

Microbiological and chemical processes associated with the production of *burukutu* a traditional beer in Ghana

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Abstract

Burukutu is the lesser known of two types of traditional sorghum beers produced in Ghana, *pito* being more common. *Burukutu* production involves malting, mashing, souring and alcoholic fermentation. Microbiological and chemical changes which occur during souring and alcoholic fermentations were investigated through analysis of samples obtained from production sites. Lactic acid bacteria (LAB) responsible for the souring were *Lb. fermentum* (33.3%), *Lb. plantarum* (25%), *Lb. acidophilus* (16.7%), *Lc. lactis* subsp. *lactis* (16.7%) and *Lb. brevis* (8.3%). Their activities resulted in a reduction in pH from 4.64 to 4.05 during a 6-7 h holding period. Souring also occurred concurrently with the alcoholic fermentation reducing the pH further to 2.88 and a final titratable acidity content of 0.82 %. *Saccharomyces cerevisiae* was responsible for the final alcoholic fermentation which resulted in an alcohol yield of 4.47% in 120 h. When *burukutu* was challenged with about 10⁵ CFU/ml of some enteric pathogens at the start of the alcoholic fermentation, *Salmonella typhimurium* could no longer be detected at 16 h, *E. coli* at 20 h, *Staphylococcus aureus* at 36 h and *Streptococcus faecalis* at 40 h. All isolates of the dominant LAB, *Lb. fermentum*, also demonstrated antimicrobial activity against *S. aureus*, *E. coli* and *Salmonella typhimurium*. This is the first detailed study of *burukutu* production in Ghana.

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Introduction

Brewing of alcoholic beverages is a traditional process carried out in most African countries. These traditional beers are produced from sorghum, millet and maize. They differ from European beers in being opaque and having a thick consistency since they are not filtered clear. The African beers are also sour because they are allowed to undergo an acidification step. They are also not bitter because hops are not added during production. Interestingly, the African beers are consumed in an active state of fermentation as no attempt is made to arrest fermentation; hence they are effervescent in appearance. These traditional beers are generally called African opaque beers. The basic unit operations involved in the production of the African beers are malting, mashing, straining, souring, boiling, and alcoholic fermentation. Procedures however vary from place to place and even from producer to producer (Holzapfel, 1991).

The African opaque beers include *Gwalo* in Congo, *Bouza* in Ethiopia, *Busaa* in Kenya and Uganda, *Merissa* in Sudan, *Pombe* or *Bwalwa* in Tanzania, *Bojalwa ja Setswana* in Botswana, *Yalwa* or *joule* in Lesotho and South Africa, *Utshwala* or *Tshwala* in South Africa, *Zezuru* in Zimbabwe, *Pito*

and *Burukutu* in Nigeria and Ghana, *Chibuki* in Zimbabwe, *Dolo* in Burkina Faso, Ivory Coast, Mali, Niger, Togo and *Tchapalo* in Ivory Coast (Faparusi *et al.*, 1973; N'Da and Coulibaly, 1996; Sefa-Dedeh *et al.*, 1999; Togo *et al.*, 2002; Sawadogo-Lingani *et al.*, 2007).

In Ghana, West Africa, two types of traditional beers are produced, *burukutu* and *pito*, the latter being more common. In a study on *pito* in Ghana, Sefa-Dedeh (1991) reported four major types *Dagarti*, *Kokomba*, *Togo* and *Nandom pito* with variations in their production methods and product characteristics. Though *pito* has been the subject of scientific study (Demuyakor and Ohta, 1991; Sefa-Dede *et al.*, 1999; Glover *et al.*, 2005, 2009; Sawadogo-Lingani *et al.*, 2007, 2008) there is no information on *burukutu* produced in Ghana in the international literature. Information on *burukutu* is limited to the product from Nigeria (Ekundayo, 1969; Faparusi, 1970; Faparusi *et al.*, 1973; Banigo, Aina and Ossa, 1987; Oke and Ijeboor, 1997; Obot, 2000; Jideani and Osume, 2001; Kolawole *et al.*, 2007; Egemba and Etuk, 2007; Yabaya and Jatau, 2009; Eze *et al.*, 2011). *Burukutu* has been described as a popular alcoholic beverage of a vinegar-like flavour prepared from sorghum grains and fermented guinea

