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Utilization of *Saccharomycodes ludwigii* and *Torulaspora delbrueckii* in production of Ale-type beers with low alcoholic contents

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A B S T R A C T

This study aimed to produce and investigate Ale-type barley-spelt beers with low-alcohol levels by utilizing two different non-*Saccharomyces* yeasts, *Torulaspora delbrueckii* and *Saccharomycodes ludwigii*, and compare them to Ale-beers fermented with commercial Ale-yeast (*Saccharomyces cerevisiae*). The effects of both primary and bottle fermentation on sugar utilization, ethanol yield, and volatile compound profiles were examined. Volatile compound profile and contents were determined after primary and bottle fermentations with GC-MS. Wort contained high initial levels of maltose (62.5 g/L), maltotriose (42.3 g/L), fructose (22.0 g/L), and sucrose (17.9 g/L). Fermentation with *S. cerevisiae* resulted in extensive sugar consumption, leaving only small amounts of maltose, maltotriose and fructose. By contrast, *T. delbrueckii* and *S. ludwigii* retained most of the maltose and maltotriose, but no fructose was detected. *S. cerevisiae* produced the highest ethanol concentrations (3.39 % v/v), whereas *T. delbrueckii* and *S. ludwigii* yielded significantly lower levels (1.24 % v/v and <1.0 % v/v, respectively). *S. cerevisiae* increased the contents of many volatile compounds after both fermentation types, while only a few volatile compounds were detected in significant quantities in the non-*Saccharomyces* fermentations. Furthermore, non-*Saccharomyces* fermentations generated higher sulfur compound levels that could impact beer aging. In conclusion, *S. cerevisiae* demonstrated high efficiency in sugar assimilation and ethanol formation, while *T. delbrueckii* and *S. ludwigii* showed limited fermentation performance, indicating their potential in producing low-alcohol beers with potentially distinct and milder sensory properties.

1. Introduction

Craft breweries have become an important part of the brewing sector by introducing new beer styles and by using different raw materials and fermentation methods. Because of their small scale and flexibility, craft breweries can quickly respond to changing consumer expectations, toward more local, sustainable, or healthier products (Baiano, 2021; Gobbi et al., 2024). At the same time, the smaller breweries need to act to changing markets to survive in the competition against other craft breweries and large multinational breweries. One trend has been the increasing use of alternative and regionally grown raw materials.

Ale-type beers are among the most traditional and diverse beer categories, typically produced with top-fermenting *Saccharomyces cerevisiae* strains at 15–25 °C, yielding fruity and estery flavor profiles. This category includes numerous substyles such as Pale Ale, India Pale Ale (IPA), Porter, and Stout, which differ mainly in malt composition, hopping, and fermentation characteristics. Ale-style beers and their variations are especially popular among craft breweries because they allow rapid production cycles, recipe flexibility, and the creative use of local ingredients, enabling differentiation and adaptation to chances in consumer preferences (Carisma & Calingacion, 2025; Villacreces et al., 2022).

Another clear trend in the brewing industry is the growing interest in non-alcoholic and low-alcohol beers. Their global demand has increased rapidly, driven by consumer awareness of health, moderation, and responsible drinking. To achieve low ethanol contents while retaining desirable beer flavor, breweries employ several approaches, including limited or arrested fermentation, dealcoholization of finished beer by thermal or membrane techniques, and the use of maltose-negative or low-attenuating yeast strains. Craft breweries have also begun to experiment with these techniques to develop flavorful low-alcohol alternatives that align with modern health and lifestyle preferences (Bellut & Arendt, 2019; Brányik et al., 2012).

Old cereal varieties, such as spelt (*Triticum spelta* L.), have received interest in both research and practice, especially in specialty beers (Salanță et al., 2020). Spelt has a long agricultural history and is known for its characteristic flavor and good nutritional properties. It has increased interest in baking, pasta, and brewing, due to its nutritional

Abbreviations: SC, *S. cerevisiae*; TD, *T. delbrueckii*; SL, *S. ludwigii*; P, Primary fermentation; B, Bottle fermentation; K, Mashing batch.

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value, unique flavor, and possible health benefits. Spelt has similar starch, fiber, ash, and lipid content, but higher protein content, compared to common wheat (Deyalage et al., 2024). Spelt is not an easy raw material for beer processing due to its low malting quality (Suriano et al., 2025), but earlier studies has shown it can be used in brewing if combined with barley malt (Fujita et al., 2020; Muñoz-Insa et al., 2013). Differences in malt quality between the spelt varieties has also been reported (Suriano et al., 2025). Even though, the malting of spelt has been studied (Muñoz-Insa et al., 2013; Munoz-Insa et al., 2016a, 2016b), studies focusing on the beers made using spelt are scarce (Rübsam et al., 2013; Sterczyńska et al., 2021). Utilization of spelt malt with barley malt can lead to lower alcohol production, because of lowered sugar content (Laureys et al., 2023). Wheat addition and especially the amount of added wheat has been reported to have significant impact on the volatile compound composition and, thus, altering the aroma of the beer (De Flaviis, Santarelli, Giuliani, et al., 2024, 2024b). Furthermore, the sunstruck flavor is a well-known off-flavor in beers originating from hops. Formation of sunstruck flavor is affected by many compounds, mostly by humulones and their isomers. Other compounds can act as initiators or inhibitors. Use of spelt may have positive effect on the possible formation of sunstruck off-flavor by addition of inhibitor compounds to brew (Munoz-Insa et al., 2016a)

Yeast selection plays a key role in developing new types of beer, especially when aiming for low alcohol content. Traditionally only *Saccharomyces*-yeasts are used to ferment beer. Especially *S. cerevisiae* is utilized to ferment ale type beers and *S. pastorianus* to ferment lager beers. Growing trend of low and non-alcoholic beer consumption has enhanced research of utilization of non-conventional yeasts in beer fermentation. These alternative yeasts may naturally limit ethanol formation due to their weaker ability to ferment sugars of malts, such as maltose and maltotriose, while still forming interesting and pleasant aromas (Sileoni et al., 2023; Vaštík et al., 2022). *Torulaspora delbrueckii* has been more studied in wine fermentations (e.g., Ramírez and Velázquez (2018) and others) and beer fermentations because of its good fermentation abilities and known effects on the aroma complexity (Drosou et al., 2023; Michel et al., 2016b). In beers, *T. delbrueckii* has been presented notable lower alcohol production capacity as pure (Canonico et al., 2016; Mestre Furlani et al., 2024; Ogawa et al., 2022; Vaštík et al., 2022) or sequential fermentation with *S. cerevisiae* (Mestre Furlani et al., 2024) compared to *S. cerevisiae*. Some *T. delbrueckii* strains have reported to produce higher and more complex volatile compound contents compared to *Saccharomyces*-yeast fermentations (Mestre Furlani et al., 2024; Michel et al., 2016a), but also contrary results have been reported (Canonico et al., 2016; Ogawa et al., 2022; Vaštík et al., 2022). Callejo et al. (2019) study with non-*Saccharomyces* strains, especially with *T. delbrueckii,* showed potential them to be utilized in craft beer brewing and beer conditioning.

Saccharomycodes ludwigii is commonly used to produce low and non-alcoholic beers in some countries (Michel et al., 2016b). It has been considered as a spoilage yeast in wines because of its capability to grow in high SO_2 and ethanol concentrations. It has high fermentation activity and some strains can produce complex aroma profiles and low diacetyl contents to beers (De Francesco et al., 2015). *S. ludwigii* cannot use maltose sugars, the main wort sugars, making it excellent yeast to produce low or non-alcoholic beers (De Francesco et al., 2015; Jackowski et al., 2023; Michel et al., 2016b). The final ethanol content is depended on the used *S. ludwigii* strain and fermentation temperature, if wort composition is not considered (Michel et al., 2016b). Sileoni et al. (2023) investigated flavor stability of unpasteurized and cold-stored low-alcohol beer fermented with *S. ludwigii*, and the beer remained stable over time with preferred sensory properties.

This study aimed to produce and investigate Ale-type beers with low-alcohol levels by utilizing two different non-*Saccharomyces* yeasts, *S. ludwigii* and *T. delbrueckii*, and compare the results to Ale-beers fermented with commercial Ale-yeast in microbrewery facilities. Utilizing different grains with barley malt in beer production has gained popularity, and wheat beer is commonly found in stores. Spelt is an ancient wheat, scarcely utilized in beer production. In addition, the previous studies focus more on the malting properties of spelt and how it affects beer quality. To our best knowledge, this is the first research focusing on utilization of non-*Saccharomyces* yeasts to ferment barley-spelt beer. Our research presents changes in volatile compound composition of barley-spelt beer between primary and bottle fermented beers. Parts of the barley malts were substituted with spelt. To investigate sugar consumption of yeasts and produced ethanol contents, sugars were analyzed with liquid-chromatograph coupled with evaporative light scattering detector (HPLC-ELSD) and ethanol with gas-chromatograph coupled with flame ionization detector (GC-FID). The volatile compounds were analyzed using head space-solid phase microextraction with gas-chromatography-mass spectrometry (HS-SPME-GC-MS). The results of this study demonstrate how the selected non-*Saccharomyces* strains have potential in small-scale brewing, especially when there is no access to dealcoholizing equipment.

2. Materials and methods

2.1. Yeast strains

The study utilized three different yeast strains: *Saccharomycodes ludwigii* (**SL**; WSL17, Hefebank Weihenstephan, München, Germany), *Torulaspora delbrueckii* (**TD**; BIODIVA Level™ TD 291, Lallemand, Edwardstown, Australia), and *Saccharomyces cerevisiae* (**SC**; SafAle S-04, Fermentis, Lesaffre, Marcq en Baroeul, France). TD and SC yeasts were added in ration recommended by the producer: TD 0.25 g/L (corresponds 2.5×10^9 CFU/mL) of wort and SC 0.40 g/L (corresponds 4.0×10^9 CFU/ml), when 1 g contains 10^{10} CFU. Active dry yeasts were not pre-cultivated to imitate microbrewery environment and, thus the initial viable cell count was not measured. SL was pre-grown twice in yeast extract peptone dextrose (YPD) broth, because it was not active dry yeast, but it was provided in an agar tube. Briefly, 1 μL loop was used to scoop yeast from the agar tube and moved to 100 mL of YPD broth, grown 24 h at 22 °C temperature with 300 rpm continue mixing. Ten milliliters of yeast broth were moved to fresh 100 mL YPD broth and let to grow as previously. From the second pre-grow medium, 20 mL of broth (10^8 CFU/mL) was added to 2 L of wort.

