

West African sorghum beer fermented with *Lactobacillus delbrueckii* and *Saccharomyces cerevisiae*: fermentation by-products

C. Djameh,^{1*} W.O. Ellis,¹ I. Oduro,¹ F.K. Saalia,² K. Haslbeck³ and G.A. Komlaga¹

Physicochemical quality parameters and volatile fermentation by-products were determined in West African sour sorghum beer (*pito*) fermented with pure cultures of *Lactobacillus delbrueckii* and *Saccharomyces cerevisiae* compared with *pito* prepared by traditional spontaneous fermentation. Levels of by-products were also compared with those found in similar beer types. Similar levels of apparent extract, alcohol, pH, lactic acid and bitterness were obtained for pure culture and traditional fermentations, although differences were observed in colour and turbidity. Significant statistical differences were obtained for all of the volatile aroma compounds analysed. The pure culture approach resulted in a higher level of total volatile compounds (353 mg/L) of which higher alcohols accounted for 88%, predominately *n*-propanol. The traditional approach had total volatiles of 229 mg/L with 86% higher alcohols but with *iso*-amyl alcohol predominating. Ester levels were low in the pure culture beer but with a relatively high level of acetaldehyde. Fermenting *pito* with pure cultures yielded a product with similar physicochemical quality as traditional *pito* but with a suggestion of a more pronounced aroma whose impact on the overall product quality will require consumer acceptance and sensory evaluation. © 2019 The Institute of Brewing & Distilling

Keywords: *pito*; sorghum beer; starter culture; physico-chemical analysis; volatile fermentation by-products; product quality

Introduction

Pito is a popular traditional sour sorghum beer widely consumed in Ghana and elsewhere in West Africa. In addition to *pito*, it is known as *dolo* (Burkina Faso, Niger and Mali) and *chapalo* (Ivory Coast, Togo and Benin). It is sold and drunk while still fermenting and is considered a refreshing drink. *Pito* brewing is a major income generating activity. It is produced from local sorghum cultivated by subsistence farmers and has economic potential for industrial commercialisation (1). However, the production process is uncontrolled and unpredictable, resulting in inconsistent product quality that undermines industrial production.

There are two fermentations involved in the production of *pito*, a lactic acid fermentation followed by an alcoholic fermentation. The lactic acid fermentation which is responsible for the sour taste is performed spontaneously by microflora on the sorghum from the field and from the brewing environment. The alcoholic fermentation is carried out by numerous types of yeasts and other microflora introduced through back-slopping inoculation. Various studies have been carried out with the application of starter cultures of lactic acid bacteria and yeasts isolated from the drink to control the process and achieve product consistency. However, matching or improving the quality of the traditionally brewed *pito* has been challenging for several reasons. Traditional or natural fermentation methods initiated by endogenous flora, yield products that have unique or particular quality attributes (2). Starter cultures for indigenous fermented foods and beverages need to be isolated from the products so as to facilitate their adaptation to the medium (1,3–5). According to Jimoh *et al.*, (6) when indigenous traditional technologies are adopted for industrial application, the methods of preparation are altered and this leads to a product of

altered flavour and poor acceptability. Demuyakor and Ohta (7) reported differences in sensory characteristics of *pito* brewed with various starter cultures of microorganisms isolated from *pito* and traditionally brewed *pito*. They also observed that improvements in traditional processing can lead to changes in product quality. Industrial production of *pito* calls for the use of stable commercial starter cultures. In this study, to assess the possibility of product quality being altered using commercial starter cultures, two parameters that influence beer quality and flavour perception were monitored. Accordingly, physicochemical parameters and volatile by-products of *pito* fermented with pure commercial cultures of *Lactobacillus delbrueckii* and *Saccharomyces cerevisiae* were compared with those of *pito* fermented spontaneously with mixed microflora.

Physical and chemical analyses of beers involve measurement of analytical parameters which can be conveniently measured in quality assurance (8). They are used to define the requirements of regulatory bodies and generally provide an assessment of the fermentation performance and an indication of the quality of the

* Correspondence to: Clement Djameh, Kwame Nkrumah University of Science and Technology. Department of Food Science and Technology. Kumasi, Ghana. E-mail: cldjameh@yahoo.co.uk

¹ Department of Food Science and Technology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

² Department of Food Process Engineering, University of Ghana, Accra, Ghana

³ Research Center Weihenstephan for Brewing and Food Quality, Technical University of Munich, Freising, Germany

product. The values of these parameters are specific to the beer type and are influenced by the nature of the raw material and processing method. In the process of beer production, sugars in the wort are fermented to ethanol and carbon dioxide. This also results in the formation of fermentation by-products which impact on the taste, aroma and other characteristic properties of the beer (9).

This study involves the assessment of pure cultures for the development of a controlled process supporting the possible industrial production of *pito* with quality comparable with that of the traditional product.

