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*Full Length Research Paper*

# Biogenic amine producing bacteria associated with three different commercially fermented beverages in Botswana

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**The microbial quality and predominant bacterial groups of three fermented beverages was investigated. The bacteria associated with sorghum beer, sour milk (*madilalamasi*) and sour maize beverage (*mageu/mahewu*) were determined using standard microbiological techniques. The predominant microorganisms were screened for the production of four biogenic amines using decarboxylase broth. Sorghum beer had the highest bacterial counts with sour maize beverage having the least bacterial counts. *Hafnia alvei* isolated from sour milk was found to be a major histamine and putrescine producer with 21.2 and 17.56 mg/100 ml respectively. On the other hand, the most important cadaverine producers were *Pantoea citrea* and *Hafnia alvei* with19.27 and 18.75 mg/100 ml respectively. *Enterococcus faecium* and *Enterococcus faecalis* isolated from sour milk were found to be prolific tyramine producers with 35.5 and 20.07 mg/100 ml respectively. The *Bacillus* species isolated from all fermented food products were found to be weak histamine producers. The study also revealed that production of biogenic amines was not a widely distributed property among the lactic acid bacteria as previously documented for other fermented food products. Based on the results, it was concluded that sour maize beverage was the safest fermented food product in terms of the microbial quality and presence of biogenic amine producing bacteria.**

**Key words:** Biogenic amines, biogenic acid producing bacteria, fermented beverages, decarboxylase activity, histamine, lactic acid bacteria.

## INTRODUCTION

Fermented foods are major dietary constituents in numerous developing countries in Africa primarily because of their longer keeping quality under ambient conditions, and also for their safety and traditional, organoleptic acceptability (Holzapfel, 2002; McMaster et al., 2005). There are several traditional fermented foods and beverages that are produced at household level in Botswana and in the southern African region as a whole. These include non-alcoholic cereal-based beverages, sour maize beverage (known as *mageu / mahewu*), fermented maize/sorghum porridges (*ting*), fermented milk (*madila*) and alcoholic beverages from sorghum or millet malt (*bojalwa jwa setswana*). Sorghum beer, sour maize beverage (*mageu/mahewu*) and sour milk (*madila/amasi*) are some of the commonly consumed traditional fermented foods that have since been commercialized and are currently easily accessible to the urban and rural market.

Sour maize beverage (*mageu/mahewu*) is a popular fermented non-alcoholic beverage that is readily available on food retail stores in Botswana and South Africa. Traditionally sour maize beverage was produced by the spontaneous fermentation of leftover maize meal porridge. Water is used to macerate the maize meal into a thin gruel and small quantities of wheat flour or

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sorghum malt is blended in to provide the microbial flora to initiate the fermentation process (Gadaga et al., 1999; Holzapfel and Taljaard, 2004). Fermentation is carried out by predominantly Lactic acid bacteria at ambient temperature to yield the characteristic sour taste that is synonymous with the product. Industrial production has seen the use of pure starter cultures such as *Lactobacillus brevis* and *Lactobacillus bulgaricus* var *delbrueckii* in the fermentation process (Holzapfel and Taljaard, 2004; McMaster et al., 2005). The product then is pasteurized to extend the shelf life to 21 days when refrigerated.

Sour milk is traditionally produced by the spontaneous fermentation of raw milk from cattle (Gran et al., 2003). This traditional preparatory method is still widely employed in rural areas of Botswana, South Africa and Zimbabwe (Gadaga et al., 1999; Beukes et al., 2001). Milk is kept in traditional storage vessels such as clay pots and calabashes. Fermentation is carried out over a period of 1 to 3 days by random microbial flora present in the milk, vessels used for fermentation and the environment. After coagulation the whey is drained from the vessel to leave the thick curd which is topped up daily with excess milk from the household, with continued drainage of the whey (Beukes et al., 2001; Gran et al., 2003). Commercial production has seen the introduction of starter cultures with *Lactococcus lactis* subsp *diacetylactis* and *L. lactis* subsp *cremoris* being the strains of choice in industry (Beukes et al., 2001).

Sorghum beer is an opaque beer with a pinkish-brown tinge and a thicker consistency than the conventional beers due to the suspended solids derived from ingredients used in the brewing process. Although brewers might employ slight variations in the industrial production, maize meal or sorghum meal is used as the main form of starch and the malt is primarily derived from sorghum or millet. The beer has a distinct sour taste due to subsequent fermentation by Lactic acid bacteria inherent in the grain used as raw material. Sorghum beer is served while actively fermenting. Industrial production of a popular brand known as *Chibuku* is expansively covered in the article by Kutyauripo et al. (2009).