2.2. Wort preparation

Beer samples were made in Kupittaa Campus Brewery located in the Turku University of Applied Sciences (Turku, Finland). The brewing was conducted using a pilot scale (20 L batch) brewing equipment in triplicate (K1, K2, or K3). All worts at an original gravity of 11°Plato. Pilsner (67.5%; Pilsner malt, EBC 3-4.5, Viking Malt, Lahti, Finland), Caramel Pale (12.5%; Caramel malt, EBC 8 ± 2, Viking Malt, Lahti, Finland) malts, and Organic spelt (20%; Birkkala, Suomusjärvi, Finland) malts were milled with malt mill. The mashing process was conducted using a 20 L Braumeister mashing- and boiling kettle (Speidels Braumeister, item No 47070, SPEIDEL Tank-und Behälterbau GmbH, Germany) equipped with temperature control capabilities. The malt was mixed with 25 L of water at 50 °C, mashing program included single step infusion mashing with 69 °C for 30 min to allow for enzymatic activity to convert starches into fermentable sugars. Then the mash was heated to 79 °C for mash out, held for 10 min to halt enzymatic processes. The wort was then lautered where the spent grains were separated from the liquid wort. The lautering process was carefully controlled to ensure a clear wort run-off, with a total of two sparges using hot water at 79 °C to extract maximum sugars. The clear wort collected was boiled for 60 min in Braumeister mashing/boiling kettle. The bitterness hops (Southern Passion hops, Humlegårdens Ekolager AB, Sweden) was added as pellets (25 g) to each 20 L batch 15 min before the end of the boil with target of 17 IBU. Prior the yeast pinching, the wort was quickly cooled to 20 °C using a plate heat exchanger. The cooling process was monitored to

ensure rapid temperature reduction to prevent any microbial contamination. The prepared wort was then transferred to fermenters under aseptic conditions to begin the fermentation process.

2.3. Yeast fermentations

Fermentations were carried out on a laboratory scale using 2-liter glass bottles equipped with airlock (1:1 water:ethanol) to ensure CO_2-outlet and inhibiting O_2-inlet at 22 °C. Two liters of cooled 20 °C wort were transferred to each fermentation bottle in two parts. Yeast was pitched directly in the fermentation vessel on the surface of the wort, using active dried yeast (SC and TD) or liquid yeast (SL). Yeast was added during the first part of the filling of the vessel and then added the rest of the wort on top to ensure good hydration of the dried yeast. Primary fermentations (**P** samples) were conducted for seven days, fermentation temperatures strictly controlled at 19 °C. Following the primary fermentation, the beers were conditioned in bottles for 2 weeks at 4 °C (**B** samples). All fermentations were done in duplicate from each prepared wort.

2.4. Analysis of ethanol

Ethanol content was determined using a Shimadzu Corp. (Kyoto, Japan) GC-2010Plus gas chromatograph (GC) equipped with a flame ionization detector (FID) as described before by (Saini et al., 2024). Before the analysis, the samples were filtered using 0.45 μm regenerated cellulose (RC) membrane filters. 0.2 μL of sample was injected in split mode with a split ratio of 1:25. Injection port temperature was set to 230 °C. The compound separation was performed using an HP-Innowax column (30 m length, 0.25 mm inner diameter, 0.25 μm film thickness, HewlettPackard, Avondale, PA, USA). Initial temperature of the column oven was set to 40 °C and held for 5 min, then increased at a rate of 40 °C/min to 240 °C and held for 5 min. FID temperature was set to 280 °C. Carrier gas was helium with a constant linear velocity of 35 cm/s. Ten-point calibration curve (R2 = 0.99) was constructed using standards between 0.11% and 7% (v/v).

2.5. Analysis of sugars

Beer samples were first centrifuged at 4500×*g* for 10 min at 22 °C. Then SC samples were diluted with acetonitrile:water (1:1, v/v) in 1:10 rate (v/v), and TD and SL samples in 1:100 rate. Prior to analysis, samples were filtered using 0.2 μm PTFE syringe filters. Five ten-point calibration curves (fructose, glucose, saccharose, maltose, and maltotriose) were prepared in 0.2–5.0 mg/mL range and analyzed.

Sugar composition was determined using an Ultra-High-Performance Liquid Chromatograph (UHPLC; Agilent Infinity II, Agilent Technologies Inc., Palo Alto, CA) consisting of a G7120A 1290 High Speed Pump, a G7129B 1290 Vial Sampler, a G7130A ICC Column Heater and a G1390B 1200 UIB II Universal Interface Box, equipped with an ELSD (ELSD-LT III; Shimadzu, Kyoto, Japan) by using method from Waters Co. (2019). The system was controlled using OpenLabs software. The method was based on an application brief published by the Waters Corporation in 2019. The mobile phases used were acetonitrile:water (80:20) containing 0.2% triethylamine (A) and acetonitrile:water (30:70) containing 0.2% of triethylamine (B). The following mobile phase B gradient was used: 10%, 0–15 min; 30%, 15–16 min; 30% 16–25 min; 10%; 25–25.5 min; 10%, 25.5–30 min. The flow rate was 0.13 mL/min. The compounds were separated using Acquity Premier BEH Amide 1.7 μm (100 × 2.1 mm; Waters Corporation, Milford, MA) column. The injection volume was 1.3 μL. The oven temperature was set to 35 °C. The gas pressure of ELSD was set to 350 kPa, the drift tube temperature was set to 40 °C, and the signal amplification factor was set to 4.

2.6. Analysis of volatile compounds

The samples were analyzed in triplicate using headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME-GC-MS). Two milliliters of each sample and 0.2 g of sodium chloride were placed in a 20 mL glass vial, and 10 μL of 4-methyl-2-pentanol solution (1 ppm in methanol) was added as an internal standard. The volatile compounds were extracted from the headspace with a 2 cm DVB/CAR/PDMS fiber (50/30 μm, Supelco, Bellefonte, PA, USA) at 45 °C for 30 min after 10 min of incubation. The fiber was conditioned at 250 °C prior to the sample extraction. After the extraction, the SPME fiber was immediately transferred to the injection port of a Trace 1310 gas chromatograph equipped with a ISQ 7000 mass spectrometer (Thermo Fisher Scientific, MA, USA) to be thermally desorbed in splitless mode at 240 °C for 5 min. A DB-WAX polar capillary column (60 m × 0.25 mm i.d. × 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA) was used to separate the volatile compounds of the samples. Helium was used as a carrier gas at a flow rate of 1.6 mL/min. The initial column temperature was set to 50 °C and held for 2.5 min. Afterwards, the temperature was increased to 240 °C at a rate of 3 °C/min and held at 240 °C for 3 min. Mass spectra were detected in electron impact (EI) mode at 70 eV, with a scan range from m/z 40 to m/z 350. The MS transfer line and the ionization source temperatures were 250 and 260 °C, respectively.

The RIs of the volatiles were calculated via co-injection with an alkane mixture (C7–C21, Sigma-Aldrich, St. Louis, MO, USA). Volatiles were identified by matching the obtained mass spectra with the standard NIST14 library and by comparing the retention indices (RIs) to those of the compounds reported in the literature and the NIST Webbook (https://webbook.nist.gov/chemistry). Moreover, the identification of a selected number of volatile compounds was confirmed by comparing the retention indices and mass spectra with those of the authentic reference compounds. The areas of identified peaks were collected and normalized with the area of internal standard peak. Table 1 shows the measured RIs and those reported in the literature and the occurrence of volatile compound in the beers fermented with different yeasts. normalized areas of identified volatile compounds. Table S1 includes the normalized areas of volatile compounds.

2.7. Statistical analyses

Unsupervised multivariate PCA and supervised PLS regression models were constructed for the volatile data with SIMCA v. 18.0.0 (Sartorius AG, Göttingen, Germany). For multivariate analysis, data was scaled with unit variance and mean centered. Differences of sugars, ethanol, and total amounts of volatile compound groups in samples were analyzed using ANOVA with Tukey's post-hoc test ($p < 0.05$; IBM SPSS software version 24, IBM Corp., Armonk, NY). Three-way ANOVA was used was used separately within beer samples to observe impact of wort, fermentation, yeast and their two-way interactions to ethanol and sugar contents.

3. Results and discussion

3.1. Differences in ethanol production

Various recent studies have reported that non-*Saccharomyces* yeasts are suitable for producing low alcoholic or non-alcoholic beer production (Jackowski et al., 2023; Mestre Furlani et al., 2024; Ogawa et al., 2022; Vaštík et al., 2022). In our study, two non-*Saccharomyces* yeast strains produced ethanol at statistically significantly lower levels than SC fermentations (Table 2). SL resulted in the lowest ethanol contents (0.77%), while TD resulted in lower contents (1.24%) compared to control yeast SC (3.39%). The findings are in accordance with Jackowski et al. (2023). However, their fermentations did not result as low ethanol content (2.58% with TD, 2.50% with SL compared to 2.95% with SC

Table 1
Identification and occurrence of volatile compounds in beer samples.