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corn and consumed in the Northern Guinea Savanna region of Nigeria by Haard *et al.* (1999). This work was carried out to characterize *burukutu* produced in Ghana with respect to its chemical, biochemical and microbiological composition and changes which occur in the composition during the lactic acid and alcoholic fermentations.

Materials and Methods

Brief survey and sampling

A brief survey was conducted in the Accra and Tema metropolis to observe *burukutu* production and collect samples for laboratory analysis. The sites selected for the study were; Tema (5° 37' 0" North, 0° 10' 0" West), Zenu (5° 43' 0" North, 0° 30' 0" West), Ashaiman (5° 42' 0" North, 0° 20' 0" West) and Accra Newtown (5° 5' 0" North, 3° 5' 0" West).

One production site was selected for sampling at each of the four selected areas. Samples of *burukutu* at various stages of production, including raw materials through to the finished product, were collected from each production site on three separate occasions between October 2011 and July 2012.

Ten *burukutu* processors were interviewed all of whom were women, eight being nationals from the neighbouring country Togo. All the processors used only sorghum to produce *burukutu* and also used the same processing method. Most of them processed either 50 or 100 kg of sorghum per batch and a few either 150 or 200 kg per batch. The spent sorghum was sold as animal feed. A batch production took 24 h but was retailed over a 5 to 7 day period during which fresh wort was periodically added to the brew to sustain the fermentation and also control the extent of sourness. The procedure used by all ten processors interviewed to produce *burukutu* is shown in Figure 1.

Enumeration of microorganisms

Ten grams (10 g) of sample was added to 90 ml sterile Salt Peptone Solution (SPS) containing 0.1% peptone and 0.85% NaCl, (with pH adjusted to 7.2) and homogenized in a stomacher (Lad Blender, Model 4001, Seward Medical) for 30 sec at normal speed. After serial dilution, aerobic mesophiles were enumerated by pour plate on Plate Count Agar (Oxoid CM325; Oxoid Ltd., Basingstoke, Hampshire, UK), incubated at 30°C for 72 h in accordance with NMKL No. 86, 2006. Yeasts were enumerated by spread plate on Dichloran-Rose Bengal Chloramphenicol Agar (DRBC) (Oxoid CM0727; Oxoid Ltd., Basingstoke, Hampshire, UK) to which Chloramphenicol supplement had