Microorganisms play an integral role in acquiring the desired taste in fermented food products. However, contamination by undesirable non-pathogenic and pathogenic microorganisms can occur if good hygienic and manufacturing practices are not implemented during the production process. Apart from affecting the sanitary quality, unwittingly, these microorganisms and some of the microorganisms used as naturally mixed starter cultures may produce toxins such as biogenic amines when the conditions are conducive for their formation. Biogenic amines are renowned for causing adverse health effects when consumed in excessive quantities. They include Histamine and tyramine, which are the main causes of numerous cases of food intoxication; other amines such as putrescine, cadaverine, and phenylethylamine are also important because they may intensify the undesirable effects of histamine (Stratton et al., 1991).

In a study that we have previously published, we reported the detection and quantification of four biogenic amines in sorghum beer, sour milk (*madila, amasi*) and sour maize beverage (*mageu/mahewu*) (Magwamba et al., 2010). In that study, putrescine was found to be the most common biogenic amine in sorghum beer and sour milk. It occurred in approximately 60% of the samples. Seventy percent of the sour maize beverage samples had some amount of cadaverine. Although in that study most of the samples in all the three categories of fermented beverages had biogenic amines concentrations all were within acceptable limits. However, one sorghum beer sample had a histamine content above the limit approved by the US Food and Drug Administration (FDA 1992). The current study is a follow up to the investigation and reports on the bacterial groups isolated from the fermented products and their capacity to produce biogenic amines. The study aims to add to the existing knowledge base of biogenic amine producing bacteria. Such a data base can be used to advise the beverage fermentation industry on appropriate strains that can be used as safe starter cultures.

## MATERIALS AND METHODS

### Sample numbers and sources

Seventy nine (79) sour maize beverage (*mageu/mahewu*), eighty four (84) sour milk (*madila/amasi*) and eighty seven (87) samples of commercial sorghum beer samples were procured from randomly selected retail markets in Gaborone from May 2007 through September 2008. Samples were transported to the laboratory in an ice cooled box and analyzed within 4 h.

### Bacteriological analysis

For isolation and enumeration of bacterial groups, serial dilutions were performed in 0.1 % (w/v) peptone water (CM9 Oxoid, Basingstoke, UK) as deemed necessary. All plates with 30-300 colony forming units after incubation were recorded as recommended in FAO Food and Nutrition paper. 14/4 Rev. 1 (Andrew, 1992).

### Total mesophilic and psychrotrophic bacterial counts

To determine the extent of quality deterioration, total aerobic mesophilic bacterial counts and psychrotrophic bacterial counts were performed by the pour plate method using plate count agar (PCA) (CM325 Oxoid, Basingstoke, UK) as described by Trytinopoulou et al. (2002) and Biaxias-Nogueras et al. (2005). Optimal incubation temperatures for mesophilic and psychrotrphic bacteria were at 30°C and 10°C for 48 h and 7 days, respectively.

### Endospore forming bacteria

For endospore enumeration and isolation a 1: 10 food dilution sample was performed in 0.1 % (w/v) peptone water (Oxoid, CM9,

Basingstoke, UK) and heated to inactivate vegetative bacterial cells in a water bath for 10 min at 80°C–Further serial dilutions were performed using 0.1% (w/v) of peptone water when found necessary. Counts of aerobic endospore forming bacteria were performed by the spread plate method on plate count agar (Oxoid, CM325) and counts for anaerobic endospore forming bacteria were performed on plate count agar (Oxoid, CM325) using the pour plate method. Plates were incubated anaerobically at 30°C for 48 h in an anaerobic jar with a $H_2$+$CO_2$ generating kit (Oxoid, BR38).

After evaluation of colonies by spore staining, suspect *Bacillus* species were subjected to the following tests to demonstrate proteolytic and lypolytic activity: PCA supplemented with 0.5% casein and gelatin was used to demonstrate proteolytic activity and lipolytic activity was demonstrated by using PCA supplemented with 0.5% tributyrin. Growth at different temperatures and concentrations of NaCl was also determined. Utilization of citrate, reduction of nitrate and production of acid and gas from glucose was determined using standard microbial assessment methods.