Compound	Abbreviation[a]	Meas. Ri[b]	Lite. Ri[c]	Identification method[d]	SCP[e]	SCB	TDP	TDB	SLP	SLB
Esters (EST)										
Ethyl acetate	EST1	881	887	RI, MS, STD	x	x	x	x	x	x
Ethyl propanoate	EST2	956	952	RI, MS	x	x	x	x	x	x
Propyl acetate	EST3	975	971	RI, MS	x	x	x	x	x	x
2-Methylpropyl acetate	EST4	1015	1014	RI, MS, STD	x	x	x	x	x	x
Ethyl butanoate	EST5	1036	1036	RI, MS	x	x	x	x	x	x
3-Methyl-1-butanol acetate	EST6	1122	1126	RI, MS, STD	x	x	x	x	x	x
Ethyl hexanoate	EST7	1235	1248	RI, MS, STD	x	x	x	x	x	x
Hexyl acetate	EST8	1274	1276	RI, MS, STD	x	x	x	x	x	
Ethyl (E)-3-hexenoate	EST9	1302	1292	RI, MS	x	x	x	x		
Ethyl heptanoate	EST10	1335	1342	RI, MS	x	x	x	x	x	x
2-Methylpropyl hexanoate	EST11	1355	1353	RI, MS	x	x				
Heptyl acetate	EST12	1376	1377	RI, MS	x	x	x	x	x	
Unknown ethyl ester	EST13	1391		RI, MS	x	x	x	x		
Methyl octanoate	EST14	1392	1387	RI, MS, STD	x	x	x	x	x	x
Ethyl octanoate	EST15	1437	1446	RI, MS	x	x	x	x	x	x
3-Methylbutyl hexanoate	EST16	1462	1460	RI, MS, STD	x	x				
Octyl acetate	EST17	1478	1475	RI, MS	x	x	x	x	x	x
Propyl octanoate	EST18	1522		MS	x	x				
Ethyl nonanoate	EST19	1539	1545	RI, MS	x	x	x	x	x	x
2-Methylpropyl octanoate	EST20	1555	1551	RI, MS	x	x				
Ethyl (3E)-3-nonenoate	EST21	1588		MS	x	x	x	x		x
Methyl decanoate	EST22	1598	1599	RI, MS	x	x	x	x	x	x
3-(Methylthio)propyl acetate	EST23	1634	1627	RI, MS	x	x	x	x	x	
Ethyl decanoate	EST24	1642	1639	RI, MS, STD	x	x	x	x	x	x
3-Methylbutyl octanoate	EST25	1662	1658	RI, MS	x	x	x	x		x
Ethyl 9-decenoate	EST26	1695		MS	x	x	x	x	x	x
Ethyl undecanoate	EST27	1745	1737	RI, MS	x	x				
Methyl dodecanoate	EST28	1806	1804	RI, MS	x	x	x	x	x	x
2-Phenylethyl acetate	EST29	1822	1813	RI, MS, STD	x	x	x	x	x	x
Ethyl dodecanoate	EST30	1848	1843	RI, MS	x	x	x	x	x	x
3-Methylbutyl decanoate	EST31	1867		MS	x	x	x	x	x	x
Ethyl hexadecanoate	EST32	2249	2260	RI, MS	x	x	x	x	x	x
Ethyl 9-hexadecenoate	EST33	2275	2283	RI, MS	x	x	x	x	x	x
Alcohols (ALC)										
Propanol	ALC1	1043	1046	RI, MS	x	x	x	x	x	x
2-Methyl-1-propanol	ALC2	1100	1091	RI, MS, STD	x	x	x	x	x	x
2-Methyl-1-butanol	ALC3	1214	1209	RI, MS	x	x	x	x	x	x
3-Methyl-1-butanol	ALC4	1216	1214	RI, MS, STD	x	x	x	x	x	x
Pentanol	ALC5	1257	1256	RI, MS, STD			x	x	x	x
Hexanol	ALC6	1361	1359	RI, MS	x	x	x	x	x	x
6-Methyl-2-heptanol	ALC7	1382	1379	RI, MS	x	x	x	x	x	x
3-Ethoxy-1-propanol	ALC8	1384	1389	RI, MS	x	x	x	x	x	x
1-Octen-3-ol	ALC9	1456	1454	RI, MS, STD	x	x	x	x	x	x
Heptanol	ALC10	1463	1461	RI, MS, STD	x	x	x	x	x	x
2-Nonanol	ALC11	1527	1525	RI, MS	x	x	x	x	x	x
Octanol	ALC12	1566	1564	RI, MS, STD	x	x	x	x	x	x
[S-(R*,R*)]-2,3-butanediol	ALC13	1588	1584	RI, MS						
2-Furamethanol	ALC14	1669	1661	RI, MS	x	x	x	x	x	x
3-(Methylthio)-1-propanol	ALC15	1728	1721	RI, MS	x	x	x	x	x	x
Decanol	ALC16	1772	1768	RI, MS	x	x	x	x	x	x
Phenylethyl aolcohol	ALC17	1923	1926	RI, MS, STD	x	x	x	x	x	x
Aldehydes (ALD)										
Acetaldehyde	ALD1	732	712	RI, MS	x	x	x	x	x	x
3-Methyl butanal	ALD2	915	923	RI, MS	x	x	x	x	x	x
Hexanal	ALD3	1080	1080	RI, MS, STD	x	x	x	x	x	x
2-Methylbenzaldehyde	ALD4	1651		MS		x	x	x	x	x
Volatile acids (VAC)										
Acetic acid	VAC1	1461	1459	RI, MS			x	x	x	x
2-Methyl propanoic acid	VAC2	1582	1571	RI, MS	x	x	x	x	x	x
Hexanoic acid	VAC3	1863	1860	RI, MS, STD	x	x	x	x	x	x
Octanoic acid	VAC4	2078	2083	RI, MS, STD	x	x	x	x	x	x
Decanoic acid	VAC5	2283	2276	RI, MS, STD	x	x	x	x	x	x
Terpenes (TER)										
β-Myrcene	TER1	1160	1165	RI, MS, STD			x	x	x	x
Linalool	TER2	1553	1549	RI, MS, STD	x	x	x	x	x	x
Humulene	TER3	1673	1677	RI, MS			x	x	x	x
Methyl geranate	TER4	1699	1701	RI, MS	x	x	x	x	x	x
Geranyl acetate	TER5	1761	1752	RI, MS	x	x	x	x	x	
Citronellol	TER6	1775	1773	RI, MS	x	x	x	x	x	x
β-Damascenone	TER7	1829	1838	RI, MS, STD	x	x	x	x	x	x
(E)-Nerol	TER8	1857	1851	RI, MS		x	x	x	x	x
Humulenen epoxide I	TER9	2027	2045	RI, MS	x	x	x	x	x	x
(E)-Nerolidol	TER10	2047	2047	RI, MS	x	x	x	x	x	x

(*continued on next page*)

Table 1 (*continued*)

Ketones (KET)										
3-Hydroxy-2-butanone	KET1	1289	1287	RI, MS	x	x	x	x	x	x
6-Methyl-5-heptene-2-one	KET2	1340	1341	RI, MS, STD	x		x	x	x	x
Others (OTH)										
Dimethyl disulphide	OTH1	754	734	RI, MS	x	x	x	x	x	x
Styrene	OTH2	1256	1254	RI, MS	x	x	x	x	x	x
1,1-Dimethyl-4-methylenehexahydro-1H-cyclopenta[c]furan (Hop ether)	OTH3	1371	1375	RI, MS	x	x	x	x	x	x
2-Methoxy-4-vinylphenol	OTH4	2200	2200	RI, MS	x	x	x	x	x	x
2,4-Di-tert-butylphenol	OTH5	2306		MS	x	x	x	x	x	x
Internal standard										
4-Methyl-2-propanol		1174	1168	RI, MS						

a Abbreviations used in Fig. 2 and S1; b Retention index (RI) calculated according to Kovat's equation in a DB-WAX column; c; d STD identification by comparison of GC and mass spectra with reference compounds, MS tentatively identified by library mass spectral match, RI identification by comparison of calculated and reference literature RI; e x marking occurrence in two out of three brews. SC *S. cerevisiae*, TD *T. delbrueckii*, SL *S. ludwigii*, K brew replicate, P primary fermentation, B bottle fermentation.

Table 2
Contents of ethanol (%) and sugars (g/L) in the beer and wort samples.

		Ethanol (v/v)	Maltose	Maltotriose	Glucose	Fructose	Sucrose	Sum sugars	pH
Wort		n.d.	62.51 ± 5.07c	42.25 ± 0.35b	31.82 ± 0.24b	22.02 ± 0.03b	17.86 ± 0.12	176.5	5.68 ± 0.04c
Beers (P and B)	SC	3.39 ± 0.65c	4.23 ± 0.22a	5.15 ± 0.47a	2.77 ± 1.29a	2.46 ± 0.29a	n.d.	14.6	4.53 ± 0.07a
	TD	1.24 ± 0.68b	54.91 ± 8.70b	42.04 ± 0.39b	n.d.	n.d.	n.d.	97.0	4.59 ± 0.10a
	SL	0.77 ± 0.23a	65.41 ± 6.75c	42.31 ± 0.51b	n.d.	n.d.	n.d.	107.7	4.71 ± 0.04b
Primary (P)	SC	3.33 ± 0.67c	4.34 ± 0.21a	5.28 ± 0.53a	n.d.	2.24 ± 0.02	n.d.	11.9	4.50 ± 0.06a
	TD	1.48 ± 0.77b	57.04 ± 8.52b	42.07 ± 0.32b	n.d.	n.d.	n.d.	99.1	4.60 ± 0.12 ab
	SL	0.82 ± 0.26a	67.84 ± 7.12b	42.40 ± 0.39b	n.d.	n.d.	n.d.	115.7	4.69 ± 0.01b
Bottle (B)	SC	3.46 ± 0.66b	4.11 ± 0.16a	5.01 ± 0.38a	2.77 ± 1.29	2.69 ± 0.27	n.d.	9.1	4.57 ± 0.06a
	TD	1.01 ± 0.50a	52.79 ± 8.58b	42.00 ± 0.48b	n.d.	n.d.	n.d.	94.8	4.58 ± 0.09a
	SL	0.73 ± 0.20a	62.99 ± 5.53b	42.22 ± 0.38b	n.d.	n.d.	n.d.	105.2	4.74 ± 0.04b
Brew batch (K)		ns	<0.001	ns	ns	ns	-		<0.001
Fermentation(P vs B)		ns	0.004	ns	-	<0.001	-		ns
Yeast		<0.001	<0.001	<0.001	-	-	-		<0.001
K*F		ns	ns	ns	-	ns	-		ns
K*Y		ns	0.005	ns	-	-	-		ns
F*Y		ns	ns	ns	-	-	-		ns

n.d., not detected; SC, *S. cerevisiae*; TD, *T. delbrueckii*; SL, *S. ludwigii*. Sum of sugars is based on means of individual compounds. Wort compared to beers in general using one-way ANOVA; three-way ANOVA (brew K, fermentation F, yeast Y and their two-way interactions) was used separately within beer samples (ns, not significant at $p < 0.05$). Tukey's posthoc test was used to compare the yeasts in general and the wort brews or within primary or bottle fermentations, and the differences are shown with letters a-c. F- values (and the degrees of freedom) of the ANOVA models are shown in Table S2.

strain) as in our study. Using *S. ludwigii*, it is possible to get as low as 0.50% ethanol content when temperature is 10 °C (Vaštík et al., 2022). Drosou et al. (2023) used the same *S. cerevisiae* and *T. delbrueckii* strains as in our study to produce Pale ale beer. In their study, they observed similarly that *T. delbrueckii* produced lower level of ethanol compared to *S. cerevisiae*.

