Microbiological Characteristics of Ghanaian Traditional Fermented Milk Product, *Nunu*

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Abstract: *Nunu* is a spontaneously fermented yoghurt-like milk product consumed as a staple food commodity in parts of the Saharan West Africa. Its production and consumption derives much food security and economic benefits to the rural people in the region. However, the process characteristics result in products which are not appealing to many people, have very short shelf-life and could have food safety concerns. In a framework of research to improve the product quality and increase consumption, a study was conducted to determine the process characteristics and elucidate the predominant microflora associate with the production of the product. A survey was done on the technology of *nunu* production in three major towns of the Upper East region, during which fermenting *nunu* were taken from 15 processors for laboratory analysis. pH, and titratable acidity were determined, as well as isolation and identification of the predominant microorganisms. Processing of *nunu* in northern Ghana takes place at ambient conditions in calabashes or plastic containers, and spontaneously. No pre-fermentation heating of the milk is done. The duration of fermentation is 24 to 48 hours. Several microorganisms were isolated from the fermenting samples, including LAB classified as lactobacillus, Leuconostoc, Lactococcus, Enterococcus, and Streptococcus; yeasts classified as *Saccharomyces cerevisiae*, *Saccharomyces pastorianus*, *Candida kefyr*, *Yarrowia lipolytica*, *Candida stellata*, *Kluyveromyce maxianus*, *Zygosaccharomyces bisporus*, *Zygosaccharomyces rouxii*. The enterobacteriaceae, which were associated with the early stages of fermentation but eliminated as fermentation progressed were identified as *Enterobacter*, *Klebsiella*, *Escherichia*, *Proteus vulgaris,* and *Shigella*. The fermentation of *nunu* is spontaneous. Potential pathogens may be present in the milk fermented, but are eliminated as the fermentation progresses to 48 hours, increasing the safety of the product. [Nature and Science 2010;8(9):178-187]. (ISSN: 1545-0740).

Keywords: *Nunu,* fermented milk, spontaneous fermentation, lactic acid bacteria.

1.0 INTRODUCTION

Milk has been preserved since early times by fermentation. Many traditional fermented milk products were made in Asia, Africa, the Middle East, and northern and eastern Europe (Savadogo *et al*., 2004). Studies on the microbiological characteristics of several fermented milk products have been carried out in countries such as Indonesia (Yodoamijoyo *et al.*, 1983; Hosono *et al.*, 1989); Zimbabwe (Feresu and Muzondo, 1990,Mutukumira, 1995; Mutukumira *et al*., 1995;Gadaga *et al*., 1999); South Africa (Keller and Jordan, 1990; Beukes *et al.*, 2001); Morocco (Hamama, 1992); Tanzania (Isono *et al.*, 1994); Burkina Faso (Savadogo *et al*., 2004); Sudan (Abdelgadir *et al*., 1998 and 2001); Nigeria (Eka and Ohaba 1977, Atanda and Ikenebom 1989); and Ethiopia (O'Connor *et al*., 1993; O'Mahony, 1988). The nature of fermented products varies from one region to another. It depends on the local indigenous microflora, which in turn reflects the climatic conditions of the area (Savadogo *et al*., 2004). Thus traditional fermented milk in regions with a cold climate contained mesophilic bacteria such as *Lactococcus* and *Leuconostoc spp.*, whilst thermophilic bacteria, which include mostly *Lactobacillus* and *Streptococcus*, prevailed in regions with a hot, subtropical or tropical climate (Thomas, 1985; Tamine and Robinson, 1988; Kurmann, 1994).

Fermented milk products fulfill multiple purposes in rural developing communities (O'Mahony, 1988). They are consumed as food and beverages and the market value and storage life are improved over that of raw milk (Motarjemi and Nout, 1995). Milk products are also used as cosmetics by rural people. For example, milk butter is used as hair and body oil in the rural areas. They have been acclaimed both by popular wisdom and some research findings as being more nutritious and health-promoting than fresh milk. Platt (1964) stated that

fermented milk is a good source of the B vitamins, including vitamin B_{12}. There are also claims that the digestibility of the milk proteins is improved by fermentation (Marshal, 1986).

