Sourdough derived strains of *Saccharomyces cerevisiae* and their potential for farmhouse ale brewing

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ABSTRACT: The Finnish farmhouse ale sahti is unique in that it is fermented with baking, rather than brewing strains of *Saccharomyces cerevisae*. The custom of maintaining farmhouse yeast cultures is however no longer practiced in Finland, and much yeast derived diversity in sahti beers has presumably been lost as a consequence. Here, the brewing potential of a number of sourdough derived strains was tested with respect to a number of different fermentation traits. Seven strains originally isolated from Finnish or Italian sourdough cultures were used to ferment high gravity sahti wort (20°P), and fermentation performance together with production of volatile compounds were assessed and compared with a reference baking yeast. Strains differed in terms of fermentation rate, yield, yeast viability and beer flavour profile. All were maltotriose positive, but utilisation varied so that alcohol yield could be greater or lower than that of the reference strain, with values ranging from 6.6 to 7.9% (v/v). Production of aroma compounds was also variable so that it was possible to identify strains producing high levels of esters and those with lower production, which could be used to emphasise flavours originating from raw materials. All strains generated 4-vinyl guaiacol and so would be suitable for other beers where this is a part of the normal flavour profile. Results suggest that sourdough isolates of *S. cerevisiae* are suitable for sahti production, but could also be applied to other beer styles as a way to differentiate products. © 2020 The Authors. Journal of the Institute of Brewing published by John Wiley & Sons Ltd on behalf of The Institute of Brewing & Distilling

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Introduction

Sahti is a traditional farmhouse beer produced in Finland, the brewing process of which differs in a number of respects compared to other beer styles. Notable features are extended mash times, the use of juniper branches for flavouring; the absence or minimal use of hops; a unique lautering process, the lack of wort boiling; very high gravity fermentations (with Plato values above 20° being typical); and a short primary fermentation, typically of around two days *(1)*. While some of these features are found in other farmhouse ales of the Baltic and Nordic regions, sahti is unique in that fermentation is carried out exclusively with baking yeast strains rather than brewing yeast, and this feature is stipulated in the style's EU Traditional Speciality Guaranteed description (Commission Regulation (EC) No 244/2002). Brewing of farmhouse ales in other Baltic countries can also involve baking yeast, but this is optional and other yeast types are also utilised. Traditional house cultures of yeast ('harvested yeast' according to the appellation) are permitted in sahti brewing, though the custom of maintaining farmhouse yeast in this manner has not persisted in Finland as it has done in other countries such as Norway *(2)*. In effect, this means that a beer cannot be described as sahti unless it has been fermented with baker's yeast.

While sahti character can vary depending on the region, brewer, and raw materials used, the yeast derived qualities are largely consistent due to the use of commercial baker's yeast to start fermentations, with one particular Finnish brand being used in most cases. It may be assumed that much of the style's variability and local character was lost when the practice of maintaining

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house cultures died out. This assumption is supported by the differences in character of Norwegian maltøl beers that arise depending on which yeast strain is employed *(2)*.

To evaluate the feasibility of re-introducing diversity to the sahti beers via yeast strain selection, a number of *Saccharomyces cerevisiae* yeast strains were collected. These were all derived from sourdough cultures. In the past, Finnish 'house' cultures would have been used for both baking and brewing and, for this study, sourdough was therefore considered an appropriate source of yeast. It is also conceivable that the yeast lineages present now in traditional Finnish sourdoughs are the same as those used previously for sahti brewing.

Previous studies have demonstrated that sourdough derived *S. cerevisiae* strains, and even sourdough itself, can be used as starter cultures to ferment brewer's wort *(3–7)*. There is, however, no information on how different strains can be used to differentiate beers - either beers in general, or farmhouse ales in particular. Here we characterise the assembled strains with respect to brewing related features such as fermentation, alcohol yield, yeast viability, aroma production and stress tolerance. The main aim was to determine the extent to which sahti beer character can be altered depending on the strain used and, additionally, to determine if the commercial baker's yeast that is invariably used in sahti brewing today is uniquely suited to this task, or if its widespread use is more related to its availability than any special brewing properties it might possess. While the current study is parochial in nature, results are expected to be broadly applicable to a number of beer styles. The work is in line with a general trend for the use of non-conventional yeast in brewing, and re-purposing of yeasts from different fermentation systems in brewery fermentations *(8)*.