3.2. Differences in attenuation

Contents of maltose, maltotriose, glucose, fructose and sucrose were determined in the wort and beer samples after the primary (P) and bottle (B) fermentations. The results showed clear differences in how each yeast strain fermented the initial wort sugars (Table 2). Further analysis using a three-way ANOVA model showed that the yeast selection was the main factor contributing to the differences among the samples.

SL showed the most restricted sugar metabolism (Table 2), fermenting only the monosaccharides and leaving maltose and maltotriose unfermented. This behavior was expected for SL, because *S. ludwigii* is known for its maltose-negative properties (De Francesco et al., 2015; Gutiérrez et al., 2018; Myncke et al., 2025). SL resulted in the highest levels of residual sugars (Table 2), which is consistent with earlier reports (Jackowski et al., 2023). These results highlight how differences in sugar metabolism among yeast species can be strategically used to control ethanol yield and residual sweetness in brewing. TD efficiently metabolized the simple sugars but left substantial amounts of maltose and nearly all maltotriose. This is consistent with previous findings that its maltose utilization varies between strains and depends on fermentation conditions (Canonico et al., 2016; Ogawa et al., 2022; Vaštík et al., 2022). TD left a notable high concentration of residual maltose (~5.9 g/L) after primary fermentation. Previous studies have shown that maltose utilization in TD strains can vary considerably depending on the exact strain and fermentation conditions (De Francesco et al., 2015; Michel et al., 2016b). In addition, this *T. delbrueckii* strain has reported to have prolonged lag phase, if maltose is present (Drosou et al., 2023). It is worth noting that in our setup, the fermentation temperature was only monitored but not actively controlled, which may have affected the sugar metabolism particularly in the TD and SL strains, whose performance has been reported to be sensitive to fermentation temperature and stress conditions (Michel et al., 2016b; Vaštík et al., 2022). Variability in temperature between fermentations may therefore partly explain the higher residual maltose levels observed compared to those reported in some previous studies.

As expected, SC fermented almost all the available sugars (Table 2). The total residual sugar content was only 11.84 g/L, which is consistent with previous reports of high attenuation and efficient consumption of the main wort sugars (De Francesco et al., 2015; Michel et al., 2016b). On the other hand, residual sugars potentially leave more body and sweetness in the final beer product. These results confirm that SC is a good choice for full attenuation, whereas TD and SL have potential to be used for low- or non-alcoholic beers where some sweetness may be a desired property (Kelanne et al., 2025). SL especially shows promise for non-alcoholic beer production due to its consistent maltose-negative

character.

3.3. Volatile compounds in beer samples

A total of 76 volatile compounds were identified in the beer samples. Of these compounds 33 were esters, 4 aldehydes, 17 higher alcohols, 5 volatile acids, 10 terpenes, and 5 classified as others. Three yeasts showed some differences in the volatile compound profile and content (Table 1). On average, TDB showed the highest number of volatile compounds (70), following both fermentation types of SC (69), TDP (68), SLP (66), SLB (65).

In this study, spelt beers were fermented from three replicated brews. However, beers from the third brew showed notable difference in multivariate analysis done with PCA (Fig. S1) and, thus, beer samples fermented from the third brew were further excluded from the result discussion. Batch-to-batch issues are common in small and microbreweries, which do not have similar resources as large breweries. In addition, adjuncts, such as spelt in our study, and other ingredients may cause unpredictability in brewing.

3.3.1. Volatile compounds originating from spelt

Twenty percent of unmalted spelt was used as adjunct with barley malt in brewing. Addition of spelt has likely affected significantly the volatile compound profile of studied beer samples. Utilization of spelt in beers has been only scarcely studied and most of the studies are focused on the effects of the wheat adjuncts on the components in mash. De Flaviis et al. (2024a, 2024b) reported that the addition of wheat drastically changed the volatile compound composition of beer and has a high impact on the sensory properties. In addition, they reported that changed wheat concentration was the main source of volatile compound variation and that the wheat variety affected the observed odor active volatiles in Blanche beers.

The increment of unmalted wheat has been reported to lead increased levels of longer-chain ethyl esters, such as ethyl heptanoate, ethyl nonanoate, and ethyl decanoate (De Flaviis et al., 2024a), which were all detected in all beer types in our study. In addition, ethyl 9-decenoate, ethyl dodecanoate, ethyl hexadecanoate, and ethyl 9-hexadecenoate were detected in all beer samples, and ethyl undecanoate in SC samples. However, adjunct spelt malt may have different effects on the volatile compound composition due to the different amino acid content and composition compared to common wheat (Laureys et al., 2023).

3.3.2. The total volatile contents and volatile profile

Both SC fermentations have statistically significantly higher concentration of every volatile group, except aldehydes and ketones, compared to TD and SL fermentations (Fig. 1).

Esters are fruity and floral aroma compounds in many food products. Esters are formed by alcohol and acid condensation. Acetates are formed by the reaction of acetic acid and higher alcohols (Olaniran et al., 2017). The contents of acetates in wine has reported to depend on the precursor content in grape must (Dennis et al., 2012). In beers, esters are mostly produced by yeast during primary fermentation. Raw material, original wort gravity, and yeast affect the final ester composition and content in beer (Olaniran et al., 2017). Furthermore, De Flaviis et al. (2024a) observed that contents of longer-chain ethyl esters increased with the higher unmalted wheat concentration in wort. In our study, the total ester content was 24-times higher in SC compared to SL and 9-times higher compared to TD after bottle fermentation. Similarly, the total acetate contents in SC bottle fermented beers were 10-times and 4-times higher compared to SL and TD, respectively. Similar results were reported by Canonico et al. (2016), comparing *S. cerevisiae* fermented beers to *T. delbrueckii,* and Bellut et al. (2018), comparing *S. cerevisiae* fermented beers to *T. delbrueckii* and *S. ludwigii* fermented ones. However, Mestre Furlani et al. (2024) reported contrary results with *T. delbrueckii* isolated from enological environments. They reported more than double total amount of studied esters after *T. delbrueckii* fermentation compared to *S. cerevisiae*. In addition, ethyl and acetyl transferase activity has been reported different between the strains of *S. ludwigii* (De Francesco et al., 2015). In our study, the total ester contents decreased after bottle fermentation, contrary to the total acetate contents which increased after bottle fermentation with TD and SL. The increase of acetate esters may be caused by higher acetic acid content (Table S1) in TD and SL fermentations. However, SC fermentations had also more different individual acetate esters and statistically significantly ($p < 0.05$) higher total content of acetates compared to either of non-*Saccharomyces* fermentation (Fig. 1). In addition, acetic acid was not present after SC fermentations (Table 1), but it was present after primary and bottle fermentations with non-*Saccharomyces* yeasts. This may indicate that used SC had higher alcohol acetyl transferase activity compared to used non-*Saccharomyces* yeasts (Etschmann et al., 2008), leading to less acetic acid and higher acetate contents and possible more fruity flavors in beer. However, Satora and Pater (2023) reported contrary results: they observed higher amounts of acetate esters in *T. delbrueckii* fermented beers compared to studied *S. cerevisiae*. Furthermore, clear difference between the yeasts was observed in the ester profiles: in average, SC samples had 34 esters after both fermentations (Table 1), TD fermentations had on average 28 and 30 esters after primary and bottle fermentations, respectively, and SL fermentations in average 26 and 24 esters, respectively.

Yeast produces higher alcohols from many different sources, e.g. amino acids, sugars, and lipids are precursors for higher alcohols. Thus, the raw material has high impact on the composition and total content of higher alcohols (Olaniran et al., 2017). Many studies has also shown that yeast specie (Ogawa et al., 2022; Rodríguez Madrera et al., 2021; Satora

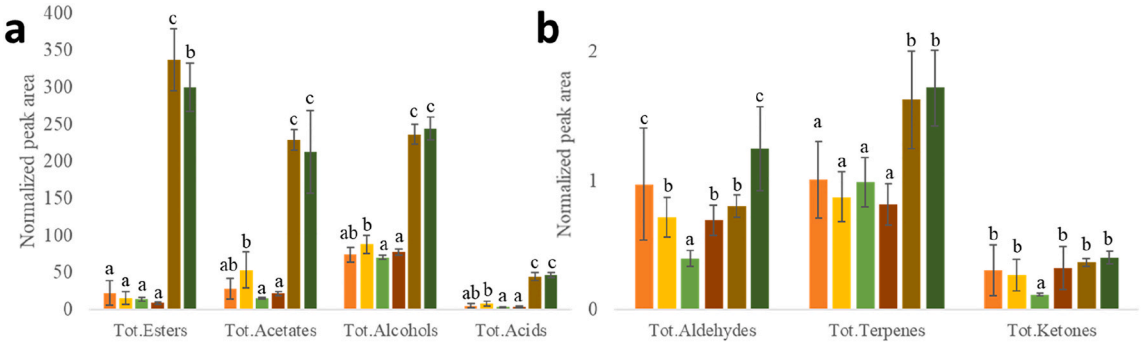

Fig. 1. The total volatile compounds content presented in sum of normalized peak areas and averaged by biological replicates (n = 2) in the beer samples (n = 2 brews × 3 yeasts × 2 fermentation schemes=12) fermented from brew 1 and 2. Different letter inside the volatile compound group means statistically significant difference calculated by ANOVA and Tuckey's test ($p < 0.05$; F-values in Table S3). Orange *T. delbrueckii* primary fermentation, yellow *T. delbrueckii* bottle fermentation, light green *S. ludwigii* primary fermentation, dark brown *S. ludwigii* bottle fermentation, light brown *S. cerevisiae* primary fermentation, dark green *S. cerevisiae* bottle fermentation.

