively. Phenethyl alcohol was the most abundant alcohol. This volatile compound is produced by *T. delbrueckii* and is responsible for fruity notes and floral aroma (Canonico et al., 2017; Comitini et al., 2011; Drosou et al., 2023); and showed the highest content in beer T2 and can be considered key aroma compounds as results from Table 5. Dodecanol, which is known for its coconut-like and banana-like aroma, exhibited the lowest value in the TC control sample (0.474 mg/L), and the highest in the T2 beer (1.262 mg/L).

The class of aldehydes consists of seven compounds with a total content ranged from 13.259 to 19.213 μg/L; the lowest value was observed in beer sample T1, followed by TC and T3, which showed similar values. Nonanal and decanal were the most abundant. Among aldehydes, decanal, undecanal, and dodecanl contributed to the aroma of the analysed beers resulting above their perception threshold (Table 5). Particularly, decanal and undecanal resulted higher in T3 beer compared to the others samples, while dodecanal displayed higher content in T1 and TC.

Esters are compounds produced during the fermentation process by yeasts that significantly contribute to the fruity character of fermented products like beer (Verstrepen et al., 2003). In the examined beer samples, 41 ester compounds were detected, and their overall amount varied from 8.170 to 10.534 mg/L. The smallest value was found in T2 beer sample; T1 and T3 had very similar values. The active compounds that contributed to the aroma of the beers were mainly ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, and ethyl hexanoate (Table 5). Ethyl decanoate and β-phenylethyl acetate are compounds that are often found in wheat beers and contribute to the fruity aromas of the fermented beverage, as evidenced by previous works in the literature (De Flaviis et al., 2021; De Flaviis et al., 2022b; Francesca et al., 2023; Gugino et al., 2024; Gugino et al., 2025, Li et al., 2012). From Table 5 and Fig. 2a high amount of esters responsible for fruity and floral aromas resulted in T1 and T2 beers.

Table 4 shows a low concentration of ketones in the examined beers, with a total content ranging from 0.446 to 0.639 mg/L. The lowest value was found in T2 beer, while the highest in the TC one. Only (*E*)-β-damascenone and nerylacetone contribute to the floral character of the beers as reported in Table 5 (Yi et al., 2019). The concentration of (*E*)-β-damascenone ranged from 0.224 to 0.394 mg/L in TC and T1, respectively; the presence of the *T. delbrueckii* strain appeared to have a positive effect on the amount of this compound, resulting in higher levels in T1 and T2 beers.

The terpene class comprises compounds derived from hops that contribute to the hoppy character of beers. Limonene, linalool, citronellol, nerol, isogeraniol, and (*E*)-nerolidol have been identified. The total content of these compounds did not show any statistically significant differences between the analyzed beer samples; this was expected

Table 4
Volatile aroma compounds identified in beers.