Materials and methods

Strains

Strains used in the study included *Saccharomyces cerevisiae* sourdough isolates from the VTT Culture Collection. Strains Y15, Y17, and Y23 were isolated from Italian sourdough and deposited in the Culture collection of the Department of Agriculturel, Food and Environment, University of Catania, Italy. The commercial Finnish baker's yeast strain Suomen Hiiva, was isolated from a fresh commercial culture. In this case, a single cell culture was obtained using a Singer MSM 400 dissection microscope, and its identity was confirmed by ITS sequencing as described in Pham et al. *(9)*.

Wort preparation

Sahti wort was prepared following a traditional step mashing protocol involving long mash duration, and wort filtration using a specialised lauter tun (kuurna). Mashing was carried out in a 120L steam-jacketed, stainless steel kettle. A starting temperature of 40°C was achieved by mixing 5 L boiling water with 15 L cold water. Milled malt consisting of 15 kg sahti malt (Viking Malt, Finland) and 1.5 kg rye malt (Laihian Mallas, Finland) was mixed into the water. After 1h, a further 7 L boiling water was added to raise the temperature to 52 °C. An additional 7L boiling water was added after another hour to raise the temperature to 63°C. After 80 minutes at 63°C, 8 L boiling water was added to bring the temperature to 72°C. After 1h at this temperature the vessel was then heated to 80 °C, at which point the thick mash was removed manually and transferred to an open, horizontal, semi-cylindrical, lauter tun. This kuurna-style lauter tun contained a bed of fresh juniper branches for flavouring. Wort was collected by means of a tap at one end of the vessel. This wort was added back to the vessel until the wort leaving the tap became visibly transparent. At this point, the 24° Plato wort was collected in a sterile stainless steel keg for storage at 0°C.

Fermentation

Yeast cultures were propagated by inoculation of 50 ml YPM (1% w/v yeast extract, 2% w/v peptone, 4% w/v maltose) in 100 ml Erlenmeyer flasks directly from agar plate cultures. The 50 ml cultures were incubated overnight at room temperature with shaking (120 rpm). The cultures were centrifuged (10 min, 10,000 g), washed in sterile reverse osmosis treated water and resuspended to give a 20% (w/v) slurry. A 5ml volume (1 g fresh yeast) was used to inoculate 1L of 15°P all malt wort in a 3L Erlenmeyer flask. The cultures were incubated for 48h before centrifugation, and resuspension in spent wort to achieve a 20% (w/v) yeast suspension.

Prior to fermentation, wort was diluted to 20°P and aerated to 10 mg/L dissolved oxygen. Wort (1.5 L) was transferred to 2 L stainless steel tall tubes. The eight *S. cerevisiae* strains were inoculated into duplicate fermenters at a pitching rate of 1 g/L fresh yeast, and fermentations proceeded at 20°C for 8 days. After the primary fermentation, the fermenters were cooled to 4°C for 1 week, after which time samples were taken for yeast viability and aroma volatile analysis.

Wort and beer analyses

Aseptic sampling from fermenters was done periodically over 8 days. Samples (25 mL) were centrifuged and supernatants used for wort/beer analyses after manual degassing. The specific gravity, alcohol (%, v/v) and pH of samples were determined from centrifuged (10 min, 10,000 g) and degassed wort and fermentation samples using an Anton Paar Density Meter DMA 5000 M with Alcolyzer Beer ME and pH ME modules (Anton Paar GmbH, Austria).

The yeast fresh mass content (i.e. yeast in suspension) was determined by washing the centrifuged yeast pellets twice with 25 mL deionised H_2O in a pre-weighed centrifuge tube and calculating the mass of the pellet after removal of supernatant. For viability evaluation, 10 µl of each sample were added to 990 µl EDTA (10 µM) in 1.5 ml Eppendorf tubes and measured with NucleocounterR YC-100™ to calculate non-viable cells. The solution (50 µl) was transferred to 450 µl Lysis Buffer in a new Eppendorf tube and measured with NucleocounterR YC-100™ to calculate total cells for each sample.