Materials and methods

Preparation of samples

The *pito* samples were brewed with 10 kg of sorghum malt prepared using the malting process described by Djameh et al. (10) from *kadaga* red sorghum, a variety commonly used in brewing *pito* in Ghana. The malt was milled together with the rootlets and shoots in a hammer mill fitted with 0.4 mm sieve and mashed with 45 L of water at ambient temperature (28–32°C) in a 50 L stainless steel vessel. The mash was left to sediment but without the addition of pounded clarifying barks employed in larger-scale preparation. The clear supernatant was decanted after 60 min and retained for subsequent conversion of starch in the residual thick mash, which was first gelatinised by boiling. The thick residual mash was boiled for 30 min and then recombined with the supernatant. The temperature was adjusted to 62°C, held for 60 min and raised to 72°C for saccharification. After starch conversion the mash was divided into two portions of 20 L for fermentation with the traditional *pito* process and with pure commercial starter cultures.

Traditional *pito* fermentation

The portion of saccharified mash for the traditional *pito* was left to undergo spontaneous lactic acid fermentation at ambient temperature (28–32°C). After achieving the typical sour point of the traditional *pito* process (pH 4.25, 0.33% lactic acid), the mash was filtered through a fine mesh nylon filter cloth. The pH was measured with a Hanna digital pH meter (H198190, Hanna Instruments, USA) according to the manufacturer's instructions. Lactic acid was measured as described by Kunze (9). The filtrate (wort) was boiled for 90 min and cooled to ambient temperature then left for 120 min to sediment and clarify. The clarified wort (15 L) was transferred to a 20 L glass fermenting vessel and 15 g of harvested dried yeast (locally known as *dambile*) from a traditional *pito* brewer's brew was added to it to initiate alcoholic fermentation at ambient temperature of 28–32°C.

Pure culture *pito* fermentation

The saccharified mash was filtered after starch conversion and 15 L of wort was boiled for 30 min in a 20 L stainless steel pressure cooker to sterilise it and then filled hot into a 20 L sanitised stainless steel fermenting vessel and cooled to 45°C. It was then inoculated with 1500 mL of a starter culture of *L. delbrueckii* 'H1' with cell population density of 2.22×10^7 CFU/mL and fitted with a fermentation lock. *L. delbrueckii* 'H1' was obtained from Biological Laboratory, Versuchs- und Lehranstalt für Brauerei in Berlin (VLB) e.V., Berlin, Germany. The vessel was left for 12 h at 45°C in a thermostatic water bath to undergo lactic acid fermentation. It was then

cooled to 24°C and pitched with 15 g of dried Anchor Brewer's Yeast (*S. cerevisiae* from Rymco (Pty) Ltd, South Africa) for fermentation at a controlled temperature of 24°C.

Analysis

Physicochemical analysis

Samples (400 mL) of *pito* fermented with pure cultures and spontaneously were cooled to 20°C and degassed by transferring into 1 L Erlenmeyer flasks and shaking. The decarbonated sample was clarified by filtering through Whatman no.12 fluted filter paper. The temperature of the filtrate was adjusted to 20°C and specific gravity (°P) determined using an Anton Paar DMA 4500 M. For turbidity measurement, the sample was unfiltered and measurement carried out with a Hanna turbidity meter model H193703-11 according to the procedure described in Analytica EBC (11). Lactic acid was measured as described previously (9). Colour was measured by a spectrophotometric method (11). The analyses were in triplicate and mean values are reported.

Volatile fermentation by-products

Samples of *pito* fermented with pure cultures and the traditional process were transferred to 330 mL bottles and pasteurised at 62°C for 25 min. The determination of volatile aroma compounds was carried out according to the headspace method for fermentation by-products (12).

Three bottles of starter culture *pito* and three bottles of traditional *pito* were analysed as fresh samples and then again after four weeks of storage at 28–30°C to assess the stability of the fermentation by-products. The samples were analysed using a Perkin Elmer Clarus 580 gas chromatograph GC-FID system with a flame ionisation detector, a Turbo Matrix 40 (HS) autosampler (2 mL sample in a 20 mL vial) and an INNOWAX column (dimension 60 m long, 0.25 mm i.d. and 0.5 µm thickness). The carrier gas was helium 5.0 ECD quality split 20 mL/min. Vials containing the samples were equilibrated to 60°C for 25 min. One minute after injection at 50°C the temperature was increased at 7°C/min to 85°C. The internal standard was *p*-cymene and the software was TotalChrom. The concentration of compounds was calculated from calibration curves created from peak areas of compounds and internal standards.

Statistical analysis

The determination of volatile aroma compounds was carried out in duplicate with the calculation of mean and standard deviation. One-way analysis of variance (ANOVA) with Statgraphics Centurion XVI Version 16.1.11 was used to analyse the volatile components in the pure culture *pito* and traditional *pito* samples followed by Fisher's least significant difference procedure to determine statistically significant differences between the means at the 95.0% confidence level. Principal component analysis was also carried out using Minitab 14 software to reduce the volatile compounds into two main components which would explain the total variation in the data.