Compounds	Odor	LRI	TC	T1	T2	T3	References
Acids (mg/L)							
Acetic acid	acidic	1456	0.013 ± 0.000^{a}	0.110 ± 0.002^{d}	0.062 ± 0.002^{c}	0.025 ± 0.001^{b}	2,5
2-Methyl propanoic acid	sweaty	1569	0.003 ± 0.000^{a}	0.005 ± 0.000^{c}	0.004 ± 0.000^{b}	0.005 ± 0.000^{bc}	9
Butanoic acid	acidic	1631	0.002 ± 0.000^{a}	0.002 ± 0.000^{b}	0.002 ± 0.000^{b}	0.002 ± 0.000^{a}	2,5,6,8,9
3-Methylbutanoic acid	sweaty	1671	0.005 ± 0.000^{a}	0.008 ± 0.000^{c}	0.007 ± 0.000^{b}	0.010 ± 0.000^{d}	2,5,8,9
Hexanoic acid	balsamic, almond	1845	0.087 ± 0.002^{b}	0.102 ± 0.005^{c}	0.058 ± 0.002^{a}	0.086 ± 0.003^{b}	3,4
2-Ethylhexanoic acid	earthy	1947	0.002 ± 0.000^{b}	0.002 ± 0.000^{b}	0.002 ± 0.000^{b}	0.001 ± 0.000^{a}	8,9,17
Heptanoic acid	fatty	1951	0.004 ± 0.000^{a}	0.006 ± 0.000^{b}	0.006 ± 0.000^{b}	0.004 ± 0.000^{a}	2,5,9
Octanoic acid	fatty	2058	0.667 ± 0.013^{b}	0.806 ± 0.041^{c}	0.477 ± 0.024^{a}	0.711 ± 0.014^{b}	2,5,9
Nonanoic acid	waxy	2164	0.015 ± 0.000^{a}	0.029 ± 0.001^{d}	0.023 ± 0.000^{c}	0.019 ± 0.000^{b}	2,5,9
Decanoic acid	fatty	2271	0.025 ± 0.001^{a}	0.111 ± 0.002^{c}	0.112 ± 0.003^{c}	0.040 ± 0.002^{b}	2,5,8,9
Dec−9-enoic acid	waxy	2336	0.011 ± 0.000^{c}	0.003 ± 0.000^{b}	nd^{a}	nd^{a}	9
Total			$\boldsymbol{0.834 \pm 0.043^{b}}$	$\boldsymbol{1.183 \pm 0.023^{c}}$	$\boldsymbol{0.753 \pm 0.015^{a}}$	$\boldsymbol{0.904 \pm 0.018^{b}}$	
Alcohols (mg/L)							
2-Methyl−1-propanol	ethereal	1101	3.140 ± 0.161^{b}	3.328 ± 0.066^{b}	4.202 ± 0.215^{c}	2.757 ± 0.055^{a}	1,3,9
2-Pentanol	fruity	1128	0.003 ± 0.000^{a}	0.224 ± 0.007^{b}	0.277 ± 0.014^{c}	0.003 ± 0.000^{a}	8
3-Pentene−2-ol	herbal, green	1182	0.003 ± 0.000^{a}	0.003 ± 0.000^{a}	0.080 ± 0.002^{b}	0.003 ± 0.000^{a}	8,18
Isoamyl alcohol	fruity	1212	42.248 ± 0.853^{a}	47.694 ± 0.944^{a}	52.878 ± 1.610^{b}	41.282 ± 2116^{a}	8,19
3-Methylpentanol	earthy	1326	0.038 ± 0.002^{b}	0.038 ± 0.001^{b}	0.047 ± 0.002^{c}	0.033 ± 0.001^{a}	8,2
1-Hexanol	fruity	1351	0.205 ± 0.004^{a}	0.253 ± 0.013^{b}	0.192 ± 0.004^{a}	0.172 ± 0.009^{a}	8,9
1-Octen−3-ol	earthy	1446	0.942 ± 0.048^{b}	0.980 ± 0.03^{b}	1.100 ± 0.056^{c}	0.822 ± 0.016^{a}	3,4
Heptanol	floral, herbal	1452	0.265 ± 0.005^{b}	0.339 ± 0.017^{c}	0.416 ± 0.013^{d}	0.178 ± 0.005^{a}	3,4,9
Nonan−2-ol	citrus, orange	1515	0.147 ± 0.008^{a}	1.187 ± 0.024^{d}	0.772 ± 0.040^{c}	0.285 ± 0.006^{b}	8
Octanol	citrus, orange	1554	$3385 \pm 0.103b^{c}$	3.600 ± 0.110^{c}	2.119 ± 0.109^{a}	3.094 ± 0.159^{b}	1,9
2-Decanol	coconut	1616	0.100 ± 0.002^{a}	0.115 ± 0.006^{b}	0.170 ± 0.009^{c}	0.129 ± 0.003^{b}	8,9
2-Undecanol	sweety, coconut	1717	0.285 ± 0.006^{a}	1.572 ± 0.081^{d}	1.246 ± 0.025^{c}	0.504 ± 0.010^{b}	8,9
Decanol	floral	1759	2.714 ± 0.139^{a}	3.701 ± 0.075^{b}	5.217 ± 0.159^{c}	2.459 ± 0.126^{a}	1,3,4,9
Phenethyl alcohol	floral, rose-like	1910	69.975 ± 1.414^{a}	73.253 ± 3.755^{a}	96.106 ± 2.927^{b}	69.204 ± 2.10^{7a}	8,9
Dodecanol	coconut, banana	1964	0.474 ± 0.024^{a}	0.662 ± 0.013^{b}	1.262 ± 0.025^{d}	0.729 ± 0.015^{c}	8,9
Total			$\boldsymbol{123.925 \pm 2.480^{a}}$	$\boldsymbol{136.950 \pm 2.740^{b}}$	$\boldsymbol{166.085 \pm 3.321^{c}}$	$\boldsymbol{121.655 \pm 2.433^{a}}$	
Aldehydes (μg/L)							
Hexanal	aldehydic	1153	0.213 ± 0.011^{d}	0.191 ± 0.004^{c}	0.132 ± 0.007^{b}	0.110 ± 0.002^{a}	8,9
Octanal	aldehydic	1291	0.522 ± 0.016^{ab}	0.673 ± 0.021^{c}	0.481 ± 0.025^{a}	0.547 ± 0.017^{b}	3,4,8,9
Nonanal	aldehydic, cardboard	1395	4.503 ± 0.231^{b}	3.724 ± 0.191^{a}	5.493 ± 0.109^{c}	5.823 ± 0.298^{d}	1,9
Decanal	aldehydic, citrus	1500	6.800 ± 0.137^{ab}	7.28 ± 0.144^{b}	6.009 ± 0.183^{a}	11.248 ± 0.577^{c}	1,3,4,8,9
Undecanal	aldehydic, citrus	1605	0.357 ± 0.018^{b}	0.332 ± 0.007^{ab}	0.286 ± 0.015^{a}	0.599 ± 0.018^{c}	8,9
Dodecanal	fatty	1711	0.575 ± 0.011^{c}	0.539 ± 0.028^{c}	0.405 ± 0.008^{a}	0.467 ± 0.024^{b}	8,9
2-Dodecenal	herbal	1752	0.332 ± 0.017^{a}	0.52 ± 0.016^{c}	0.607 ± 0.031^{d}	0.419 ± 0.008^{b}	8,9,20
