Research article

Institute of Brewing & Distilling

Received: 18 February 2015 Revised: 20 May 2015 Accepted: 10 June 2015 Published online in Wiley Online Library: 11 September 2015

(wileyonlinelibrary.com) DOI 10.1002/jib.246

Physicochemical characterization of sahti, an 'ancient' beer style indigenous to Finland

Jukka Ekberg,[1] Brian Gibson,[1]* Jussi J. Joensuu,[1] Kristoffer Krogerus,[1,2] Frederico Magalhães,[1,2] Atte Mikkelson,[1] Tuulikki Seppänen-Laakso[1] and Arvi Wilpola[1]

Sahti, a strong, unhopped farmhouse beer flavoured with juniper, is still actively brewed in rural areas in Finland. Presented here is the first comprehensive analysis of the physical and chemical properties of this unique beer style. Twelve sahti samples from the southwest of Finland were analysed and, while properties varied, the beers generally had high levels of alcohol (mean = 7.9% ABV) and high residual extract (mean = 9.5°P). Foam stability was negligible, as is typical for the style, and glycerol concentrations at 3.1 – 4.7 g L^{-1} were higher than in reference beers (commercial lager, wheat beer and porter). These features may be attributed to the very high gravity conditions employed in brewing sahti beers. Bitterness levels were relatively low (3–13 IBU) owing to the absence or moderate use of hops. All samples contained detectable levels of the clove-like compound 4-vinylguaiacol owing to the use of baker's rather than brewer's yeast for brewing. Concentrations of higher alcohols and esters were high, with many individual aroma compounds being above the normal flavour thresholds. Results have highlighted the uniqueness of this style of beer in comparison to commercially available beers and have contributed to our understanding of the reasons for the particular sensorial properties of this traditional beer style.

Keywords: sahti; Finland; beer; flavour

Introduction

Despite being a unique and distinctive part of Finnish cultural heritage and one of the few primitive beer styles remaining in production in Europe, sahti beer has never been subject to comprehensive chemical or physical analysis. International interest in sahti has been due to a number of factors including, firstly, promotion by connoisseurs such as the late Michael Jackson *(1)* and, secondly, the beer's unique method of preparation and distinctive sensorial properties. Sahti wort is prepared using malted barley along with malted or unmalted grains of rye (typically ~10% of the grain bill) and sometimes also wheat or oats. Malting and mashing for sahti production have traditionally been carried out in domestic saunas, which afforded some measure of temperature control as well as facilities for boiling water. Wort production is by infusion mashing, usually over a long period (4 h or more), and typically involves addition of heated water to the mash. In some cases heated stones may be introduced to produce *kivisahti*, a form of Steinbier *(2)*. In contrast to other beer styles, hops are an optional ingredient and sahti beers are typically more sweet than bitter. The titular 'ancient' is used here in reference to the fact that the style pre-dates the use of hops as a bittering agent. Another distinctive feature of sahti brewing is that wort is not boiled and as such is prone to microbial contamination, a feature that limits commercialization somewhat. Uniquely, sahti production involves branches (and sometimes berries) of juniper, which add a spice flavour to the brew and have an antimicrobial effect. These branches lie on a filter bed composed of straw (rye typically) at the bottom of a *kuurna* (a trough-shaped, aspen-hewn vessel) through which the sahti wort is filtered. The first wort prepared is the strongest and may be collected separately to the later wort. The weaker, sugar-poor wort was traditionally reserved for preparation of a mild table beer, while the stronger wort was used to ferment the full-strength sahti normally containing 7–8% alcohol. Another unique feature of the process is the use of baker's yeast, rather than brewing yeast, to carry out fermentation *(3)*.

While the origin of sahti is not known, it has been brewed in Finland for several hundred years at least. The first detailed written description of sahti appears to be the doctoral dissertation of Carl Hellenius of Åbo Academy in 1780. Until the prohibition years (1919–1932), the Finnish government's attitude to domestic brewing was quite liberal and Finns enjoyed a freedom to maintain their brewing traditions in a way that was not possible for citizens of other European countries *(4)*. In addition, urbanization came relatively late to Finland, further maintaining the tradition of sahti brewing, which is predominantly carried out in rural areas. Sahti was, and still is, brewed particularly for celebratory events such as Easter, Midsummer, harvest celebrations, Christmas and wedding ceremonies. Sahti brewing is mostly associated with the south and southwestern parts of Finland with the northern and eastern parts of the country having the least activity, probably

* Correspondence to: B. Gibson, VTT Technical Research Centre of Finland, Tietotie 2, PO Box 1000, FI-02044 VTT, Espoo, Finland.
E-mail: brian.gibson@vtt.fi

[1] VTT Technical Research Centre of Finland, Tietotie 2, PO Box 1000, FI-02044 VTT, Espoo, Finland

[2] Department of Biotechnology and Chemical Technology, Aalto University, School of Chemical Technology, Kemistintie 1, Aalto, PO Box 16100, FI-00076 Espoo, Finland

owing to lack of suitable land for arable farming *(3)*. Production now is carried out in much the same way as in the traditional process except that stainless steel vessels are more common than the traditional wooden vessels and straw filter beds are rarely used. In the EU, sahti is protected by the 'Traditional Speciality Guaranteed' label, implying that commercial use of the name is restricted to beer produced according to the traditional, registered production method *(5)*. A small number of commercial breweries produce sahti in Finland, including Lammin Sahti Oy and Finlandia Sahti Oy. National and international distribution is, however, limited owing to the microbiological instability of the beer and the style is little known outside of Finland and neighbouring countries.

While sensorial properties vary regionally, sahti is typically strong in alcohol, sweet, cloudy and with strong yeast-derived fruit flavour notes. Rye and juniper flavours are often quite prominent *(6)*. We present here data on the physicochemical characteristics of 12 'farmhouse' sahti beers produced in various locations throughout Finland and compare these properties with those of modern, commercially available ale and lager beers.

Methodology

Sahti samples

Twelve freshly brewed sahti beers produced in various locations around the country (Fig. 1) were sampled in August 2014. All samples were maintained at 4°C after sampling and during transport. Samples for chemical analysis were centrifuged to remove solid material and aliquots were frozen (−20°C) for later analysis. The brewers of donated sahti samples were interviewed for their manufacture process. Each brewer had some specific details in their process. Generally, the malt bill consisted about 90% of a special sahti blend [Viking Malt, Lahti, Finland (majority pils malt, minority of dark and enzyme malt, http://www.polttimo.com/filebank/993-SAHTI_MALLAS.pdf)] and 10% dark Tuoppi rye malt (Laihian mallas, Laihia, Finland). Some brewers also used minor amounts of special malts or unmalted grain products. The malt to extract (w:v) ratio varied from 0.9 to 0.34. Most of the brewers used the traditional hot water steeping process for mashing. Aliquots of near-boiling water were used to raise the temperature of the mash gradually from 30 to 70°C. The duration of mashing was long and averaged around 6 h. Before lautering the mash was typically transferred to another vessel with a heat source and outmashed between 85°C and boiling point. All the brewers lautered the mash in a *kuurna*, a log-shape vessel traditionally carved from an aspen trunk, although most of the wooden kuurnas have now been replaced by stainless steel counterparts. The mash was rinsed with hot water until a desired volume of wort was collected. Only a few of the brewers measured the actual gravity of their wort. A cooled wort was pitched with fresh baker's yeast (Suomen Hiiva Oy, Lahti, Finland). Pitching rates varied from 1 to 2.5g of fresh yeast per litre of wort. The brewers of large (over 100 L) batches typically used a starter culture, while the smaller batches were inoculated directly with yeast. The primary fermentation was carried out at room temperature and lasted 24–48 h after which the fermentation was continued at cooler temperatures (5–10°C). Here, the variation between brewers was large – some finished the fermentation in the same vessel and others transferred the wort to another vessel for secondary fermentation. Also the duration of secondary fermentation varied a lot, from a few days to a few weeks. When the fermentation was complete, sahtis were stored at cool temperatures (around 5°C) before the sampling process. Only a small number of the brewers used hops in the brewing process. All brewers used juniper twigs or berries, in either the mashing or the lautering process.