Results and discussion

Physicochemical analysis

The analysis of the pure culture *pito* beer compared favourably with the traditional beer with the exception of turbidity. Here, the traditionally brewed *pito* had a higher turbidity value (225 FTU) compared with 39 FTU of the pure culture beer. The analytical values are reported in Table 1. A similar study by Orji *et al.* (14) brewed *pito* in the laboratory with pure cultures of *L. plantarum* together with *S. cerevisiae*, *Pediococcus halophilus* and *Candida tropicalis* isolated from a local beer. Analysis showed the pH, colour, titratable acidity, alcohol content and specific gravity to compare favourably with those of *pito* produced by the traditional process.

The comparatively high turbidity of 225 FTU for traditionally brewed *pito* may reflect the presence of non-flocculating yeast such as *Torulopsis*, which have small cells and are therefore slow to sediment (15). The turbidity of 39 FTU for pure culture *pito* is considered to be 'slightly hazy' but is exceptionally clear for a traditional African sorghum beer. Most of such beers are typically opaque as they are kept at warm ambient temperature, which does not encourage sedimentation of the yeasts, together with the presence of unconverted starch granules.

Sefa-Dedeh *et al.* (16) also reported the quality metrics of *pito* produced using *S. cerevisiae* as a single starter microorganism which compared favourably with the traditional approach. However, the alcohol content of the single strain pure culture fermentation was slightly lower than that of the control traditional beer. This was in agreement with the findings of Lyumugabe *et al.* (17)

and Demuyakor and Ohta (7) where traditional sorghum beer *ikigage* and *pito* fermented with mixed culture of microorganism produced more alcohol than in the fermentation with single strain starter culture. This was also reflected in the higher apparent extract of the pure culture *pito* with the implication of lower degree of fermentation as both brews started with the same original extract of 13°P. This could be attributed to the intrinsic characteristics of the pure strain yeast, Anchor Brewer's Yeast. Again, in agreement with their findings, the mixed culture fermentation produced more titratable acid (determined as lactic acid) and had lower pH, which could possibly reflect the presence of acetic acid bacteria, a typical spoilage bacterium found in *pito* which is responsible for spoilage by rendering the product too sour to drink (18).

In the absence of the use of hops to provide bitterness in *pito* brews, the measured bitterness can be attributed to polyphenol, protein and yeast bitterness (9). As the source of yeast bitterness is the only difference between the two brews, it can be concluded from the same value for bitterness that the yeasts have no significant effect on the bitterness of *pito*.

The colour of the of *pito* fermented with single pure cultures of lactic acid bacteria and yeast was lower (45 EBC) than of the control brew fermented with the traditional process of mixed cultures (51 EBC). Both values were higher than the values (17 ± 3 and 18 ± 2 EBC respectively) obtained by Avicor *et al.* (19) in a similar study. In their work, malt from *chireh*, a white non-tannin sorghum variety, was used rather than the red sorghum, *kadaga*, used here. The red pigment in the testa of the grain may have leached into the mash, accounting for the comparatively high colour of the experimental beers.

Table 1. Analytical values of *pito* fermented with pure cultures of *Lactobacillus delbrueckii* 'H1' and Anchor Brewer's Yeast (*Saccharomyces cerevisiae*) (SC *Pito*) and *pito* fermented with the traditional mixed population of microflora (Trad *Pito*)

Parameter	Trad <i>Pito</i> , mean	Trad <i>Pito</i> , STDEV	SC <i>Pito</i> , mean	SC <i>Pito</i> , STDEV	Range (13)
Original extract (°P)	13	0	13	0	8.0 — 14.0
Apparent extract (°P)	5.2	0.2	6.4	0.2	2.0 — 7.0
Rest extract (°P)	6.8	0.2	7.8	0.1	3.0 — 8.0
Alcohol (% v/v)	4.2	0.3	3.6	0.1	2.0 — 5.0
pH	3.5	0.1	3.6	0.2	3.1 — 3.8
Lactic acid (% w/v)	0.8	0	0.6	0	0.4 — 0.9
Colour (EBC)	50.7	0.2	45.6	0.9	15 — 80
Turbidity (FTU)	225	5.6	39.1	1.2	N/A

Table 2. Concentration of fermentation aroma compounds in fresh and 4 week stored *pito* traditionally fermented with mixed population of microflora (Trad *Pito*) and *pito* fermented with pure starter cultures of *L. delbrueckii* and Anchor Brewer's Yeast (SC *Pito*)