Total			$\boldsymbol{13.302 \pm 0.269^{a}}$	$\boldsymbol{13.259 \pm 0.68^{a}}$	$\boldsymbol{13.413 \pm 0.408^{a}}$	$\boldsymbol{19.213 \pm 0.585^{b}}$	
Esters (mg/L)							
Ethyl acetate	ethereal	895	0.259 ± 0.013^{ab}	$0.32 \pm 0.006c$	$0.28 \pm 0.014b^{c}$	0.254 ± 0.005^{a}	1
Ethyl propanoate	ethereal, fruity	963	0.007 ± 0.000^{b}	0.005 ± 0.000^{a}	0.009 ± 0.000^{c}	0.010 ± 0.001^{c}	9,11
Isobutyl acetate	fruity, banana	1017	0.014 ± 0.000^{a}	0.016 ± 0.001^{b}	0.019 ± 0.001^{c}	0.013 ± 0.000^{a}	8,9
Ethyl butanoate	fruity, tropical fruit	1039	0.062 ± 0.001^{a}	0.072 ± 0.004^{b}	0.058 ± 0.001^{a}	0.062 ± 0.001^{a}	8,9
Ethyl 2-methylbutanoate	fruity, pineapple	1054	0.003 ± 0.000^{a}	0.003 ± 0.000^{b}	0.0032 ± 0.0001^{b}	0.002 ± 0.000^{a}	8,9
Ethyl 3-methylbutanoate	fruity, pineapple	1069	0.001 ± 0.000^{b}	0.001 ± 0.000^{a}	0.001 ± 0.000^{a}	0.001 ± 0.000^{a}	8,9
Isoamyl acetate	fruity, banana	1121	0.434 ± 0.022^{b}	0.513 ± 0.010^{c}	0.433 ± 0.009^{ab}	0.405 ± 0.008^{a}	1,2,4,5,8,9
Ethyl pentanoate	fruity, papaya	1131	0.004 ± 0.000^{a}	0.006 ± 0.000^{b}	0.004 ± 0.000^{a}	0.004 ± 0.000^{a}	8,9
Isoamyl propionate	fruity, pineapple	1186	0.003 ± 0.000^{c}	0.002 ± 0.000^{a}	0.002 ± 0.000^{a}	0.002 ± 0.000^{b}	8,9
Ethyl hexoate	fruity, banana	1231	0.705 ± 0.021^{bc}	0.756 ± 0.023^{c}	0.408 ± 0.021^{a}	0.657 ± 0.020^{b}	4,9
Hexyl acetate	fruity, banana	1271	0.008 ± 0.000^{a}	0.009 ± 0.000^{b}	0.01 ± 0.000^{b}	0.008 ± 0.000^{a}	8,9
Ethyl 3-hexenoate	fatty	1301	0.002 ± 0.000^{a}	0.002 ± 0.000^{a}	0.003 ± 0.000^{b}	0.002 ± 0.000^{a}	8,9
Propyl hexanoate	fruity, blackberries	1317	0.003 ± 0.000^{b}	0.005 ± 0.000^{c}	0.001 ± 0.000^{a}	0.001 ± 0.000^{a}	8,9,21
Ethyl heptanoate	fruity, banana	1331	0.031 ± 0.001^{a}	0.034 ± 0.002^{bc}	0.036 ± 0.001^{c}	0.030 ± 0.002^{ab}	10
Ethyl 3-pentenoate	fruity, pineapple	1335	0.002 ± 0.000^{ab}	0.002 ± 0.000^{ab}	0.002 ± 0.000^{b}	0.002 ± 0.000^{a}	8,9
Ethyl lactate	fruity, strawberry	1345	0.001 ± 0.000^{a}	0.004 ± 0.000^{b}	0.001 ± 0.000^{a}	0.001 ± 0.000^{a}	8,9
2-Methylpropyl hexanoate	fruity	1349	0.001 ± 0.000^{b}	0.001 ± 0.000^{a}	0.001 ± 0.000^{a}	0.001 ± 0.000^{a}	6,7,8,9
Heptyl acetate	fruity, pear	1372	0.003 ± 0.000^{a}	0.005 ± 0.000^{b}	0.005 ± 0.001^{b}	0.003 ± 0.000^{a}	8,9
Methyl octanoate	fruity, orange-like	1389	0.002 ± 0.000^{c}	0.002 ± 0.000^{c}	0.001 ± 0.000^{a}	0.001 ± 0.000^{b}	8,9
Ethyl octanoate	fruity	1439	5.502 ± 0.109^{b}	4.886 ± 0.250^{b}	3.316 ± 0.067^{a}	5.129 ± 0.104^{b}	2,5,6,9
Octyl acetate	coconut	1474	0.005 ± 0.000^{a}	0.014 ± 0.000^{d}	0.011 ± 0.000^{c}	0.007 ± 0.000^{b}	8
Propyl octanoate	coconut, cacao	1517	0.004 ± 0.000^{a}	0.006 ± 0.000^{c}	0.004 ± 0.000^{a}	0.005 ± 0.000^{b}	8
Ethyl nonanoate	floral, rose-like	1534	0.230 ± 0.012^{c}	0.131 ± 0.003^{a}	0.210 ± 0.004^{b}	0.205 ± 0.004^{b}	8,9
Isobutyl octanoate	fruity	1551	0.016 ± 0.000^{c}	$0.01 \pm 0.000a$	0.010 ± 0.000^{a}	0.013 ± 0.000^{b}	8,9
Ethyl decanoate	sweety	1641	1.196 ± 0.061^{a}	1.474 ± 0.029^{b}	2.124 ± 0.109^{c}	1.402 ± 0.028^{ab}	9,16
Isoamyl octanoate	fruity	1657	0.063 ± 0.002^{c}	0.046 ± 0.001^{b}	0.041 ± 0.002^{a}	0.062 ± 0.002^{c}	8,9
Ethyl 4E-decenoate	fruity, pineapple	1665	0.007 ± 0.000^{a}	0.011 ± 0.001^{c}	0.009 ± 0.000^{b}	0.009 ± 0.000^{b}	13,9
Diethyl succinate	ethereal	1676	0.002 ± 0.000^{a}	0.005 ± 0.000^{c}	0.008 ± 0.000^{d}	0.002 ± 0.000^{b}	9
Ethyl 9-decenoate	fruity	1690	1.332 ± 0.068^{d}	0.408 ± 0.008^{b}	0.220 ± 0.011^{a}	0.509 ± 0.015^{c}	1,9
Geranic acid methyl ester	fruity, floral	1694	0.019 ± 0.000^{a}	0.022 ± 0.001^{b}	0.019 ± 0.000^{a}	0.023 ± 0.001^{b}	12,9
Ethyl 9-decenoate	fruity	1703	0.003 ± 0.000^{b}	0.002 ± 0.000^{a}	0.002 ± 0.000^{a}	0.002 ± 0.000^{a}	1,9
Propyl decanoate	waxy	1722	0.001 ± 0.000^{a}	0.001 ± 0.000^{b}	0.002 ± 0.000^{c}	0.001 ± 0.000^{b}	8,9
Ethyl undecanoate	waxy	1739	0.010 ± 0.001^{d}	0.003 ± 0.000^{a}	0.008 ± 0.000^{c}	0.006 ± 0.000^{b}	8,9
Methyl salicylate	sweety	1777	0.001 ± 0.000^{a}	0.001 ± 0.000^{a}	0.003 ± 0.000^{c}	0.002 ± 0.000^{b}	22