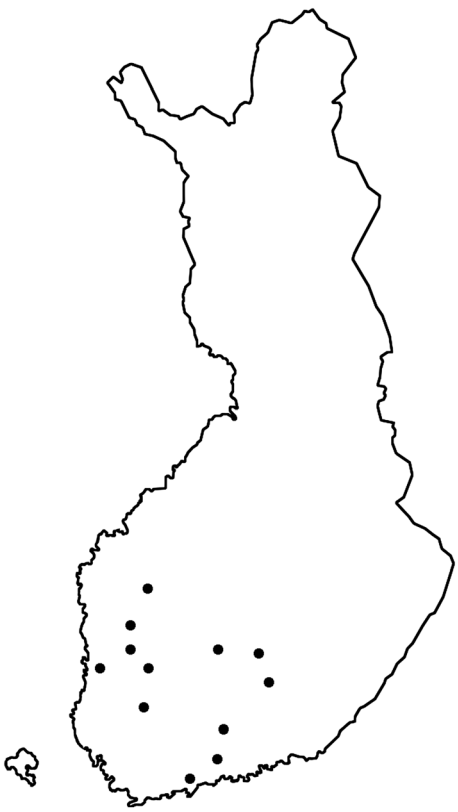

Figure 1. Locations in Finland from which sahti samples originated.

Beer analyses. Colour (EBC Method 9.6) and bitterness (EBC Method 9.8) were determined as described by the European Brewery Convention. Specific gravity, alcohol content and pH were determined with an Anton-Paar DMA 5000M density meter equipped with Alcolyser and pH module. Beer foam stability was measured according to Analytica-EBC method 9.42 *(7)* using a NIBEM foam stability meter (Haffmans, Holland).

Fermentable sugars, glycerol, lactic acid and acetic acid were quantified with Waters Alliance 2690 HPLC system (Waters, Milford, MA, USA) where the injection volume was 20 μL. An Aminex HPX-87H Organic Acid Column (300 × 7.8 mm; Bio-Rad, Hercules, CA, USA) linked to a Fast Acid Analysis column (100 × 7.8 mm; Bio-Rad, USA) was used as a stationary phase in the HPLC. Columns were maintained at 55°C and 5 mM H_2SO_4 (Titrisol, Merck, Germany) was used as an eluent with the flow rate of 0.3 mL min^{-1}. A Waters 2414 Differential Refractometer (Waters, Milford, MA, USA) was used for detection.

Total phenolic acid content. Sahti samples (0.5 mL) were spiked with heptadecanoic acid (C17:0 FFA, 50 μg) and hydrolysed with 2 M NaOH (1.1 mL per sample) in the dark, at room temperature, for 16 h. After hydrolysis the samples were acidified with 5 M HCl (700 μL, pH $<$ 4) and extracted with 5 mL of ethyl acetate by vortexing gently for 10 min. The samples were centrifuged (2500 rpm for 10 min) and the ethyl acetate layers were separated. The extraction was repeated and the ethyl acetate layers were combined. Ethyl acetate was evaporated under nitrogen flow and the residue was dissolved into 50 μL of

dichloromethane and transferred into GC sample vials. The samples were trimethylsilylated with MSTFA (25 μL, *N*-methyl-*N*-trimethylsilyltrifluoro-acetamide, Pierce, Rockford, IL, USA) containing 0.1% trimethylchlorosilane at 80°C for 20 min. An Agilent 7890A GC combined with a 5975C mass selective detector was equipped with a DB-5 silica capillary column (30 m, 0.25 mm i.d., phase thickness of 0.25 μm, Restek, Bellefonte, PA, USA) and the temperature programme was from 70°C (1 min) to 240°C (15 min) at 10°C min^{-1}. The split ratio was 20:1 and the data was collected at a mass range of 50–600 *m/z*. A Gerstel Maestro MPS 2 sampling system (Gerstel GmbH&Co.KG, Mühlheim am der Ruhr, Germany) was used for injection of the samples (1 μL aliquots). Identification of the compounds was based on retention times and library comparison (NIST '08, Scientific Instrument Services Inc., Ringoes, NJ, USA).

GC/MS analysis of volatiles. Sahti samples were analysed for volatiles from pentane extracts. Samples (5 mL) were extracted with pentane (3 mL) by vortexing for 5 min, the extraction was repeated and the pentane layers combined. The samples were concentrated to 2 mL, dried with sodium sulphate, transferred into GC vials and concentrated to 0.5 mL under nitrogen flow. The analyses were performed by GC-MS fitted with an FFAP silica capillary column (25 m, 0.20 mm, 0.3 μm, Agilent). The injection volume was 2 μL, the split ratio 15:1, and the oven programme from 40°C (1 min) to 200°C (6 min) at 7°C min^{-1}. The data was collected at a mass range of 30–600 *m/z*.

4-Vinyl guaiacol was analysed using HPLC-PAD based on methods described by Coghe et al. *(8)* and McMurrough et al. *(9)*. The chromatography was carried out using a Waters Alliance HPLC system consisting of a Waters e2695 Separations Module equipped with a XTerra® MS C_{18} column (5 μm, 4.6 × 150 mm) and a Waters 2996 Photodiode Array Detector. The mobile phase consisted of H_2O–CH_3OH–H_3PO_4 (64:35:1, v/v) and the flow rate was 0.5 mL min^{-1}. The diode array detector was used at 190 - 400 nm. The 4-vinyl guaiacol was quantified at 260 nm using standard curves of the pure compound (0.3 – 30 μg mL^{-1}).

Yeast-derived aroma compounds were determined by headspace gas chromatography with flame ionization detector (HS-GC-FID) analysis. Four-millilitre samples were filtered (0.45 μm) and incubated at 60°C for 30 min, and then 1 mL of gas phase was injected (split mode; 225°C; split flow of 30 mL min^{-1}) into a gas chromatograph (HP 6890 Series; Palo Alto, CA, USA) combined with an FID detector and headspace autosampler (Headspace Autosampler 7000 HT, Tekmar-Dohrmann, USA). Analytes were separated on a HP-5 capillary column (50 m × 320 μm × 1.05 μm column, Agilent, USA). The carrier gas was helium (constant flow of 1.4 mL min^{-1}). The temperature programme used was 50°C for 3 min, 10°C min^{-1} to 100°C, 5°C min^{-1} to 140°C, 15°C min^{-1} to 260°C and then isothermal for 1 min. Compounds were identified by comparison with authentic standards and were quantified using standard curves. The compound 1-butanol was used as the internal standard.

Results

Alcohol, extract, residual sugar

Sahti samples had high levels of alcohol with a mean value of 7.9% (ABV, v/v), although values varied from 3.7 to 10.5% (Fig. 2). Amongst the reference beers, the strongest was the commercial sahti at 9.2%, followed by the porters (both at 7.4%). The lagers and wheat beers had levels ranging from 4.5 to 5.4%. Extract levels were high relative to the non-sahti reference beers, with the lowest value for a sahti (5.0°P) being close to the highest value for the reference beers (5.2°P). The mean extract value for the sahti beers was 9.5°P and in several samples the final extract values were close to starting values of many beers, that is, around 11–12°P. In one exceptional case the extract value was 18.2°P. The commercial sahti included in the analysis had values for extract and alcohol that were comparable to the farmhouse sahtis (6.6°P and 9.2% ABV). Samples with the highest extract values generally had the lowest alcohol content (Fig. 2). The values for extract and alcohol suggest that the starting extract would have been around 25°P for many of the fermentations. High extract values were due to the presence of unfermented sugars, which ranged from 18 to 43 g L^{-1} for maltotriose and from 0 to 115 g L^{-1} for maltose (Table 1). These values were an order of magnitude higher than in the non-sahti