Gram positive, catalase negative, endospore forming bacilli, suspected to be *Clostridium* species were subjected to the following tests; Saccharolytic and lipolytic activities of isolates were determined using PCA supplemented with 0.5% starch and tributyrin, respectively. Reduction of nitrate using nitrate broth and production of acid from inositol, lactose, mannose, sorbitol and sucrose were additional tests that were carried out. Based on the results obtained above, the Bergey's Manual of Systematic Bacteriology (Sneath et al., 1986) was used to identify the isolates to species level.

**Lactic acid bacteria counts**

Counts of lactic acid bacteria were performed by the pour plate count method on deMan Rogosa Sharpe (MRS) agar (Oxoid, CM361) as described by Pons-Sanchez-Cascado et al. (2005) and incubated anaerobically at 30°C for 48 h in an anaerobic jar with a $H_2$+$CO_2$ generating kit (Oxoid, BR38).

The presumptive Gram positive, non-spore forming and catalase negative lactic acid bacteria (LAB) were further subjected to phenotypic tests as described by Holzapfel and Schillinger (1992), Axelsson (1993), Wood and Holzapfel (1995) and Leisner et al. (2000). In addition to the above tests, all Gram positive cocci isolates were streaked on Bile Aesculin Agar (Oxoid, CM888). The presumptive *Streptococcus* species which produced a dark brown/ black complex on the agar were further characterized using the API 20 Strep (BioMerieux, France) galleries. API 50CHL galleries (BioMerieux S.A., Marcy-l'Etoile, France) were also used to identify other LAB to species level.

**Enteric bacterial counts**

Counts of enteric bacteria were performed using violet red bile glucose agar (Oxoid, CM485) as described by Pons-Sanchez-Cascado et al. (2005). *Enterobacteriaceae*-like Gram-negative, oxidase-negative, and catalase-positive isolates were tested for formation of gas from glucose, and fermentation of trehalose using standard tests. Fermentative isolates were characterized with API 20E (BioMerieux, France) galleries.

***Pseudomonas* spp. counts**

Gram negative oxidase-positive bacteria suspected to be *Pseudomonas* species were streaked on *Pseudomonas* Agar Base (Oxoid, CM 559). The isolates were tested for liquefaction of gelatin, production of acid from maltose, production of indole from tryptophane, and fluorescence on King's B agar as described by Palleroni (1984) and Tryfinopoulou et al. (2002). API 20E (BioMerieux, France) galleries were used to identify the *Pseudomonas* species.

**Gram-positive catalase-positive coccal counts**

Counts of Gram-positive catalase-positive cocci were performed on plates of mannitol salt agar (Oxoid, CM85) as described by Pons-Sanchez-Cascado et al. (2005). The suspected *Staphylococcus aureus* isolates were streaked on Baird-Parker agar (Oxoid, CM275) and characteristic jet-black colonies were subjected to standard biochemical tests for the anaerobic utilization of glucose and mannitol, coagulase test and lysostaphin sensitivity.

**Confirmation of the identity of isolates to species level**

In addition to the above physiological and biochemical tests, the Biolog Metabolic Fingerprinting (Biolog Inc., Hayward, California.) was used to confirm the identity of *Streptococcus, Enterobacteriaceae, Staphylococcus*, *Pseudomonas, Bacillus* and some members of the Lactic acid bacteria to species level.

**Screening of identified microorganisms for decarboxylase activity**

Strains identified to species level were prepared for testing by subculturing in nutrient broth (NB CM3, Oxoid, Basingstoke, UK) supplemented with 0.4% each of the amino acids histidine, tyrosine, lysine, or ornithine and incubated at 25°C for 48 h (da Silva et al., 2002). A loop of each culture was spread on the decarboxylation agars which were then incubated at 25°C for 24 - 48 h. All strains were incubated aerobically except LAB and *Clostridium* species which were incubated anaerobically in an anaerobic jar with a $H_2$+$CO_2$ generating kit (Oxoid, BR38). A purple halo was interpreted as positive for amine production on both media. The exception however, was of the decarboxylation media containing tyrosine because it produced a clear area surrounding the colonies indicating a positive reaction, as observed in other studies, such as that proposed by Joosten and Northholt (1989).

Medium used for preliminary screening of decarboxylating strains was prepared as described by Niven et al. (1981). Additionally, the LAB-specific decarboxylation agar proposed by Maijala (1993), a modified version of the medium described by Joosten and Northolt (1989), was used to detect decarboxylating LAB strains. Both media were autoclaved for 10 min at 121°C to avoid excessive hydrolysis of the agar at low pH. The decarboxylation agar proposed by Maijala (1993) had Tween-80 0.5 g/L, $MgSO_4$ 0.02 g/L, $MnSO_4$ 0.005 g/L and $FeSO_4$ 0.004 g/L, added to it in order to enhance the growth of LAB strains.