(*continued on next page*)

Table 4 (*continued*)

Compounds	Odor	LRI	TC	T1	T2	T3	References
Ethyl phenylacetate	floral,rose-like	1785	0.011 ± 0.000^{b}	0.014 ± 0.001^{c}	0.011 ± 0.001^{b}	0.009 ± 0.000^{a}	9,11,15
β-Phenylethyl acetate	floral, rose-like	1815	0.381 ± 0.008^{a}	0.494 ± 0.025^{c}	0.611 ± 0.012^{d}	0.408 ± 0.008^{b}	3,8,20,23,24
Ethyl dodecanoate	sweety	1843	0.183 ± 0.009^{c}	0.105 ± 0.002^{a}	0.26 ± 0.008^{d}	0.126 ± 0.006^{b}	9,16
(*Z*)-Ethyl pentadec−9-enoate	fruity	1857	nd^{a}	0.014 ± 0.001^{c}	0.009 ± 0.000^{b}	nd^{a}	8,9
Isoamyl decanoate	fruity	1860	0.003 ± 0.000^{b}	0.002 ± 0.000^{a}	0.005 ± 0.000^{c}	0.003 ± 0.000^{b}	8,9
Ethyl hydrocinnamate	fruity, sweety	1883	0.003 ± 0.000^{a}	0.011 ± 0.000^{d}	0.004 ± 0.000^{b}	0.008 ± 0.000^{c}	8,9
Ethyl−10-undecenoate	waxy	1895	0.020 ± 0.001^{d}	0.010 ± 0.000^{c}	0.007 ± 0.000^{b}	0.006 ± 0.000^{a}	9,14
Total			$\boldsymbol{10.534 \pm 0.321^{b}}$	$\boldsymbol{9.427 \pm 0.287^{a}}$	$\boldsymbol{8.170 \pm 0.419^{a}}$	$\boldsymbol{9.396 \pm 0.286^{a}}$	
Ketones (mg/L)							
2-Pentanone	floral	985	0.075 ± 0.004^{b}	0.001 ± 0.000^{a}	0.001 ± 0.000^{a}	0.071 ± 0.004^{b}	8,9
6-Methyl−5-heptene−2-one	citrus	1339	0.025 ± 0.001^{c}	0.013 ± 0.000^{b}	0.009 ± 0.000^{a}	0.023 ± 0.001^{c}	2,5,9
2-Nonanone	fatty	1390	0.061 ± 0.003^{c}	0.006 ± 0.000^{a}	0.006 ± 0.000^{a}	0.016 ± 0.001^{b}	8,9
(*E*)-β-Damascenone	fruity	1818	0.224 ± 0.004^{a}	0.394 ± 0.020^{d}	0.289 ± 0.006^{c}	0.246 ± 0.013^{b}	7,9
Nerylacetone	fatty	1853	0.254 ± 0.013^{d}	0.169 ± 0.005^{b}	0.1478 ± 0.0072^{a}	0.237 ± 0.005^{c}	8,9
Total			$\boldsymbol{0.639 \pm 0.013^{b}}$	$\boldsymbol{0.584 \pm 0.030^{b}}$	$\boldsymbol{0.446 \pm 0.014^{a}}$	$\boldsymbol{0.594 \pm 0.018^{b}}$	
Terpenes (mg/L)							
Limonene	citrus	1193	0.022 ± 0.001^{c}	0.008 ± 0.000^{b}	0.003 ± 0.000^{a}	0.007 ± 0.000^{b}	8,9
Linalool	floreal, citrus	1544	0.071 ± 0.002^{a}	0.071 ± 0.002^{a}	0.088 ± 0.005^{b}	0.076 ± 0.004^{a}	7,9,10
Citronellol	floreal, citrus	1763	0.019 ± 0.000^{a}	0.027 ± 0.001^{b}	0.018 ± 0.001^{a}	0.020 ± 0.000^{a}	8,9,10
Nerol	floral, rose-like/citrus	1798	0.008 ± 0.000^{ab}	0.008 ± 0.000^{b}	0.007 ± 0.000^{a}	0.007 ± 0.000^{a}	8,9
Isogeraniol	spicy	1810	0.004 ± 0.000^{a}	0.006 ± 0.000^{c}	0.005 ± 0.000^{b}	0.006 ± 0.000^{bc}	25
(*E*)-Nerolidol	woody	2036	0.004 ± 0.000^{a}	0.012 ± 0.001^{c}	0.010 ± 0.000b	0.011 ± 0.000^{c}	26
Total			$\boldsymbol{0.128 \pm 0.007^{a}}$	$\boldsymbol{0.131 \pm 0.003^{a}}$	$\boldsymbol{0.131 \pm 0.003^{a}}$	$\boldsymbol{0.127 \pm 0.003^{a}}$	
Other compounds (mg/L)							
Styrene	balsamic, almond	1259	0.017 ± 0.000^{a}	0.461 ± 0.014^{c}	0.3503 ± 0.0071^{b}	0.515 ± 0.010^{d}	3,4
γ-Nonalactone	coconut	2027	6.192 ± 0.317^{a}	9.971 ± 0.197^{c}	8.501 ± 0.436^{b}	6.421 ± 0.127^{a}	2,5,6,8
4-Vinylguaiacol	spicy/cloves/phenolic	2196	0.060 ± 0.002^{a}	33.515 ± 1.021^{d}	22.027 ± 1.129^{c}	8.829 ± 0.269^{b}	8,9
Total			$\boldsymbol{6.269 \pm 0.321^{a}}$	$\boldsymbol{43.947 \pm 2.253^{d}}$	$\boldsymbol{30.885 \pm 0.612^{c}}$	$\boldsymbol{15.765 \pm 0.808^{b}}$	

