Process Characteristics and Microbiology of *Fura* Produced in Ghana

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Abstract: Majority of traditional cereal-based foods consumed in Africa are processed by spontaneous fermentation and are important as weaning foods for infants and as dietary staples for adults. Detailed knowledge of traditional processing is a prerequisite for investigating ways to improve both the nutritional and microbiological qualities of the corresponding product. In this study, the traditional processing of millet into *fura*, a popular millet-based dumpling consumed throughout West Africa, particularly Nigeria, Burkina-Faso and Ghana was investigated in a range of production units in northern Ghana. Microbiology of the processing was also investigated. Processing steps included soaking or steeping the grains, washing, dehulling, wet milling with the addition of aromatic ingredients, dough fermentation (optional), initial moulding, cooking, pounding into sticky cohesive mass and final moulding. The process was dominated by lactic acid bacteria (LAB) and yeasts. The isolated organisms were identified based on morphological, physiological and biochemical characteristics as *Lactobacillus* spp. (51.42%), *Pediococcus* spp. (21.4%), *Streptococcus* spp. (14.3%), *Leuconostoc* spp. (8.5%), and *Enterococcus* spp. (4.3%) in the case of LAB and *Issatchenkia orientalis* (26%), *Saccharomyces cerevisiae* (22%), *Pichia anomala* (16%), *Candida tropicalis* (16%), *Saccharomyces pastorianus* (10%), *Yarrowia lipolytica* (6%), and *Galactomyces geotricum* (4%) in respect of yeasts. Enterobactriaceae were also isolated and identified. The development of starter culture from the dominating organisms is important for the potential production of standardized *fura* at a commercial, small industrial scale, and for the improvement of its acceptability, microbiological stability and hygienic safety. [Nature and Science 2010;8(8):41-51]. (ISSN: 1545-0740).

Key words: *fura*, traditional processing, lactic acid bacteria, Yeasts.

1. Introduction

The role of the small-scale food processing as a sub-sector that can contribute significantly to the development of the rural economy is increasingly being realized (Dietz, 1999). Small-scale food processing industry is important for stimulating sustainable development in the rural and peri-urban areas of developing countries and for making food available to the increasing populations in urban areas. It provides a source of income and a means of poverty alleviation and contributes to variety in the diet and the food security of millions. Small-scale food industry also provides linkages to local suppliers of agricultural raw materials and to income-generating activities such as the manufacture of machinery, packaging and ingredients (FAO, 1997). Throughout Africa, fermentation is a traditional part of cereal, cassava and dairy processing. A wide range of cereal based fermented foods exist including *ogi* and *mahew* in Benin, *kenkey* in Ghana, *injera* in Ethiopia, *poto-poto* in Congo, *ogi* and *kunu-zaaki* in Nigeria, *uji* and *togwa* in Tanzania, *kisra* in Sudan (Tomkins *et al*., 1988, Hounhouigan *et al*., 1993, Oyewole, 1997, and Blandino *et al*., 2003). The northern regions of Ghana are home to several forms of grain-based and seed fermented foods, such as *pito* (from sorghum), *dawadawa* (from bambara groundnuts), *kantong* (from silk-cotton seeds), *hausa koko* and *fura* (from millet). *Fura* is a semi-solid dumpling millet-based meal (Jideani and Wedricha, 1994) or cereal porridge. It is a traditional staple food in West Africa particularly in Nigeria, Ghana and Burkina Faso (Jideani *et al*., 2001) produced mainly from millet blended with spices and water, compressed into dough balls and cooked (Kordylasi, 1990, Jideani *et al*., 2001). The cooked dough balls are broken up and made into porridge by mixing with yoghurt (*nunu*), fresh milk or water (Kordylasi, 1990). Sugar may be added to taste. The mixture of fermented milk and cooked spiced millet (*fura de nunu*) is almost a complete food with milk serving as a source of protein while the cooked spiced millet provides energy. The sour taste is known to be particularly suited for quenching thirst.

Traditional *fura* processing has been developed largely as an art handed down from one generation to the other, rather than through scientific principles. Although procedures and equipment used for *fura* processing are relatively simple, the microbiology and biochemistry aspects have not been adequately researched. Physical aspects (temperature, time, relative humidity and level of agitation and aeration) of the processing of millet into *fura* are poorly controlled, and production techniques are not standardized. The process therefore results in products of variable quality. Poor hygienic practices and improper handling during *fura* production, post-fermentation processing (pounding with mortar and pestle, molding with bare hands), and at the point of sale, may render the products susceptible to contamination. The fermentation process in traditional *fura* processing, like many other traditional fermentation processes occurs spontaneously and difficult to control. The process is not predictable in terms of length of fermentation and quality of product. It can produce unwanted products or products with a short shelf life and may not be safe since they are liable to contamination by pathogens. If *fura* is to meet its full potential benefits and be able to compete favorably with imported and industrially processed foods, there is the need to upgrade processing technologies to add value and ensure safety. This requires much more research on the product. Published data on the microbiology of the traditional processing of *fura*, and the effect of variations in processing techniques on the quality characteristics of the final product is not available. This paper, therefore, reports on the traditional processing of *fura* in northern Ghana and the associated microorganisms. This was done with the aim that the data generated will provide a rational basis for the improvement of processing techniques and thus the nutritional and microbiological qualities of *fura*.

2. Materials and Methods

2.1 Study Area

The study was undertaken in three townships in the Upper East Region of Ghana. These included Bolgatanga, Navrongo and Paga which shares borders with the south of Burkina Faso.

2.2 Survey

A total of 15 traditional processing units (TPUs) for *fura* production were identified and surveyed using questionnaire and direct observations. The purpose was to describe the different processing steps for *fura* production (for the development of a flow diagram) and to determine variations that existed during processing.