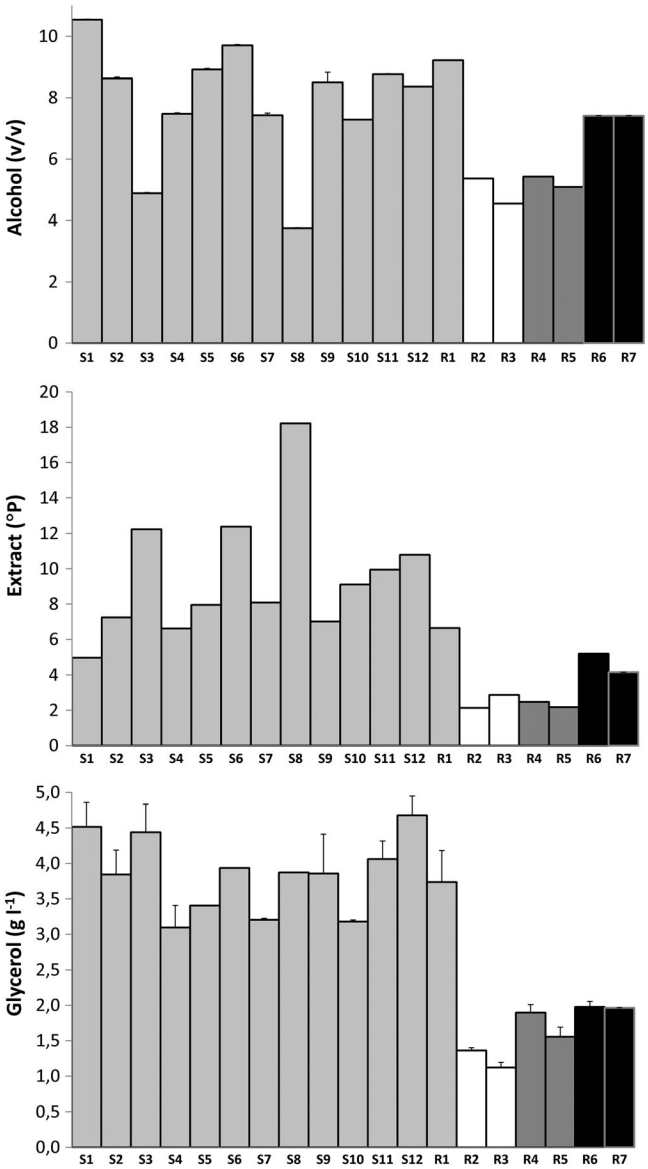

Figure 2. Alcohol (v/v, %) and extract (°Plato) sahti samples and reference beers as determined by Anton Paar DMA5000M density meter and alcolyser. Beers include sahti samples (S) and Reference samples (R) including a commercial sahti (R1), two pale lagers (R2/3), two wheat beers (R4/5) and two porters (R6/7). Values are means of two technical replicates and error bars where visible indicate half the range.

Institute of Brewing & Distilling

Table 1. Residual sugars in sahti samples and reference samples including one commercial sahti, two pale lagers, two wheat beers and two porters. Values are means of two technical replicates ± half the range

Beer sample	Maltotriose (g L^{-1})	Maltose (g L^{-1})	Glucose (g L^{-1})
Sahti 1	28.6 ± 0.3	12.0 ± 0.2	0.3 ± 0.1
Sahti 2	27.9 ± 1.3	8.3 ± 0.1	0.4 ± 0.1
Sahti 3	31.7 ± 2.3	72.7 ± 5.5	0.5 ± 0.1
Sahti 4	21.1 ± 0,5	4.0 ± 0,1	0.4 ± 0,1
Sahti 5	26.6 ± 0.0	7.4 0.0	0.2 ± 0.0
Sahti 6	27.7 ± 0.0	9.4 ± 0.0	0.5 ± 0.0
Sahti 7	17.7 ± 0.3	6.0 ± 0.1	0.6 ± 0.2
Sahti 8	36.8 ± 0.0	115.4 ± 0.0	1.2 ± 0.0
Sahti 9	24.0 ± 1.9	8.3 ± 0.6	0.6 ± 0.3
Sahti 10	23.1 ± 3.1	0.0 ± 0.0	0.6 ± 0.3
Sahti 11	42.6 ± 3.6	12.0 ± 0.6	0.5 ± 0.3
Sahti 12	28.7 ± 1.1	23.1 ± 0.8	0.7 ± 0.2
Reference 1 (sahti)	28.6 ± 1.5	8.1 ± 0.3	0.8 ± 0.0
Reference 2 (lager)	1.3 ± 0.1	0.0 ± 0.0	0.2 ± 0.0
Reference 3 (lager)	1.5 ± 0.0	1.2 ± 0.1	0.1 ± 0.0
Reference 4 (wheat)	1.7 ± 0.1	0.0 ± 0.0	0.2 ± 0.0
Reference 5 (wheat)	1.8 ± 0.0	3.1 ± 0.1	0.1 ± 0.0
Reference 6 (porter)	6.5 ± 0.1	3.3 ± 0.0	0.6 ± 0.0
Reference 7 (porter)	1.7 ± 0.1	0.5 ± 0.1	0.2 ± 0.0

reference beers, which had mean values of 2.4 and 1.3 g L^{-1} for maltotriose and maltose, respectively. Values for residual glucose were low, with only the least attenuated sahti beer (sample 8) containing >1 g L^{-1} (Table 1). The glycerol contents of farmhouse sahtis and commercial reference sahti ranged from 3.1 to 4.5 g L^{-1}, and were higher than those found in the non-sahti reference beers (1.1–2.0 g L^{-1}).

pH and organic acids

At a pH of 4.4, the mean value for farmhouse sahti beers was only slightly less acidic than that of the non-sahti commercial reference beers at pH 4.3 (Fig. 3). There was, however, greater variation within the sahti beers (pH 3.9–4.8) than in the non-sahti reference beers (pH 4.2–4.4). The lowest pH values for sahti beers (sahti samples S3, S8 and R1) coincided with the highest concentrations of lactic acid (2.2–2.8 g L^{-1}), and in the case of samples S3 and S8, also the highest concentrations of acetic acid (1.2 and 2.0 g L^{-1}, respectively).

Foam stability

Traditional sahti is characterized by an absence of foam and the results of this study show a clear difference in foam stability between the sahti samples and the six non-sahti reference beers, where the time required for a 3 cm collapse in beer foam varied from 4 to >5 min (Fig. 4). The commercial sahti maintained foam stability for <1 min and in all farmhouse sahti samples the collapse in foam was immediate.

Colour and bitterness

All sahtis were turbid in appearance and red in colour, with shades ranging from bright oranges to deep reds. EBC colour values

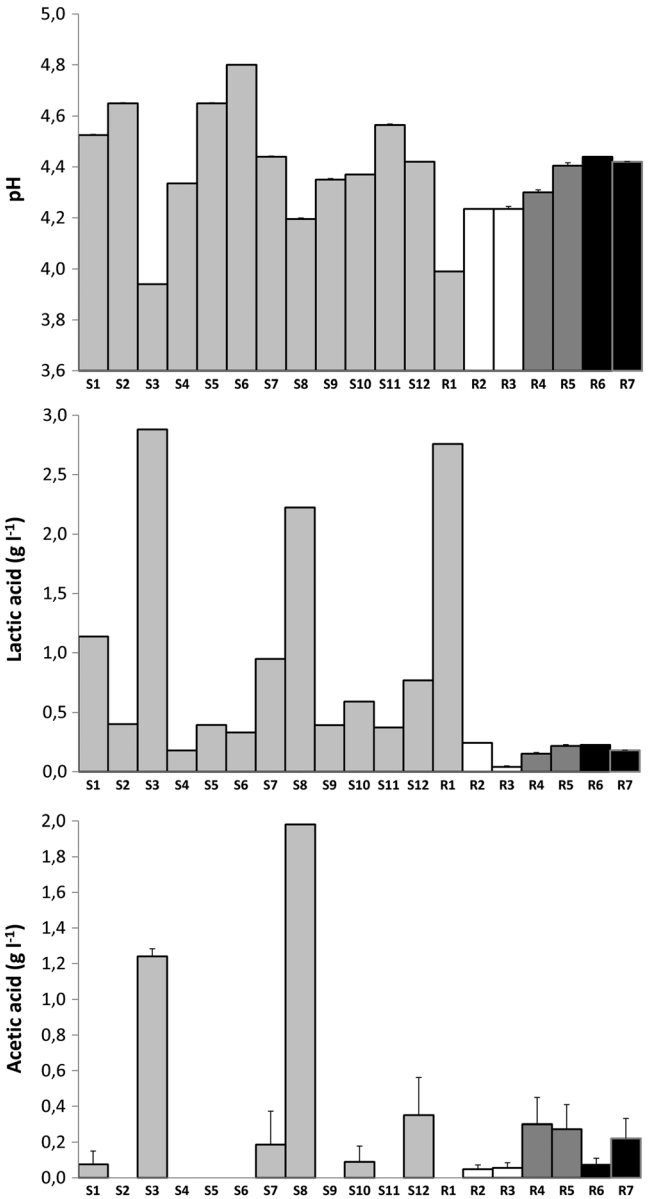

Figure 3. pH, lactic acid and acetic acid concentration in sahti samples and reference beers. See legend of Fig. 2 for coding. Values are means of two technical replicates and error bars where visible indicate half the range.