Component (mg/L)	Trad <i>Pito</i> , fresh	SC <i>Pito</i> , fresh	Trad <i>Pito</i> , 4 weeks storage	SC <i>Pito</i> , 4 weeks storage
Acetaldehyde	2.0 ± 0.1 ^a	32.7 ± 2.1 ^b	1.2 ± 0.2 ^a	34.4 ± 1.2 ^b
<i>n</i> -Propanol	23.9 ± 0.2 ^a	134.2 ± 1.4 ^b	23.8 ± 0.7 ^a	135.4 ± 3.4 ^b
<i>Iso</i> -butanol	33.1 ± 0.5 ^a	75.8 ± 0.1 ^b	33.2 ± 0.7 ^a	76.9 ± 1.2 ^b
<i>Iso</i> -amyl alcohol	140.1 ± 1.9 ^a	100.9 ± 0.2 ^b	141.4 ± 5.7 ^a	101.7 ± 0.9 ^b
Ethyl acetate	28.9 ± 1.5 ^a	9.4 ± 0.5 ^b	29.2 ± 1.9 ^a	9.7 ± 0.2 ^b
<i>Iso</i> -amyl acetate	1.1 ± 0.1 ^a	0.2 ± 0.0 ^b	1.1 ± 0.1 ^a	0.3 ± 0.0 ^b
Diacetyl (total)	0.2 ± 0.0 ^a	0.2 ± 0.0 ^b	0.2 ± 0.0 ^a	0.2 ± 0.0 ^b
Total volatiles	229 ± 4.3 ^a	353.1 ± 1.2 ^b	229.8 ± 9.3 ^a	358.4 ± 6.8 ^b

Values are means of two determinations. Mean values with same superscript in a row are not significantly different ($p < 0.05$).

Volatile fermentation by-products

The levels of aldehydes, vicinal diketones, higher alcohols and esters influence the sensory perception of beer (aroma and taste) and hence quality. The values of these compounds in the test and control *pito* brews are reported in Table 2.

There was a significant statistical difference in the levels of all the volatile fermentation compounds in the test and control brews of *pito*. The differences can be explained by the diversity of the different strains of yeasts producing these compounds, as reported by Zhu *et al.* (20). Yeast strains differ greatly with regard to their formation of fermentation by-products, in particular higher alcohols and esters and the ratio of the floral esters to the higher aliphatic alcohols (9). Romano *et al.* (21) have reported that, in the fermentation of wine, each yeast determines the concentration of flavour compounds in the final wine and noted that the significant strain variability in the species may cause a loss of characteristic aroma and flavour determinants when starter cultures are used for fermentation.

There was no significant difference between the level of volatile fermentation by-products in the fresh *pito* samples and samples stored for four weeks at 28–30°C to accelerate ageing. This implied aroma stability of at least four weeks. This can be explained by the absence of any further metabolic activity by yeast in the samples as they were pasteurised.

Acetaldehyde is an important aldehyde in beer and is an intermediate in fermentation (9). The value in pure culture *pito* of 33 mg/L was high and above the threshold value of 2–20 mg/L reported by Ma *et al.* (22) for ales – the style of beer *pito* resembles through its fermentation conditions. It was also high compared with the 0.019 mg/L obtained in experimental traditional sorghum beer *ikigage* of Rwanda brewed with a pure culture of *S. cerevisiae* and *L. fermentum* in the study of Lyumugabe *et al.* (17). The traditional *ikigage* contained 0.076 mg/L acetaldehyde. However, the 2 mg/L in traditionally brewed *pito* was closer to the lower end of the range of 2–20 mg/L reported by Ma *et al.* (22). The higher fermentation temperature of traditional *pito*, 30°C compared to 24°C for pure culture *pito*, favours the reduction of aldehydes and can explain its lower value in traditional *pito* compared with starter

culture *pito*. The effect of the warmer fermentation temperature can similarly explain the lower level of diacetyl in traditional *pito* as this is favoured by higher fermentation temperatures. The higher alcohols found were *n*-propanol, *i*-butanol and *iso*-amyl alcohol (2,3-methyl butanol). These accounted for 311 mg/L (88% of the total volatile compounds) in the *pito* brewed with pure cultures in comparison with 197 mg/L (86%) in traditionally brewed *pito*. These results are consistent with the observations of Ashraf *et al.* (23) that, of the secondary metabolites formed by yeasts, the higher alcohols are produced in the highest concentrations during fermentation, with propanol, isobutyl alcohol and isoamyl alcohol the predominant aroma compounds. The high concentration of higher alcohols in the *pito* brew is also in agreement with the report of Greenshields (24) that the concentration of higher alcohols in home brewed beers and wines is at least 10 times higher than those in the commercial products. The concentrations of *n*-propanol (134 mg/L) and isoamyl alcohol (101 mg/L) in the pure culture *pito* were high in comparison with the values of 11 mg/L for *n*-propanol in pure culture *S. cerevisiae* and 53 mg/L for isoamyl alcohol in a mixed culture of *S. cerevisiae* and *C. tropicalis* obtained by Coulibaly *et al.* (25) in their study on the influence of freeze dried starter cultures on volatile compounds of *tchakpalo*, the Ivorian sorghum beer equivalent of *pito*.