Yeast derived flavour compounds were determined by headspace gas chromatography with flame ionisation detection (HS-GC-FID). Samples (4 mL) were filtered (0.45 µm), incubated at 60°C for 30 minutes and 1 mL of the gas phase injected (split mode; 225 °C; split flow of 30 mL/min) into a gas chromatograph equipped with a FID detector and headspace autosampler (Agilent 7890 Series; Palo Alto, CA, USA). Analytes were separated on a HP-5 capillary column (50m × 320 µm × 1.05 µm column, Agilent, USA). The carrier gas was helium (constant flow of 1.4 mL/min). The temperature program was 50°C for 3 min, 10°C/min to 100 °C, 5°C/min to 140°C, 15°C/min to 260°C and then isothermal for 1 min. Compounds were identified by comparison with authentic standards

and were quantified using standard curves. 1-Butanol was used as internal standard (246 mg/L).

Assessment of stress tolerance and phenolic off flavour production

Stress tolerance tests were performed on the strains using spot plates. Colonies of the sourdough-derived strains, and reference strains, were inoculated into 25 mL YPD (1% w/v yeast extract, 2% w/v peptone, 2% w/v glucose) and incubated for 2 days. Optical density at 600nm was measured and corrected to 0.5 and a ten-fold dilution series was carried out. 5 µL of each dilution was spotted onto YPD plates. Temperature tolerance tests were conducted on standard YPD plates (4% w/v agar) and incubated at 4, 25, 37and 40°C. Ethanol tolerance was tested on YPD plates supplemented with 10% v/v ethanol and incubated at 25°C. To assess osmotic tolerance, YPD plates supplemented with 20% v/v sorbitol were used and incubated at 25°C. For POF analysis, 25ml YPD containing 100 mg/L ferulic acid were inoculated directly from an agar plate (stock was 10g/L trans-ferulic acid in ethanol) and incubated statically at 25°C for 4 days. The presence of the characteristic clove-like 4-vinyl guaiacol was assessed by smelling. The reference yeast strains were A15 (POF⁻ lager) and A62 (POF⁺ ale) (Table 1).

Statistical analysis of data

One-way analysis of variance was used to assess statistical differences between values ($P < 0.05$). Fisher's protected least significant difference (PLSD) test was used to directly compare individual values after signifant differences were detected by one-way ANOVA. Analysis was performed using Statview software.

Results

Fermentation performance of strains

All yeast strains were capable of fermenting the 20°P wort with varying degrees of efficiency. The reference baking strain displayed the fastest fermentation rate in the first two days of fermentation, and achieved an alcohol concentration of 7% (v/v) 96 hours after pitching, a concentration that did not increase over the next 4 days of fermentation (Fig. 1). The sourdough strains generated different levels of ethanol, with two (C117 and C118) producing significantly lower levels compared to the other strains (ca. 6.6%), and two (B352 and C207) having a similar yield to the reference strain, while three strains had significantly higher concentrations atapproximately 7.5% (both Y15 and Y23) and 7.9% (Y17). The alcohol production was related to sugar utilisation, with residual maltotriose concentrations in beer correlating inversely with alcohol level (Fig. 2). While all the strains were able to utilise maltotriose, the uptake varied significantly from about 3 g/L (for strain C117) to 30 g/L (for strain Y17). Maltose consumption was likely to have had an impact on fermentation rate, but had little effect on overall productivity, with all beers containing 3-4 g/L residual maltose (Fig. 2). Overall, the results indicate that a shorter fermentation time of between 4 and 6 days is adequate for fermentation under these conditions.

For the majority of yeast strains a similar pH profile was observed during fermentation (Fig. 3). The lowest values (pH 4.3 to 4.4) were measured 48 hours after yeast inoculation and increased to pH 4.5 to 4.6 towards day 6 or 8 of fermentation. The exception was strain C118, in which a pH of 4.3 was maintained between days 2 and 8. The lower pH of C118 may be linked to the relatively slow fermentation in this strain. Continuous metabolic activity may also be linked to the yeast maintaining relatively high levels of yeast mass in suspension. Conversely, the C117 strain had the lowest mass in suspension and the highest pH value in beer (Fig. 3).

After one week of secondary fermentation, there was no further fermentation, with alcohol levels remaining unchanged relative to those in the green beer (Table 2). At this stage, the viability of the remaining yeast was highly variable, with values ranging from 1% in the B352 strain, to between 50 and 60% in the most tolerant strains (C118, Y17).