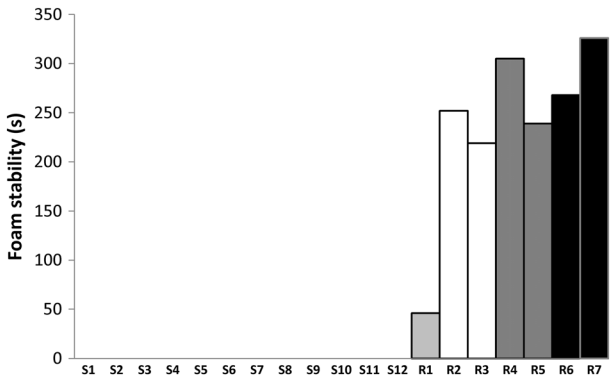

Figure 4. Foam stability of sahti samples and reference beers as determined by NIBEM foam stability meter. Values represent the time in seconds for beer to collapse by 30 mm. See legend of Fig. 2 for coding. No value indicates that foam collapse was immediate.

ranged from 33 to 105 and, as expected, were higher than in the reference lager and wheat beers (mean values of 8 and 16, respectively; Fig. 5). The porters had a mean value of 210 EBC. Bitterness values varied somewhat within the sahti group but, with a mean value of 8.9 IBU, were lower than for the reference lager, wheat beer and porter samples, which had mean values of 14, 12 and 30 IBU, respectively.

Phenolic acids

The sum of the phenolic compounds (caffeic acid, cinnamic acid, ferulic acid, sinapic acid and vanillic acid) was higher for the sahti samples (mean of 50 mg L^{-1}; range of 30–62 mg L^{-1}) than the lager (mean 19.5 mg L^{-1}), wheat beer (29 mg L^{-1}) and porter (47 mg L^{-1}) samples (data not shown). Mean values for vanillic (5.2 mg L^{-1}) and ferulic (30 mg L^{-1}) acid in sahti beers (including the reference) were similar to those of the porters (4.1 and 32 mg L^{-1}, respectively) and at least 2-fold higher than the concentrations in the lagers and wheat beers (Fig. 6). Sinapic acid concentration was highly variable amongst the sahti strains but mean values at 9.2 mg L^{-1} were higher than the mean values for all other beer types (2.3–7.4 mg L^{-1}). Caffeic acid values were highest in sahti and wheat beers at 2.3 mg L^{-1} each, compared with the lager and porter beers styles (mean values of 1.7 and 1.4 mg L^{-1}).

The volatile phenol derived from ferulic acid, 4-vinylguaiacol (phenolic/bitter/clove aroma) was present in all sahti samples above the flavour threshold of 0.3 mg L^{-1} (Fig. 7). Values were, however, variable, ranging from a minimum of 0.4 to a maximum of 3.2 mg L^{-1}. Levels of 4-vinylguaiacol above the flavour threshold were only found in the wheat beer references. In the lagers and porters the values were either low or below the analytical detection limit.

In this study, all classes of yeast-derived aroma compound (higher alcohols, acetate esters and ethyl esters) were found at high concentrations, with mean concentrations of individual flavour compounds being present at concentrations 2- to 5-fold greater in sahtis than in the reference beers (Figs. 8 and 9). Concentrations were in many cases above the flavour thresholds *(10,11)*. Notable examples were the honey/rose aroma phenylethanol (at or above threshold levels in three sahtis; Fig. 8), the fruit/solvent aroma ethyl acetate (above threshold levels in 10 sahtis and the non-lager reference beers), the banana/pear aroma 3-methylbutyl acetate (at or above threshold levels in all but one sahti and in three reference beers), the apple aroma ethyl caprylate (at or above threshold levels in two sahtis) and the apple/aniseed aroma ethyl caproate (above threshold levels in 11 sahtis and one reference beer; Fig. 9).

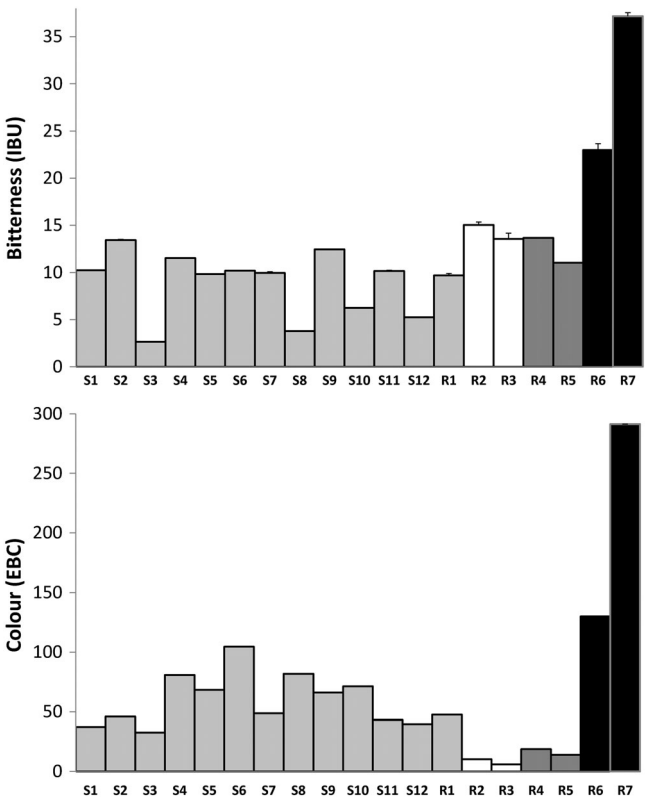

Figure 5. Colour and bitterness of sahti samples and reference beers. See legend of Fig. 2 for coding. Values are means of two technical replicates and error bars where visible indicate half the range.

Discussion

Sahti beers are traditionally brewed to be strong and sweet *(3)* and the 'farmhouse' sahtis sampled and analysed here were no exception, having alcohol contents as high as 10.5% (ABV, v/v), while retaining high levels of fermentable sugars. The initial wort extract levels for these beers are mostly not known, but the alcohol and residual extract values would suggest a starting gravity for all beers in the very high gravity range (~25°P). Where alcohol levels were considerably lower than the mean value, for example, samples 3 and 8, there remained particularly high levels of unfermented maltose and glucose. The relatively low alcohol levels are therefore due to a low level of attenuation as opposed to a lack of fermentable sugar. Consumption of incompletely fermented sahti is not unusual in parts of Finland. The consistently high levels of maltotriose would suggest an inability of the fermenting yeast to use this sugar under the prevailing conditions. This is probably due to the inhibitory effect of high maltose concentrations rather than an inability to transport and metabolize maltotriose *(12)*. The high levels of glycerol in the sahti beers would suggest that the yeast was experiencing osmotic stress under the very high gravity brewing conditions employed. Glycerol is produced by yeast in a response to stresses caused by high osmotic pressure in an effort to balance the water potential between the cell and the environment *(13)* in a process mediated by the high osmolarity glycerol pathway *(14)*. In comparison, the non-sahti reference beers contained 1–2 g L^{-1} of glycerol, which is within the normal range for beers *(15)*.