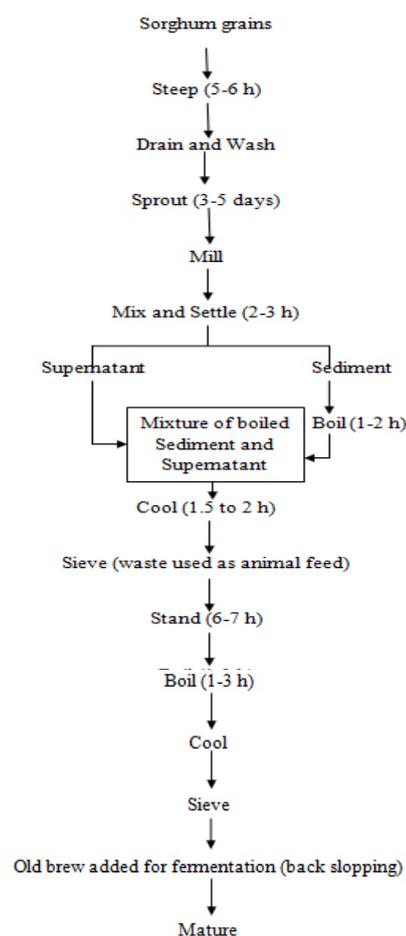


Figure 1. Flow chart for the main steps involved in *burukutu* production

been added to inhibit bacteria growth. The pH was adjusted to 5.6 and incubated at 25°C for 48-120 h in accordance with ISO 21527-1:2008. Lactic acid bacteria were enumerated by pour plate on deMan, Rogosa and Sharpe Agar (MRS, Oxoid CM361), pH 6.2 to which had been added 0.1% cycloheximide to suppress yeast growth and cystein HCl to achieve anaerobic conditions. Plates were incubated in an anaerobic jar at 30°C for 120 h. Total coliforms and *E. coli* were enumerated by pour plate on Trypton Soy Agar (Oxoid CM131), pH 7.3 overlaid with Violet Red Bile Agar (Oxoid CM107), pH 7.4 and incubated at 37°C for 24 h for total coliforms and at 44°C for 24 h for *E. coli*. Colonies for total coliforms were confirmed on Brilliant Green Bile Broth (Oxoid CM31), pH 7.4 incubated at 37°C for 24 h according to NMKL No. 44 (2004) and *E. coli* using EC Broth (Oxoid CM853), pH 6.9, followed by Trypton Water (Oxoid CM87), pH 7.5, all incubated at 44°C for 24 h as described by NMKL. No. 125 (2005).

Isolation, characterization and tentative identification of yeasts and lactic acid bacteria isolates

About 15 colonies from a segment of the highest dilution or suitable DRBC or MRS plate were sub-

cultured by streaking repeatedly on agar till pure colonies were obtained. Yeast isolates were examined by colony and cell morphology and tentatively identified by determining their pattern of utilisation of various carbohydrates in ID 32 C galleries (Bio Mérieux S.A., Marcy-l'Etoile, France).

Isolates on MRS were examined by Gram reaction and catalase test. Isolates which were Gram-positive catalase-negative regular rods, coccoid or cocci were assumed to be lactic acid bacteria and further examined by gas production in MRS broth with Durham tube and also in MRS broth in which glucose was replaced with gluconate as sole carbon source, growth at 10°C and 45°C, growth at pH 4.4 and 9.6, and growth in 6.5 and 18% (w/v) NaCl. The species of lactic acid bacteria were tentatively identified by determining their pattern of carbohydrate fermentation in API 50 CHL galleries (Bio Mérieux S.A.). Total coliform bacteria and *E. coli* isolates which were Gram negative and oxidase negative were identified using API 20E (BioMérieux, France).

Challenge testing and antimicrobial activity of isolates

The ability of different enteric pathogens to survive in fermenting *burukutu* was studied as described by Mante *et al.* (2003). The enteric pathogens used were *Escherichia coli* NC10418, *Staphylococcus aureus* NC06571, and *Salmonella typhimurium* NC12023 all obtained from Health Protection Agency (HPA) Culture Collection, UK and also a laboratory strain of *Streptococcus faecalis*. The inhibitory potential of isolates of the dominant LAB against *Escherichia coli* NC10418, *Staphylococcus aureus* NC06571, and *Salmonella typhimurium* NC12023 was investigated using the Agar Well Diffusion method as described by Schillinger and Lücke (1989) and Olsen *et al.* (1995).

Enzymatic analysis

Isolates of lactic acid bacteria were tested for amylase production on starch agar as described by Almeida *et al.* (2007). Sorghum grains, malted grains, supernatant and sediment were tested for α -amylase activity as described by Bernfeld (1955) and Terlabie *et al.* (2005).