and Pater, 2023) and strain (Canonico et al., 2016; De Francesco et al., 2015; Satora and Pater, 2023) impact on the composition and contents of higher alcohols. Higher alcohols affect the beer flavor by making it more harmonious and making beer more full-bodied (Sun and Xiao, 2018). In our study, SC fermentations had statistically significantly ($p < 0.05$) higher contents of total higher alcohols compared to non-*Saccharomyces* fermentations (Fig. 1). Furthermore, TDB had statistically significantly higher content of total higher alcohols compared to SL fermentations. Pentanol and 2,3-butanediol were not present in SC (Table S1). 2,3-butanediol, as well as 3-hydroxy-2-butanone, is a reduction product of 2,3-butanedione (*sweet*, *butter*; not detected), which has notable lower flavor detection threshold compared to 2, 3-butanediol and, thus, can cause undesirable flavor properties in lower concentration. Einfalt (2021) reported contrary results for 2, 3-butanediol production compared to ours. In their study, *T. delbrueckii* produced significantly lower amount of 2,3-butanediol compared to *S. cerevisiae* and *Metschnikowia pulcherrima* during barley-sorghum beer fermentation.

Volatile acids are also produced via many metabolomic pathways during alcoholic fermentation. Commonly volatile acids have relative high detection thresholds, and they cause different kind of flavors, such as *waxy*, *goaty*, and *soapy* (Michel et al., 2016b). Volatile acids take part in the ester formation, making them important flavor precursors. SC fermentations had statistically significantly higher amount of total volatile acids compared to non-*Saccharomyces* fermentations (Fig. 1). Previously, *S. ludwigii* strains has reported to produce significantly less some volatile acids compared to *S. cerevisiae* (Rodríguez Madrera et al., 2021) and the same *T. delbrueckii* as used in our study to produce statistically significantly less studied volatile acids compared to other *T. delbrueckii* strain (Toh et al., 2020). Acetic acid was the only volatile acid not found in the SC samples (Table 1). The wort composition impacts to acetic acid content. Li and Liu (2015) reported increased rice mash proportion in wort to decrease acetic acid content in bottom-fermented beer. In our case, the added spelt malt may have affected the acetic acid content. In addition, used SC may have more acetyl transferase activity resulting in higher acetate ester content and lower acetic acid content. All volatile acids were present in beers fermented with non-*Saccharomyces* yeasts. Hexanoic, octanoic, and decanoic acids were detected in statistically significantly ($p < 0.05$) higher concentration in SC samples compared to TD and SL beers.

Aldehydes affect the sensory properties and stability of beer. Barley variety, malting, and brewing conditions affect the formation of aldehydes, which originate, among others, from amino acids, proteins, and reducing sugars. Aldehydes have low flavor thresholds, making them easily detected by humans (Filipowska et al., 2021). The composition and contents of aldehydes can be adjusted, for example, by replacing part of the malt with adjuncts, such as rice and corn (Maia et al., 2023). During the alcohol fermentation, yeast reduces aldehydes to their corresponding higher alcohols, reducing aldehydic off-flavors (Piornos et al., 2023). Total aldehyde contents had statistically significant ($p < 0.05$) differences between the beer samples (Fig. 1). Surprisingly, TDP sample was the only primary fermentation sample with higher total aldehyde content compared to corresponding bottle fermentation. SLP had the lowest total aldehyde content and statistically significantly lower than corresponding bottle fermented sample. SCB had the highest total amount of aldehydes. The main aldehyde in all samples was acetaldehyde. Acetaldehyde is undesirable carbonyl compound produced during beer fermentation. It is product of sugar metabolism and it is converted mostly to ethanol during the fermentation (Michel et al., 2016b). 3-Methylbutanal is one of the aldehydes giving wort off-flavor to beer (Gernat et al., 2020). TDP had statistically significantly ($p < 0.05$) higher content of 3-methylbutanal compared to TDB and SL samples.

3.3.3. Comparison of volatile compounds in beers by Saccharomyces and non-Saccharomyces yeasts

Principal component analysis (PCA) model was constructed with the beer samples from two replicated fermentations from the first two brews as samples ($n = 24$) and volatile compounds as variables ($n = 75$; ALC13 excluded) to further observe differences between the samples (Fig. 2a). First two components explained 77.1% of data variance (PC1 70.4% and PC2 6.9%), showing good explanation rate. PC1 separated SC samples from TD and SL. In addition, SCB samples are clearly separated from the SCP samples on PC2. Even though, primary and bottle fermentations of TD and SL are not so clearly separated on different sides of PC2, they show similar separation pattern as SC samples and primary fermentations are located upper and bottle fermented on lower part of PC2. Still, especially both types of TD samples are located near to each other on PC2 compared to SL and SC samples, indicating less changes happened during TD bottle fermentations compared to other two yeasts.

Most of the volatile compound variables are positively correlating with SC samples on the Loadings plot (Fig. 2a). The most distinct compounds separating primary and bottle fermented SC samples were hexanal, 2-methylbenzaldehyde, humulene epoxide I, ethyl (3E)-3-nonenoate, and ethyl nonanoate correlating with primary fermentation, and 2,4-di-tert-butylphenol, acetaldehyde, ethyl 9-decenoate, styrene, 6-methy-2-heptanol, and 2-methylpropanoic acid correlating with bottle fermented samples (Fig. 2a).

Certain variables show positive correlation to TD and SL samples in PCA (Fig. 2a): (E)-nerol, β-myrcene, and humulene are positively correlating with SLP from both brews, K2TDP, K1TDB, and with one SLB from both brews on the negative side of PC2. This indicates that primary fermentation, especially with SL, has preserved the terpenes, but the second fermentation in bottles has decreased all terpene contents. Generally, these three terpenes are not present in the SC fermented samples (Table 1). Pentanol, 3-ethoxy-1-propnaol, 3-(methylthio)-1-propanol, acetic acid, heptanol, dimethyl disulfide, hexanol, and 2-methoxy-4-vinylphenol are clearly correlating with three primary and bottle TD fermentations and two SL bottle fermentations on the positive side of PC2.

2-Methoxy-4-vinylphenol, 4-vinylphenol, and styrene are produced from free phenolic acids by the yeast during fermentation. In other type beers than wheat beer, 2-methoxy-4-vinylphenol and 4-vinylphenol are considered as phenolic off-flavors (Rahman et al., 2020) and styrene is reported to have a significant negative impact on consumer liking of beer (Gonzalez Viejo et al., 2019). 2-Methoxy-4-vinylphenol has a *clove-like* and *spicy* aroma notes and it is especially characteristic volatile compound for wheat beers (Langos et al., 2013). In our study, 20% of malt was spelt, which is considered as ancient wheat. The increased wheat content has been reported to result in increased 2-methoxy-4-vinylphenol content (De Flaviis et al., 2024a). Langos and Granvogl (2016) studied different *S. cerevisiae* strains in different process steps of wheat beer. They observed that *S. cerevisiae* strains have different enzymatic activity rates to produce 2-methoxy-4-vinylphenol and 4-vinylphenol. They also studied production of styrene, which yeast is enzymatically produced from cinnamic acid. They further observed that the production rate of styrene was linked to the production rates of 2-methoxy-4-vinylphenol and 4-vinylphenol. In our study, styrene is positively correlating with SCB samples in PCA (Fig. 2a), but no statistically significant difference was observed with one-way ANOVA ($p < 0.05$) between all samples. In addition, 2-methoxy-4-vinylphenol had a clear positive correlation with TDB samples in PCA, but no statistically significant difference between all samples. This indicates that TD used in this study has a high enzymatic activity towards ferulic acid conversion to 2-methoxy-4-vinylphenol and low production rate for styrene and, thus, to be suitable for wheat beer fermentation. Drosou et al. (2023) also reported that the same *T. delbrueckii* strain to have high 2-methoxy-4-vinylphenol production rate. 4-Vinylphenol was not detected in our study.

Sulfur compounds in beer originate from raw material (malt, hops)