Variation in pH of the sahti beers appeared to coincide with variation in the levels of lactic and acetic acid in the beer. These organic acids are most likely due to the presence of acidifying bacteria in the fermentation. As sahti wort is not boiled and a baker's yeast is used for pitching, there is a resident microflora of lactic acid bacteria, and such 'contamination' can be considered a normal part of the sahti brewing process and may contribute in a positive way to the typical sensorial properties of sahti beer, as it does in lambic beers and certain other beer styles *(15)*. However, the high levels of acetic acid in samples S3 and S8 would suggest that the bacterial count or composition was not optimal and may have limited the fermentation performance in these cases. The most likely cause of the higher acetic acid levels is ingress of oxygen during or after fermentation. This oxygenation would have allowed the proliferation of the obligate aerobic bacteria that

Institute of Brewing & Distilling

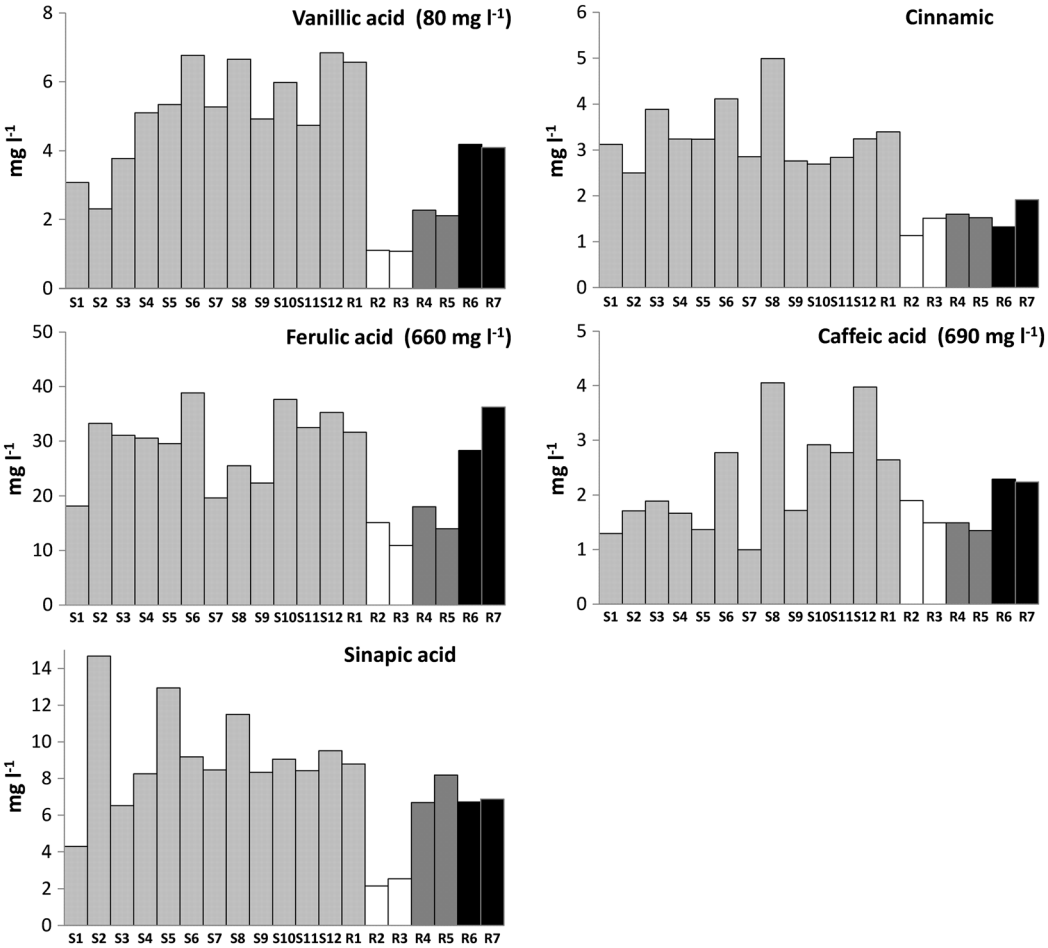

Figure 6. Total phenolic acid content of sahti samples and reference beers. See legend of Fig. 2 for coding. Values in parentheses represent flavour threshold values (where known) as determined by Meilgaard *(11)*.

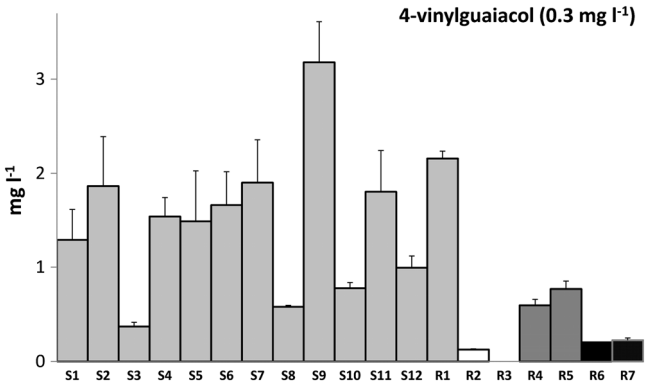

Figure 7. Concentrations of 4-vinylguaiacol in sahti samples and reference beers. See legend of Fig. 2 for coding. Value in parentheses represents the flavour threshold as determined by Meilgaard *(11)*. Values are means of two technical replicates and error bars where visible indicate half the range.

oxidize ethanol to acetic acid *(16)*. The metabolism of these organisms may have contributed to the lower alcohol contents of the affected beers.

Farmhouse sahtis, like many English ales, have a characteristically low level of carbonation and foam production. Multiple factors determine the propensity of a beer to generate and maintain a head of foam *(17)* and it is likely that the lack of foam stability in sahti is due to a combination of factors including carbonation, yeast activity during fermentation and raw material composition. Carbon dioxide bubbles arising from nucleation sites in beer provide the impetus for foam production at dispense and in the glass, and in sahti the lack of foaming is certainly influenced by the low level of carbonation. Stability of foam is determined by foam stabilizers such as grain-derived polypeptides and hop-derived iso-acids. These stabilizers ensure that the CO_2 rising through the beer becomes trapped at the surface within numerous small bubbles that restrict gas loss and prevent beer in the foam draining back down to the liquid beer. The principal protein involved in foam stabilization is the water-soluble albumin lipid transfer protein (LTP1) *(18)* and there is no reason to suspect that sahti wort is particularly deficient in this protein. However, LTP1 is known to be most effective after wort boiling owing to denaturation and consequent exposure of hydrophobic amino acids *(19,20)*. As sahti wort is not boiled, it may be expected that the protein remains in the native state and therefore contributes little to foam stability. This property will be compounded by the fact that the yeasts used for sahti fermentation are likely to release significant levels of the vacuolar aspartic enzyme proteinase A *(21)*, which is known to impair foam stability by degrading foam-promoting polypeptides, including LTP1 *(22)*. Proteinase A release is normal in high-gravity wort fermentations *(23,24)* where yeast are exposed to a number of stresses including osmotic stress and