The concentrations of volatile compounds varied considerably between strains (Table 2), with significant differences ($P<0.05$) observed between strains for each compound analysed. 3-methylbutyl acetate (pear/banana), which is characteristic of sahti beers *(10)*, was found in six beers to be 3-5 times higher than the flavour threshold of 1.6 mg/L *(11)*. In just two beers (those produced with C117 and C118) the values were lower than the flavour threshold. A relatively low production of esters by these two yeasts seemed to be typical, and this was observed for both acetate

Table 1. *Saccharomyces cerevisiae* strains and reference strains used in the study

| Code	Other	Species	Collection	Notes
Suomen Hiiva	SH	*S. cerevisiae*	Commercially available strain	Commercial Finnish baker's yeast and standard sahti brewing strain
VTT C-81117	C117	*S. cerevisiae*	VTT Culture Collection	Finnish sourdough isolate from 1981
VTT C-81118	C118	*S. cerevisiae*	VTT	Finnish sourdough isolate from 1981
VTT C-94207	C207, NCYC 2937	*S. cerevisiae*	VTT	Finnish sourdough isolate from 1994
VTT B-19352	B352, Maisa	*S. cerevisiae*	VTT	Finnish sourdough isolate from 2019, Virrat
Y15		*S. cerevisiae*	University of Catania Culture Collection	Traditional Sicilian Maiorca flour sourdough isolate, Maletto
Y17		*S. cerevisiae*	University of Catania	Traditional Sicilian Maiorca flour sourdough isolate, Maletto
Y23		*S. cerevisiae*	University of Catania	Traditional Sicilian Maiorca flour sourdough isolate, Catania
VTT A-63015	A15	*S. pastorianus*	VTT	Reference lager strain, Frohberg-type lager yeast strain, low flocculence, POF⁻
VTT-A81062	A62	*S. cerevisiae*	VTT	Reference ale strain, flocculent, POF⁺
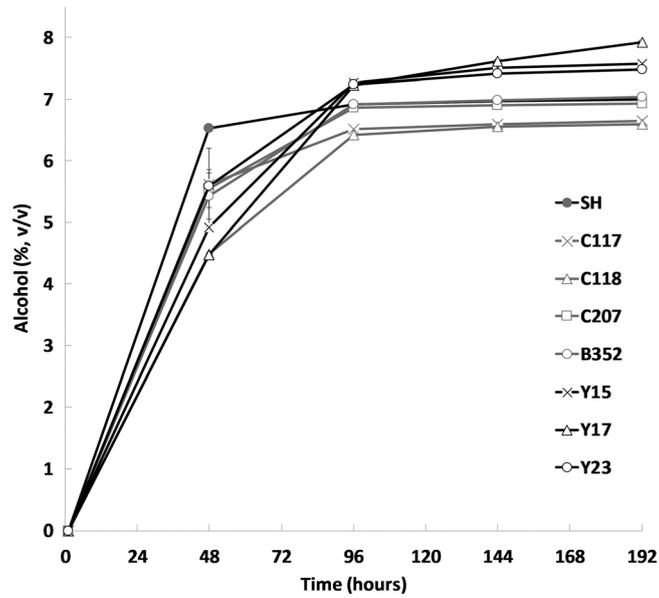
Figure 1. Alcohol production during fermentation of 20°P wort at 20°C using eight different baking or sourdough strains. Values are means of two independent replicates. Error bars, where visible, represent range.
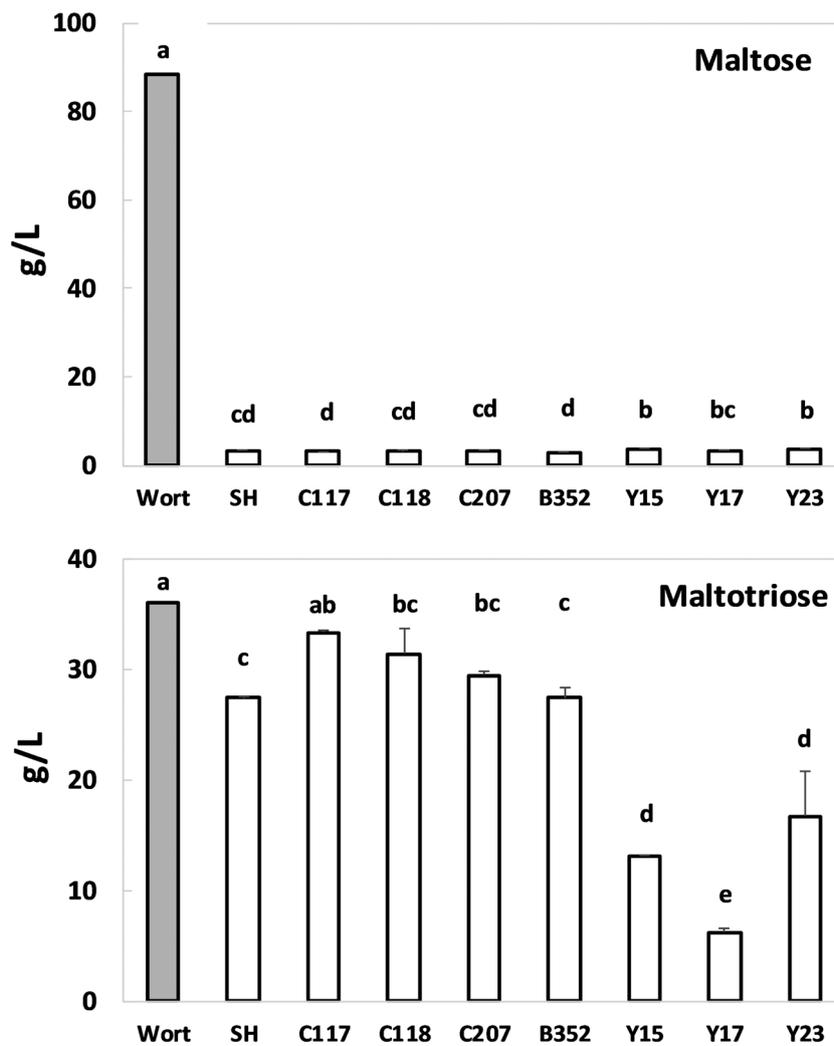