2.3 Samples and Sample Collection

Six different samples made up of three differently-prepared doughs and the *fura* made from each of them were collected from the surveyed TPUs. Dough sample (1) was made from millet grains that were soaked for 24 hours before milling and was designated as soaked grain fermented dough (SGFD); dough sample (2) was made from millet grains that were dehulled, wet milled and fermented for about 12 hours, designated as dehulled grains fermented dough (DGFD); dough sample (3) was made from millet grains that were dehulled, wet milled but without fermentation and designated as dehulled grains unfermented dough (DGUD). Samples were collected in sterile rubber bags and transported in cooler boxes to the laboratory for analysis. The samples were prepared by the traditional *fura* processing methods as described in Figure 1.

2.4 Determination of pH of Samples

Ten grams of sample was homogenized with 20 ml of distilled water in a stomacher bag mixer (Selecta, Buch and Holm A/S) for 30 seconds and the pH of the homogenate determined using the digital pH meter (Crison Basic 20 model) calibrated with standard buffer solutions (Crison).

2.5 Enumeration and Isolation of Microorganisms

Duplicate 10g-samples were homogenized with 90ml sterile peptone physiological saline solution [5g bactopeptone, 8.5g NaCl, 100ml distilled water, pH 7.0 ± 0.2). The homogenate was serially diluted to the 10^{-9} concentration and 1ml aliquots of the appropriate dilutions directly inoculated into Petri plates containing various isolation media. Aerobic mesophilic bacteria were enumerated on Plate count agar (PCA) (Oxoid Ltd, Basingstoke, Hamsphire, England), pH 7.0 and incubated at a temperature of 32°C for 48hrs. Pour plates of deMan, Rogosa, Sharpe (MRS) agar (Oxoid) (de Man *et al.*, 1960) pH 6.4 were used for enumeration of total Lactic Acid Bacteria (LAB). Plates were incubated anaerobically using BBL Gas Pack (Anaerocult 'A') (Merck, Darmstadt, Germany) at 35°C for 48hours. Purity was checked by subculturing selected distinct colonies from countable plates on MRS agar with incubation at 35°C for 48hours. Pure isolates were cultivated in MRS broth at 30°C for 18 hours. Sabourand Dextrose Agar (Merck), supplemented with 250mg/100ml chloramphenicol (selective supplement, Oxoid) with pH adjusted to 3.5 with tartaric acid was used for enumeration and isolation

of yeast. Inoculated plates were incubated at 25°C for 5 days. Purity was checked by selecting colonies with distinct morphological differences such as colour, shape and size, and re-streaking them on the same medium. Pure isolates were stored on slants at 4°C. Pour plates of violet red bile glucose agar (VRBGA) (Oxoid), pH 7.4 (Mossel *et al*., 1962) were used for enumeration and isolation of Enterobacteriaceae. Purity was checked by re-streaking well isolated colonies on the same medium. The pure isolates were then stored on slants of PCA at 4°C.

2.6 Characterization and Identification of Isolates

The LAB were characterized by microscopic examination and by conventional biochemical and physiological tests. Isolates were examined for colony and cell morphology; motility, cell arrangements, Gram reaction; catalase reaction; growth in broth at 10, 15 and 45°C; growth in the presence of 6.5% NaCl; production of ammonia from arginine; starch hydrolysis; dextran formation from sucrose; and carbon dioxide production (Harrigan, 1998). Their fermentation pattern for the sugars--raffinose, melibiose, arabinose, cellobiose, gluconate, lactose, salicin, and sucrose was determined. Identification was based on morphological, physiological and biochemical characteristics, and by reference to Bergey's Manual of Systematic Bacteriology (Sneath *et al.,* 1986) and Wood and Holzapfel (1995).

Yeasts were identified using the Simplified Identification Method (SIM) described by Deak and Beuchat (1996), with additional standard taxonomical methods (Kurtzman, 1998). For SIM, tests were carried out according to the descriptions of Yarrow (1998) including fermentation patterns among D-glucose, raffinose, maltose, galactose, sucrose, lactose, fructose, trehalose; and the liquid assimilation (of carbon and nitrogen compounds) patterns among glucose, galactose, sucrose, lactose, maltose, melibiose, raffinose, trehalose, α-methyl-D-glucoside, cellobiose, erythritol, citrate, nitrate, L-lysine, and cadavarine. Other tests included growth at 37°C, cycloheximide resistance, urease activity, growth in 50% glucose-yeast extract, growth in vitamin free medium, and growth in the presence of 16% NaCl. The formation of pseudohypha and mycelium was examined by microscopy (Harrigan, 1998), and cell morphology determined from wet mounts.

Enterobacteriaceae were identified based on their morphological and biochemical characteristics. Microscopy (including Grams staining, motility, cell shape) and the ability to produce gas (from glucose, lactose, sucrose, D-mannitol, L-arabinose, raffinose) were done according to Harrigan (1998). Other tests included production of indole from tryptophan, methyl red test, Voges-Proskauer test, utilization of citrate, and hydrogen sulfide production.

2.7 Statistical Analysis

All analyses were conducted in duplicate and five replicates of each sample were collected for analysis. Data obtained were subjected to Analysis of Variance (ANOVA) and means were separated by Tukey's family error rate multiple comparison test ($p<0.05$) using the MINITAB statistical software package (MINITAB Inc. Release 14 for windows, 2004).