Chemical analysis

The pH of samples homogenized with the same volume of distilled water in a stomacher were determined using a pH meter (Radiometer PHM 92; Radiometer Analytical A/S, Bagsvaerd, Denmark). Titratable acidity was determined as described by Amoa-Awua *et al.* (2006). The percentage of alcohol by volume from specific gravity was determined

according to AOAC method (1990). 100 ml of the sample was diluted with 50 ml of distilled water. After collecting about 100 ml of distillate, its specific gravity was determined by dividing the weight of 25 ml of the distillate by the weight of an equal volume of water using a 25 ml specific gravity bottle at 20°C and referring to the reference table. Soluble solids of samples were determined using a refractometer (Model RB32, Hanna Instruments).

Results

Microbial population at the various stages of burukutu production

Table 1 shows the population of aerobic mesophiles, yeasts, lactic acid bacteria, coliform bacteria and *E. coli* present in samples collected at various stages of burukutu production from the production sites. The resident microbiota on the sorghum grains was high for all the different types of microorganisms including coliform bacteria and *E. coli*. Considerable increases in counts were recorded during malting. The highest increase in counts during malting was from 10¹ to 10⁷ CFU/g for LAB. The yeast population also increased from 10⁵ to 10⁷ CFU/g. In the supernatants and sediments which were collected after the malted flour had been mixed with water and allowed to settle for 2-3 h, tenfold increases were recorded in the LAB and *E. coli* counts.

In the wort which was obtained after the sediment had been boiled, remixed with the supernatant, allowed to cool, strained and held for 6 to 7 h (Figure 1), a two log units reduction was recorded in the population of LAB, three log units reduction in yeasts and coliform bacteria and four log units reduction in aerobic mesophiles and *E. coli*. The reduction in the microbial counts could be attributed to the boiling of the sediment which destroyed its microbiota before remixing with the supernatant. The relatively higher LAB population in the soured wort could be attributed to growth of lactic acid bacteria during the 6-7 h holding period. The mean pH of samples of supernatant and sediment collected from all four production sites were 4.63 and 4.66 respectively and that of the wort obtained at the end of the holding period 4.05, confirming acidification or souring of the wort during the holding period. A further reduction in the microbial numbers was recorded after the soured wort had been boiled and cooled for 1-3 h. Coliform bacteria and *E. coli* were no longer detected in the concentrated wort samples (Table 1).

Changes which occurred during the alcoholic fermentation

There was a gradual increase by three log units

Table 1. Mean microbial counts in log₁₀ CFU/ml or g during the processing of sorghum grains into *burukutu* at four production sites

SAMPLES	AEROBIC MESOPHILES	YEASTS	LACTIC ACID BACTERIA	COLIFORMS	<i>E. COLI</i>
Sorghum grains	6.62 ± 0.82	5.23 ± 0.57	1.37 ± 1.66	5.73 ± 0.94	3.36 ± 2.64
Malted grains	8.59 ± 0.93	7.55 ± 0.72	7.75 ± 0.76	7.78 ± 0.56	5.54 ± 1.09
Supernatant	8.88 ± 0.57	7.35 ± 0.82	8.11 ± 0.59	7.75 ± 0.82	6.19 ± 0.95
Sediment	9.17 ± 0.47	7.52 ± 0.62	8.34 ± 0.43	7.80 ± 0.93	6.34 ± 1.05
Soured wort	6.03 ± 1.24	4.89 ± 0.65	6.40 ± 1.84	3.58 ± 1.16	2.27 ± 1.51
Concentrated wort	4.52 ± 0.46	3.46 ± 0.85	3.61 ± 0.50	0.00 ± 0.00	0.00 ± 0.00
Old brew (for backslopping)	8.14 ± 0.55	7.89 ± 0.49	7.62 ± 0.56	1.82 ± 3.64	1.29 ± 2.58
Fermentation					
0 h	7.14 ± 0.34	6.82 ± 0.34	6.66 ± 0.36	0.88 ± 1.76	0.47 ± 0.94
12 h	7.95 ± 0.45	7.49 ± 0.54	7.64 ± 0.60	0.13 ± 0.26	0.00 ± 0.00
24 h	8.37 ± 0.41	7.86 ± 0.30	7.75 ± 0.28	nd (not detected)	nd
36 h	8.65 ± 0.38	7.94 ± 0.40	7.94 ± 0.44	nd	nd
48 h	8.82 ± 0.43	8.16 ± 0.34	8.00 ± 0.42	nd	nd
72 h	8.69 ± 0.06	8.23 ± 0.29	7.87 ± 0.40	nd	nd
96 h	8.54 ± 0.21	8.15 ± 0.24	8.26 ± 0.35	nd	nd
120 h	8.41 ± 0.38	8.18 ± 0.43	8.14 ± 0.41	nd	nd