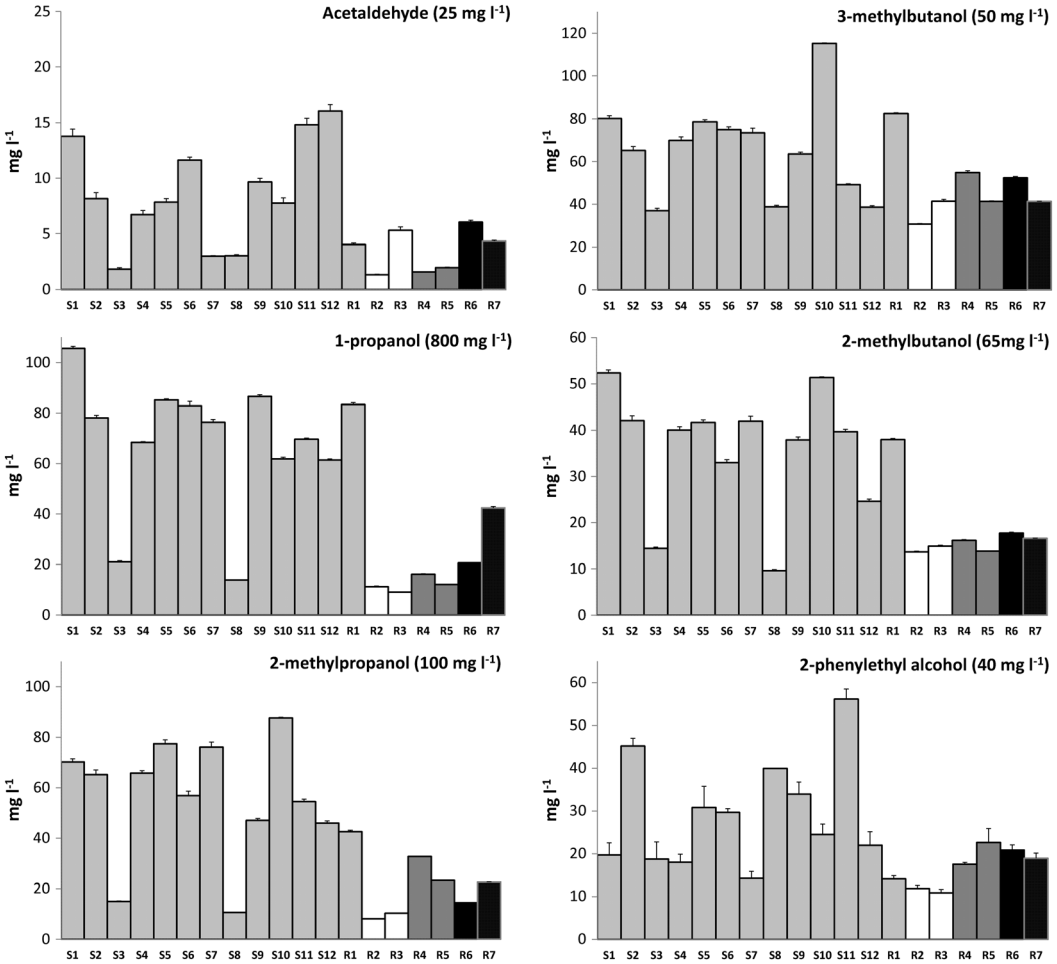

Figure 8. Concentrations of acetaldehyde and higher alcohols in sahti samples and reference beers. See legend of Fig. 2 for coding. Values in parentheses represent flavour threshold values as determined by Meilgaard *(11)*. Values are means of three technical replicates and error bars where visible indicate half the standard deviation.

ethanol toxicity *(25)*. The extreme fermentation conditions employed in brewing sahti will almost certainly promote the release of proteinase A from stressed cells, particularly as baking yeast, which have a low level of ethanol tolerance, are used for fermentation.

Iso-α-acids originating from hops are known to stabilize foams by promoting hydrophobicity *(26)*. As hops are only used in small amounts in sahti brewing, this foam fortification does not normally occur. Of note here is the fact that the commercial sahti used as a reference in this study (and the only sahti from which foam stability was measurable) was brewed with hops. The high ethanol content of sahti may also aggravate the foam instability *(27)*.

While phenolic acids can contribute an astringent flavour to foods and beverages, their concentrations in beer are rarely high enough to be detected sensorially *(11)*. Rather, their significance lies in the fact that they can in some cases act as precursors for volatile phenols such as 4-vinylguaiacol (see below) as well as having antioxidant properties in beer. The anti-radical activity of phenolic acids in beer is known to contribute to flavour stability *(28–30)* and has been suggested to have health-promoting effects *(31)*. Phenolic compounds are derived from the malt and hops used in beer production and concentrations in beer are highly variable *(32,33)* and dependent on barley variety *(34)* and process parameters such as malting, milling and mashing conditions as well as wort boiling *(30,35–38)*. Levels of total free phenolic compounds (sum of caffeic, cinnamic, ferulic, sinapic and vanillic acids) were highest in the sahti beers and in the porters, where mean concentrations for these beer styles were around 50 mg L^{-1} in both cases, considerably higher than the mean lager and wheat beer concentrations (around 20 and 30 mg L^{-1}, respectively). As the contribution of hops and wort boiling to sahti phenolic acid concentrations is likely to be low, it may be assumed that the phenolic acids are derived from the malt predominantly and that the relatively high levels in the sahti beer samples are due to the high extract levels in sahti wort. The sum of the phenolic compounds (caffeic acid, cinnamic acid, ferulic acid, sinapic acid and vanillic acid) was higher for the sahti samples (mean of 50 mg L^{-1}; range of 30–62 mg L^{-1}) than the lager (mean 19.5 mg L^{-1}), wheat beer (29 mg L^{-1}) and porter (47 mg L^{-1}) samples (data not shown). The compound α-terpineol, which is derived from juniper, was detected in five of the farmhouse sahtis analysed by GC/MS (sahti samples 1, 2, 6, 10 and 11). These compounds were not detected in the non-sahti reference beers (data not shown).

The volatile phenolic compound 4-vinylguaiacol was present in all sahti beers at concentrations above the flavour threshold of 0.3 mg L^{-1} *(11)*. This compound is a vinyl derivative of free ferulic acid and characteristic of yeast strains that express phenylacrylic acid decarboxylase. Strains that produce these flavours are phenolic off-flavour positive. This trait is not typical for brewing yeast, with the exception of those yeasts that are utilized to produce wheat beers *(39)*, and this was shown in the analysis of the reference

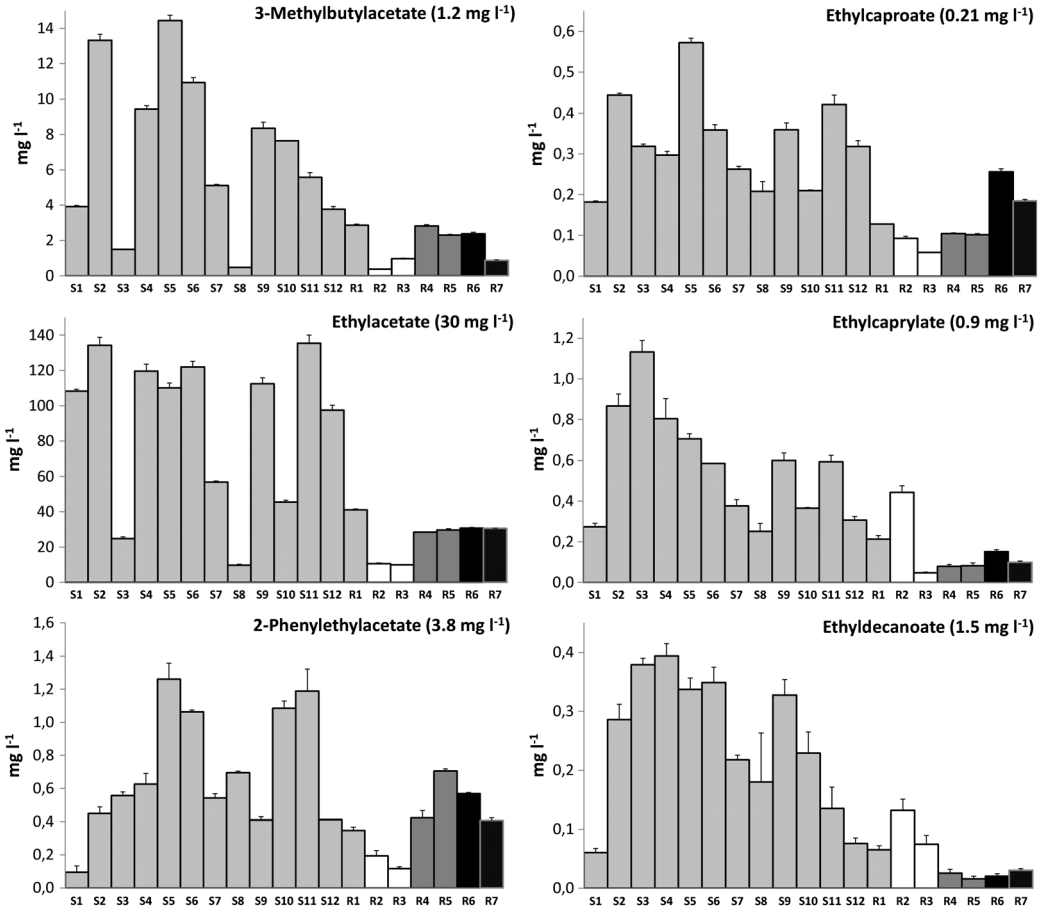

Figure 9. Concentrations of acetate and ethyl esters in sahti samples and reference beers. See legend of Fig. 2 for coding. Values in parentheses represent flavour threshold values as determined by Meilgaard *(11)*. Values are means of three technical replicates and error bars where visible indicate half the standard deviation.

beers in this study. It is almost certain that the presence of these phenolic flavours is due to the traditional use of baker's yeast for sahti fermentation *(3)*. Our own unpublished work has shown that the commercial baker's yeasts available in Finland do indeed express phenylacrylic acid decarboxylase. While it is known that 4-vinylguaiacol can be produced from ferulic acid in the absence of yeast, this process is associated with high-temperature treatment *(40)* and is not expected to occur during sahti production, where wort boiling is not normally employed.