3. Results

3.1 Characteristics of *Fura* Processing

The main ingredients for *fura* processing in Ghana are the pearl millet (*Pennisetum* spp.*)* and spices such as pepper, cloves, mint and ginger. The scales of operation for the processing units surveyed varied based on the quantity of millet processed daily. The amounts ranged from about 6 kg to 27 kg, with an average of about 12 kg. There were also some significant variations in the processes, as regards the techniques used and the parameters involved. Figure 1 summarizes the traditional *fura* process, with the variations observed. As the first step, some processors dehull the millet grains while others soak the grains without dehuling. The duration of soaking varies, ranging from about 18 hours to 28 hours, and with an average of about 23.3 hours. At all the processing units visited, washing of the grains before milling was practiced. This constitutes the second major step in processing millet into *fura.* The extent of washing apparently depends on the quantity and quality of the raw material (millet). Following washing, wet milling is done using the plate attrition mill. It is during this time that the ingredients (pepper, mint, cloves, and ginger) are added. Some processors ferment the dough formed. Depending on the variations in the processes as depicted in Figure 1, three different doughs result; the dehulled grain unfermented dough (DGUD), the soaked grain fermented dough (SGFD), and the dehulled grain fermented dough (DGFD). Once the doughs are produced, they are hand-moulded into balls of about 10cm in diameter and then cooked for about 30 minutes. The cooked millet balls are pounded with a mortar and pestle. They are finally moulded into much smaller balls for sale. The balls may be coated with maize flour before being packed for sale. The shelf stability of the final product at ambient conditions was noted to vary from 1 to 6 days. The

duration varied depending on the producer's expertise and processing techniques. Indicators of spoilage included mold growth, caking, and excessive souring resulting from continuous fermentation after processing. All unit operations were observed to be performed under uncontrolled, open environmental conditions. The dehulling and milling are done at small commercial community milling centers. The main by-product of *fura* processing is the chaff resulting from the partial dehulling and winnowing which consists of the hulls and sometimes the germs of the millet grains, and is used as animal feed.

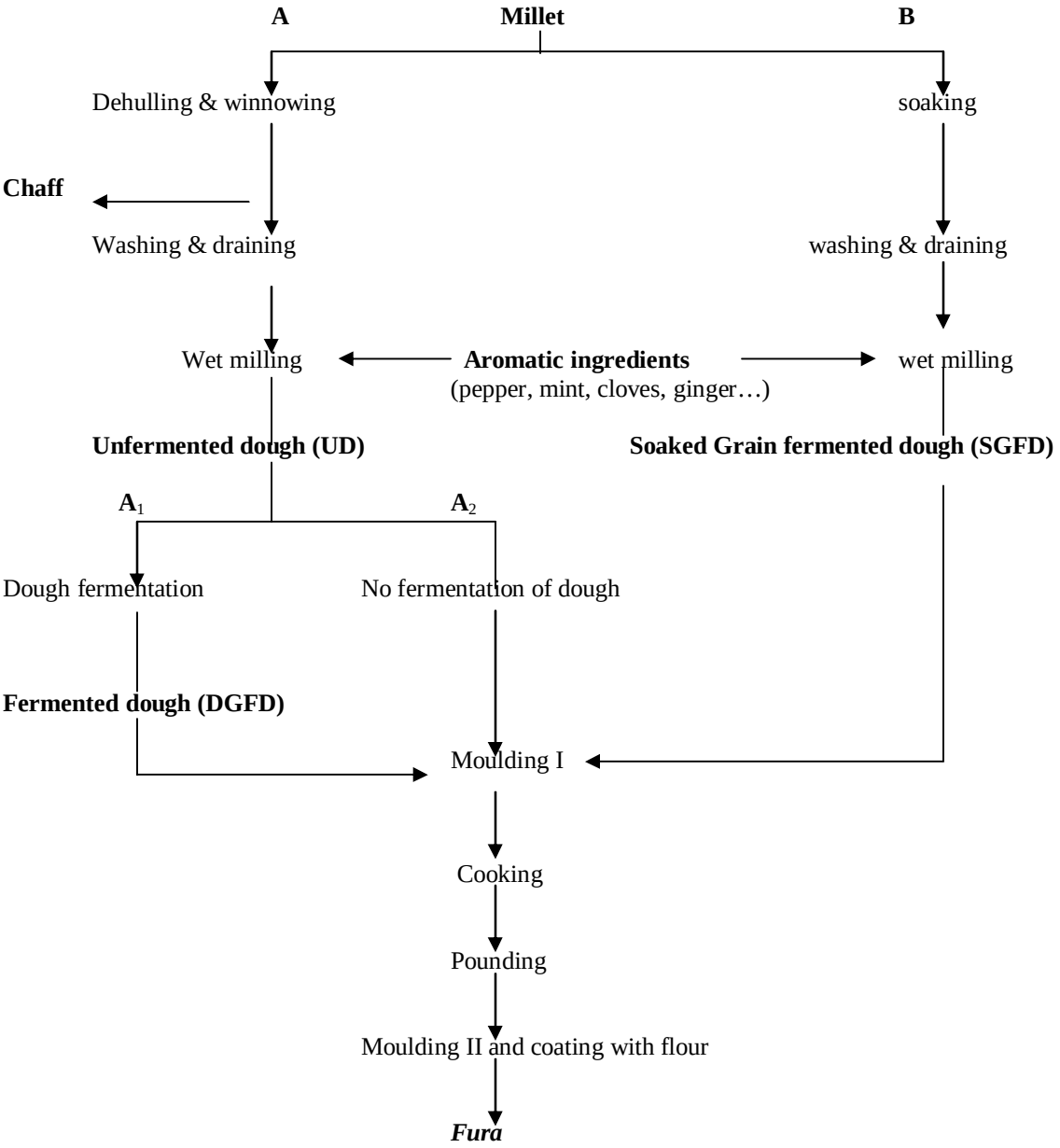

Figure 1: Flow diagram for the traditional processing of *fura* using different millet dough preparations

3.2 pH and Microbial Counts

The pH and microbial counts of samples taken at various stages of the processing (SGFD, DGFD, DGUD) are presented in Table 1. Aerobic mesophilic counts ranged between averages of 4.18 and 6.80 logcfu/g. Lactic acid bacteria and yeasts counts were higher than Enterobacteraceae counts in both SGFD and DGFD samples. However, for the

DGUD, Enterobacteriaceae recorded higher average count (4.64 logcfu/g) as compared to LAB and yeast which recorded average counts of 4.18 logcfu/g and 3.54logcfu/g respectively. The samples of *fura* had average pHs ranging between 4.77 and 5.55. Aerobic mesophiles and LAB had the highest counts whereas Enterobacteriaceae had the least average counts (Table 2). There was however, a generally higher Enterobacteriaceae count in the final *fura* as compared to the dough samples.