Figure 2. Concentration of fermentable sugars in 20°P wort, and beers after fermentation at 20°C using eight different baking or sourdough strains. Values are means of two independent replicates. Bars sharing the same letters are not significantly different, as determined by Fisher's PLSD test. Error bars, where visible, represent range.
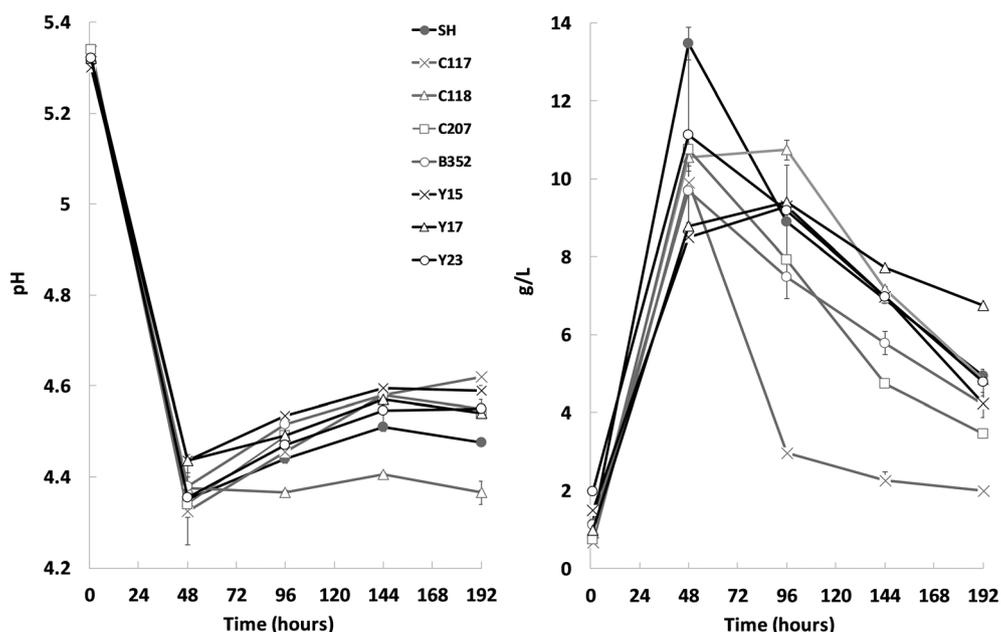
Figure 3. pH and fresh yeast mass in suspension (g/L) during fermentation of 20°P wort at 20°C using eight different baking or sourdough strains. Values are means of two independent replicates. Error bars, where visible, represent range.