Nunu is processed by collecting fresh cow milk and allowing it to ferment for a day or two. The Fulanis in Ghana ferment the milk in calabashes, or rubber buckets. *Nunu* is yoghurt-like in taste (a sharp acid taste) and can be consumed alone or with sugar and *fura*. The latter is made of millet dough mixed with spices, compressed into balls and cooked for about 30 minutes (Owusu-Kwarteng *et al*., 2010). The cooked *fura* is crumbled in a bowl of *nunu* into what is called *fura de nunu*. The production of fermented milk in northern Ghana does not involve the use of starter cultures, suggesting that the fermentation arises spontaneously from microbes originating from the environment, processing equipments, or processors.

Nunu production and consumption are much more practiced in the northern parts of Ghana than in the south. It, however, does not appeal to majority of the people because of the apparent unhygienic conditions under which it is prepared, and also its short shelf-life (Yahuza, 2001). Knowledge of the biochemical and microbial changes that are associated with its processing will obviously enhance the production and consumption on a larger scale.

The main purpose of this study therefore was to isolate, identify and characterize the microorganisms present in *nunu* produced in northern Ghana.

2.0 MATERIALS AND METHODS

2.1 Samples and Sample collection

Fifteen duplicate samples of *nunu* were donated by producers. Samples were collected at 0, 12, 24, 36, and 48hours of fermentation between the months of February and April 2009. All samples were collected in sterile containers and transported immediately in an ice chest to the laboratory for analysis.

2.2 Determinations of pH and Titratable acidity.

pH of samples were determined using a pH meter (Crison basic 20, Barcelona). Total Titratable acidity was measured by titrating a mixture of 10ml of sample and 90 ml of distilled water against 0.1 M sodium hydroxide (NaOH) solution using phenolphthalein as indicator.

2.3 Enumeration and Isolation of Microorganisms

Ten milliliters of each sample was homogenized in 90 ml sterile diluent [1% peptone (Difco, Detroit, Michigan, USA), 0.85% NaCl, pH 7.0] using a stomacher (Stomacher- Bagmixer, Buch and Holm) for 30 s, at a preset speed. Tenfold serial dilutions (10^{-1} to 10^{-9}) were made with same diluent and 0.1 ml aliquot of each appropriate dilutions was spread-plated while 1ml was pour-plated in duplicates on various media for enumeration of isolates. Aerobic mesophilic bacteria were enumerated on pour plates of Plate Count Agar (PCA) (Oxoid Ltd, Basingstoke, Hampshire, England) incubated at 30^{o}C for 48 -72 hours. Lactic acid bacteria were enumerated on pour plates of de Man, Rogosa and Sharpe (MRS) agar (Oxoid Ltd, Basingstoke, Hampshire, England), incubated at 35^{o}C for 2 days anaerobically using the Anaerocult A pack (Merck, Darmstadt, Germany). Yeast were enumerated on spread plates of Sabouraud Dextrose Agar (SDA) (Oxoid Ltd, Basingstoke, Hampshire, England), pH 5.6±0.2 with chloramphenicol (100 mg/l) (Oxoid) added and incubated at 25^{o}C for 3-5 days. Enterobacteriaceae were enumerated on pour plates of Violet Red Bile Agar (VRBA) (Oxoid Ltd, Basingstoke, Hampshire, England), incubated at 37^{o}C for 24 hours. Representative colonies on MRS agar, SDA and VRBA were picked from a sector of the plates and further sub-cultured by streaking on fresh media until pure cultures were obtained. The purified isolates were stored on MRS agar, Malt Extract Agar (MEA) (Oxoid Ltd, Basingstoke, Hampshire, England) and Nutrient agar (NA) (Oxoid Ltd, Basingstoke, Hampshire, England) slants respectively at 4^{o}C until required for identification.

2.4 Phenotypic characterization

Colonies on individual culture plates were examined for shape, size, elevation, surface characteristics, and edges.

The hanging drop technique was used to observe cell motility and arrangements while Grams stain was used for cell shape.

2.5 Characterization of Lactic acid bacteria

Growth at 10^{o}C and 45^{o}C was determined by culturing isolates on MRS broth and observing visually for turbidity after 72 hours of incubation. Gas production from glucose was determined according to the methods described by Harrigan (1998), using MRS broth as basal medium.