Table 1: pH and microbial counts (logCFU/g) of millet dough samples used for *fura* processing

[1]Sample	pH	Aerobic mesophiles	LAB	Yeast	Enterobacteraceae
			SGFD		
SGFD 1_1	4.86	6.5	5.7	6.1	2.0
SGFD 1_2	4.50	6.2	6.2	7.2	1.2
SGFD 1_3	5.00	7.0	7.3	7.4	2.6
SGFD 1_4	4.53	6.8	7.8	6.3	2.2
SGFD 1_5	4.10	7.5	7.4	6.5	1.9
[2]Mean±SD[3]	**4.6±0.4[a]**	**6.80±0.5[a]**	**6.88±0.9[a]**	**6.70±0.6[a]**	**1.98±0.5[a]**
			DGFD		
DGFD 2_1	4.18	8.5	8.7	5.0	1.0
DGFD 2_2	3.37	7.7	9.6	5.2	0.0
DGFD 2_3	4.07	7.0	6.7	5.4	2.1
DGFD 2_4	4.01	8.0	8.0	4.2	1.7
DGFD 2_5	3.85	7.6	7.9	5.3	0.0
Mean±SD	**3.9±0.3[b]**	**7.76±0.6[b]**	**8.18±1.1[a]**	**5.02±0.5[b]**	**0.96±0.9[a]**
			DGUD		
DGUD 3_1	6.68	4.3	3.5	2.9	5.7
DGUD 3_2	6.40	4.5	4.3	3.1	4.3
DGUD 3_3	6.51	3.5	3.9	4.0	4.8
DGUD 3_4	5.80	4.6	4.8	3.5	5.2
DGUD 3_5	6.50	4.0	4.4	4.2	3.2
Mean±SD	**6.4±0.3[c]**	**4.18±0.4[c]**	**4.18±0.5[b]**	**3.54±0.6[c]**	**4.64±0.9[b]**

[1]Key: SGFD = soaked grain fermented dough; DGFD = dehulled grain fermented dough; DGUD = dehulled grain unfermented dough. Subscripts of sample number represent replicates of the same sample.
[2]Means with same letters as superscripts in a column are not significantly different.
[3]± represents standard deviations (SD)

Table 2: pH and microbial counts (logCFU/g) of *fura* processed from different dough preparations

[1]Sample	[2]pH	Aerobic mesophiles	LAB	Yeast	Enterobacteria
SGFD-F	4.85±0.60	4.40±0.57	4.80±0.46	0.74±1.66	2.88±1.10
DGFD-F	4.77±0.63	4.24±0.89	4.86±0.98	0.00±0.00	1.96±1.43
DGUD-F	5.55±0.49	4.82±1.10	4.20±0.81	1.26±1.74	3.56±1.51

Key: [1]SGFD-F = soaked grain fermented dough *fura*; DGFD-F = dehulled grain fermented dough *fura*; DGUD-F = dehulled grain unfermented dough *fura*.
[2]Values are means of five replicates of samples with standard deviations

3.3 Characterization and Identification of Microorganisms

3.3.1 Characteristics of identified lactic acid bacteria

A total of seventy (70) lactic acid bacteria (LAB) isolates were characterized and identified. All isolates were Gram positive and catalase negative. Among these, rods accounted for 51.4% (36 isolates) whereas cocci accounted for 48.6% (34 isolates). About 30% of the isolates were able to produce gas from glucose, 58.5% grew at 45°C, 24% tolerated 6.5% NaCl, and 57.2% hydrolyzed arginine of which 17.2% were *Lactobacillus*. Dextran formers were 27.1%. About 8.6% were able to hydrolyze starch and were identified as *L. plantarum*. The LAB were grouped into five genera belonging to *Lactobacillus* (51.42%), *Pediococcus* (21.4%), *Streptococcus* (14.3%), *Leuconostoc* (8.5%), and *Enterococcus* (4.3%). The dominant *Lactobacillus* species were further identified as belonging to *Lactobacillus (L) plantarum* (27.1%), *L. brevis* (11.4%), *L. delbrukii sps. delbrukii* (7.1%), and *L. fermentum* (5.7%).

3.3.2 Characteristics of Identified Yeasts

A total of fifty (50) yeast isolates were characterized during *fura* processing and were identified as *Issatchenkia orientalis* (26%), *Saccharomyces cerevisiae* (22%), *Pichia anomala* (16%), *Candida tropicalis* (16%), *Saccharomyces pastorianus* (10%), *Yarrowia lipolytica* (6%), and *Galactomyces geotricum* (4%). None of the isolates identified fermented lactose. The seemingly general rule that when a yeast strain ferments a carbohydrate it is also able to grow in it (or assimilate it) was consistent with the tested isolates. The reverse, however, did not hold true because strains identified as *Yarrowia lipolytica* assimilated glucose and galactose but could not ferment either of these sugars. All isolates could grow at 37°C except *Saccharomyces pastorianus*.

3.3.3 Characteristics of Identified Enterobacteriaceae

All isolates were gram negative rods. About 32% were non-motile whiles 68% were motile. The identified species included *Enterobacter aerogenes* (28%), *Klebsiella pneumonia* (22%), *Proteus vulgaris* (16%), *Enterobacter sakazakii* (12%), *Serratia liquefaciens* (12%), and *Escherichia coli* (10%).