Higher alcohols are formed through the catabolic Ehrlich pathway from amino acids and from anabolic route from sugars through biosynthesis. Their formation is dependent upon the fermentation temperature; an increase in temperature results in increased concentrations of higher alcohols, except for *n*-propanol. Brown and Hammond (26) reported the taste thresholds for higher alcohols to be 2–100 mg/L. Typical levels of isoamyl alcohol have been found to be 38–100 mg/L (26). The value of isoamyl alcohol in starter culture *pito* fell within these established ranges.

Esters contribute to the overall flavour of beer and are important aroma compounds, but abnormally high levels may be regarded as off-flavours. According to Hough *et al.* (15), wild yeasts *Hansenula* and *Pichia* produce large quantities of ethyl acetate by aerobic fermentation and they reported that lambic and gueuze beers contain 33–167 mg/L of ethyl acetate. These beers are sour beers produced by spontaneous fermentation similar to traditional

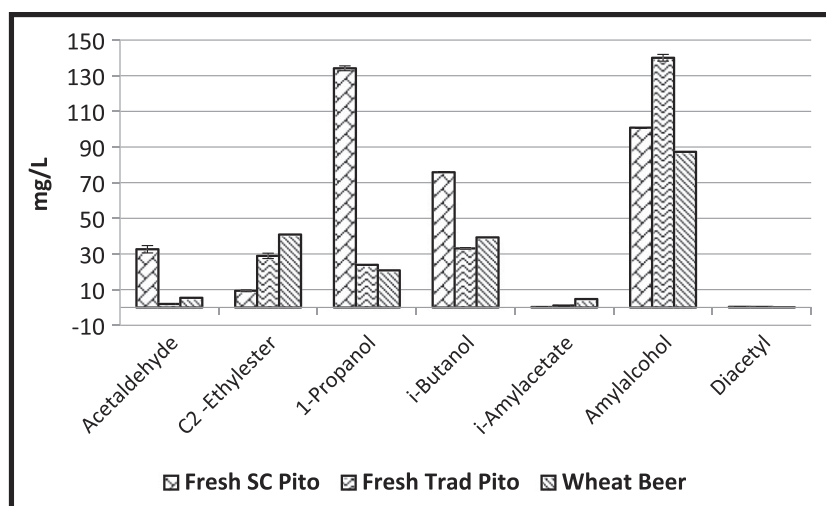


Figure 1. Levels of volatile fermentation by-products in *pito* fermented with pure strain starter culture of *Lactobacillus delbrueckii* and Anchor Brewer's Yeast (Fresh SC *Pito*), *pito* fermented traditionally with mixed microflora (Fresh Trad *Pito*) and in sour German wheat beer (Wheat Beer).

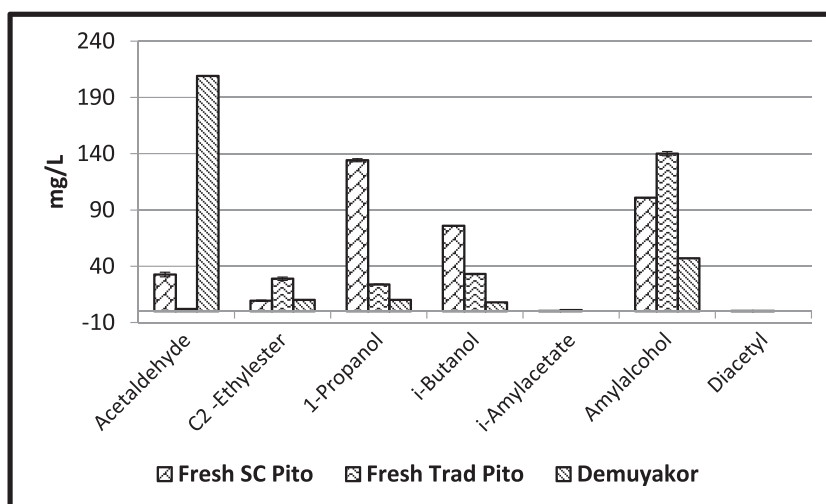


Figure 2. Levels of volatile fermentation by-products in *pito* fermented with pure strain starter culture of *L. delbrueckii* and Anchor Brewer's Yeast (Fresh SC *Pito*), *pito* fermented traditionally with mixed microflora (Fresh Trad *Pito*) and in the study of Demuyakor and Ohta (7) fermented with mixed culture microflora of the traditional process (Demuyakor).