Salt tolerance test was done using MRS broth containing 6.5% (w/v) NaCl. Tubes were then inoculated and incubated for 4 days at 37^{o}C. A positive result was detected by visual inspection for

an increase in the turbidity of the solution. Tween agar medium (10g peptone; 0.1g calcium chloride; 5g sodium chloride; 10g tween; 15g agar; 1liter distilled water, pH 7.0) was used to test for lipolysis. Each plate of the tween agar was inoculated by streaking once across the center and incubated at 35°C for 4 days. An opaque zone surrounding microbial growth indicated a positive lipolytic activity.

2.6 Characterization of Enterobacteriaceae

The Triple Sugar Iron (TSI) (Oxoid Ltd, Basingstoke, Hampshire, England) Agar test was used to determine sugar formation, CO_2 and H_2S production (Harrigan 1998). Urea hydrolysis was tested using Christensen's urea agar according to Christensen (1946). The IMViC (Indole, Methyl Red, Voges- Proskauer, and Citrate) Test was done to determine the various end products using standard procedures.

2.7 Characterization of yeast

The ability to ferment glucose, galactose, sucrose, maltose, lactose and raffinose was tested using the methods described by Harrigan (1998). A positive result was indicated by accumulation of gas in the Durham tubes. For liquid assimilation of carbon compounds, 5 ml of distilled water were distributed into tubes and sterilized by autoclaving at 121° C for 15 min. An aliquot (0.5 ml) of filter-sterilized yeast nitrogen base, containing 5 % of the compound under test was aseptically added to the tubes. The tubes were inoculated by aseptically adding 0.1ml of a visible suspension in peptone solution (Oxoid) of an actively growing culture and then incubated at 25°C for 3 weeks. A positive reaction was determined by visual inspection for an increase in the turbidity of the solution. The carbon compounds tested were galactose, glucose, sucrose, lactose, L-arabinose, maltose, D-mannitol, raffinose, soluble starch, α-methyl-D-glucoside, citrate and DL-lactate.

For the determination of the assimilation of nitrogen compounds, the same procedure was used as in the carbon compound except that yeast nitrogen base was replaced by yeast carbon base. The nitrogen compounds tested were nitrate, ethylamine hydrochloride, L-lysine and cadavarine. Growth on vitamin-free medium was tested on vitamin- free yeast base medium prepared by dissolving 16.7g into 100 ml of distilled water (Yarrow, 1998).

Growth in the presence of 0.1% and 0.01% Cycloheximide was tested on liquid Bacto yeast Nitrogen base with D-glucose, as for the assimilation test. Cycloheximide was added to give a final concentration of 0.1% or 0.01% (w/v). Growth was recorded after 12 days of incubation at 25° C. A positive reaction was detected by visual inspection for an increase in the turbidity of the solution. Growth at 19ºC and 40ºC was tested on glucose-peptone- yeast extract broth: 10g of glucose, 5g of peptone and 2.5g of yeast extract were dissolved in 500 ml of distilled water. The medium was distributed into tubes and sterilized by autoclaving at 121°C for 15minutes.The tubes were inoculated and incubated at 19°C and 40°C and inspected for growth after 1-3 weeks.

Lipolysis and urea hydrolysis were tested using the methods described by Harrigan (1998). Tween agar medium was used to test for lipolysis. An opaque zone surrounding microbial growth indicated a positive lipolytic activity. Urea hydrolysis was tested using Christensen's urea agar. Positive result was indicated by a change in the color of the medium from yellow to intense pink.

2.8 Statistical analysis

Results were statistically analyzed by 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).

3.0 RESULTS

3.1 *Nunu* Production in Upper East Region of Ghana

A total of 29 *nunu* producers were identified in the three towns surveyed in the Upper East Region (Paga, Navrongo and Bolgatanga). The processors were more concentrated in Paga which is a border town between Ghana and Burkina Faso. All the producers identified were females and Fulani migrants. Of the 29 processors, 24 of them did not have any formal education, 3 had primary and 2 Arabic education. The age range of these people varied but majority of them were between 21 and 40 yrs of age.