Beers produced by micro-brewing trials with fermentation protocols: TC (control beer with US-05 strain), T1 (beer with co-inoculation of US-05 and *T. delbrueckii* strain), T2 (beer with *T. delbrueckii* strain inoculation and subsequent addition of US-05 strains after 48 h), and T3 (beer with *T. delbrueckii* strain inoculation during refermentation). LRI= Linear retention index; nd=not detected. Values in the same line followed by different letters are statistically different ($p < 0.05$).
Reference: (1) De Flaviis et al., (2022a)*; (2)* Medina et al., (2023)*; (3)* Langos et al., (2013)*; (4)* Mascia et al., (2014)*; (5)* Li et al., (2012)*; (6)* De Flaviis et al., (2022b)*; (7)* De Flaviis et al., (2021)*; (8)* (Zunkel et al., 2011)*; (9)* (The Good Scents Company © 1980-2021,)*; (10)* Takoi et al., (2016)*; (11)* Hoff et al., (2013)*; (12)* (Adamenko and Kawa-Rygielska, 2022)*; (13)* Dresel et al., (2015)*; (14)* Thompson Witrick et al., (2017)*; (15)* Steyer et al., (2017)*; (16)* Canonico et al., (2014)*; (17)* Witrick et al., (2020)*; (18)* Oussou et al., (2022)*; (19)* Riu-Aumatell et al., (2014)*; (20)* Eyres et al., (2007)*; (21)* Yang et al., (2022)*; (22)* Rong et al., (2016)*; (23)* Toh et al., (2020)*; (24)* Toh et al., (2018)*; (25)* Iglesias et al., (2022)*; (26)* (King and Dickinson, 2000).

Table 5
Relative Odour Activity Value (ROAV) of the main volatile compounds found in beer.

Chemical class	Compounds	OTV* (ppm))	Odor type	TC	T1	T2	T3
Acid	Octanoic acid	0.500	fatty	1.33^{b}	1.61^{b}	0.95^{a}	1.42^{b}
Alcohol	Isoamyl alcohol	30.000	fruity	1.41	1.59	1.76	1.38
Alcohol	Octanol	0.900	green	3.76^{b}	4.00^{b}	2.35^{a}	3.44^{b}
Alcohol	Phenethyl alcohol	14.000	floral	5.00^{a}	5.23^{a}	6.86^{b}	4.94^{a}
Aldehyde	Decanal	0.006	aldehydic	1.16^{ab}	1.19^{b}	1.03^{a}	1.97^{c}
Aldehyde	Undecanal	0.00035	aldehydic	1.07^{b}	0.97^{ab}	0.86^{a}	1.76^{c}
Aldehyde	Dodecanal	0.0004	fatty	1.41^{c}	1.42^{c}	1.03^{a}	1.23^{b}
Ester	Ethyl acetate	0.030	estery	8.62^{a}	10.67^{ab}	9.34^{a}	8.45^{a}
Ester	Ethyl butanoate	0.020	fruity	3.09	3.58	2.91	3.11
Ester	Ethyl 2-methylbutanoate	0.000013	fruity	199.65^{a}	236.64^{b}	241.75^{b}	180.34^{a}
Ester	Ethyl 3-methylbutanoate	0.000013	fruity	61.10^{a}	100.5^{b}	60.19^{a}	60.66^{a}
Ester	Isoamyl acetate	0.030	fruity	14.47^{a}	17.08^{b}	14.45^{a}	13.50^{a}
Ester	Ethyl hexanoate	0.005	fruity	141.03^{b}	151.13^{c}	81.67^{a}	131.34^{b}
Ester	Ethyl decanoate	0.200	fruity	5.98^{a}	7.37^{b}	10.62^{c}	7.01^{b}
Ester	β-Phenylethyl acetate	0.250	floral	1.52^{a}	1.98^{a}	2.44^{ab}	1.63^{a}
Keton	(*E*)-β-Damascenone	0.000006	floral	37372.60^{a}	65743.08^{c}	48175.55^{b}	41001.65^{b}
Keton	Nerylacetone	0.060	floral	4.24^{b}	2.81^{a}	2.34^{a}	3.95^{b}
Terpens	Linalool	0.008	floral	8.84^{a}	8.82^{a}	10.99^{b}	9.54^{b}
Others	Styrene	0.020	Balsamic	0.85^{a}	23.06^{c}	17.87^{b}	25.76^{c}
Others	4-Vinylguaiacol	0.3	Spicy	0.20^{a}	111.72^{d}	73.42^{c}	29.43^{b}

Beers produced by micro-brewing trials with fermentation protocols: TC (control beer with US-05 strain), T1 (beer with co-inoculation of US-05 and *T. delbrueckii* strain), T2 (beer with *T. delbrueckii* strain inoculation and subsequent addition of US-05 strains after 48 h), and T3 (beer with *T. delbrueckii* strain inoculation during refermentation).* Odor Threshold Value from literature. Values in the same line followed by different letters are statistically different ($p < 0.05$).

since the beers were produced from the same production batch, with the same variety and quantity of hops. Linalool was found to be a key aroma compound (Table 5), its content varied from 0.071 and 0.088 mg/L, with the lowest value in samples T1 and TC, followed by T3, while the highest was in sample T2. The use of *T. delbrueckii* could influence the final profile of these compounds due to its ability to transform hop terpenoids (King and Dickinson, 2003).