Table 2. Changes in pH, and percentage titratable acidity, soluble solids and alcohol content during fermentation of *burukutu* after backslopping with old brew

Fermentation time (h)	pH	Titratable acidity (%)	Soluble solids (%)	Alcohol content (%)
0	3.36 ± 0.16	0.54 ± 0.20	7.50 ± 0.71	0.99 ± 0.21
12	3.25 ± 0.14	0.65 ± 0.11	6.33 ± 0.54	2.18 ± 0.67
24	3.19 ± 0.16	0.67 ± 0.10	5.70 ± 0.06	2.75 ± 0.29
36	3.09 ± 0.22	0.69 ± 0.11	5.13 ± 0.48	3.15 ± 0.53
48	3.11 ± 0.17	0.69 ± 0.13	4.88 ± 0.75	3.27 ± 0.51
72	3.05 ± 0.22	0.66 ± 0.13	3.88 ± 0.63	3.53 ± 0.72
96	2.94 ± 0.31	0.74 ± 0.11	3.50 ± 0.41	3.95 ± 0.33
120	2.88 ± 0.31	0.82 ± 0.11	3.33 ± 0.35	4.47 ± 0.96

Table 3. Antimicrobial activity of lactic acid bacteria isolates from *burukutu* against three common enteric pathogens

Isolates of lactic acid bacteria	Indicator strains		
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Salmonella typhimurium</i>
<i>Lb. Fermentum</i> 1	++	+++	++
<i>Lb. Fermentum</i> 2	++	+++	++
<i>Lb. Fermentum</i> 3	++	++++	++
<i>Lb. Fermentum</i> 4	+	++	+
<i>Lb. plantarum</i> 1	++	-	-
<i>Lb. plantarum</i> 2	-	+	-
<i>Lc. Lactis</i> 1	++	+	-
<i>Lc. Lactis</i> 2	+	+	-
<i>Lb. acidophilus</i> 1	-	++	-
<i>Lb. acidophilus</i> 2	+	-	-
<i>Lb. acidophilus</i> 3	-	++	-
<i>Lb. brevis</i> 1	-	+++	+

-: no inhibition
 +: 2-3 mm inhibition zone
 ++: 3-4 inhibition zone
 +++: 4-5 inhibition zone
 ++++: > 5 mm inhibition zone

in the population of aerobic mesophiles, yeasts and lactic acid bacteria following backslopping of the wort with old brew and fermentation for 120 h (Table 1). Unfortunately, backslopping also re-introduced coliform bacteria including *E. coli* into the brew, but these soon disappeared during fermentation. The final concentration of both yeasts and LAB were at 10⁸ CFU/ml.