3.2 Environmental and Hygienic Conditions during *Nunu* Production

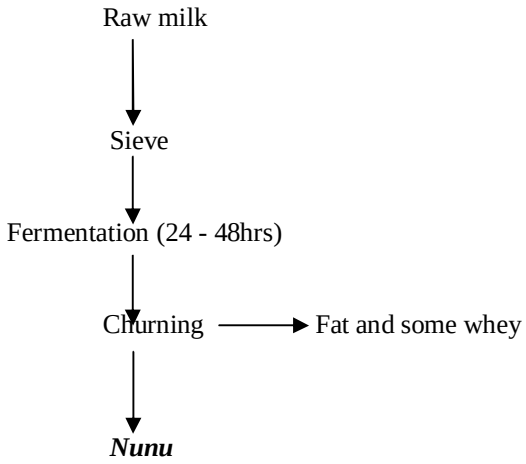
Milk production practices influenced the level of contamination at the farm level. Most farmers did not tie the cow's tail during milking, had no appropriate milking place, did not wash hands before milking, did not cover the milk and had no potable water for washing hands and utensils. Tying of the tail is important in the local setting because cows

carry a lot of dust or mud from the stable on their body. During milking, a lot of this dust is dislodged by the constant waving of the tail to drive away flies. This constitutes one of the most direct means of milk contamination.

3.3. *Nunu* production process

Figure 1 illustrates the production process of *nunu* in northern Ghana. Typically, fresh cow milk is collected in the morning in calabashes, sieved and left to ferment for a minimum of 24 hours or a maximum of 48 hours depending on the season. During the hot season which is usually from March to June, high ambient temperatures of 35°C promote acidification of the milk within 12–24 hours yielding the desired product, while in the cold season (October to February) where temperature of 15-17°C are recorded, the fermentation takes up to 48 hours. Sometimes, in the dry season when there is not enough grass for grazing of the animals, smaller quantities of milk are obtained and these are added to the previous day's batch depicting a form of back-slopping. The fermentation occurs spontaneously without starter cultures and at ambient temperatures. The fermented milk is then churned using a wooden ladle. Fat accumulates as a result of the churning and is removed. Excess whey is drained off to obtain a product with a thick consistency which is the *nunu*, consumed alone or with *fura*

Figure 1: A Flow diagram of *nunu* processing



3.3 Microflora Associated with *Nunu*

Table 1 shows the microbial counts of fermented milk samples collected from *nunu* producers through 0 hrs to 48 hrs of fermentation. The fermentation process was dominated by LAB and yeasts. LAB counts ranged between 4.00 and 9.00logcfu/ml while yeast numbers were between 1.0 and 7.00 log cfu/ml. Whereas LAB numbers increased, those of yeast decreased in all samples, with increasing fermentation time. Enterobacteriaceae numbers decreased with increasing fermentation time from 5.19 to 0.0log cfu/ml.

Table 1: Microbial counts of *nunu*

Fermentation time (hours)	TPC Log cfu/ml	LAB Log cfu/ml	Yeast Log cfu/ml	Enterobacteriaceae Log cfu/ml
0	7.37±1.05	4.69±0.37	6.63±0.85	5.19±0.63
12	6.92±0.77	6.43±0.44	6.27±0.63	4.66±0.65
24	6.68±1.19	7.91±0.36	3.64±0.73	3.09±0.69
36	6.62±0.52	8.33±0.28	2.43±1.11	1.53±1.36
48	5.59±0.69	8.82±0.32	1.22±1.34	0.00±0.00

Key: TPC = total plate count, LAB = lactic acid bacteria, cfu = colony forming units

3.4 Identification of Microorganisms

LAB isolated from *nunu* were identified based on their morphological, physiological and biochemical characteristics. All isolates were gram positive, catalase negative, and non-motile. Rods/coccobacilli accounted for 53.52% while cocci accouted for 46.48%. A total of 61.97% possessed the ability to produce gas from glucose (heterofermentatives). The genera identified included *Lactobacillus* (53.52%), *Leuconostoc* (15.49%), *Lactococcus* (9.86%), *Enterococcus* (15.49%) and *Streptococcus* (2.82%). Lactobacilli were further differentiated into *Streptobacterium, Thermobacterium* and *Betabacterium.*