esters and ethyl esters (Table 2). 3-methylbutanol, which also adds a banana aroma, was found to be close or above the flavour threshold of 70 mg/L in all beers. Ethyl acetate, which imparts a fruity or solvent aroma, depending on concentration, was found to be high in five beers, at the flavour threshold in two beers, and below the threshold in C117 beer (Table 2). Ethyl hexanoate, typically described as apple, aniseed or cherry like in flavour, was below the flavour threshold of 0.23 mg/L in three beers (SH, C117, C118), close to the flavour threshold in three (C207, M, Y23) and above the threshold in Y15 and Y17 beers. In particular, the ethyl hexanoate level in the Y17 beer at 0.5 mg/L was more than double the normal threshold value. In beers produced with C207 and M, there was a relatively high concentration of ethyl octanoate and ethyl decanoate (often associated with tropical flavour notes), relative to other beers. All yeasts were found to produce the spice/clove-like aroma of 4-vinyl guaiacol (Table S1).

Spot plates tests showed that some differences existed with respect to temperature tolerance (Fig. S1). All *S. cerevisiae* strains grew well under the control condition (25°C) and at 37°C. The *S. pastorianus* reference strain was the only one not capable of growth at or above 37°C. All other strains showed some growth at 40°C, though growth was impaired in the Finnish sourdough strains C117 and C118 as well as the *S. cerevisiae* ale reference strain. Growth at low temperature (4°C) was limited for all strains, with the psychrophilic *S. pastorianus* showing the greatest ability to grow at this temperature. At this temperature the Y17 strain and reference *S. cerevisiae* ale strain appeared to be particularly sensitive. The strains were capable of growth under conditions of ethanol and osmotic stress (Fig. S2), though some reduction in growth at 10% ethanol was seen for all strains.

Discussion

All sourdough strains showed ability to ferment sahti wort, with alcohol yields comparable to those for the baking yeast routinely used for sahti fermentations. The sourdough strains, like the reference strain, were found to be both maltotriose positive and POF positive, and to generally display similar characteristics with respect to pH change and yeast mass in suspension during fermentation. Results suggest therefore that sourdough strains of *S. cerevisiae* could be used in sahti brewing, and may introduce yeast derived diversity to sahti beers.

The use of sourdough strains in wort fermentations has been demonstrated previously [3–7] and this approach is consistent with a growing interest in the potential value of non-conventional yeasts in brewing [12]. For example, strains of *S. cerevisiae* that have been re-purposed for brewing include the probiotic strain *S. cerevisiae* var *S. boulardii* [13], an Andean chicha strain [14], and Brazilian cachaça strains [15–17]. The use of baking or sourdough yeasts in beer production may be seen as appropriate given the similarity of the baking and brewing systems, i.e. the fermentation by yeast of grain flour mixed with water, where the main sugars are similar in both cases (genes for maltose utilisation are over-represented in the genomes of both brewing and baking yeasts) [18]. The phenotypic similarities of brewing and baking yeasts stem not only from the similar environmental conditions encouraging convergent evolution, but also from the genetic relatedness of the two groups. Historical accounts of Pliny the Elder (1st century AD), and Olaus Magnus (16th century), describe the collection of flocculated brewing yeasts for use in baking, and the use of baking yeast to start brewing fermentations [1,19]. Also, in ancient Egypt, beers were apparently brewed from a breadbased wort [20], a practice that is still used in the kvass production of Eastern Europe and Russia [21]. The historical connection of the beer yeast and bread yeast lineages is also clear from phylogenetic comparisons, where these two groups show closer relationships to each other than to other production yeast groups [22–25].

While there are clear phenotypic similarities between the two lineages, differences should also be acknowledged. Baking yeasts are invariably POF positive, and produce the clove-like aroma of 4-vinyl guaiacol [22]. This trait is only seen in certain brewing yeast strains (those used to make wheat beers and various Belgian ale

Table 2. Fermentation and aroma characteristics of beers produced with sourdough isolates of *S. cerevisiae* after fermentation of 20°P wort at 20°C. Fermentations were conducted at 2L scale. Yeast viabilities and aroma were measured after one week secondary fermentation at 5°C. Values are means of two independent replicates. The range about the mean is indicated. Values in rows sharing the same letter are not statistically different, as determined by Fisher's PLSD test. Volatile values in bold are at, or above, typical flavour thresholds for beer *(11)*.