Table 2 shows the chemical changes which occurred in the wort during the alcoholic fermentation. The pH of the samples decreased during the 120 h fermentation period from a mean value of 3.36 to 2.88, whilst titratable acidity increased from 0.54 to 0.82%. The soluble solids content decreased from 7.50 to 3.33% whilst the alcohol content increased from 0.99 to 4.47%. Thus a concurrent alcoholic and lactic acid fermentation of the wort occurred after

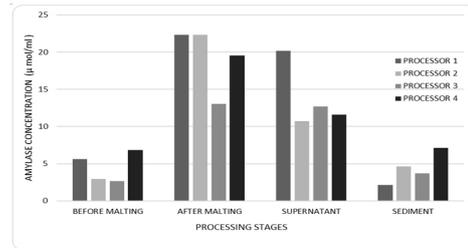


Figure 2. Alpha amylase activity of sorghum grains, sorghum malt and mash during *burukutu* production

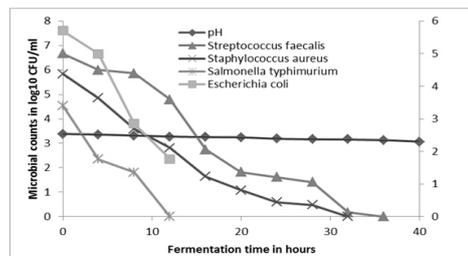


Figure 3. Survival of some common enteric pathogens when inoculated into *burukutu* during fermentation

backslopping with the old brew.

Identification of dominant lactic acid bacteria and yeasts

One hundred and eighty isolates of lactic acid bacteria from the various production sites at the different production sites were grouped based on their biochemical characteristics. The dominant species were able to grow at 45°C but not at 15°C. They also grew in 6.5% NaCl and at pH 4.4 and 9.6, but not in 18% NaCl. In the API galleries they fermented mainly galactose, D-glucose, D-fructose, D-mannose, arabinose, D-ribose, maltose, lactose, melibiose, saccharose and D-raffinose. Based on these characteristics this dominant lactic acid bacteria was tentatively identified as *Lactobacillus fermentum*. It accounted for 33.3% of the total lactic acid bacteria population. The second most dominant species grew at 15°C but not at 45°C nor in 18% NaCl and 6.5% NaCl. They also grew at pH 4.4 and 9.6 and were able to ferment L-arabinose, ribose, galactose, D-glucose, D-fructose, D-mannose, mannitol, sorbitol, α-methyl-D-mannoside, N-acetyl glucosamine, amygdaline, arbutin, esculin, salisin, cellobiose, maltose, lactose, melibiose, saccharose, trehalose, melezitose, D-raffinose, β-gentiobiose, D-turanose and gluconate. This species was identified as *Lactobacillus plantarum* and accounted for 25% of the LAB population. The other lactic acid bacteria identified in the API galleries were *Lactococcus lactis* subsp. *lactis* (16.7%), *Lactobacillus acidophilus* (16.7%) and *Lactobacillus brevis* (8.3%).

With regards to yeasts, 180 isolates were initially classified based on their cell and colony morphology as well as growth in liquid medium. Tentative

identification of the isolates based on assimilation of carbon compounds in API 32 C showed the dominant yeast to be *Saccharomyces cerevisiae*. These isolates were able to assimilate glucose, galactose, sucrose, maltose, raffinose and trehalose but could not assimilate lactose. The other yeast species identified was *Candida krusei*. These isolates were able to assimilate glucose, glycerol, glucosamine and lactic acid but not arabinose, raffinose, maltose and trehalose.

Enzymatic analysis

The alpha amylase activity of sorghum grains obtained from the four production sites ranged from 2.65 to 6.85 $\mu\text{mol/ml}$ (Figure 2). In malted sorghum samples obtained from the same production sites, drastic increases in the amylase activity were recorded ranging from 13.05 to 22.35 $\mu\text{mol/ml}$. Alpha amylase activity in supernatant and sediment samples obtained from the production sites ranged from 11.60 to 20.15 $\mu\text{mol/ml}$ and 2.15 to 7.15 $\mu\text{mol/ml}$, respectively. The reduced activity levels recorded in these samples compared to the malted grains were attributed to the effect of mixing the malt flour with 3 times its volume of water thus diluting the concentration per ml or g.