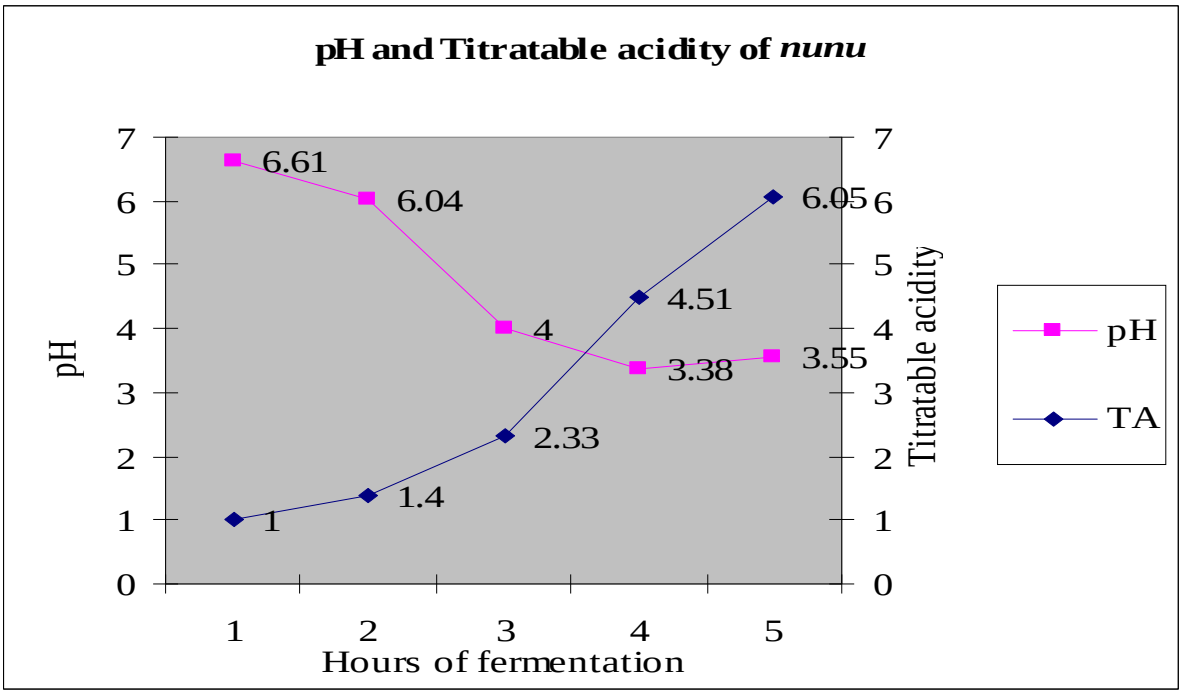
A total of 48 isolates of yeast were isolated and identified as *Saccharomyces cerevisiae* (35.42%), *Saccharomyces pastorianus* (4.17%), *Candida kefyr* (33.33%), *Yarrowia lipolytica* (4.17%), *Candida stellata* (14.58%), Kluyveromyces *maxianus* (4.17%), *Zygosaccharomyces bisporus* (2.08%), and *Zygosaccharomyces rouxii* (2.08%).

A total of 40 Enterobacteriaceae were isolated and identified as: *Enterobacter* (47.5%), *Klebsiella* (17.5%), *Escherichia coli* (20%), *Proteus vulgaris* (12.5%) and *Shigella* (2.5%).

3.5 pH and Titratable Acidity of *nunu* samples

Figure 1 shows the pH and Titratable acidity values of *nunu* samples recorded for 0-48hours of fermentation period. Generally, the pH decreased with fermentation time while Titratable acidity increased. The pH of the milk became very acidic, decreasing from 6.6 to about 3.4 and Titratable acidity increased from 1.0 to 4.5 (% lactic acid) after 36 hours fermentation. The pH increased between 36 and 48 hours while the TA continued to increase over the same period.

Figure 1: pH and Titratable acidity of *nunu* samples.



Key: for horizontal-axis, 1=0Hrs, 2=12hrs, 3=24hr, 4=36hrs, 5=48hrs
TA = Titratable acidity

4.0 DISCUSSION

The Fulani in northern Ghana produce their fermented milk by traditional methods. Fresh cow milk is left to chance fermentation in calabashes or rubber containers. The fermentation is spontaneous (without starter cultures) and occurs at ambient temperatures in a typical Fulani hut. Fermentation time varies from one producer to the other resulting in products of variable quality and stability. The spontaneous fermentation (without starter cultures) may also contribute to variable quality and stability in the product.