Styrene and 4-Vinylguaiacol (4VG) were identified, too. Styrene is a typical aromatic compound found in wheat beers (Gugino et al., 2024). This compound enhances the balsamic aroma, almond flavour, and phenolic profile of wheat beers. In the analysed beer samples, styrene had an OTV between 17 and 25 in samples T1, T2, and T3, with a content

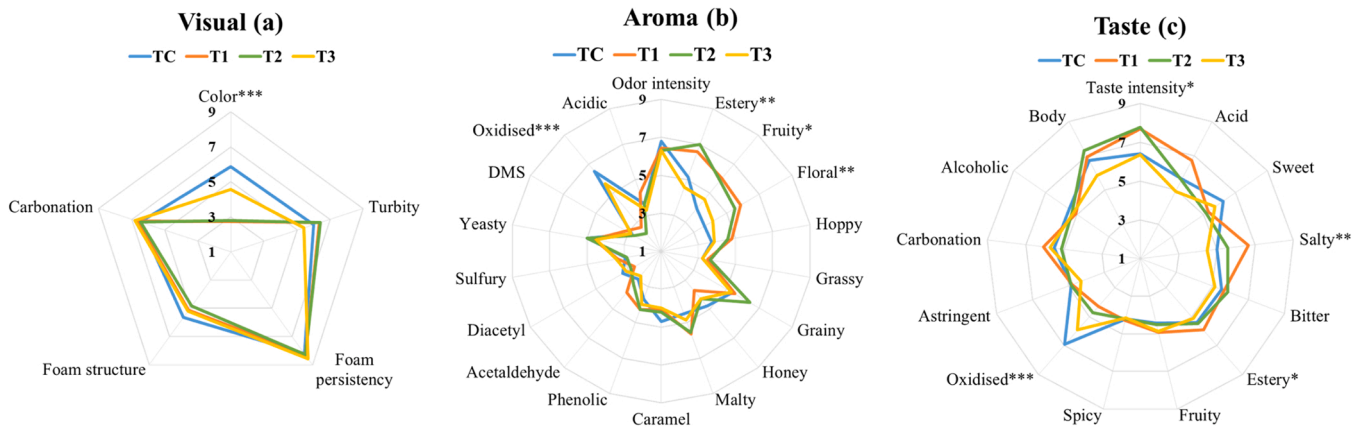

Fig. 2. Sensory analysis performed on visual (a), aroma (b) and taste (c) of beers: spider plot of average scores for aroma determined by judges during tasting sessions. Beers subjected to varying *T. delbrueckii* strain inoculation methods and compared with beer produced through fermentation with the commercial *S. cerevisiae* US-05 strain. TC (control beer fermented with the US-05 strain), T1 (beer co-inoculated with US-05 and *T. delbrueckii*), T2 (beer inoculated with *T. delbrueckii* with subsequent addition of US-05 after 48 h), and T3 (beer inoculated with *T. delbrueckii* during refermentation). Symbols: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

of 0.350 mg/L in beer T2 and 0.515 mg/L in beer T3, respectively. 4VG is a phenolic compound commonly found in wheat beers (Xu et al., 2020). It is formed during fermentation through the decarboxylation of ferulic acid, which is typically released during mashing (McMurrough et al., 1996). The content of this compound ranged from 0.060 to 33.515 mg/L in the TC and T1 samples, respectively. The use of *T. delbrueckii* increase its amount in T1, T2 and T3 beers compared to the control beer where only the saccharomyces yeast strain was used. The compound exceeded its OTV by 111, 73 and 29 times in samples T1, T2 and T3 respectively, while in the TC sample it remained close to the perception threshold.

3.7. Sensory evaluation

The sensory analysis results for the experimental beers were presented through radar graphs (Fig. 2). Trained tasters assessed the descriptive properties and categorized them to identify variations in visual appearance, aroma, and taste (Fig. 2a–c). Analysis of the appearance (Fig. 2a) revealed colour differences among the beer samples studied. No significant differences were observed in attributes such as turbidity, foam persistency, and foam structure. Specifically, the colour was darker in TC and T3 samples as compared to those from T1 and T2. Based on this initial evaluation, it can be inferred that *T. deulbreukii* yeast might provide bio-protection against oxidation phenomena that occur during beer production (Simonin et al., 2018). The appearance of the beers in T2, T1, T3, and TC was favoured in that order. Statistical analysis of aroma descriptors (Fig. 2b) showed significant differences between the experimental beers in terms of estery, fruity, floral, and mainly oxidised. In terms of oxidized aroma, the beer with the highest rating was TC, whereas T2 received the lowest rating. As a general trend, the beers TC and T3 exhibited a higher level of oxidation aroma compared to T1 and T2. The panel favored the taste of T2 beer over T3, which was found to be less palatable (Fig. 2). Beer T2 exhibited the highest value for estery attribute, whereas the lowest value was observed in beer T3. The highest value for the floral attribute was observed in T1 beers followed by T2, whereas the lowest values were found for T3 and TC. This trend was also observed for the fruity descriptor. The rise in these descriptors in beers fermented with *T. delbreukii* corresponds with the findings in the literature, which indicate an increase in the compounds responsible for these aromas (Magalhães et al., 2017).

The analysis of taste descriptors was reported in Fig. 2c. Significant differences in taste intensity ($p < 0.05$), estery ($p < 0.05$), salty attribute ($p < 0.01$), and oxidation ($p < 0.001$) were observed. T1 and T2 beers exhibited stronger taste intensity than TC and T1. The T1 beer had the highest salty attribute score, followed by T2, TC, and T3 in descending order.