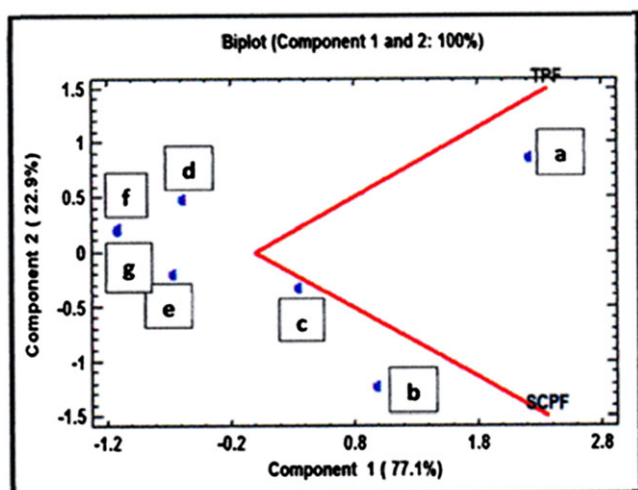


Figure 3. Principal component analysis loadings for volatile fermentation compounds in fresh starter culture *pito* (SCPF) and fresh traditional *pito* (TPF). Key: a, iso amylalcohol; b, *n*-propanol; c, *i*-butanol; d, ethyl acetate; e, acetaldehyde; f, diacetyl; g, isoamylacetate. [Colour figure can be viewed at wileyonlinelibrary.com]

pito and are fermented by a diversity of microorganisms. The value of ethyl acetate at 29 mg/L in the traditional *pito* stored for four weeks fell close to the lower limit of the range but lambics are stored for 1–3 years before consumption, during which period the ester content is increased by esterification of alcohols. The levels of esters (9.4 mg/L for ethyl acetate and 0.2 mg/L for iso-amylacetate) in the pure culture *pito* were low compared with the levels in traditional *pito* (29 mg/L for ethyl acetate and 1.1 mg/L for iso-amyl acetate) but much higher than the 0.34 and 0.07 mg/L, respectively, for ethyl acetate and iso-amyl acetate obtained in the *pito* style Rwandan sour sorghum beer *ikigage* brewed with pure cultures reported by Lyumugabe *et al.* (17).

Esters are formed during fermentation by esterification of fatty acids and also in small amounts by esterification of higher alcohols (9). The lower level of higher alcohols found in traditional *pito* and the higher level of esters found in pure culture *pito* suggest that

the diverse yeasts present in the traditional *pito* may collectively possess a higher ability to convert higher alcohols into esters than the single strain yeast used in fermenting the starter culture *pito*.

The ester content of beer, however, depends on beer type. Bottom fermented beers contain esters up to 60 mg/L and top fermented beers contain up to 80 mg/L (9). Wei *et al.* (27) reported that the content of ethyl acetate and isoamyl acetate in extruded white sorghum beer in the ranges 8–50 and 0.86–6 mg/L gave the beer a typical aroma. As the value of ethyl acetate in the starter culture *pito* fell within this range, the typical sorghum aroma would be expected. The formation of esters is increased with the attenuation during the fermentation (9). Attenuation is the conversion of extract to alcohol and the percentage apparent attenuation – calculated by dividing the fermented extract by the initial extract and multiplying by 100 – was 60% for the traditionally brewed *pito* and 51% for *pito* brewed with starter cultures. This could explain the higher total level of esters in the traditionally brewed test *pito*.

According to Meilgaard (28) the concentration of iso-amyl acetate found in lambic sour beers is much lower than the concentrations in regular beers and iso-amyl acetate can be in the ranges 1.2–2.8 mg/L in lagers and 0.7–3.3 mg/L in ales. The values of 0.2 and 1.1 mg/L measured in starter culture and traditional *pito*, respectively, are therefore consistent with the levels in the sour beer category.

A comparison of volatile fermentation by-products in the *pito* brews and a sour German wheat beer is presented in Fig. 1. Starter culture *pito* showed higher concentrations of acetaldehyde, propanol and iso-butanol than the other two beers while traditional *pito* showed the highest concentration of amyl alcohol. The German wheat beer which is particularly known for the classic 'banana' notes aroma has higher levels of iso-amyl acetate, which is responsible for the aroma.

A comparison of volatile compounds in the experimental *pito* brews and a *pito* fermented in a study by Demuyakor and Ohta (7) with mixed culture microflora of the traditional process is shown in Fig. 2. The level of acetaldehyde was markedly higher in the mixed culture fermentation of Demuyakor and Ohta (7). The level exceeded 150 mg/L which according to Šmogrovičová

(29) may cause an aldehydic character. The variations in the levels obtained in the comparison may also be partially due to differences in the fermentation conditions, in particular the temperature and yeast pitching rate.

The volatile compounds in the fresh *pito* brews were reduced to two main components by PCA in Fig. 3. The two components accounted for 100% of the total variation in the data (component 1, 77.1% and component 2, 22.9%). The two brews were very much separated from each other by their predominant volatile compounds. The predominant higher alcohol in the pure culture *pito* was *n*-propanol and in the traditional *pito* iso-amyl alcohol. Iso-butanol also contributed positively to component 1. Component 2 was characterised by ethyl acetate and acetaldehyde.