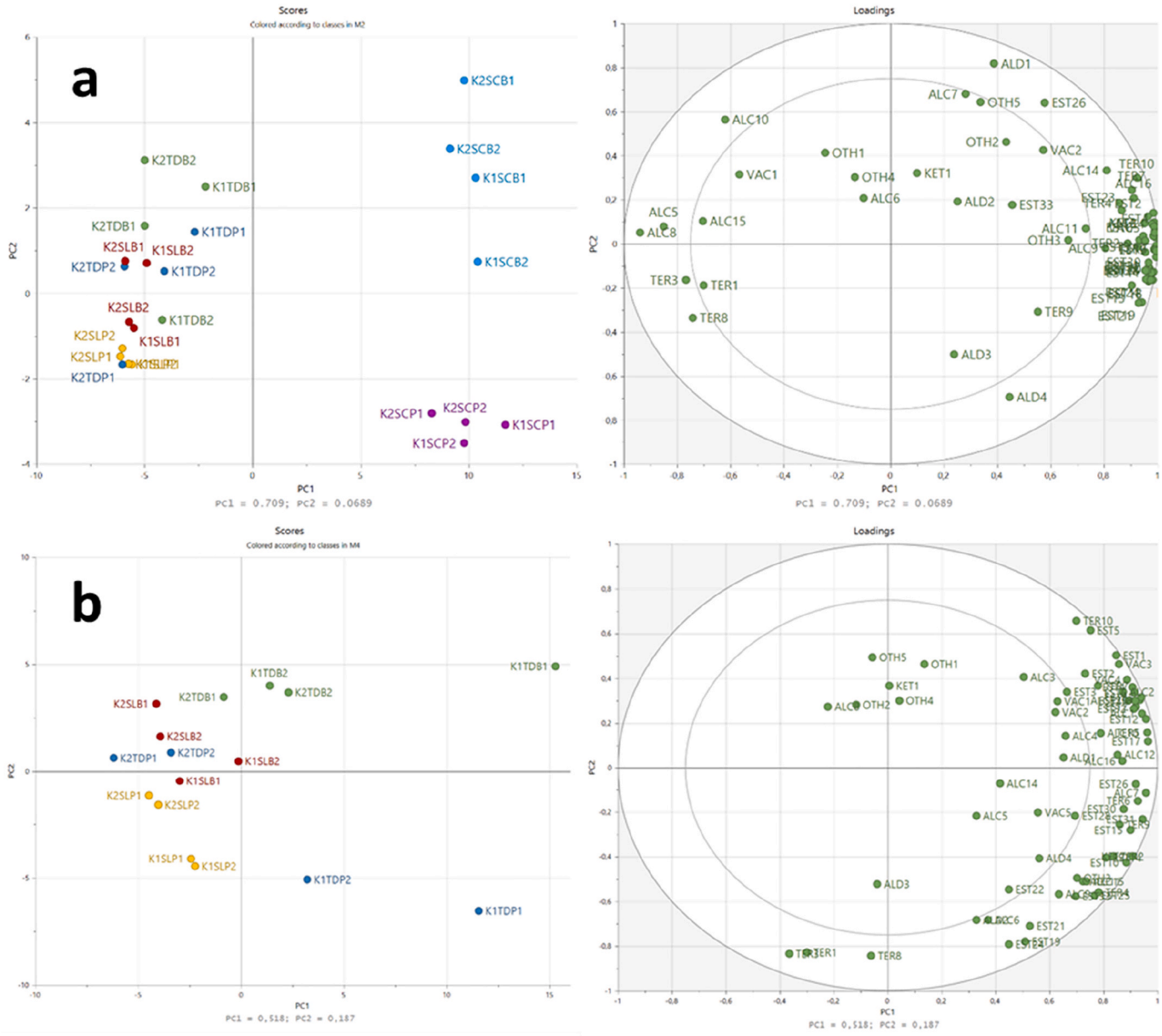

Fig. 2. Principal component analysis constructed with a) all primary and bottle fermented beers as samples (n = 24) and volatile compounds as variable (n = 75; ALC13 was excluded), or b) non-*Saccharomyces* yeast fermented primary and bottle fermented beers as samples (n = 16) and volatile compounds as variable (n = 68; EST9, 11, 13, 16, 18, 20, and 22 were excluded). Abbreviations refer to Table 1.

or yeast metabolism during fermentation. Sulfur compounds can have positive or negative effect on the sensory properties of beer. They have low flavor detection threshold making them easily be detected by human but not by instrument (Olaniran et al., 2017; Rettberg et al., 2018). The flavor threshold of dimethyl disulfide is reported as low as 7.5 μg/L and it was described as *sulfurous*, *vegetable*, and *onion* by Miracle et al. (2005). In our study, dimethyl disulfide positively correlates mostly with bottle fermented TD samples on PCA (Fig. 2a). However, it is detected in some rate in every sample (Table 1). Satora and Pater (2023) reported similar results when they observed that *T. delbrueckii* produced dimethyl disulfide in beer fermentation, but *S. cerevisiae* did not produce any. Other sulfur compounds detected in our study were 3-(methylthio)-1-propanol and 3-(methylthio)-1-propyl acetate, which are both derived from methionine, a sulfur amino acid (Etschmann et al., 2008). 3-(Methylthio)-1-propanol is one of the precursors of dimethyl trisulfide, a fresh onion-like aroma compound, in aged beers (Gijs et al., 2000). *S. cerevisiae* strain (Etschmann et al., 2008), fermentation condition (Sun et al., 2024), and raw material composition (Etschmann et al., 2008) have reported to affect production rate of 3-(methylthio)-1-propanol and 3-(methylthio)-1-propyl acetate. Here, 3-(methylthio)-1-propanol was positively correlating with primary fermented non-*Saccharomyces* yeast and 3-(methylthio)-1-propyl acetate with bottle fermented SC samples (Fig. 2a). In addition, statistically significant difference ($p < 0.05$) in 3-(methylthio)-1-propanol content was detected between TDP or SLP and the other samples and SCB had statistically significantly lower amount of 3-(methylthio)-1-propanol compared to other samples. Furthermore, 3-(methylthio)-1-propyl acetate content is statistically significantly ($p < 0.05$) higher in SCP and SCB samples compared to the non-*Saccharomyces* samples. This result indicates that used non-*Saccharomyces* yeasts produce more sulfur compounds to the beers, which may have negative effect on the sensory properties, especially after bottle aging.

Terpenes in beer originates mostly from hops (Rettberg et al., 2018). In our study, β-myrcene, humulene, and (E)-nerol, were only detected

after non-*Saccharomyces* yeast fermentation, and after primary fermentations higher contents were detected compared to bottle fermentations (Table S1). On the other hand, all other terpenes were positively correlated with SC samples (Fig. 2a), indicating higher concentrations in these samples. Linalool was detected in the highest concentration of all terpenes in all samples. β-damascenone was one of the terpenes positively correlating with bottle fermented SC samples. It has been previously reported as one of the key aroma compounds in beer, giving beer *sweet*, *fruity*, and *plum* aromas (Satora and Pater, 2023). In the same study by Satora and Pater (2023), humulene and methyl geranate were not detected after yeast fermentations, but they were detected in the hopped wort. However, in our study, both terpenes were detected after non-*Saccharomyces* yeast fermentations. Yeasts are able to convert terpenes to other terpenes during the fermentation, for example humulene is converted to caryophyllene (King and Dickinson, 2003). However, caryophyllene was not detected in any of our samples, indicating used yeasts may have converted it further. Furthermore, yeasts can convert geraniol to citronellol (King and Dickinson, 2003). In our study, citronellol was detected in all sample types. In the PCA (Fig. 2a), it positively correlates with K1SCB samples, and the citronellol concentrations were approx. 4–8 times lower in non-*Saccharomyces* yeasts fermented beers (Table S1). Similar results were reported by King and Richard Dickinson (2000). They studied biotransformation of certain monoterpenes by *S. cerevisiae*, *T. delbrueckii*, and *Kluyveromyces lactis*. They observed that *S. cerevisiae* was able to convert geraniol to citronellol during fermentation, whereas *T. delbrueckii* was not.

Geranyl esters are reported to be in higher concentration in late-hopped lagers (King and Dickinson, 2003). Yeasts are also reported to produce esterified terpenes. However, King and Dickinson (2003) reported difference in esterification of terpenes by lager- and ale-yeasts. They observed that used Ale-yeast strain could not produce esterified terpenes, such as geranyl acetate and methyl geranate, but Lager-yeast produced them. In our study contrary results were observed, when Ale-type yeast was used and both geranyl acetate and methyl geranate was detected after SC fermentations. Furthermore, their contents were statistically significantly ($p < 0.05$) higher after SC bottle fermentation compared to non-*Saccharomyces* yeast fermentations. SL fermentations had the lowest contents of these esterified terpenes.

3.3.4. Comparison of volatile compounds in beers by non-Saccharomyces yeasts

To further compare non-*Saccharomyces* yeast fermented beer samples to each other, a second PCA was constructed using TD and SL primary and bottle fermented beers as samples (n = 16) and volatile compounds as variables (n = 68; EST9, 11, 13, 16, 18, 20, and 22 were excluded; Fig. 2b). PCA shows clearer clustering by fermentation type and yeast compared to previous PCA (Fig. 2a). Bottle fermented samples are located on the positive side of PC2 and primary fermentation on more negative side. In addition, samples show clustering by the brew. TD samples are the most different from each other by locating further away on PC2 and some separation is also observed on PC1. The difference between the TD samples can indicate that the fermentation with TD is not as repeatable as with SL, which may have been caused by the different inoculation methods: TD was inoculated as an active dry yeast and SL as pre-growth inoculate. In addition, most of the volatile variables positively correlate with K1TDP1 and K1TDB1 samples. However, similar correlation between certain variables and SL samples can be seen as in Fig. 2a: β-myrcene, humulene, and (E)-nerol positively correlate with SLP samples. Dimethyl disulphide, styrene, 2,4-di-tert-butylphenol, 3-hydroxy-2-butanone, and 3-ethoxy-1-propanol positively correlate with almost all bottle fermented beer samples.

A PLS-DA model was constructed with the same samples and variables as in Fig. 2b to further observe Variance Importance in Projection (VIP; Table 3 and Fig. S2) of volatile compound variables in non-*Saccharomyces* beer samples. Twenty-two volatile compounds had VIP >1. PLS-DA confirmed that most of the volatile compounds positively correlate with TD samples, when non-*Saccharomyces* yeasts are compared, indicating possibly more complex aroma compared to SL fermented beers. The five compounds with highest VIPs were humulene (VIP 1.56), ethyl decanoate (1.52), 3-methyl butanal (1.51), ethyl nonanoate (1.45), and β-myrcene (1.39), of which only humulene and β-myrcene correlated with SLP, 3-methyl butanal with TDP, and ethyl decanoate and ethyl nonanoate with TDB. Longer-chain ethyl esters have been previously reported linked to unmalted wheat in brewing (De Flaviis et al., 2024a), humulene and β-myrcene are originated from hops, and 3-methyl-butanal is a product of Strecker degradation and it can cause wort off-flavor in beer (Gernat et al., 2020). Humulene and β-myrcene are positively correlated with SLP, and 3-methyl butanal, ethyl nonanoate, and ethyl (3E)-3-nonenoate with K1TDP samples.

Table 3
Variance Importance in Projection values from PLS model constructed only with non-*Saccharomyces* yeasts ($X = 72$, $Y = 4$).