4. Discussion

The process characteristics of *fura* in northern Ghana look similar to other traditional processes for the production of cereals into fermented dough and dumplings in Nigeria, Benin, and Burkina Faso (Odunfa and Adeyele, 1985; Blandino *et al*., 2003; Hounhouigan *et al*., 1993; and Tomkins *et al*., 1988). Perhaps the major difference between the *fura* process and the others is that, while the fermentation or cooking is the last step during the production of most of these other foods, *fura* undergoes further processing (pounding and moulding) after the fermentation and cooking steps. Like in the other traditional processes, the fermentation starts from the soaking of grains and continues in the dough, and it is caused spontaneously by the natural flora of the raw materials, process utensils, water, the environment, etc. Processing conditions, including time and temperature are uncontrolled resulting in product of variable quality and stability, which is often peculiar to most traditional processes.

The results revealed the predominance of LAB and yeasts. The dominance and association of LAB and yeasts are common in several traditional cereal-based fermented foods and beverages, including *ogi* (Odunfa, 1985), *kenkey* (Halm *et al*., 1993; Hayford and Jakobsen, 1999), *togwa* (Mugula *et al*., 2001), Nigerian *fufu* (Adekoge and Babaola, 1988) and *mawe* (Hounhouigan *et al*., 1993). Different works have however given different counts of these organisms in different fermented foods depending on the length of the fermentation process, initial number of microflora present, nature of substrate for the

fermentation, and sometimes the ambient temperature of the local region where the fermentation process took place. Counts of Yeast and Lactic Acid bacteria in the millet dough samples and *fura* were similar. They were lower in the soaked grain fermented dough after a fermentation period of about 24 hours than in the dehulled grains fermented dough which was fermented for about 12 hours. This observation may be consistent with the fact that soaking the grains in water does not provide favorable conditions for the growth of lactic acid bacteria which have fastidious nutritional requirements. However, in the dehulled grain fermented dough nutrients such as proteins and vitamins from the millet grains could have been made more easily available to LAB through reduction in size during grinding of the grains and also possibly by the autolysis of yeasts or other biological factors. This therefore might have resulted in the higher counts of LAB and yeasts in the dehulled grain fermented dough although fermentation was for only 12hours on the average. Consistent with the fermentation patterns, the dehulled grains unfermented dough had lower counts of LAB and yeasts. Enterobacteriaceae count was however, significantly higher in the dehulled grain unfermented dough than the fermented doughs. This observation is in agreement with other works where fermented dough has higher antimicrobial properties than unfermented dough due to the lowering of pH and the production of other antimicrobial compounds during the fermentation process (Mensah *et al.,* 1991; Holzapfel *et al*., 1995). A decrease in the number of Enterobacteriaceae in fermented millet dough is in accordance with their death kinetics reported in similar other spontaneously fermented plant materials (Nout *et al*., 1989; Mensah *et al*., 1990). Samples of the millet dough which had pH values of less than 4.5 had very low to undetectable numbers of Enterobacteriaceae. Nout (1991) and Mensah *et al.* (1990) also reported their disappearance as pH comes below 4.5, although Mensah *et al.* (1991) suggested that the antimicrobial effect of fermented maize dough porridge was not due to pH per se but probably due to the presence of other antimicrobial compounds. Kingamkono *et al*. (1994) also reported on the antimicrobial effect of *togwa* on several enteropathogens. Inhibitory effect of yeasts has also been shown to be due to substrate competition, but inhibition of spore germination might also occur due to the production of concentrations of organic acids. As observed, the doughs are cooked at a higher temperature which further reduces the microbial counts (Lei and Jakobsen, 2004; Kingamkono *et al*., 1994; and Nout and Motarjemi, 1997). Most of the samples of the final product (*fura*) had high Enterobacteriaceae counts. This could be due to the processes which followed the fermentation and cooking of doughs (pounding with mortar and pestle, and moulding with bare hands). The survival and growth of Enterobacteriaceae suggests that the antimicrobial properties of the *fura* were not enough to control the post-cooking contamination. Numerous studies have shown that bacterial pathogens do not survive well when added to a pre-fermented food where the LAB have grown to large numbers and the pH is already low (Mensah *et al.,* 1990, 1991; Nout *et al.,*1989; Simango and Rukure, 1991; Svanberg *et al.,* 1992). However, when LAB and a pathogen were inoculated simultaneously into a model weaning food, even when the LAB outnumbered the pathogen *(Escherichia coli)* by more than 5 log cycles, the pathogen was still able to grow for 5 h, increasing in number by 2 log cycles (Yusof *et al.,* 1993). This emphasizes the fact that antimicrobial effect of fermentation should be seen as an adjunct to good hygienic practices and not as a substitute for them. Pace (1975) indicated that coliform counts higher than 10^2/g in delicatessen food products is indicative of dangerous contamination. Most samples of *fura,* however, had Enterobacteriaceae counts higher than 10^2/g and therefore could indicate dangerous contamination.

The lactic acid bacteria types isolated from the samples taken during *fura* processing included members of the genera *Lactobacillus, Pediococcus, Streptococcus*, *Leuconostoc*, and *Enterococcus*. *Lactobacillus (L) plantarum, L. brevis, L. delbrueckii sps. delbrueckii*, *L. fermentum* and species of *Pediococcus*, *Streptococcus, Leuconostoc*, and *Enterococcus* have been isolated from several indigenous fermented foods. The most commonly isolated specie is *L. plantarum* (Olasupo *et al*., 1997). *L. plantarum* has been identified as the dominant organism at the end of several natural lactic acid fermentations (Mugula *et al*., 2003; Kunene *et al*., 2000; Olasupo *et al*., 1997; Brauman *et al.,* 1996; Mbugua, 1984; Nout, 1980), probably due to its acid tolerance (Fleming and McFeters, 1981) and superior ability to utilize the substrate (Oyewale and Odunfa, 1990), including dextrins (Akinrele, 1970). Giraud *et al*., (1994) isolated an amylolytic *L. plantarum* strain with the ability to break down cassava raw starch that has not been subjected to preliminary physicochemical treatment. Some of the lactic acid bacteria isolated during *fura* were able to hydrolyze starch. *L. plantarum* together with *Pediococcus pentosaceus* have been isolated from sorghum powder and fermented sorghum porridge samples (Kunene *et al.,* 2000). *Lactobacillus* spp. were predominant, constituting 51% while *Pediococcus*