Production of aroma compounds by yeast during fermentation is critically important for the beer flavour profile. While the flavours vary depending on the compound in question, aroma compounds are typically perceived as fruity or floral. A range of factors are known to influence the concentration of aroma compounds in beer. These include yeast strain, the presence of contaminant microorganisms, original gravity and nutritional status of the wort, the availability of flavour precursors, and process conditions such as oxygen availability, temperature and pitching rate *(41–47)*. While it is not possible to determine the exact reasons for the concentrations of aroma compounds found in each sahti beer, it is likely that the high levels were influenced by the high fermentation temperatures and original gravities that are typically employed for sahti fermentation. It is known that even small increases in temperature can have a profound influence on the concentration of aroma compounds in beer *(43,44,48–50)*. The sahti beers included in this study were fermented without temperature control during the summer of 2014 (mean temperature in Finland in July 2014 was ca. 19°C). The low fermentation temperatures of lager brewing would likewise explain why the reference lager beers in this study had the lowest concentrations of many aroma compounds analysed. All of the sahti fermentations could be described as very high gravity fermentations, that is, with an original gravity above 20°P. Such fermentations are known to contain disproportionately high levels of esters relative to the amount of alcohol produced *(25,42,44)*. It is probable that both factors, high temperature and original gravity, had a major impact on the flavour profiles of the sahti beers tested.

Under the conditions employed to produce the sahti beers, especially where traditional wooden brewing equipment was used, the presence of some wild yeast would not be unexpected. Such yeast, for example, *Hanseniaspora* spp., *Torulaspora* spp. or *Dekkera bruxellensis* are known to produce high levels of aroma compounds and if present could have influenced the flavour profiles of some of the beers included here *(51–53)*. It is also certain that the fermentations would have contained some lactic acid bacteria, as these are present in the commercially available baker's yeast products used to ferment sahti wort and are known to influence flavour via ester synthesis *(54)*.

The sensorial attributes reported here show clearly that sahti occupies a unique position in the beer family. Interpretation of the results has, however, been mainly through *post-hoc* analysis of the data and there is a clear need for further study to determine the exact relationship between sahti brewing procedures and organoleptic properties. In particular, the contribution of flavour

compounds derived from juniper requires more detailed analysis as does the contribution of the complex microflora associated with sahti fermentation. Such knowledge is essential if sahti is to be faithfully recreated and properly experienced outside of the restricted geographical area with which it has traditionally been associated.

Acknowledgements

The authors are grateful for the support of Sirpa Kääriäinen of Lammin Sahti Oy (Lammi) for her assistance in sample collection and critical reading of the manuscript and to the individual sahti brewers for donating their beers and providing descriptions of their brewing procedures. Liisa Änäkäinen and Airi Hyrkäs are thanked for skilful technical assistance and Riikka Juvonen is thanked for critical appraisal of the manuscript prior to submission.