Conclusions

The physicochemical parameters of *pito* fermented with pure cultures of *L. delbrueckii* ('H1') and *S. cerevisiae* (Anchor Brewer's Yeast) were similar to those of *pito* fermented using the traditional spontaneous process with diverse microflora. The quality parameters of both beers fell well within the physicochemical ranges of traditional *pito* reported by UNIDO (29) indicating the similarity of the two products. The pure culture *pito* was brighter and had a more intense colour from the variety of sorghum used. A significant difference was found in the concentration of the volatile aroma compounds determined in the two experimental beers. In the beer fermented with pure single cultures, the level of the fermentation by-products was accounted for by the fermentation characteristics of *L. delbrueckii* and Anchor Brewer's Yeast (*S. cerevisiae*). On the other hand, in the traditional brew fermented with a varied (and unknown) population of microflora, the level of the fermentation by-products was influenced by numerous individual microorganisms. There was a higher level of total volatile aroma components in the pure culture *pito*, suggesting a more intense aroma compared with the traditional *pito*. The concentrations of the volatile compounds in both types of *pito*, however, were consistent with those found in similar sour spontaneous fermented beers. There was no significant change in the concentration of compounds after a forced aging period of four weeks, suggesting flavour stability for that duration. It can be concluded that fermenting *pito* with pure commercial cultures of *L. delbrueckii* and *S. cerevisiae* will yield a product that will have a similar physicochemical quality to *pito* fermented with the traditional process but with possibly a more pronounced aroma. The possible influence of this on the overall quality of the product would need to be evaluated in a consumer study and associated sensory analysis.

References

- Glover, R. L., Abaidoo, R. C., Jakobsen, M., and Jespersen, L. (2005) Biodiversity of *Saccharomyces cerevisiae* isolated from a survey of *pito* production sites in various parts of Ghana, *Syst. Appl. Microbiol.* 28, 755–761. <https://doi.org/10.1016/j.syapm.2005.05.003>
- Obire, O. (2005) Activity of *Zymomonas* species in palmsap obtained from three areas in Edo State, Nigeria, *J. Appl. Sci. Environ. Mgt* 9, 25–30. <http://www.bioline.org.br/pdf?ja05005> (last accessed 20th January 2019).
- Jespersen, L. (2003) Occurrence and taxonomic characteristics of strains of *Saccharomyces cerevisiae* predominant in African indigenous fermented foods and beverages, *FEMS Yeast Res.* 3, 191–200. [https://doi.org/10.1016/S1567-1356\(02\)00185-X](https://doi.org/10.1016/S1567-1356(02)00185-X)