**Confirmation of amine forming capacity by HPLC analysis**

Biogenic amine production of the isolates was confirmed using decarboxylase broth according to the method developed by Bover-Cid and Holzapfel (1999). The decarboxylase broth for LAB was prepared as proposed by Maijala (1993) without agar while that for other microorganisms was prepared as per Niven et al. (1981) description without any agar. Strains were prepared for testing using the method of da Silva (2002). The positive reactions were recorded when a purple color occurred in the decaboxylase broth. A simultaneous inoculation of the strain on decarboxylase broth without an amino acid precursor was performed to eliminate false positive reactions due to formation of other alkaline compounds. Two randomly selected positive broth tubes for each bacterium

**Table 1.** Average bacterial counts and biogenic amine content in sorghum beer, sour milk and sour maize.

| Food type | Average bacterial counts $log_{10}$ (CFU/ml) | | | | | | | [a]Mean biogenic amine (mg/100 ml) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | TMC | PBC | LAB | ENT | ANA | AER | MIC | HIS | PUT | CAD | TYR |
| Sorghum beer | 8.3 | 5.7 | 7 | 6 | 4.5 | 4 | ND | 0.94 | 1.58 | 1.01 | 2.08 |
| Sour milk | 6.5 | 5.6 | 5.3 | 3.8 | 3.6 | 3.7 | ND | 0.31 | 2.02 | 0.87 | 3.2 |
| Sour maize beverage | ND | ND | ND | ND | ND | ND | ND | 0.01 | 0.12 | 0.09 | 0.002 |

[a-]Mean biogenic amine data published in Magwamba et al. (2010); ND-Not detected TMC- Total mesophilic count; PBC - Pyschrotrophic bacterial count; LAB - Lactic Acid Bacteria; ENT- Enterobacteria; ANA - Anaerobic endospore formers; AER- aerobic endospore formers; MIC – Micrococcaceae; HIS-histamine; PUT- putrescine; CAD- cadavarine; TYR-tyramine.

were analyzed for biogenic amine production by high-performance liquid chromatography (HPLC) as described previously (Magwamba et al., 2010). An average content of a given biogenic amine was then calculated.

#### Determination of biogenic amine concentration from the fermented foods

The concentration of histamine, putrescine, cadaverine and tyramine from sorghum beer, sour milk and sour maize beverage samples was determined as described by Magwamba et al. (2010).

#### Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Sciences, SPSS Version 10.0 for windows (SPSS Inc., Chicago, IL, USA). The Pearson correlation was carried out to determine the relationship between specific bacterial counts and biogenic amine concentrations in the each beverage. Value of $P < 0.05$ was used to indicate significant deviation.

## RESULTS

The microbial quality of three fermented food products was investigated by looking at six parameters, mesophilic bacteria, psychrotrophic bacteria, lactic acid bacteria, enterobacteria, endospore formers, most of which are associated with microbial quality deterioration. Sour maize beverage was found to have the best sanitary quality with the least bacterial counts on all parameters investigated (Table 1). The mean total mesophilic, psychrotrophic and lactic acid bacterial count for sorghum beer and total mesophilic count for sour milk was found to be higher than 5 $log_{10}$ cfu/ml. The exception was in the case of sorghum beer which had a mean enterobacterial count of 5 $log_{10}$ cfu/ml.

There was no relationship between the biogenic amine content and various microbial counts investigated except for a positive correlation ($r = 0.54$) between cadaverine concentration and enterobacterial counts in sour milk. There was a weak positive correlation ($r = 0.35$) between mesophilic bacterial counts and cadaverine content in sour milk. However, the low average bacterial counts in sour maize beverage corresponded with low biogenic amine content (Table 1).

The identity of the representative isolates are given in Table 2 and the number of strain isolated across all samples indicated. Therefore, the number of strains isolated per food type gives an approximate figure of the frequency of isolation of the various strains from each product. *Bacillus subtilis* was the most predominant species isolated across all three fermented food products (Table 2).