The estery descriptor was observed to be more pronounced in the T1 beer than in the other beers subjected to sensory evaluation. As reported in Table 5, certain compounds, including ethyl acetate, ethyl butanoate, ethyl 3-methylbutanoate, isoamyl acetate, and ethyl hexanoate, which contribute to the positive perception of estery and fruity notes, were found to be present in greater concentrations in the T1 and T2 beers than in the other beers. The content of these compounds is influenced by the metabolism of the *T. delbluecki* yeast strain, as reported in previous studies (Canonico et al., 2017; Comitini et al., 2011; Van Breda et al., 2018). In addition, as observed in the aroma analysis, the TC and T3 beers exhibited a higher degree of oxidation than the other samples under consideration. The T2 beer received a higher overall acceptance score for visual odor and taste, according to the panelists' ratings (Fig. 3).

3.8. Impact of yeast strains and fermentation methods on the organoleptic profile of beers

The hierarchical clustering (Fig. 4) on both the aroma compound and sample axes reveals distinct groupings that allow for the interpretation of similarities and differences in aroma profiles. At the top of the clustergram, the dendrogram clearly shows two primary clusters separating the samples. TC and T3 form one group, while T1 and T2 are clustered together, indicating that the aroma profiles of the control and bottle refermentation treatments are similar, but distinct from the beers inoculated with *T. delbrueckii* during primary fermentation. This suggests that the early introduction of *T. delbrueckii* in T1 and T2 leads to significant shifts in aroma compound production compared to both the control (TC) and the bottle refermentation strategy (T3). The closeness of T1 and T2 further suggests that the timing of *S. cerevisiae* inoculation (simultaneous vs. sequential) does not result in drastically different aroma profiles, though subtle differences may exist. Conversely, the large distance between the TC/T3 cluster and the T1/T2 cluster indicates that *T. delbrueckii*, when present during the main fermentation stages, plays a key role in modulating the aromatic landscape of the beer. On the vertical axis, the dendrogram groups the aroma compounds into distinct clusters, indicating that certain groups of volatiles co-occur or show similar trends across samples.

The red cluster includes volatile compounds such as neryl acetone, which is generally responsible for floral aromas and sensory descriptors such as, "honey", "caramel", "diacetyl", "oxidised", and "yeasty". Except

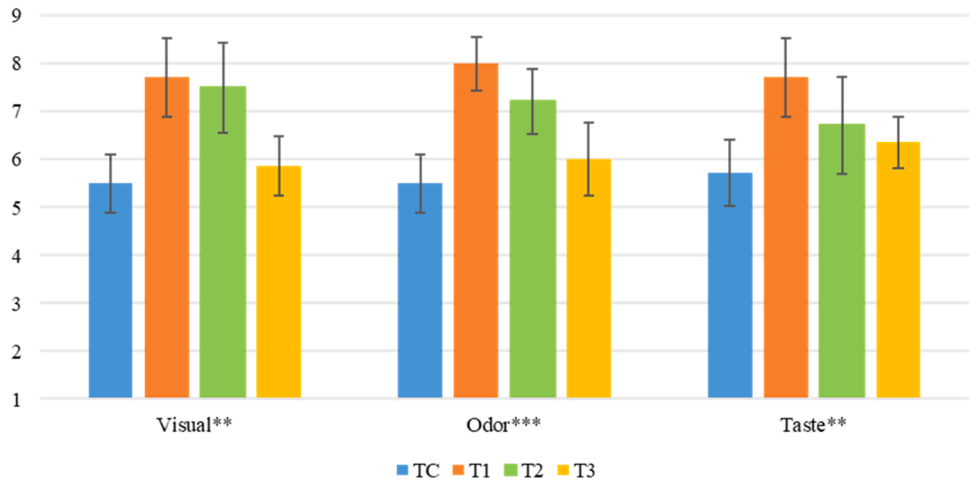

Fig. 3. Overall acceptance of beers. Barr graph of average scores for overall acceptance determined by judges during tasting sessions. Symbols: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

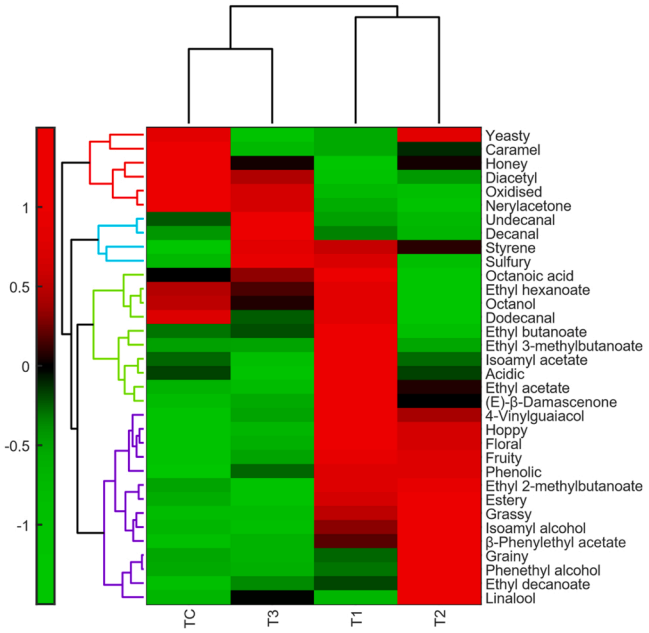

Fig. 4. Clustergram of beer quality parameters. Data source: GC-MS data as the average total amount of ROAV and sensory analysis data on aroma of experimental beers.

for the first two attributes, the other descriptors traditionally associated with buttery, sweet, and oxidative notes show higher intensity in TC and T3, as evidenced by the red coloration. This suggests that beers fermented with *S. cerevisiae* only (TC) or with bottle refermentation (T3) are more prone to develop these flavors. The high levels of diacetyl and oxidized compounds in these samples could potentially be undesirable, as they can lead to off-flavors associated with fermentation management or oxygen exposure.