Compound	VIP	Correlation[a]
Humulene	1.56	SLP
Ethyl decanoate	1.52	TDB
3-Methyl butanal	1.51	TDP
Ethyl nonanoate	1.45	TDB
β-Myrcene	1.39	SLP
(E)-Nerolidol	1.37	TDB
Ethyl (3E)-3-nonenoate	1.32	TDB
2,4-Di-tert-butylphenol	1.30	TDB
Ethyl butanoate	1.26	TDB
Ethyl acetate	1.24	TDB
Decanol	1.23	TDB
2-Methyl propanoic acid	1.22	TDB
Citronellol	1.16	TDB
Methyl decanoate	1.14	TDB
2-Methylbenzaldehyde	1.13	TDB
3-(Methylthio)propyl acetate	1.11	TDB
(E)-Nerol	1.11	SLP
3-Methyl-1-butanol acetate	1.09	TDB
Hexanoic acid	1.09	TDB
2-Methylpropyl acetate	1.09	TDB
Hexanal	1.08	SLP
3-Methylbutyl octanoate	1.08	TDP
Ethyl 9-hexadecenoate	1.06	TDP
Ethyl octanoate	1.06	TDP
2-Methylbenzaldehyde	1.04	TDB
Acetaldehyde	1.04	TDB
β-Damascenone	1.04	TDB
Octyl acetate	1.04	TDB
2-Methyl-1-butanol	1.01	TDB
Geranyl acetate	1.01	TDB
Heptyl acetate	1.00	TDB
Ethyl hexadecanoate	1.00	TDB

[a] Sample type the compound is correlating. Abbreviations refer to Table 1.

4. Conclusion

This study presented results from utilization of *Saccharomyces cerevisiae*, *Torulaspora delbrueckii* and *Saccharomycodes ludwigii* to ferment barley-spelt wort for the first time. Study showed difference in the yeast attenuation and the volatile compound composition between the barley-spelt beers fermented with different yeasts. *T. delbrueckii* and *S. ludwigii* did not consume maltotriose, whereas *T. delbrueckii* showed some maltose consumption. Studied non-*Saccharomyces* yeasts presented lower alcohol fermentation capacity compared to *S. cerevisiae*, making them possible yeast to produce low alcoholic barley-spelt beer. Results highlight how differences in sugar metabolism among yeast species can be strategically used to control ethanol yield and residual sweetness in brewing.

Multivariate data analysis (PCA) showed clear correlation between most of the volatile compound variables and *S. cerevisiae* fermented beers, and only a few compounds positively correlated with non-*Saccharomyces* yeast fermented beers. *T. delbrueckii* showed the clearest

positive correlation with 2-methoxy-4-vinylphenol, a compound highly desired in wheat beers, indicating used strain to be suitable for spelt beer fermentation. Furthermore, *S. cerevisiae* produced the highest content of styrene, an undesired compound in wheat beer. Non-*Saccharomyces* yeast fermentations showed higher concentration of sulfur compounds, which could cause off-flavors during the aging of beer. Some of the differences in volatile compounds between yeasts may have been caused by utilization of spelt with barley in brewing process. Furthermore, this study utilized active dry yeasts *S. cerevisiae* and *T. delbrueckii* in the inoculation rates recommended by the producer and living *S. ludwigii* with pre-growing and adjusted inoculation rate. The differences in inoculation dosages may have affected the development of volatile compound profiles and contents.

Future studies should also focus on investigation of sensory properties of barley-spelt beers. Maltose intolerant non-*Saccharomyces* yeasts have potential for low and non-alcoholic beer production. Previous studies have shown that consumers prefer sweeter low and non-alcoholic beers, which could be produced using the non-*Saccharomyces* yeasts investigated in the present study. Furthermore, our study demonstrated that non-*Saccharomyces* yeasts could be also suitable in beer production from brewer's spent grain, which has low content of maltose sugars. Furthermore, spelt could be suitable adjunct utilized with brewer's spent grain in regular or low alcoholic beer production because it possibly adds fermentable sugars and compounds important for beer flavor development, such as free ferulic acid and amino acids.

CRediT authorship contribution statement

Niina Kelanne: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Eija Kulju:** Writing – original draft, Visualization, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Mira Vähäsalo:** Investigation, Formal analysis. **Marika Kalpio:** Writing – review & editing, Methodology. **Baoru Yang:** Writing – review & editing, Resources. **Oskar Laaksonen:** Writing – review & editing, Visualization, Supervision, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Niina Kelanne reports financial support was provided by Finnish Cultural Foundation. If there are other authors, they 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2026.108737.

Data availability

Data will be made available on request.

References

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

Bellut, K., & Arendt, E. K. (2019). Chance and challenge: Non-*saccharomyces* yeasts in nonalcoholic and low alcohol beer brewing – A review. *Journal of the American Society of Brewing Chemists, 77*, 77–91. https://doi.org/10.1080/03610470.2019.1569452

Bellut, K., Michel, M., Zarnkow, M., Hutzler, M., Jacob, F., De Schutter, D. P., Daenen, L., Lynch, K. M., Zannini, E., & Arendt, E. K. (2018). Application of non-*saccharomyces* yeasts isolated from kombucha in the production of alcohol-free beer. *Fermentation, 4*, 66. https://doi.org/10.3390/fermentation4030066

Brányik, T., Silva, D. P., Baszczyński, M., Lehnert, R., & Almeida e Silva, J. B. (2012). A review of methods of low alcohol and alcohol-free beer production. *Journal of Food Engineering, 108*, 493–506. https://doi.org/10.1016/j.jfoodeng.2011.09.020

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

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

Carisma, N. A. S., & Calingacion, M. N. (2025). Metabolomics and (craft) beers – Recent advances. *Food Research International, 205*, Article 116010. https://doi.org/10.1016/j.foodres.2025.116010

De Flaviis, R., Santarelli, V., Giuliani, M., Neri, L., & Sacchetti, G. (2024). Influence of wheat content and origin on the volatilome of craft wheat beer: An investigation by combined multivariate statistical approaches. *Food Research International, 191*, Article 114709. https://doi.org/10.1016/j.foodres.2024.114709

De Flaviis, R., Santarelli, V., Grilli, S., & Sacchetti, G. (2024b). An integrative multi-omics approach aimed to gain insight on the effect of composition, style, yeast, and wheat species on wheat craft beer flavour. *Food Chemistry, 441*, Article 138387. https://doi.org/10.1016/j.foodchem.2024.138387

De Francesco, G., Turchetti, B., Sileoni, V., Marconi, O., & Perretti, G. (2015). Screening of new strains of *Saccharomycodes ludwigii* and *Zygosaccharomyces rouxii* to produce low-alcohol beer. *Journal of the Institute of Brewing, 121*, 113–121. https://doi.org/10.1002/jib.185

Dennis, E. G., Keyzers, R. A., Kalua, C. M., Maffei, S. M., Nicholson, E. L., & Boss, P. K. (2012). Grape contribution to wine aroma: Production of Hexyl acetate, octyl acetate, and benzyl acetate during yeast fermentation is dependent upon precursors in the must. *Journal of Agricultural and Food Chemistry, 60*, 2638–2646. https://doi.org/10.1021/jf2042517

Deyalage, S. T., House, J. D., Thandapilly, S. J., & Malalgoda, M. (2024). Nutritional characteristics and physicochemical properties of ancient wheat species for food applications. *Food Bioscience, 62*, Article 105397. https://doi.org/10.1016/j.fbio.2024.105397

Drosou, F., Anastasakou, K., Tataridis, P., Dourtoglou, V., & Oreopoulou, V. (2023). Evaluation of commercial strains of *Torulaspora delbrueckii* in beer production. *Journal of the American Society of Brewing Chemists, 81*, 211–220. https://doi.org/10.1080/03610470.2021.2025327

Einfalt, D. (2021). Barley-sorghum craft beer production with *Saccharomyces cerevisiae, Torulaspora delbrueckii* and *Metschnikowia pulcherrima* yeast strains. *European Food Research and Technology, 247*, 385–393. https://doi.org/10.1007/s00217-020-03632-7

Etschmann, M. M. W., Kötter, P., Hauf, J., Bluemke, W., Entian, K.-D., & Schrader, J. (2008). Production of the aroma chemicals 3-(methylthio)-1-propanol and 3-(methylthio)-propylacetate with yeasts. *Applied Microbiology and Biotechnology, 80*, 579–587. https://doi.org/10.1007/s00253-008-1573-4

Filipowska, W., Jaskula-Goiris, B., Ditrych, M., Bustillo Trueba, P., De Rouck, G., Aerts, G., Powell, C., Cook, D., & De Cooman, L. (2021). On the contribution of malt quality and the malting process to the formation of beer staling aldehydes: A review. *Journal of the Institute of Brewing, 127*, 107–126. https://doi.org/10.1002/jib.644

Fujita, A., Simsek, S., & Schwarz, P. B. (2020). Observations on the malting of ancient wheats: Einkorn, emmer and spelt. *Fermentation, 6*, 125. https://doi.org/10.3390/fermentation6040125

Gernat, D. C., Brouwer, E., & Ottens, M. (2020). Aldehydes as wort off-flavours in alcohol-free beers—origin and control. *Food and Bioprocess Technology, 13*, 195–216. https://doi.org/10.1007/s11947-019-02374-z

Gijs, L., Perpète, P., Timmermans, A., & Collin, S. (2000). 3-Methylthiopropionaldehyde as precursor of Dimethyl trisulfide in aged beers. *Journal of Agricultural and Food Chemistry, 48*, 6196–6199. https://doi.org/10.1021/jf0007380

Gobbi, L., Stanković, M., Ruggeri, M., & Savastano, M. (2024). Craft beer in food science: A review and conceptual framework. *Beverages, 10*. https://doi.org/10.3390/beverages10030091

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

Gutiérrez, A., Boekhout, T., Gojkovic, Z., & Katz, M. (2018). Evaluation of non-*saccharomyces* yeasts in the fermentation of wine, beer and cider for the development of new beverages. *Journal of the Institute of Brewing, 124*, 389–402. https://doi.org/10.1002/jib.512