constituted 21.4%. Nche *et al.* (1994) reported that *Pediococcus* species predominate in the latter stage of corn dough fermentation. *L. brevis* has also been often found to occur in fermenting plant material (Corsetti *et al.*, 2001) and have been isolated from fermented maize dough (Halm *et al.*, 1993, Hounhouigan *et al*., 1993), and fermented cassava dough (Amoa-Awua and Appoch, 1996). *L. fermentum* and *L. brevis* have been reported to dominate in the intermediate and final stages of the fermentation of *fufu* and to produce the flavor typical of the product (Adekoge and Babaola, 1988). Lactobacilli in general have been reported to be responsible for acid production and flavor development in *ogi* (Akinrele, 1970) and *gari* (Ngaba and Lee, 1979). Only 9% of the isolated strains were identified as *Leuconostoc* spp. *Leuconostoc* may contribute to the development of flavor quality attributes of fermented products but their lower percentage in *fura* could be explained by their complex nutritional requirements. *Leuconostoc* have also been shown to exhibit a weak competitive ability during the fermentation of milk (Wood and Hozapfel, 1995).

Among yeast isolates *Issatchenkia orientalis* dominated in *fura* processing, followed by *Saccharomyces cerevisiae, Pichia anomala, Candida tropicalis, Saccharomyces pastorianus, Yarrowia lipolytica*, and *Galactomyces geotricum. Candida krusei* is considered to present the anamorphic form of *I. orientalis* because the type strain, as well as other isolates of both species, showed significant (93-100%) DNA base sequence complementarity (Kurtzman *et al*., 1980b). *I. orientalis* and its anamorph *C. krusei* have been isolated from a wide variety of habitats. Although Hurley *et al.* (1987) discussed clinical evidence to support the concept that *C. krusei* should be considered pathogenic rather than a transient saprophyte, the significant role of this specie in cereal fermentation is equally evident (Annan *et al*., 2003; Halm *et al*., 1996). *I. orientalis* and *S. cerevisiae* have been isolated from acidic fermentation of plant substrates (Nout, 1980; Hounhouigan *et al*., 1993; Gobbetti *et al*., 1994; Nago *et al*., 1998; Mugula *et al*., 2003). Mugula *et al.* (2003) reported the dominance of *I. orientalis, S. cerevisiae* and *C. tropicalis* in *togwa*. Halm *et al.* (1993) also reported the dominance of *Candida* spp. followed by *Saccharomyces* spp. in fermented maize dough. *Candida tropicalis*, *Yarrowia lipolytica* (anamorph of *C. lipolytica*), and *S. pastorianus* have also been isolated from *sobia*, a fermented beverage in the western province of Saudi Arabia (Gassem, 2002), and some traditional fermented milk products (Gadaga *et al.*, 2000). *C. tropicalis* has been identified as an important yeast pathogen of humans (Ahearn, 1998) and its presence in the traditionally fermented cereal products (e.g. *fura*) and fermented milk indicates that these fermented foods could be a source of pathogenic microorganisms. This observation therefore emphasizes the need to develop starter cultures for controlled fermentation in combination with Good Hygienic Practices (GHP) in order to produce safer fermented food products. *Pichia anomola* (synonym: *Hansenula anomola*) also represents the anamorph of *Candida pelliculosa.* This specie was the third most dominant isolate from *togwa* (Mugula *et al.*, 2003) and has also been found to produce fusel alcohols in mixed yeast culture with *S. cerevisieae* in wheat sourdoughs (Damiani *et al.*, 1996). Yeasts have been reported to make a useful contribution to the improvement of flavor and acceptability of fermented cereal gruels (Odunfa and Adeyele, 1985; Baningo *et al.*, 1974). Akinrele (1970) reported the contribution of *S. cerevisiae* and *C. mycoderma* to the flavor acceptability of *ogi*.

The species of Enterobacteriaceae isolated from millet doughs and *fura* especially coliforms, are associated with poor hygiene and their occurrence in the product may indicate a potential health risk which is of public health concern (Beukes *et al.*, 2001).

5. Conclusion and Recommendations

A study of the traditional processing of pearl millet into *fura* in northern Ghana has for the first time allowed a detailed flow diagram to be established and the various steps involved to be characterized, providing a rational basis for further investigations to ensure reproducible conditions for the production of constant food quality.

The results also indicated that a wide variety of microorganisms, notably lactic acid bacteria and yeasts, are associated with *fura* production. These microorganisms spontaneously come from raw materials, the environment, processing equipments and persons involved in the production. Further work (which is underway in our laboratories) is required using modern molecular techniques to fully characterize the predominant microorganisms and to establish their technological roles and contribution to product quality and safety. The development of starter culture from these organisms is important for the potential production of *fura* on a commercial, small industrial scale, and for the improvement of its acceptability, microbiological stability and hygienic safety. We finally recommend the establishment of national culture collection of lactic acid bacteria and yeasts from Ghanaian fermented foods as it will open the way for gene banks for future studies.

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References

[1] Dietz HM. Opportunity and potential for processing of horticultural products by small entrepreneurs. Project 95.2254.1-001.00, Promotion of horticulture, Malawi, March/April, 1999: 34

[2] FAO. The agro-processing industry and economic development. In: The State of Food and Agriculture, FAO, Rome, 1997: 221–265.

[3] Tomkins A. Alnwick D. Haggerty P. Fermented foods for improving child feeding in eastern and southern Africa. In: Alnwick D. Moses S. Schmidt OG, eds. Inproving young child Feeding Feeding in eastern and southern Africa, Household level technology, Ottawa International Development Research Center 1988; 136-167

[4] Hounhouigan DJ. Nout MJR. Nago CM. Houben JH. Rombout FM. Microbiological changes in mawe during natural fermentation. World Journal of Microbiology and Biotechnology 1993; 10: 410-413

[5] Oyewole OB. Lactic fermented foods in Africa and their benefits. Food Control 1997; 8: 289-297.