All four isolates of *Lb. fermentum* tested for amylase activity on starch agar flooded with iodine produced clear zones ranging from 2.0 – 4.2 mm in diameter. The two isolates of *Lb. plantarum* produced 3.0 and 12.0 mm clear zones. With *Lc. lactis*, one isolate produced a clear zone of 5.0 mm whilst the other tested negative for amylase production. None of the three isolates of *Lb. acidophilus* and the isolate of *Lb. brevis* tested produced amylase.

Survival of enteric pathogens in fermenting Burukutu

Figure 3 shows the antimicrobial activity of burukutu when challenged with four common enteric pathogens at the start of the alcoholic fermentation. *Salmonella typhimurium* was no longer detected in 25 g of the sample at 16 h (pH 3.26). *E. coli* was not detectable at 20 h and *Staphylococcus aureus* at 36 h. *Streptococcus faecalis* was detected in the sample until 40 h (pH 3.06).

Discussion

This study has documented the main steps involved in the production of *Burukutu*, a traditional beer in Ghana. The production procedure observed at the four different production sites was the same but different from the method reported for *burukutu* production in Nigeria. In the Nigerian process

lumps of *gari* (roasted cassava granules) are added during mashing as adjunct, but no such addition was observed at any of the production sites visited in the Accra and Tema Metropolis. In the Ghanaian process ‘excess’ sorghum malt was used compared to the comparative product *pito*, hence could account for the extra source of starch represented by cassava granules as adjunct in the Nigerian process. In *pito* mash the ratio of sorghum malt to water is 1:5, whilst in *burukutu* mash it is 1:3. This accounts for why most of the consumers considered *burukutu* as both food and as an alcoholic beverage. Also whereas in the *pito* process the malted grains are dried before milling, in *burukutu* production the malted grains are milled without drying and used immediately.

The present work has confirmed that as is the case with all African opaque beers, three important activities occur during *burukutu* production; hydrolysis of starch into fermentable sugars which can be utilized by the yeasts for alcoholic fermentation, souring of the product by lactic acid fermentation and the final production of alcohol by yeasts.

Souring of burukutu

The gradual reduction in pH and increases in titratable acidity which occurred throughout production shows that souring/acidification is an important activity in *burukutu* production as has been reported for most African traditional beers (Sawadogo-Lingani, 2007, 2008). Unlike in *pito* production, where souring is restricted to one processing step, souring of *burukutu* occurred also during the alcoholic fermentation. This could be attributed to the backslopping of the wort with old brew to initiate alcoholic fermentation. The old brew contained high counts of both yeasts and LAB. In *pito* brewing the wort is pitched with only yeasts which are usually trapped from the previous brew using a woven belt (Sefa-Dedeh, 1991).

Lactobacillus fermentum was found to be the dominant lactic acid bacteria responsible for the souring of *burukutu*. The LAB population included *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactococcus lactis* subsp. *lactis* and *Lactobacillus brevis*. Sawadogo-Lingani *et al.* (2007) in their studies on *pito/dolo* in northern Ghana and Burkina Faso reported *Lb. fermentum* as the dominant species during the acidification of *pito*. Kayode *et al.* (2006) also isolated *Lb. fermentum* among others from opaque sorghum beer in Nigeria. Marcellin *et al.* (2009) isolated *Lb. fermentum* as the dominant yeasts in tchapalo, a traditional beer in Ivory Coast. Glover *et al.* (2009) used a double-strain combination of *Lb. fermentum* and *Saccharomyces cerevisiae* as a

starter culture in the production of *pito* that yielded a preferable taste and aroma comparable to the traditional product. Faparusi *et al.* (1973) isolated *Lb. plantarum* and *Lb. brevis* among others in a 24 h fermented mash-gari mixture during *burukutu* production in Nigeria. Amoa-Awua *et al.* (2006) found *Lb. plantarum* to be the dominant lactic acid bacteria in palm wine in Ghana. The presence of *Lb. acidophilus* and *Lc. lactis* subsp. *lactis* have also been reported in other traditional beers such as *chibuku* and *tchapalo* (Togo *et al.*, 2002; Marcellin *et al.*, 2009).