	SH	C117	C118	C207	B352	Y15	Y17	Y23
Alcohol (ABV)	7.0 ± 0.00[c]	6.6 ± 0.01[d]	6.6 ± 0.00[d]	6.9 ± 0.00[c]	7.0 ± 0.06[c]	7.6 ± 0.00[b]	7.9 ± 0.01[a]	7.5 ± 0.05[b]
pH	4.6 ± 0.04[b]	4.7 ± 0.01[a]	4.4 ± 0.01[c]	4.6 ± 0.00[b]	4.6 ± 0.00[b]	4.7 ± 0.00[ab]	4.6 ± 0.00[b]	4.6 ± 0.00[b]
Viability (%)	16	23	60	16	1	9	51	15
Volatiles (mg/L)*								
Acetaldehyde	17.3 ± 1.4[b]	**32.3 ± 2.7**[a]	13.3 ± 3.2[bc]	15.7 ± 1.4[b]	10.4 ± 1.8[c]	11.2 ± 0.1[bc]	9.1 ± 0.7[c]	14.1 ± 2.1[bc]
Propanol	**69.2 ± 0.5**[a]	47.6 ± 0.0[b]	44.0 ± 0.6[b]	**62.6 ± 0.3**[a]	**59.6 ± 5.1**[a]	50.1 ± 0.1[b]	**50.9 ± 0.3**[b]	**65.0 ± 7.4**[a]
2-Methylbutanol	36.5 ± 0.4[b]	25.0 ± 0.1[c]	36.8 ± 1.2[ab]	36.1 ± 0.3[b]	38.7 ± 0.7[ab]	34.8 ± 0.3[b]	34.9 ± 0.2[b]	40.9 ± 3.2[a]
3-Methylbutanol	**98.9 ± 1.3**[a]	56.7 ± 0.1[c]	67.8 ± 2.2[c]	**82.9 ± 1.0**[abc]	**83.1 ± 5.7**[a]	65.3 ± 0.4[c]	**72.8 ± 0.6**[bc]	**88.2 ± 16.3**[a]
2-Methylpropanol	64.8 ± 0.4[a]	39.4 ± 0.2[c]	54.2 ± 1.4[ab]	61.3 ± 0.5[ab]	63.7 ± 2.0[a]	47.7 ± 0.1[bc]	47.0 ± 0.2[bc]	63.8 ± 9.1[a]
2-phenylethanol	71.2 ± 1.1[a]	25.3 ± 0.4[b]	28.4 ± 1.1[b]	51.5 ± 0.4[a]	48.5 ± 9.5[ab]	32.0 ± 1.2[b]	30.5 ± 0.5[b]	53.3 ± 17.8[a]
3-Methylbutyl acetate	**4.5 ± 0.1**[c]	0.2 ± 0.0[d]	0.3 ± 0.0[d]	**7.1 ± 0.2**[b]	**8.2 ± 0.5**[a]	**7.4 ± 0.1**[ab]	**6.9 ± 0.1**[b]	**6.4 ± 0.7**[b]
2-Phenylethyl acetate	1.02 ± 0.01[ab]	0.01 ± 0.01[d]	0.27 ± 0.21[d]	1.23 ± 0.05[ab]	1.28 ± 0.06[a]	1.03 ± 0.00[abc]	0.84 ± 0.01[c]	1.15 ± 0.12[b]
Ethyl hexanoate	0.17 ± 0.00[de]	0.10 ± 0.00[e]	0.13 ± 0.00[de]	**0.23 ± 0.01**[cd]	**0.27 ± 0.03**[c]	**0.39 ± 0.00**[b]	**0.5 ± 0.01**[a]	**0.28 ± 0.07**[c]
Ethyl octanoate	0.29 ± 0.01[c]	0.09 ± 0.00[d]	0.13 ± 0.02[d]	0.47 ± 0.01[b]	0.62 ± 0.09[a]	0.28 ± 0.00[c]	0.36 ± 0.01[bc]	0.23 ± 0.06[c]
Ethyl decanoate	0.25 ± 0.00[bc]	0.09 ± 0.00[ef]	0.05 ± 0.01[f]	0.40 ± 0.01[a]	0.44 ± 0.06[a]	0.18 ± 0.01[cd]	0.18 ± 0.01[d]	0.13 ± 0.02[de]
Ethyl acetate	**36.9 ± 0.4**[c]	18.0 ± 0.1[c]	**35.0 ± 0.8**[c]	**61.4 ± 1.2**[b]	**79.0 ± 11.0**[ab]	**91.0 ± 1.9**[a]	**88.9 ± 1.0**[a]	**67.7 ± 15.0**[b]

styles). Consequently, baking yeast could only be applied in practice to certain styles of beer, though exceptions do exist *(12)*, and a recent study has shown how strains may be modified naturally to eliminate the POF phenotype *(26)*. Other beer related traits in baking yeasts (flocculation potential, repitchability etc) require more extensive investigation.