Sorghum beer had the highest microbial counts in all microbial quality parameters investigated. Sour milk had the most diverse strains with 21 different bacterial species identified compared to 9 in sorghum beer. Sour maize beverage had the least diversity with 6 bacterial species characterized. Lactic acid bacteria and endospore formers were the only bacterial groups isolated from across all three fermented food products (Table 2). Enteric bacteria were isolated from sour milk and sorghum beer samples, while no isolates were detected in sour maize beverage. A peculiar finding was that all the 64 colonies from the sorghum beer samples were found to be *Enterobacter intermedius*. One other highlight was that *Pseudomonas* spp. and members of the *Staphylococcus* were only isolated from sour milk and not the other fermented foods.

In general, the endospore formers isolated from the fermented food products were weak histamine producers, with the exception of *Bacillus subtilis* which produced 1.86 mg/100ml of histamine in sorghum beer. A *Paenibacillus azotofixans* isolate from sorghum beer was the most important putrescine producer at 4.04 mg/100 ml in decarboxylase broth. The highest cadaverine and tyramine production level was found in *Bacillus cereus* isolates from sorghum beer with 19.8 and 3.45 mg/100 ml respectively (Table 2). This was also the only food poisoning pathogen that was isolated from sorghum beer. However, no sample had more than 3 $log_{10}$ cfu/ml allowable limit as suggested by the FDA (1992). All the endospore formers isolated from sour maize beverage were found to be weak biogenic amine producers, with less than 1 mg/ 100 ml of a given biogenic amine with the exception of *Bacillus subtilis* which produced 1.07mg/ 100 ml of histamine in deacarboxylase broth.

Among the members of the enterobacteria *Citrobacter freundii* was the most predominant species comprising 21% of the isolates in sour milk. It was also a major

**Table 2.** Bacterial isolates from the fermented beverages and the amounts of biogenic amines each produced in decarboxylase broth.

| Bacteria | Sour milk | | | | | Sorghum beer | | | | | Sour maize beverage | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [a]No | BGA content (mg/100 ml) | | | | [a]No | BGA content (mg/100 ml) | | | | [a]No | BGA content (mg/100 ml) | | | |
| | | His | Put | Cad | Tyr | | His | Put | Cad | Tyr | | His | Put | Cad | Tyr |
| **Endospore formers** | | | | | | | | | | | | | | | |
| *Bacillus subtilis* | 72 | ND | 0.20 | 0.27 | 0.11 | 80 | 1.86 | 0.10 | 0.12 | 0.17 | 25 | 1.07 | 0.1 | 0.3 | 0.09 |
| *B. thermoglucosidasius* | 37 | 1.05 | 0.13 | 0.19 | 0.08 | | | | | | | | | | |
| *B. cereus* | | | | | | 20 | 1.65 | 0.26 | 19.8 | 3.45 | | | | | |
| *B. laevolacticus* | 31 | ND | 0.22 | 0.32 | 0.24 | | | | | | | | | | |
| *B. halodurans* | | | | | | 48 | 1.05 | 0.11 | 0.31 | 1.72 | | | | | |
| *B. megaterium* | | | | | | | | | | | 22 | 0.72 | 0.18 | 0.22 | 0.14 |
| *B. coagulans* | | | | | | | | | | | 10 | ND | 0.16 | 0.21 | 0.38 |
| *Paenibacillus azotofixans* | | | | | | 39 | ND | 4.04 | 2.36 | 1.88 | | | | | |
| *Clostridium* spp. | 58 | 0.54 | 0.18 | 0.25 | 0.12 | 67 | 1.11 | 0.23 | 0.42 | 1.01 | 19 | 0.91 | 0.24 | 0.15 | 0.12 |
| **Enterobacteriaceae** | | | | | | | | | | | | | | | |
| *Enterobacter aerogenes* | 56 | 1.48 | 8.74 | 1.98 | 2.31 | | | | | | | | | | |
| *E. intermedius* | | | | | | 64 | ND | 7.50 | ND | 2.02 | | | | | |
| *C. freundii* | 70 | 2.30 | 11.51 | 0.34 | 3.23 | | | | | | | | | | |
| *C. braakii* | 65 | ND | 3.44 | 0.21 | 2.86 | | | | | | | | | | |
| *Hafnia alvei* | 67 | 21.20 | 17.56 | 18.75 | 8.16 | | | | | | | | | | |
| *Escherichia coli* | 47 | ND | 0.32 | 0.11 | 0.14 | | | | | | | | | | |
| *Pantoea citrea* | 29 | ND | 6.01 | 19.27 | 5.89 | | | | | | | | | | |
| **Pseudomonads** | | | | | | | | | | | | | | | |
| *Pseudomonas fluorescens/putida* | 56 | 18.8 | 11.32 | 15.01 | 1.55 | | | | | | | | | | |
| *P.seudomonas* spp | 66 | 5.30 | 3.41 | 7.95 | 2.20 | | | | | | | | | | |
| **Staphylococcus** | | | | | | | | | | | | | | | |
| *Staphylococcus epidermis* | 52 | 1.16 | 0.48 | 0.22 | 2.02 | | | | | | | | | | |
| *S. auricularis* | 38 | ND | 0.24 | 0.19 | 0.88 | | | | | | | | | | |
| *S. arlettae* | 19 | 1.27 | 0.36 | 0.14 | 0.62 | | | | | | | | | | |
| *S. aureus* | 43 | 0.91 | 1.55 | 0.27 | 2.25 | | | | | | | | | | |
| **Lactic acid bacteria** | | | | | | | | | | | | | | | |
| *Enterococcus faecium* | 81 | ND | ND | ND | 35.5 | | | | | | | | | | |
| *Ent. faecalis* | 76 | ND | ND | ND | 20.07 | | | | | | | | | | |
| *Ent. gallinarum* | 45 | ND | ND | ND | ND | | | | | | | | | | |
| *Lactobacillus* spp | 37 | ND | ND | ND | ND | 67 | ND | ND | ND | ND | 9 | ND | ND | ND | ND |
| *Carnobacterium gallinarum* | 52 | ND | ND | ND | ND | 52 | ND | ND | ND | 21.68 | ND | ND | ND | ND | ND |
| *Streptococcus oralis* | ND | ND | ND | ND | ND | 35 | 2.34 | 4.20 | 3.81 | 8.64 | ND | ND | ND | ND | ND |
| *Lactococcus raffinolacticus* | ND | ND | ND | ND | ND | 29 | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| *Pediococcus dextrinicus* | ND | ND | ND | ND | ND | 23 | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| *Leuconostoc mesenteroides* | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 4 | ND | ND | ND | ND |