Alcoholic fermentation of *burukutu*

The most important operation in *burukutu* production was confirmed in the present work to be the alcoholic fermentation since it is what basically defines the product. The alcoholic fermentation was limited to the final processing step in which the soured concentrated wort was inoculated by backslopping with previous brew. The yeast population increased from a concentration of 10^6 to 10^8 CFU/ml in 120 h of fermentation resulting in an increase in alcohol concentration from 0.99 to 4.47%. The initial alcohol level was due to alcohol from the old stock of *burukutu* used for backslopping.

Saccharomyces cerevisiae was isolated as the yeast species responsible for the alcoholic fermentation of *burukutu* and accounted for about 70% of yeasts isolated from the four production sites. Faparusi *et al.* (1973), Ogbonna *et al.* (1983) and Achi (2005) have all isolated *Saccharomyces cerevisiae* as the dominant yeast in *burukutu* production in Nigeria. Sefa-Dedeh *et al.* (1999) isolated *S. cerevisiae* as the dominant yeast in *pito* fermentation in Ghana, whilst Glover *et al.* (2005) isolated *S. cerevisiae* as the dominant yeast in *pito/dolo* in both Ghana and Burkina Faso. *S. cerevisiae* has also been isolated as the yeast species responsible for another traditional alcoholic beverage, palm wine in Ghana (Okraqu-Ofei, 1968; Owusu, 1987; Brown, 1990, 1994; Amoa-Awua, Sampson and Tanoh-Debra, 2006). Though *Candida krusei* which is not an alcohol producer was isolated in *burukutu*, this was not surprising because its presence has been reported in several Ghanaian indigenous fermented foods. These include kenkey from maize (Halm *et al.*, 1993; Obiri-Danso, 1994; Hayford and Jakobsen, 1999; Annan *et al.*, 2003a, 2003b) palm wine (Amoa-Awua *et al.*, 2006), *agbelima* and *akyeke* from cassava (Amoa-Awua and Jakobsen, 1996; Amoa-Awua *et al.*, 1997). *Candida krusei* is therefore ubiquitous in the Ghanaian environment.

Safety of *burukutu*

It was observed during the field visits that the

basic elements of Good Manufacturing Practices were not in place at the production sites. Most of the processors did not also adhere to the basic elements of Good Hygienic Practices. The population of aerobic mesophiles, coliform bacteria and *E. coli* were therefore monitored at the different production sites as indicator organisms. Coliform bacteria including *E. coli* were found present on the sorghum grains and throughout the various stages of processing until the soured wort was boiled to concentrate it. However, backslopping reintroduced these organisms into *burukutu* but *E. coli* was no longer detected after 12 h of fermentation and coliform bacteria after 24 h. These were attributed to the lowering of pH during fermentation. Challenge testing also showed that most common enteric pathogens will eventually not survived in *burukutu*. It however took 34 h before none of the pathogens introduced into the product could no longer be detected in the sample. The present work has also shown that all isolates of the dominant LAB in *pito* *Lb. fermentum* have anti-microbial activity against *E. coli*, *Staph aureus* and *Salmonella typhimurium*.

Conclusion

Production of *burukutu* from sorghum grains in Ghana involves malting, mashing, straining, souring, wort concentration and fermentation. Both lactic acid and alcoholic fermentations are involved in yielding a sour alcoholic beverage of a thick consistency resulting from the use of a high proportion of sorghum malt. *Lb. fermentum*, *Lb. plantarum* and *Saccharomyces cerevisiae* are the predominant lactic acid bacteria and yeasts involved in the fermentations. Though high counts of coliform bacteria and *E. coli* were isolated at all four production sites, *burukutu* as well as some lactic acid bacteria isolates showed antimicrobial activity against some common enteric pathogens.

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