The light blue cluster includes undecanal, decanal, styrene, and sulfury descriptors. Both undecanal and decanal are aldehydes, typically contributing waxy, citrus, or fatty notes. These compounds often come from lipid oxidation or the breakdown of fatty acids during fermentation or storage (Filipowska et al., 2021). In the heatmap, undecanal and decanal show higher intensities (red shading) in the control (TC) and the bottle refermentation sample (T3). This suggests that the beers fermented without *T. delbrueckii* are more prone to the production of these aldehydes, which could be linked to oxidative processes. Elevated aldehyde amounts can contribute to off-flavors associated with aging or poor handling, such as "papery" or "stale" aromas, which can negatively affect the beer's freshness.

The green cluster includes volatile compounds from the classes of esters, alcohols, and ketones, such as ethyl hexanoate, ethyl butanoate, ethyl 3-methylbutanoate, isoamyl acetate, ethyl acetate, octanol, dodecanal, and other volatiles that show moderate variability across all samples. Although these compounds are more abundant in the T1 sample, their production appears to be less influenced by the presence of *T. delbrueckii*, suggesting that their levels are more stable and dependent on baseline fermentation conditions rather than specific yeast interactions.

The purple cluster includes compounds such as 4-vinylguaiacol, ethyl 2-methylbutanoate, isoamyl alcohol, β-phenylethyl acetate, phenethyl alcohol, and ethyl decanoate, along with sensory descriptors like "phenolic," "malty," "fruity," "floral," and "hoppy." These compounds and attributes are typically associated with desirable flavor and aroma characteristics in beer, contributing to the complexity and enhancing the overall sensory profile. These compounds exhibit significantly higher intensities in T1 and T2, where *T. delbrueckii* was involved early in the fermentation. The red color in these samples indicates that the co-inoculation (simultaneous or sequential) of *T. delbrueckii* enhances the production of these aroma compounds, which are often desirable in beer due to their contribution to complexity and sensory appeal.

4. Conclusions

The findings of this study provide strong evidence that the introduction of *T. delbrueckii* during primary fermentation, either through simultaneous or sequential inoculation, has a significant impact on the beer's aroma profile. Both treatments demonstrate an increase in the intensity of desirable aromatic compounds such as esters (fruity, floral), phenolics, and malty notes, while suppressing undesirable compounds including diacetyl, oxidized aromas, octanoic acid, and aldehydes. These results are particularly important for brewers looking to enhance beer complexity while reducing off-flavors.

The comparison between the simultaneous inoculation and the sequential inoculation reveals minimal differences in their aroma profiles, with both treatments showing high intensities of fruity, phenolic, and floral compounds. This suggests that the timing of *S. cerevisiae* introduction does not drastically alter the production of key volatile compounds when *T. delbrueckii* is already present. The results indicate that co-inoculation strategies, whether simultaneous or sequential, can both effectively leverage the aromatic potential of *T. delbrueckii*. However, the slight differences in aroma profiles between T1 and T2 could

reflect nuanced variations in how the yeast strains interact over the fermentation timeline, possibly influencing ester production rates or phenolic expression. The T3 sample, in which *T. delbrueckii* is introduced solely during bottle refermentation, exhibits an aroma profile that is more similar to the control (TC) than to T1 or T2. This suggests that bottle refermentation with *T. delbrueckii* has a restricted impact on the beer's volatile composition in comparison to the effect observed when *T. delbrueckii* is introduced during primary fermentation. The relatively high levels of "honey," "hiacetyl," and "oxidised" compounds in T3, coupled with the absence of elevated fruity and floral notes, indicate that *T. delbrueckii*'s ability to modulate aroma is greatly reduced when its activity is restricted to the bottle-conditioning phase.

From a brewing perspective, these results underscore the importance of fermentation timing and yeast strain selection in shaping beer aroma. *T. delbrueckii* significantly enhances the production of complex and desirable aroma compounds when introduced early in fermentation, suggesting that brewers can utilize this yeast strain in co-inoculation strategies to enhance beer complexity, especially in styles that benefit from pronounced esters and phenolic notes. Additionally, the suppression of oxidative and diacetyl related off-flavors in the *T. delbrueckii* inoculated samples may suggest a beneficial role for this yeast in improving beer stability and sensory quality. These findings are relevant for brewers aiming to create beers with greater aromatic complexity and reduced off-flavors, highlighting the potential of *T. delbrueckii* as a valuable yeast strain for innovation in brewing practices.

Ethics

The research was not approved by an ethical committee, in line with Italian law on not clinical studies. The study was conducted according to the principles established in the Declaration of Helsinki and the Code of Ethics & Standards for Sensory Project Managers defined by the Italian Sensory Science Society (https://scienzesensoriali.it/en/association/the-code-of-ethics-standards/). Informed consent was obtained from all subjects involved in the study.

CRediT authorship contribution statement

Cristina Restuccia: Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Formal analysis. **Vincenzo Alfeo:** Writing – review & editing, Visualization, Validation, Software, Methodology, Data curation. **Biagio Fallico:** Writing – review & editing, Validation, Supervision, Methodology, Formal analysis. **Ignazio Maria Gugino:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Data curation. **Gert De Rouck:** Visualization, Validation, Supervision, Formal analysis. **Fabrizio Cincotta:** Writing – review & editing, Software, Methodology, Data curation. **Lucia Paraf-ati:** Writing – review & editing, Writing – original draft, Visualization, Data curation. **Ilaria Proetto:** Writing – review & editing, Writing – original draft, Visualization, Data curation. **Aldo Todaro:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Antonino Pirrone:** Writing – review & editing, Visualization, Validation, Software, Methodology, Data curation. **Antonella Verzera:** Writing – review & editing, Validation, Methodology, Investigation, Data curation. **Marco Torre:** Writing – original draft, Data curation. **Rosa Palmeri:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Data curation. **Alessandra Currò:** Writing – original draft, Data curation.

Declaration of Competing Interest

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

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Data availability

Data will be made available on request.

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