An aim of this work was to assess the level of variability that could be introduced to sahti beer through the use of selected strains, while staying true to the style's official appellation. Strains differed with respect to both fermentation performance and flavour profile. While all yeasts tested could use maltotriose, a signature of domestication in baking and brewing yeasts *(27)*, the extent of utilisation varied, resulting in beers with quite different alcohol content. The 1.3% difference in alcohol content, with values being up to 13% higher or 6% lower than the control, were similar to a comparable study that included sourdoughderived strains. The study by Marongiu et al. *(3)* also showed about a 1.3% difference in alcohol, with values being up to 16% higher or 8% lower than the control. These differences may also, like here, have been influenced by the yeast strains' relative abilitie to utilise maltotriose. Such differences in yield amongst strains could be exploited to design beers with more or less alcohol depending on requirements. However, much greater variation was observed for the concentrations of volatile flavour compounds. These were, as expected, quite high in most beers *(10)*, but there were clear strainspecific differences. Two sourdough strains in particular (C117 and C118) produced relatively low levels of flavour volatiles, and may be suitable for production of beers with a more prominent malt or juniper flavour. Conversely, a number of strains produced flavour volatile levels at or above those seen in the reference beers. Five sourdough strains produced, for example, relatively high levels of the pear/banana-like 3-methylbutyl acetate, a characteristic flavour compound in sahti beer, and such strains could be used to accentuate this particular note. In addition, certain strains produced high concentrations of other compounds such as the ethyl hexanoate, and could likewise be used to introduce the associated apple-like flavours of this compound.

The sahti brewing process has a number of unique features relative to the conventional brewing process. The high starting gravities (sometimes higher than 25°P), would be expected to impose significant stress on yeasts through high osmotic stress early in the fermentation process and high levels of ethanol toxicity later in the fermentation. The absence of a wort boiling step, combined with the lack of the antimicrobial effect of hops, suggests that bacterial growth during fermentation is inevitable, and fast fermentation rate (despite stressful conditions) is necessary. In this respect, sahti brewing strains, similar to kveik yeast strains, must have the ability to be metabolically active in extreme conditions. Therefore, stress tolerance was considered to be a key feature for prospective brewing yeast strains. All strains tested were capable of withstanding high levels of both ethanol and osmotic stress, though viability following fermentation varied between strains. These viability values were low relative to those found in industrial yeast slurries intended for repitching. It should be noted however that, in sahti brewing, yeast is used only once, and yeasts need only be resistant enough to successfully complete the fermentation. It is of interest that a high stress tolerance was apparent in the most productive strain of the group (Y17). Assessing the sahti fermentation performance of yeasts associated with similarly stressful conditions could yield interesting results. In addition to kveik strains, saké or distilling strains could

be evaluated for their performance in the context of sahti brewing, though such an approach should take into account the style's official appellation.

Conclusions

Overall, this screening experiment, though limited in scope, successfully identified a number of strains that could feasibly be used to differentiate sahti beers, and indicated that a greater degree of diversity would be achievable with a larger cohort of strains. It may be speculated that, despite the absence of extant Finnish house yeasts, the culture of maintaining particular yeast strains for particular regional beers could still be revived. It may be that traditional sourdough cultures maintained locally still house the original sahti strains of that region, and this warrants further investigation. Results of this study, while focused on sahti beer production, are applicable to any beer style where phenolic flavour notes are permissible. This would include wheat beers, certain farmhouse ales, including saisons and other Belgian ale styles.

Author contributions

BG and MC wrote the original draft of the manuscript. AM, CR, FM, JN, KK, LJ, LS and MC carried out the investigations (experiments and analytics). BG, JN, ML and MP were involved in the initial concept development. All authors reviewed, edited and approved the final manuscript.

Conflict of interest

The authors declare there are no conflicts of interest.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.