Raw milk drawn from a healthy udder normally will contain only a few hundred to a few thousand of bacteria per milliliter, mostly from the genus

Micrococcus and the udder diptheroid *Corynebacterium bovis.* In normal non-aseptic milking, many microorganisms are introduced into even high-quality milks from the external environment, i.e from the udder surface, from bovine faeces, soil, bedding, feed and so on (Harrigan, 1998). An observation from the survey showed that most producers did not employ good milking practice to minimize contamination of the milk. For instance, they did not wash their hands prior to milking and they did not tie they cow's tail while milking. In addition, milk- handling equipments were not sufficiently cleaned. Lack of potable water and use of detergents was a major constrain to hygienic practices on the farm. Many producers did not sufficiently clean the udder before milking. Yet pre-milking udder preparation plays an important role in the contamination of milk during milking (Galton *et al* 1989). This therefore resulted in a variety of organisms being isolated including Enterobacteriaceae that have implications on the microbiological quality of the final product. Since unpasteurized milk was used for traditional fermentation in this study, it can be assumed that the isolates originated from such contamination.

The microbiological study of *nunu* revealed the dominance of lactic acid bacteria in all samples at 0,12,24,36 and 48hours of fermentation although yeasts were also present in considerably high numbers during the fermentation. The counts of LAB in *nunu* were similar to other fermented milk products. Other researchers such as Obodai and Dodd (2005) found LAB counts between 8 and 10 logcfu/ ml in a fermented milk product (*nyarmie)* in Accra-Ghana. Beukes *et al*. (2001) also found LAB counts of 8.88logcfu/ml on MRS at 42^{o} C and 8.85logcfu/ml on M17 from some fermented milks of South Africa. Savadogo *et al* (2004) also found LAB numbers between 4.3 and 8.6log cfu/ml in Fulani fermented milk. Similar results were found in Tanzanian fermented milk (Isono *et al.*, 1994), and Zimbabwean fermented milk products (Feresu and Muzondo, 1990). This high numbers of LAB, coupled with the low values of pH (3.0) and high acidity (6.55) may be responsible for the sour taste, flavor and unique aroma of *nunu*. The production of lactic acid gives the fermented product a sour taste and also results in the formation of a smooth gel. In addition to this, various flavour compounds are formed and these are responsible for the specific taste of different products. Such flavour compounds can be formed from citrate, when the important flavour compounds diacetyl, acetic acid and carbon dioxide are formed. Besides these compounds mentioned, LAB probably do not produce flavour compounds in fermented milk that are in concentrations above taste threshold levels(Narvhus *et al*., 1998; Ayad *et al*., 1999, Narvhus and Gadaga, 2003).

The genera of lactic acid bacteria identified in this study were: *Lactobacillus* (53.52%), *Leuconostoc* (15.49%), *Lactococcus* (9.86%), *Enterococcus* (15.49%) and *Streptococcus* (2.82%). The *Lactobacillus* was made up of 15.79% *Streptobacteriun*, 18.31% *Thermobacterium* and 9.86% *Betabacterium*. The genera identified are in agreement with other works. Beukes *et al*. (2001) identified similar organisms in South Africa. It is worth noting that *Lactobacillus* and *Leuconostoc* dominated at 0 and 12 hours but towards the end, (at 36 and 48 hours), the numbers of *Leuconostoc* decreased. The increase in numbers of the *Lactobacillus* corresponded with decrease in pH at 36 and 48 hours of fermentation. The dominance of the genera *Lactobacillus* especially at 36 and 48 hours of fermentation could be explained by the acidic conditions which develop at the end of fermentation, or perhaps their ability to utilize the substrate better. The acid tolerance of lactobacilli compared with lactococci is well known and, according to Siegumfeldt *et al.* (1999), it could be explained by their ability to decrease their internal pH giving a competitive advantage and hence, the predominance of *Lactobacillus.*

It is reported that traditional fermented milks in regions with a cold climate favor the growth of mesophilic bacteria such as *Lactococcus* and *Leuconostoc spp.* whereas, in warm regions, thermophilic bacteria like *Lactobacillus* and *Streptococcus* prevailed (Savadogo *et al*., 2004*).* The dominance of thermophilic bacteria in our samples could be explained by the fact that the samples were collected in the hotter months and the ambient temperatures at which the natural fermentation took place probably were high. Navrongo records the highest temperature values (about 37-42^{o}C) in Ghana during the period of sampling. This result supports the theory that the microorganisms that are found in traditionally fermented milks depend on the particular climatic region and the distribution of lactic acid bacteria depends on the nature of fermented milk or fermented food (Savadogo *et al*., 2004*).*

Yeasts appear to be commonly associated with traditional fermented dairy products and have been reported in several studies (Mathara *et* al., 2004, Isono *et al*., 1994; Gadaga *et al*., 2001; Beukes *et al*., 2001). Isono *et al.* (1994) reported the occurrence of yeasts in seven of 10 samples of traditional fermented milk in northern Tanzania with mean counts ranging from 6.0 to 8.0 logcfu /ml, while Abdelgadir *et* al.