[a]No- For each bacterial strain numbers, two randomly selected strains were tested for the production of a given biogenic amine in decarboxylase broth and an average was calculated; ND- Not detected.

histamine forming bacteria (2.3 mg/100 ml) coming only second to *Hafnia alvei* (21.2 mg/100 ml) in decarboxylase broth. The highest putrescine production level was found in *Hafnia alvei* with 17.56 mg/100 ml and the most important cadaverine producers were *Pantoea citrea* and *Hafnia alvei* with 19.27 and 18.75 mg/100 ml in decaboxylase broth respectively. *Hafnia alvei* and *Pantoea citrea* were also found to be the most important tyramine producers with 8.16 and 5.89 mg/100 ml in decarboxylase broth respectively. In general, *Hafnia alvei* was found to be an important biogenic amine producer for sour milk as it produces all four biogenic under investigation in significant quantities. The *Pseudomonas fluorescens/putida* also isolated from sour milk was found to be a prolific biogenic amine producer, producing significant amounts of all four biogenic amines assayed in the current study (Table 2).

Biogenic amine production was rare among the lactic acid bacteria isolated from all three fermented food products. From the 12 species of lactic acid bacteria identified, only *Streptococcus oralis* isolated from beer could produce all four biogenic amines under investigation, while only three lactic acid bacteria species were only capable of producing tyramine. These were *Enterococcus faecium* and *Enterococcus faecalis* isolates from sour milk and *Carnobacterium gallinarum* from sorghum beer. All these were all found to be fervent tyramine producer in decarboxylase broth (Table 2).

## DISCUSSION

On assessing the microbial quality of the three fermented beverages, the bacterial load for sorghum beer and total mesophilic count for sour milk was found to be higher than 5 $\log_{10}$ cfu/ml (FDA, 1992). However, the counts were deemed as acceptable as the lactic acid bacteria play a key role in the fermentation process and could have consequently contributed to the higher total mesophilic and psychrotrophic counts observed. The exception was in the case of sorghum beer which had a mean enterobacterial count of 5 $\log_{10}$ cfu/ml, which is marginally acceptable. This suggests that this beverage is not produced to the desired sanitary standard.