- Togo, C. A., Feresu, S. B., and Mutukumira, A. N. (2002) Identification of lactic acid bacteria isolated from opaque beer (chibuku) for potential use as a starter culture, *J. Food Technol. Africa* 7, 93–97. <https://doi.org/10.4314/jfta.v7i3.19239>
- Van Der Aa Kuhle, A., Jespersen, L., Glover, R. L. K., Diawara, B., and Jakobsen, M. (2001) Identification and characteristics of *Saccharomyces cerevisiae* strains isolated from West African sorghum beer, *Yeast* 18, 1069–1079. <https://doi.org/10.1002/yea.756>
- Jimoh, S. O., Ado, S. A., Ameh, J. B., and Whong, C. M. (2012) Characteristics and diversity of yeast in locally fermented beverages sold in Nigeria, *World J. Eng. Pure Appl. Sci.* 2, 40–46. http://rrpjournal.org/wjepas/en_wjepas_vol_2_iss_2_pg_40_44.pdf (last accessed 20th January 2019).
- Demuyakor, B., and Ohta, Y. (1993) Characteristics of single and mixed culture fermentation of *pito* beer, *J. Sci. Food Agric.* 62, 401–408. <https://doi.org/10.1002/jsfa.2740620414>
- Broderick, H. M. (1977) Beer Quality, in *The Practical Brewer – A Manual for the Brewing Industry*, (Broderick, H. M. Ed.) 2nd ed., pp. 288–301, Master Brewers Association of the Americas, Madison, WI.
- Kunze, W. (2004) *Technology Brewing and Malting*, 3rd int ed., VLB, Berlin, Germany.
- Djameh, C., Saalia, F. K., Sinayobye, E., Budu, A., Essilfie, G., Mensah-Brown, H., and Sefa-Dedeh, S. (2015) Optimization of the sorghum malting process for *pito* production in Ghana, *J. Inst. Brew.* 121, 106–112. <https://doi.org/10.1002/jib.191>
- European Brewery Convention (1987) *Analytica – EBC*, 4th ed., Brauerei und Getränke Rundschau, Zurich.
- MEBAK III. (2012) Wurze, Bier, Biermischgetranke, WBBM 2.21.1 Leichtflüchtige Gärungsnebenprodukte (Headspace). Selbstverlag der MEBAK, Freising-Weihenstephan, Germany.
- Orji, M. U., Mbata, T. I., Aniche, G. N., and Ahonkhai, I. (2003) The use of starter cultures to produce 'Pito', a Nigerian fermented alcoholic beverage, *World J. Microbiol. Biotechnol.* 19, 733–736. <https://doi.org/10.1023/A:1025172506965>
- Hough, J. S., Briggs, D. E., Stevens, R., and Young, T. W. (1982a) Microbiological contamination in breweries, in *Malting and Brewing Science – Hopped Wort and Beer*, 2nd ed., II, pp. 741–773, Chapman & Hall, London.
- Sefa-Dedeh, S., Sanni, A. I., Tetteh, G., and Sakyi-Dawson, E. (1999) Yeasts in the traditional brewing of *pito* in Ghana, *World J. Microbiol. Biotechnol.* 15, 593–597. <https://doi.org/10.1023/A:1008955300156>
- Lyumugabe, F., Uyisenga, J. P., Songa, E. B., and Thonart, P. (2014) Production of traditional sorghum beer 'kigage' using *Saccharomyces cerevisiae*, *Lactobacillus fermentum* and *Issatckenkia orientalis* as starter cultures, *Food Nutr. Sci.* 5, 507–515. <http://dx.doi.org/10.4236/fns.2014.56060>
- Lyumugabe, F., Gros, J., Nzungize, J., Bajyana, E., and Thonart, P. P. (2012) Characteristics of African traditional beers brewed with sorghum malt: A review, *Biotechnol. Agron. Soc. Environ.* 16, 509–530. <http://popups.ulg.ac.be/1780-4507/index.php?id=9217>
- Avicor, M. N., Saalia, F. K., Djameh, C., Sinayobye, E., Mensah-Brown, H., and Essilfie, G. (2015) The fermentation characteristics of single and mixed yeast cultures during *pito* wort fermentation, *Int. Food Res. J.* 22, 102–109. [http://www.ifrj.upm.edu.my/22%20\(01\)%202015/\(16\).pdf](http://www.ifrj.upm.edu.my/22%20(01)%202015/(16).pdf) (last accessed 20th January 2019).
- Zhu, J., Shi, J., Lu, Y., Liu, L., and Liu, Y. (2016) Applications of strains of *Geotrichum* spp. to increase higher alcohols and to increase esters, *J. Inst. Brew.* 122, 147–155. <https://doi.org/10.1002/jib.287>
- Romano, P., Fiore, C., Paraggio, M., Caruso, M., and Capece, A. (2003) Function of yeast species and strains in wine flavour, *Int. J. Food Microbiol.* 86, 169–180. [https://doi.org/10.1016/S0168-1605\(03\)00290-3](https://doi.org/10.1016/S0168-1605(03)00290-3)
- Ma, C., He, Y., Cao, Y., Bai, X., and Li, H. (2016) Analysis of flavour compounds in beer with extruded sorghum as an adjunct using headspace solid-phase micro-extraction and gas chromatography–mass spectrometry, *J. Inst. Brew.* 122, 251–260. <https://onlinelibrary.wiley.com/doi/pdf/10.1002/jib.330>
- Ashraf, N., Linforth, R. S. T., Bealin-Kelly, F., Smart, K., and Taylor, A. J. (2010) Rapid analysis of selected beer volatiles by atmospheric pressure chemical ionisation–mass spectrometry, *Int. J. Mass Spectrom.* (1), 47–53. <https://doi.org/10.1016/j.ijms.2010.05.007>
- Greenshields, R. N. (1974) Volatiles in home-brewed beers and wines, *J. Sci. Food Agric.* 25, 1307–1312.
- Coulibaly, W. H., N'guessan, K. F., Coulibaly, I., Cot, M., Rigou, P., and Dje, K. F. (2016) Influence of freeze-dried yeast starter cultures on volatile

- compounds of Tchapalo, a traditional sorghum beer from Côte d'Ivoire, *Beverages* 2, 35. <https://doi.org/10.3390/beverages2040035>
25. Brown, A., and Hammond, J. (2003) Flavour control in small-scale beer fermentations, *Food Bioprod. Process.* 81, 40–49. <https://doi.org/10.1205/096030803765208652>.
 26. Wei, L., Yongli, F., Jiahui, L., and Hongwei, L. (2007) Study on the influence of ester substances on the beer flavour, *Food Sci. Technol.* 8, 101–103 Cross-referenced from: *J. Inst. Brew.* (2016), 122, 251–260.
 27. Meilgaard, M. C. (1975) Flavour chemistry of beer: Part I: Flavour interaction between principal volatiles, *Tech Q Master Brew Assoc Am* 12, 107–117.
 28. Šmogrovičová, D. (2014) Beer volatile compounds, in *Dependence on Yeast Strain Used for Fermentation*. A presentation at the Brewing Science Group of the European Brewery Convention in Vienna, Austria. [http://www.ebc-symposium.org/uploads/mycms .../L09_D_Smogrovicova_EBC_BSG.pdf](http://www.ebc-symposium.org/uploads/mycms.../L09_D_Smogrovicova_EBC_BSG.pdf) (last accessed 20th January 2019).
 29. UNIDO (2007). Technical report on the industrial development of sorghum malt for the brewing industry. UNIDO/CFC Sorghum Malt Project. 2007, Accra, Ghana.