[6] Blandino A. Al-Aseeri ME. Pandiella SS. Cantero D. Webb C. Cereal based fermented foods and beverages. Food Research International 2003; 36: 527-534

[7] Jideani VA. Wedricha BL. Sorbic acid distribution in the aqueous extract of cooked millet, fura. Food Additives and Contaminants Journal 1994; 12: 16-66.

[8] Jideani VA. Nkama I. Agbo ED. Jideani IA. Survey of *fura* production in some northern states of Nigeria. Plant Food for Human Nutrition 2001; 56: 23-26.

[9] Kordylasi JM. Processing and preservation of tropical and sub-tropical foods, Macmillan, London, 1990: 112-114

[10] de Man JC. Rogosa M. Sharpe ME. A medium for the cultivation of lactobacilli. Journal of Applied Bacteriology 1960: 23, 130-135.

[11] Mossel DAA. Mengerink WHJ. Scholts HH. Use of modified MacConkey agar medium for the selective growth and enumeration of enterobacteriaceae. Journal of Bacteriology 1962; 84: 381.

[12] Harrigan WF. Laboratory methods in food microbiology, 3rd edn. Academic press, London 1998.

[13] Sneath PHA. Mair NS. Sharpe ME. Bergey`s manual of systematic bacteriology, Baltimore, Maryland: Williams and Wilkins 1986; 2

[14] Wood BJB. Holzapfel WH. The Genera of Lactic Acid Bacteria. Blackie Academic and Professional, Glasgow 1995; 2

[15] Deak T. Beuchat L. Handbook of food spoilage yeasts CRC Press, Boca Raton 1996.

[16] Kurtzman CP. Fell JW. *The Yeast, a taxonomic study*. 4th edition. Amsterdam: Elsevier Science Publisher 1998

[17] Yarrow D. Methods for the isolation, maintenance and identification of yeasts. In: Kurtzman CP. Fell WC. eds. The Yeasts, A taxonomic study, 4th edn., Elsevier, Amsterdam, 1998; 77-121.

[18] Odunfa SA. Adeyele S. Microbiological changes during the traditional production of ogi-baba, a West African fermented sorghum gruel. Journal of Cereal Science 1985; 3: 173-180.

[19] Odunfa SA. African fermented foods. In: Wood, BJ. ed. Microbiology of fermented foods, Elsevier Applied Science Publishers. London 1985; 2

[20] Halm M. Lillie A. Spreusen AK. Jakobsen, M. Microbiological and aromatic characteristics of fermented maize doughs for kenkey production in Ghana. International Journal of Food Microbiology 1993; 19: 135-143.

[21] Hayford AE. Jakobsen M. Characterization of *Candida krusei* strains from spontaneously fermented maize dough by profiles of assimilation, chromosome profile, polymerase chain reaction and restriction endonuclease analysis. Journal of Applied Microbiology 1999; 87: 29–40.

[22] Mugula JK. Nnko SAM. Sørhaug T. Changes in quality attributes during storage of *togwa*, a

lactic fermented gruel. Journal Food Science 2001; 21: 181-194
[23] Adegoke GO. Babaola AK. Characteristics of microorganisms of importance in the fermentation of *fufu* and *ogi* - two Nigerian foods. Journal of Applied Bacteriology 1988: 65, 449- 453.
[24] Mensah P. Tomkins AM. Drasar BS. Harrison TJ. Antimicrobial effect of fermented Ghanaian maize dough. Journal of Applied Bacteriology 1991; 70, 203-210.
[25] Holzapfel WH. Geisen R. Schillinger U. Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. International Journal Food Microbiology 1995; 24: 343-362.
[26] Nout MJR. Rombouts FM. Hautfast GJ. Accelerated natural lactic fermentation of infant food formulations. Food and Nutrition Bulletin 1989; 11: 65-73.
[27] Mensah P. Tomkins AM. Drasar BS. Harrison TJ. Fermentation of cereals for reduction of bacterial contamination of weaning foods. Lancer 1990; 336: 140-143.
[28] Nout MJR. Ecology of accelerated natural lactic fermentation of sorghum-based infant formulas. International Journal of Food Microbiology 1991; 12: 217-224
[29] Kingamkono R. Sjögren E. Svanberg U. Kaijser B. pH and acidity in lactic fermenting cereal gruels: effect on viability of enteropathogenic microorganisms. World Journal of Microbiology and Biotechnology 1994; 10: 664-669.
[30] Lei V. Jakobsen M. Microbiological characterization and probiotic potential of *koko* and *koko* sour water, African spontaneously fermented millet porridge and drink. Journal of Applied microbiology 2004; 96: 384-397
[31] Nout MJR. Motarjemi Y. Assessment of fermentation as a household technology for improving food safety: a joint FAO/ WHO workshop. Food Control 1997; 8: 221-226.
[32] Simango C. Rukure G. Survival of *Campylobacter jejuni* and *Escherichia coli* in *mahewu*, a fermented cereal gruel. Transactions of the Royal Society of Tropical Medicine and Hygiene 1991; 85: 399-400.
[33] Svanberg U. Sjiigren E. Lorri W. Svennerholm AM. Kaijser B. Inhibited growth of common enteropathogenic bacteria in lactic-fermented cereal gruel. World Journal of Microbiology and Biotechnology 1992; 8: 601-606.
[34] Yusof RM. Morgan JB. Adams MR. Bacteriological safety of a fermented weaning food containing L-lactate and nisin. Journal Food Protection 1993; 56: 414-417.
[35] Pace PJ. Bacteriological quality of delicatessen foods: are standards needed? Journal of Milk and Food Technology 1975; 38: 347-353
[36] Olasupo NA. Olukoya DK. Odunfa SA. Identification of *Lactobacillus* species associated with selected African fermented foods. *Z.* Naturforsch 1997; 51: 105-108.
[37] Mugula JK. Nnko SAM. Narvus JA. Sørhaug T. Microbiological and fermentation characteristics of *togwa*, a Tanzanian fermented food. International Journal of Food Microbiology 2003; 80: 187-199.
[38] Kunene NF. Geornaras I. von Holy A. Hastings JW. Characterization and determination of origin of lactic acid bacteria from a sorghum-based fermented food by analysis of soluble proteins and amplified fragment length polymorphism fingerprinting. Applied and Environmental Microbiology 2000; 66: 1084-1092
[39] Braunman A. Keleke S. Malonga M. Miambi E. Ampe F. microbiological and Biochemical characteristics of cassava retting, a traditional lactic acid fermentation for foo-foo (cassava flour) production. Applied and Environmental Microbiology 1996; 62: 2854-2855
[40] Mbugua SK. Isolation and characterization of lactic acid bacteria during the traditional fermentation of uji. East African Agricultural Journal 1984; 50: 36-43
[41] Nout MJR. Microbiological aspects of traditional manufacture of Bussa, a Kenyan opaque maize beer. Chemische Mikrobiol. Technologie Lebensmitte 1980; l6: l37-142.
[42] Flemming HP. McFeters RF. Use of microbial cultures: vegetable products. Food Technology 1981; 35: 84.
[43] Oyewole OB. Odunfa SA. Characterization and distribution of lactic acid bacteria in cassava fermentation during *fufu* production. Journal of Applied Bacteriology 1990; 68: 145–152.
[44] Akinrele IA. Fermentation studies on maize during the preparation of a traditional African starch cake. Journal of the Science of Food and Agriculture 1970: 21, 619-625.
[45] Giraud E. Champailler A. Raimbault M. Degradation of raw starch by a wild amylolytic strain of *Lactobacillus plantarum.* Applied and Environmental microbiology 1994; 60: 4319-4323
[46] Nche PF. Nout MJR. Rombouts FM. The effect of cowpea supplementation on the quality of