The fact that some Lactic acid bacteria were isolated from across all three fermented food was not unanticipated as lactic acid bacteria are deliberately introduced as starter cultures in most fermented food products. The diversity of lactic acid bacteria isolated in sour milk in the current study was lower than traditionally fermented sour milk documented by Gadaga et al. (1999) and Beukes et al. (2001). This was to be expected as specific starter cultures are used in commercially produced sour milk. However, *Lactococcus lactis* subsp *cremoris*, *Lactococcus lactis* subsp *lactis* and *Lactobacillus plantarum* which are usually isolated in traditionally fermented milk and used in commercial preparation of sour milk (Beukes et al., 2001) were not isolated in the current study. *Lactobacillus* and *Leuconostoc* species were isolated from sour maize beverage and could constitute deliberately selected starter cultures. *Lactococcus lactis* subsp *lactis* is associated with spontaneous fermentation of sour maize beverage (Steinkraus et al., 1993), while commercial production utilizes *Lactobacillus delbrueckii* (Gardini et al., 2001). However, it was disappointing that a comparative analysis could not be undertaken, as the *Lactobacillus* isolated in this study could not be identified to species level with absolute certainty due to the lack of sensitivity of the technique.

Sour maize beverage had the best sanitary quality and consequently had the lowest average biogenic amine content when compared to other fermented products. It suggest that sour maize beverage is subjected to a strict pasteurization process and accounts for the insignificant presence of thermoduric endospore formers which was found in this product. However, the presence of certain microbial species that serve as sanitary indicators suggest unsatisfactory hygienic production protocols for sour milk production. *Enterococcus faecium* and *Enterococcus faecalis* were the predominant lactic acid bacteria isolated from sour milk. Both species are frequently associated with the gastrointestinal tract of a variety of animals and suggest unsanitary manufacturing practices. This observation is supported by the presence of heat sensitive enterobacteria and *Pseudomonas* spp. as well as members of the *Staphyloccus aureus*, which suggests human contact and a lapse in good food handling practice during the manufacturing process.

In general, the endospore formers isolated from the fermented food products were weak histamine producers. These results corresponds well with those of other studies in which members of the genus *Bacillus* were identified as weak histamine-forming bacteria in fermented fish (Yatsunami and Echigo, 1991) and sufu, a Chinese soybean product (Kung et al., 2007). *Bacillus* spp. isolated from anchovies have also been found to produce low levels of histamine (Kim et al., 2004). A peculiar finding was that in this study a *Bacillus subtilis* strain isolated from sour milk was found to be a non histamine producer, whereas the one isolated from sorghum beer was a histamine producer. Previous studies have revealed that the capacity to form amines was strain-dependent, rather than species-dependent (Bover-Cid and Holzafel, 1999; Martin et al., 2006) and this could explain the aberrant observation and differences in biogenic amine production in other report. To our knowledge, this is the first report of decarboxylase activity in *Paenibacillus azotofixans*

Many members of the family *Enterobacteriaceae* have been reported to produce considerable levels of histamine (Klausen and Huss, 1987; Halasz et al., 1994). In our study *Hafnia akvei* was the most prolific biogenic amine producer in sour milk. It is therefore postulated that

*Hafnia alvei* was responsible for the bulk of biogenic amine accumulation in sour milk. Strains of *Hafnia alvei* have been reported to be prolific histamine producers and as such they are considered to be important in the hygiene of fish products (Silla Santos 1998). *Hafnia alvei* was also found to produce the highest level of putrescine, a finding that has been documented in other studies carried out on meat products (Durlu-Ozkaya et al., 2001). Though there were no justifiable reasons, sour milk was the only food product to have *Pseudomonas* species. Representative isolates were capable of producing all four biogenic amines.

Studies by Bover-Cid et al*.* (2001) have reported biogenic amine production among the pseudomonads with *Pseudomonas cepacia* and *Pseudomonas fluorescens* producing appreciable amounts of putrescine in sausages.

Staphylococcal isolates were only found in sour milk. All the representative staphylococcal isolates from sour milk were found to be producers of the four biogenic amines under investigation with the exception of *Staphylococcus auricularis* which was a non-histamine producer. Histamine producing strains of *Staphylococcus* species have previously been reported by Chen-Chang et al. (2008). This observation has also been documented in earlier studies by Hernandez-Herrero et al*.* (1999) and Yatsunami and Echigo (1991) who found that most of the *Staphylococcus* isolates were histamine producers. Putrescine production by *Staphylococcus epidermis* isolated from salt-dried sardines, have been reported by Lakshmanan et al. (2002) and histidine decarboxylase activity has been detected in *Micrococcus* and *Staphylococcus* isolates (Stratton et al., 1991; Yatsunami and Echigo, 1991). *Staphylococcus epidermis* strain isolated in this study was capable of producing all four biogenic amines. This result correlates well with the findings of Martin et al*.* (2006), who found that the diamines, putrescine and cadaverine, as well as histamine, were produced simultaneously by several strains identified as *S. epidermidis*. *Staphylococcus aureus* was the only food poisoning pathogen that was isolated from sour milk, and no sample had more than the 20 cfu/ml limit suggested by the FDA (1992).