Jackowski, M., Czepiela, W., Hampf, L., Żuczkowski, W., Dymkowski, T., & Trusek, A. (2023). Comparison of two commercially available strains, *Saccharomycodes ludwigii* and *Torulaspora delbrueckii*, for the production of low-alcohol beer. *Beverages, 9*, 66. https://doi.org/10.3390/beverages9030066

Kelanne, N., Zhu, Y., Rysä, T., Yang, B., Zhu, B., & Laaksonen, O. (2025). Characterization of commercial non-alcoholic beers in two locations, Finland and China. *Beverages, 11*, 47. https://doi.org/10.3390/beverages11020047

King, A. J., & Dickinson, J. R. (2003). Biotransformation of hop aroma terpenoids by ale and lager yeasts. *FEMS Yeast Research, 3*, 53–62. https://doi.org/10.1111/j.1567-1364.2003.tb00138.x

King, A., & Richard Dickinson, J. (2000). Biotransformation of monoterpene alcohols by *Saccharomyces cerevisiae, Torulaspora delbrueckii* and *Kluyveromyces lactis*. *Yeast, 16*, 499–506. https://doi.org/10.1002/(SICI)1097-0061(200004)16:6%3C499::AID-YEA548%3E3.0.CO;2-E

Langos, D., & Granvogl, M. (2016). Studies on the simultaneous formation of aroma-active and toxicologically relevant vinyl aromatics from free phenolic acids during wheat beer brewing. *Journal of Agricultural and Food Chemistry, 64*, 2325–2332. https://doi.org/10.1021/acs.jafc.5b05606

Langos, D., Granvogl, M., & Schieberle, P. (2013). Characterization of the key aroma compounds in two Bavarian wheat beers by means of the sensomics approach. *Journal of Agricultural and Food Chemistry, 61*, 11303–11311. https://doi.org/10.1021/jf403912j

Laureys, D., Baillière, J., Vermeir, P., Vanderputten, D., & De Clippeleer, J. (2023). The impact of 10 unmalted alternative adjuncts on wort characteristics. *Foods, 12*, 4206. https://doi.org/10.3390/foods12234206

Li, g, & Liu, F. (2015). Changes in organic acids during beer fermentation. *Journal of the American Society of Brewing Chemists, 73*, 275–279. https://doi.org/10.1094/ASBCJ-2015-0509-01

Maia, C., Cunha, S., Debyser, W., & Cook, D. (2023). Impacts of adjunct incorporation on flavor stability metrics at early stages of beer production. *Journal of the American Society of Brewing Chemists, 81*, 54–65. https://doi.org/10.1080/03610470.2021.1993054

Mestre Furlani, M. V., Vargas Perucca, M. F., Petrignani, D. B., Vergara, S. C., Leiva-Alaniz, M. J., Maturano, Y. P., Vazquez, F., & Dellacassa, E. (2024). Enhancing flavor complexity in craft beer: Sequential inoculation with Indigenous non-saccharomyces and commercial saccharomyces yeasts. *Fermentation, 10*, 657. https://doi.org/10.3390/fermentation10120657

Michel, M., Kopecká, J., Meier-Dörnberg, T., Zarnkow, M., Jacob, F., & Hutzler, M. (2016a). Screening for new brewing yeasts in the non-*saccharomyces* sector with *Torulaspora delbrueckii* as model. *Yeast, 33*, 129–144. https://doi.org/10.1002/yea.3146

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

Miracle, R. E., Ebeler, S. E., & Bamforth, C. W. (2005). The measurement of sulfur-containing aroma compounds in samples from production-scale brewery operations. *Journal of the American Society of Brewing Chemists, 63*, 129–134. https://doi.org/10.1094/ASBCJ-63-0129

Munoz-Insa, A., Gastl, M., & Becker, T. (2016a). Variation of sunstruck flavor-related substances in malted barley, triticale and spelt. *European Food Research and Technology, 242*, 11–23. https://doi.org/10.1007/s00217-015-2513-z

Munoz-Insa, A., Gastl, M., & Becker, T. (2016b). Influence of malting on the protein composition of spelt (Triticum spelta L.) 'Frankenkorn. *Cereal Chemistry, 93*, 1–9. https://doi.org/10.1094/CCHEM-12-14-0250-R

Muñoz-Insa, A., Selciano, H., Zarnkow, M., Becker, T., & Gastl, M. (2013). Malting process optimization of spelt (*Triticum spelta* L.) for the brewing process. *LWT - Food Science and Technology, 50*, 99–109. https://doi.org/10.1016/j.lwt.2012.06.019

Myncke, E., Vanderputten, D., Laureys, D., Huys, J., Schlich, J., Van Opstaele, F., Schouteten, J. J., & De Clippeleer, J. (2025). Navigating yeast selection for NABLAB production: Comparative study of commercial maltose- and maltotriose-negative strains. *Food Chemistry, 477*, Article 143486. https://doi.org/10.1016/j.foodchem.2025.143486

Ogawa, M., Vararu, F., Moreno-Garcia, J., Mauricio, J. C., Moreno, J., & Garcia-Martinez, T. (2022). Analyzing the minor volatilome of *Torulaspora delbrueckii* in an alcoholic fermentation. *European Food Research and Technology, 248*, 613–624. https://doi.org/10.1007/s00217-021-03910-y

Olaniran, A. O., Hiralal, L., Mokoena, M. P., & Pillay, B. (2017). Flavour-active volatile compounds in beer: Production, regulation and control. *Journal of the Institute of Brewing, 123*, 13–23. https://doi.org/10.1002/jib.389

Piornos, J. A., Koussissi, E., Balagiannis, D. P., Brouwer, E., & Parker, J. K. (2023). Alcohol-free and low-alcohol beers: Aroma chemistry and sensory characteristics. *Comprehensive Reviews in Food Science and Food Safety, 22*, 233–259. https://doi.org/10.1111/1541-4337.13068

Rahman, M. J., Liang, J., Eskin, N. A. M., Eck, P., & Thiyam-Holländer, U. (2020). Identification of hydroxycinnamic acid derivatives of selected canadian and foreign commercial beer extracts and determination of their antioxidant properties. *LWT, 122*, Article 109021. https://doi.org/10.1016/j.lwt.2020.109021

Ramírez, M., & Velázquez, R. (2018). The yeast torulaspora delbrueckii: An interesting but difficult-to-use tool for winemaking. *Fermentation, 4*, 94. https://doi.org/10.3390/fermentation4040094

Rettberg, N., Biendl, M., & Garbe, L.-A. (2018). Hop aroma and hoppy beer flavor: Chemical backgrounds and analytical tools—A review. *Journal of the American Society of Brewing Chemists, 76*, 1–20. https://doi.org/10.1080/03610470.2017.1402574

Rodríguez Madrera, R., Pando Bedriñana, R., & Suárez Valles, B. (2021). Evaluation of indigenous non-*Saccharomyces* cider yeasts for use in brewing. *European Food Research and Technology, 247*, 819–828. https://doi.org/10.1007/s00217-020-03665-y

Rübsam, H., Gastl, M., & Becker, T. (2013). Determination of the influence of starch sources and mashing procedures on the range of the molecular weight distribution of beer using field-flow fractionation. *Journal of the Institute of Brewing, 119*, 139–148. https://doi.org/10.1002/jib.69

Salanță, L. C., Coldea, T. E., Ignat, M. V., Pop, C. R., Tofană, M., Mudura, E., Borşa, A., Pasqualone, A., & Zhao, H. (2020). Non-alcoholic and craft beer production and challenges. *Processes, 8*, 1382. https://doi.org/10.3390/pr8111382

Satora, P., & Pater, A. (2023). The influence of different non-conventional yeasts on the odour-active compounds of produced beers. *Applied Sciences, 13*, 2872. https://doi.org/10.3390/app13052872

Sileoni, V., Maranghi, S., De Francesco, G., Perretti, G., & Marconi, O. (2023). Flavour stability of a cold-stored unpasteurized low-alcohol beer produced by *Saccharomycodes ludwigii*. *Food and Bioprocess Technology, 16*, 2471–2482. https://doi.org/10.1007/s11947-023-03061-w

Sterczyńska, M., Stachnik, M., Poreda, A., Pużyńska, K., Piepiórka-Stepuk, J., Fiutak, G., & Jakubowski, M. (2021). Ionic composition of beer worts produced with selected unmalted grains. *LWT, 137*, Article 110348. https://doi.org/10.1016/j.lwt.2020.110348

Sun, Q., Ma, J., Basit, R. A., Fu, Z., Liu, X., & Fan, G. (2024). Screening of a *Saccharomyces cerevisiae* strain with high 3-Methylthio-1-Propanol yield and optimization of its fermentation conditions. *Foods, 13*, 1296. https://doi.org/10.3390/foods13091296

Sun, Z., & Xiao, D. (2018). Review in metabolic modulation of higher alcohols in top-fermenting yeast. In H. Liu, C. Song, & A. Ram (Eds.), *Advances in applied biotechnology* (pp. 767–773). Singapore: Springer. https://doi.org/10.1007/978-981-10-4801-2_79.

Suriano, S., Moscaritolo, S., Russo, M., & Troccoli, A. (2025). Evaluation of malt quality of different Italian varieties of hulled, soft and durum wheat. *European Food Research and Technology*. https://doi.org/10.1007/s00217-025-04716-y

Toh, D. W. K., Chua, J. Y., Lu, Y., & Liu, S. Q. (2020). Evaluation of the potential of commercial non-*Saccharomyces* yeast strains of *Torulaspora delbrueckii* and *Lachancea thermotolerans* in beer fermentation. *International Journal of Food Science and Technology, 55*, 2049–2059. https://doi.org/10.1111/ijfs.14399

Vaštík, P., Rosenbergová, Z., Furdíková, K., Klempová, T., Šišmiš, M., & Šmogrovičová, D. (2022). Potential of non-*saccharomyces* yeast to produce non-alcoholic beer. *FEMS Yeast Research, 22*, foac039. https://doi.org/10.1093/femsyr/foac039

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

Waters Co. (2019). Analysis of food sugars using ACQUITY UPLC BEH amide columns. https://www.waters.com/nextgen/dk/en/library/application-notes/2009/food-sugars-using-acquity-uplc-beh-amide-columns.html, 11.12.25.