References

1. Jackson, M. (1988) *New World Guide to Beer*, Bloomsbury, London.
2. Räsänen, M. (1975) *Von Halm zum Fass*, Oy Weilin & Göös Ab, Helsinki.
3. Asplund, U. (1990) *Sahtikirja*, Suomen Sahtiseura, Lammi.
4. Ovell, P. (1996) *Finland's Indigenous Beer Culture*, Perinteisen Oluen Seura, Helsinki.
5. European Community, Commission Regulation (EC) No 244/2002 of 8 February 2002 supplementing the Annex to Regulation (EC) No 2301/97 on the entry of certain names in the 'Register of certificates of specific character' provided for in Council Regulation (EEC) No 2082/92 on certificates of specific character for agricultural products and foodstuffs. OJ L39/11.
6. Rokka, J. (2012) in Sahti, *The Oxford Companion to Beer*, (Oliver, G. Ed.) 1st ed., pp. 709–711, Oxford University Press, New York.
7. European Brewery Convention (2007) *Analytica-EBC*, Fachverlag Hans Carl, Nürnberg.
8. Coghe, S., Benoot, K., Delvaux, F., Vanderhaegen, B., and Delvaux, F. R. (2004) Ferulic acid release and 4-vinyl guaiacol formation during brewing and fermentation: Indications for feruloyl esterase activity in *Saccharomyces cerevisiae*, *J. Agric. Food Chem. 52*, 602–608.
9. McMurrough, I., Madigan, D., Donnelly, D., Hurley, J., Doyle, A.-M., Hennigan, G., and McNulty, N. (1996) Control of ferulic acid and 4-vinyl guaiacol in brewing, *J. Inst. Brew. 102*, 327–332.
10. Engan, S. (1972) Wort composition and beer flavour. 2. Influence of different carbohydrates on formation of some flavour components during fermentation, *J. Inst. Brew. 78*, 169–173.
11. Meilgaard, M. C. (1975) Flavor chemistry of beer. Part II: Flavour and threshold values of 239 aroma volatiles, *Tech. Q. Master Brew. Assoc. Am. 12*, 151–168.
12. Londesborough, J. (2001) Fermentation of maltotriose by brewer's and baker's yeast, *Biotechnol. Lett. 23*, 1995–2000.
13. Gibson, B. R., Lawrence, S. J., Leclaire, P. R., Powell, C. D., and Smart, K. A. (2007) Yeast responses to stresses associated with industrial brewery handling, *FEMS Microbiol. Rev. 31*, 535–569.
14. Maeda, T., Wurgler-Murphy, S. M., and Sato, H. (1994) A two-component system that regulates an osmosensing MAP kinase cascade in yeast, *Nature 369*, 242–245.
15. Briggs, D. E., Boulton, C. A., Brookes, P. A., and Stevens, R. (2004) *Brewing: Science and Practice*, Woodhead, Cambridge.
16. Van Vuuren, H. J. J., and Priest, F. G. (2003) Gram-negative spoilage bacteria, in *Brewing Microbiology*, (Priest, F. G., and Campbell, I. Eds.) 3rd ed., pp. 219–246, Chapman & Hall, London.
17. Bamforth, C. W. (2012) *Foam*, American Society of Brewing Chemist, St Paul, MN.
18. Sørensen, S. B., Bech, L. M., Muldbjerg, M., Beenfeldt, T., and Breddam, K. (1993) Barley lipid transfer protein 1 is involved in beer foam formation, *Tech. Q. Master Brew. Assoc. Am. 30*, 136–145.
19. Bech, L. M., Vaag, P., Heinemann, B., and Breddam, K. (1995) Throughout the brewing process barley lipid transfer protein 1 (LTP1) is transformed into a more foam-promoting form, in *Proceedings of the European Brewers Convention Congress, Brussels*, pp. 561–568, IRL Press, Oxford.
20. Van Nierop, S. N. E., Evans, D. E., Axcell, B. C., Cantrell, I. C., and Rautenbach, M. (2004) Impact of different wort boiling temperatures on the beer foam stabilizing properties of lipid transfer protein 1, *J. Agric. Food Chem. 52*, 3120–3129.
21. Lenney, J., Matile, A., Wiemken, A., Schellenberg, M., and Meyer, J. (1974) Activities and cellular localization of yeast proteases and their inhibitors, *Biochem. Biophys. Res. Commun. 60*, 1378–1383.
22. Leisegang, R., and Stahl, U. (2005) Degradation of a foam-promoting barley protein by a proteinase from brewing yeast, *J. Inst. Brew. 111*, 112–117.
23. Cooper, D. J., Stewart, G. G., and Bryce, J. H. (2000) Yeast proteolytic activity during high and low gravity wort fermentations and its effect on head retention, *J. Inst. Brew. 106*, 197–201.
24. Brey, S. E., de Costa, S., Rogers, P. J., Bryce, J. H., Morris, P. C., Mitchell, W. J., and Stewart, G. G. (2003) The effect of proteinase A on foam-active polypeptides during high and low gravity fermentation, *J. Inst. Brew. 109*, 194–202.
25. Gibson, B. R. (2011) Improvement of higher gravity brewery fermentation via wort enrichment and supplementation, *J. Inst. Brew. 117*, 268–284.
26. Evans, D. E., Finn, J. E. C., Robinson, L. H., Eglinton, J. K., Sheehy, M., and Stewart, D. C. (2011) The effects of hop-α-acids and proline-specific endoprotease (PSEP) treatements on the foam quality of beer, *J. Inst. Brew. 117*, 335–342.
27. Brierly, E. R., Wilde, P. J., Onishi, A., Hughes, P. S., Simpson, W. J., and Clark, D. C. (1996) The influence of ethanol on the foaming properties of beer protein fractions: A comparison of rudin and microconductivity methods of foam assessment, *J. Sci. Food Agric. 70*, 531–537.
28. Maillard, M.-N., Soum, M.-H., Boivin, P., and Berset, C. (1996) Antioxidant activity of barley and malt. Relationship with phenolic content, *Lebenm. Wiss. u. Technol. 29*, 238–244.
29. Szwajgier, D., and Bancarzewska, M. (2011) Changes in the phenolic acid content during wort boiling and whirlpool, *Acta Sci. Pol. Technol. Aliment. 10*, 19–22.
30. Leitao, C., Marchioni, E., Bergaentzlé, M., Zhao, M., Didierjean, L., Miesch, L., Holder, E., Miesch, M., and Ennahar, S. (2012) Fate of polyphenols and antioxidant activity of barley throughout malting and brewing, *J. Cereal Sci. 55*, 318–322.
31. Nardini, M., Natella, F., Scaccini, C., and Ghiselli, A. (2006) Phenolic acids from beer are absorbed and extensively metabolized in humans, *J. Nutr. Biochem. 17*, 14–22.
32. Ganbaatar, C., Valášek, P., Kuban, V., Fisera, M., and Hoza, I. (2010) Review on comparative analyses of different phenolic acids content in different beers, *J. Life Sci. 4*, 58–62.
33. Marova, I., Parilova, K., Friedl, Z., Obruca, S., and Duronova, K. (2011) Analysis of phenolic compounds in lager beers of different origin. A contribution to potential determination of the authenticity of Czech beer, *Chromatographia 73*, 83–95.
34. Zhao, H., Fan, W., Dong, J., Lu, J., Chen, J., Shan, L., Lin, Y., and Kong, W. (2008) Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties, *Food Chem. 107*, 296–304.
35. Szwajgier, D. (2009) Content of individual phenolic acids in worts and beers and their possible contribution to the antiradical activity of beer, *J. Inst. Brew. 115*, 243–252.
36. Szwajgier, D. (2011) Dry and wet milling of malt. A preliminary study comparing fermentable sugar, total protein, total phenolics and the ferulic acid content in non-hopped worts, *J. Inst. Brew. 117*, 569–577.
37. Szwajgier, D., Pielecki, J., and Targónski, Z. (2005) Changes of free ferulic and coumaric acid contents during malting of barley grain, *Pol. J. Food Nutr. Sci. 14*, 423–429.
38. Zhao, H., and Zhao, M. (2012) Effects of mashing on total phenolic contents and antioxidant activities of malts and worts, *Int. J. Food Sci. Technol. 47*, 240–247.
39. Vanbeneden, N., Gils, F., Delvaux, F., and Delvaux, F. R. (2008) Formation of 4-vinyl and 4-ethyl derivatives from hydroxycinnamic acids: Occurrence of volatile phenolic flavour compounds in beer and distribution of Pad1-activity among brewing yeasts, *Food Chem. 107*, 221–230.
40. Vanbeneden, N., Van Roey, T., Willems, F., Delvaux, F., and Delvaux, F. R. (2008) Release of phenolic flavour precursors during wort production: Influence of process parameters and grist composition on ferulic acid release during brewing, *Food Chem. 111*, 83–91.
41. Äyräpää, T. (1970) Der Einfluss der Temperatur auf die Bildung hoherer Alkohole durch Kulturhefen, *Brauwissenschaft 23*, 48–53.
42. Verstrepen, K. J., Derdelinckx, G., Dufour, J.-P., Winderickx, J., Thevelein, J. M., Pretorius, I. S., and Delvaux, F. R. (2003) Flavor-active esters: Adding fruitiness to beer, *J. Biosci. Bioeng. 96*, 110–118.

43. Saerens, S. M. G., Delvaux, F., Verstrepen, K. J., Van Dijck, P., Thevelein, J. M., and Delvaux, F. R. (2008) Parameters affecting ethyl ester production by *Saccharomyces cerevisiae* during fermentation, *Appl. Environ. Microbiol. 74*, 454–461.
44. Saerens, S. M. G., Verbelen, P. J., Vanbeneden, N., Thevelein, J. M., and Delvaux, F. R. (2008) Monitoring the influence of high-gravity brewing and fermentation temperature on flavour formation by analysis of gene expression levels in brewing yeast, *Appl. Microbiol. Biotechnol. 80*, 1039–1051.
45. Verbelen, P. J., Saerens, S. M. G., Mulders, S. E., Delvaux, F., and Delvaux, F. R. (2009) The role of oxygen in yeast metabolism during high cell density brewery fermentations, *Appl. Microbiol. Biotechnol. 82*, 1143–1156.
46. Mota, A., Novák, P., Macieira, F., Vicente, A. A., Teixeira, J. A., Šmogrovičová, D., and Brányik, T. (2011) Formation of flavor-active compounds during continuous alcohol-free beer production: The influence of yeast strain, reactor configuration, and carrier type, *J. Am. Soc. Brew. Chem. 69*, 1–7.
47. Lei, H., Zhao, H., Yu, Z., and Zhao, M. (2012) Effects of wort gravity and nitrogen level on fermentation performance of brewer's yeast and the formation of flavour volatiles, *Appl. Biochem. Biotechnol. 166*, 1562–1574.
48. Anderson, R. G., and Kirsop, B. H. (1974) The control of volatile ester synthesis during the fermentation of wort of high specific gravity, *J. Inst. Brew. 80*, 48–55.
49. Peddie, H. A. B. (1990) Ester formation in brewery fermentations, *J. Inst. Brew. 96*, 327–331.
50. Titica, M., Landaud, S., Trelea, I. C., Latrille, E., Corrieu, G., and Cheruy, A. (2000) Modelling of the kinetics of higher alcohol and ester production based on CO_2 emission with a view to control of beer flavor by temperature and top pressure, *J. Am. Soc. Brew. Chem. 58*, 167–174.
51. Plata, C., Millán, C., Mauricio, J. C., and Ortega, J. M. (2003) Formation of ethyl acetate and isoamyl acetate by various species of wine yeasts, *Food Microbiol. 20*, 217–224.
52. Rojas, V., Gil, J. V., Pinaga, F., and Manzanares, P. (2003) Acetate ester formation in wine by mixed cultures in laboratory fermentations, *Int. J. Food Microbiol. 86*, 181–188.
53. Viana, F., Gil, J. V., Vallés, S., and Manzanares, P. (2009) Increasing the levels of 2-phenylethyl acetate in wine through the use of a mixed culture of *Hanseniaspora osmophila* and *Saccharomyces cerevisiae*, *Int. J. Food Microbiol. 135*, 68–74.
54. Sumby, K. M., Grbin, P. R., and Jiranek, V. (2010) Microbial modulation of aromatic esters in wine: Current knowledge and future prospects, *Food Chem. 121*, 1–16.