kenkey, a traditional Ghanaian fermented food. Journal of Cereal Science 1994; 19: 191-197.
[47] Corsetti A. Lavermicocca P. Morea M. Baruzzi F. Tosti N. Gobbetti M. Phenotypic and molecular identification and clustering of lactic acid bacteria and yeast from wheat (species *Triticum durum* and *Triticum aestivum*) sourdoughs of southern Italy. International Journal of Food Microbiology 2001; 64: 95-104.
[48] Amoa-Awua WK. Appoch FE. Lactic acid fermentation of cassava dough into agbelima. International Journal of Food Microbiology1996: 31: 87– 98.
[49] Ngaba PA. Lee JS. Fermentation of cassava (*Manihot esculenta Crantz*). Journal of Food Science 1979; 44: 1570-1571.
[50] Kurtzman CP. Smiley MJ. Johnson CJ. Emendation of the genus *Issatchenkia* and comparison of species by deoxyribonucleic acid reassociation, mating reaction, and ascospore ultrastructure. International Journal of Systematic Bacteriolology 1980; 30: 503-513.
[51] Hurley R. de Louvois J, Mulhall A. Yeast as human and animal pathogens. In: Rose AH. Harrison JS. eds. The Yeasts, vol. 1, Biology of Yeasts. Academic press, London 1987: 207-281.
[52] Annan NT. Poll L. Sefa-Dede S. Plahar WA. Jakobsen M. Volatile compounds produced by *Saccharomyces cerevisiae* and *Candida krusei* in single starter culture fermentations of Ghanaian maize dough. Journal of Applied Microbiology 2003: 94, 462-474
[53] Halm M. Osei-Yaw A. Hayford AE. Kpodo KA. Amoa-Awua WKA. Experiences with the use of starter culture in the fermentation of maize for 'kenkey' production in Ghana. World Journal of Microbiology and Biotechnology 1996; 12: 531–536.
[54] Gobbetti M. Corsetti A. Rossi J. La Rosa F. De Vincenzi M. Identification and clustering of lactic acid bacteria and yeasts from wheat sourdoughs of central Italy. Italian Journal of Food Science 1994; 1: 85-93.
[55] Nago CM. Hounhouigan DJ. Akissoe N. Zanou E. Mestres C. Characterization of the Beninese ogi, a fermented maize slurry: physiological and microbiological aspects. International Journal of Food Science and Technology 1998; 33: 307-305. [58] Ahearn DG. Yeasts pathogenic for humans. In Kurtzman CP. Fell JW. The yeasts: A taxonomic study. Amsterdam Elsevier 1998: 9-12.
[56] Gassem MAA. A microbiological study of Sobia: a fermented beverage in the Western province of Saudi Arabia. World Journal of Microbiology and Biotechnology 2002; 18: 173-177.
[57] Gadaga TH. Mutukumira AN. Narvhus JA. Enumeration and Identification of yeast isolated from Zimbabwean traditional fermented milk. International Dairy Journal 2000; 10: 456-466.
[58] Aheam DG. Yeast pathogenic for humans. In Kurtzma CP. Fell JW. eds. The yeasts: A taxonomic study. Amsterdam Elsevier. 9-12
[59] Damiani P. Gobbetti M. Cossignani L. Corsetti A. Simonetti MS. Rossi J. The sourdough microflora. Characterization of hetero- and homofermentative lactic acid bacteria, yeasts and their interactions on the basis of the volatile compounds produced. Lebensmittel Wissenschaft und Technologie 1996; 29: 63–70.
[60] Baningo EOI. deMan JM. Duitschaever CL. Utilization of high-lysine corn for the manufacture of ogi using a new improved processing system. Cereal Chemistry 1974: 52, 559-572.
[61] Beukes EM. Bester BH. Mostert JF. The microbiology of South African traditional fermented milk. International Journal of Food Microbiology 2001; 63: 189-197.

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