The inability of most Lactic acid bacteria to produce the other three biogenic amines conforms to results obtained in similar studies (Bover-Cid and Holzapfel, 1999; Leuschner et al., 1999; Bover-, 2001). The tyramine producing ability of *Enterococcus faecium* and *Enterococcus faecalis* in decarboxylase broth is consistent with data reported by Bover-Cid et al. (2001), Masson et al*.* (1997), Gardini et al. (2001), Ozogul and Ozugul (2007) and Bonetta et al. (2008). *Leuconostoc mesenteroides* subsp *dextranicum* isolated from sour maize beverage was also found to be a non biogenic amine producing bacteria. This confirms the studies by Bover-Cid and Holzapfel (1999) and Straub et al*.* (1995). However, some strains of *Lactococcus* and *Leuconostoc* have been described as tyramine producers (Choudhury et al., 1990; Gonzalez de Llano et al., 1998).

The *Enterococcus gallinarum*, *Lactobacillus* spp and *Carnobacterium gallinarum* isolated from sour milk were non tyramine producers. This is in contrast to the results found by Masson et al*.* (1997), where strains of *Carnobacterium gallinarum* were found to produce considerable amounts of tyramine. It is possible that the biogenic amine producing gene(s) might be borne on plasmid(s) and that the strain we isolated might have been cured or lost its plasmid. On the other hand, *Carnobacterium gallinarum* isolated from sorghum beer was found to be a notorious tyramine producer. *Carnobacterium* spp. has been previously reported to produce tyramine from tyrosine in laboratory media and chill-stored beef (Edwards et al., 1987; Leisner et al*.*, 1994). Again, strain variability in biogenic amine production was demonstrated. It should be noted that in this study the ability of the strains to produce biogenic amines was only determined in decarboxylase media and not in original food under investigation, so the chemical composition and synergistic interactions with other microbial flora could explain the variability and inconsistency with results from other studies.

The Pearson correlation was used to establish whether there was a relationship between bacterial counts and the presence of biogenic amines. The amount of biogenic amines in foods is used to indicate the extent of food spoilage. In particular, putrescine and cadaverine can be used as indicators of toxicity in fish (Arnold and Brown, 1978; Mietz and Karmas, 1977). However, the same could not be extrapolated to this study. A positive correlation between cadaverine concentration and enterobacterial counts in sour milk was established with the sizeable amount of cadaverine produced by *Pantoea citrea* and *Hafnia alvei* enterobacterial species. On the other hand, a weak positive correlation between mesophilic bacterial counts and cadaverine content in sour milk was also evident. The former can be attributed to the sizeable amount of cadaverine produced by *Pantoea citrea* and *Hafnia alvei* enterobacterial species while the latter could also be as result of the two enterobacterial strains working in concert with *Pseudomonas fluorescens/putida* strain which was shown to produce appreciable amounts of cadaverine. Therefore, no particular microbial count parameter can be used as a reliable indicator of biogenic amines as this property seems to be strain specific.

In conclusion, sour milk had the highest diversity of bacteria. It also possessed the highest total average concentration of biogenic amines in line with previous reports by Magwamba et al. (2010). The presence of *Enterobacteriaceae*, pseudomonads and members of the *Staphylococcus* in this particular beverage suggests unsanitary production conditions or limitations in the pre-distribution pasteurization protocol. Lactic acid bacteria were found to produce low levels of biogenic amines with

the only exception being *Streptococcus oralis, Enterococcus faecium* and *Enterococcus faecalis* isolated from sour milk and sorghum beer. These bacteria could possibly have been introduced by contamination as both products were observed to also have members of the family *Enterobacteriaceae*. Sour maize beverage had the best microbial quality condition and hence the lowest total average of biogenic amines. This data suggests that with careful selection of lactic acid bacteria as starter cultures and the implementation of appropriate hurdle technology to inactivate endospores, fermented foods of good microbial quality and low biogenic amine content are a feasible prospect.

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