(2001) reported yeast counts of 10^7logcfu/ml in Sudanese *Rob*. In the current study, yeast counts ranged from 1.22 to 6.63logcfu/ml.

Yeast species identified in this study were *Saccharomyces cerevisiae* (35.42%), *Saccharomyces pastorianus* (4.17%), *Candida kefyr* (33.33%), *Yarrowia lipolytica* (4.17%), *Candida stellata* (14.58%), *Kluyveromyces maxianus* (4.17%), *Zygosaccharomyces bisporus* (2.08%), and *Zygosaccharomyces rouxii* (2.08%). According to Abdelgadir *et al.* (2001), *S. cerevisiae* and *C. kefyr* always occur together as was the case in *nunu*. *K. marximanus* (the teleomorph of C. *kefyr*) was isolated from pasteurized milk by Fleet and Mian (1987). According to the review by Tudor and Board (1993), *S. cerevsiae* has been reported to be associated with cheese, whereas *C.* kefyr has been shown to occur frequently in dairy products (Fleet, 1990). Both *K. marximanus* and *S. cerevisiae* have been isolated from blue-veined cheeses (De Boer and Kuik, 1987; van den Tempel and Jakobsen, 1998), samples of yogurt (Suriyarachchi and Fleet, 1981; Fleet and Mian, 1987), and considered as normal to *Kefir* (Iwasawa, Ueda, Miyata, Hirota, and Ahiko, 1982; Engel, Krusch, and Teuber, 1986; Marshall, 1986). *S. cerevisiae* was the most dominant yeast despite its inability to ferment and assimilate lactose. This may be due to the fact that it is able to utilize the trace amounts of glucose and galactose in milk (Rosenthal, 1991; Abdelgadir *et al*., 2001). *S. cerevisiae* appears to be involved in the fermentation of *Koumiss* and *Laben* (Oberman, 1985; Marshall, 1986). *S. cerevisiae* was also isolated from *nono*, a Nigerian fermented milk (Okagbue and Bankole, 1992) and from *mbanik* a Senegalese cultured milk (Gni ngue, Roblain, and Thonart, 1991). They may also play a positive role, e.g. in aroma formation as reviewed by Jakobsen and Narvhus (1996). The possible probiotic properties of S. *cerevisiae* were mentioned by Gedek (1991). In other works, *S. cerevisiae* has been isolated from raw milk but in low numbers (van den Tempel and Jakobsen, 1998). *C.kefyr* was the second most dominant yeast, probably because it is able to ferment lactose. *Yarrowia lipolytica* has been reported to be one of the most frequently occurring species in dairy products (Fleet, 1990; Jakobsen and Narvhus, 1996). This species is capable of degrading proteins using alkaline protease and also produces lipases (Roostita and Fleet, 1996; Wyder, 1998). It has been isolated more often from lipid and protein containing substrates, such as cheese, yoghurt or salads containing meat or shrimps as well as spoiled food (Wyder, 1998). It also metabolizes lactic acid. These are important technological properties in fermented milk. However, extensive proteolysis and lipolysis is undesirable in fermented milk (Gadaga *et al*., 2000).The high numbers of yeasts suggests that yeasts are able to multiply in the milk and may result in spoilage or, conversely, in enhancement of the flavor of the fermented milk (Gadaga *et al*. 2000). The presence of *Candida* species could indicate poor hygiene and ineffective cleaning procedures and show the need for improved sanitization procedures (Corbo *et al*. 2001). These high levels of yeast populations show that yeasts constitute a significant part of the microflora of this naturally fermented milk. At such high levels, the yeast metabolism should impact on the overall quality and acceptability of these products (Gadaga *et al.* 2000). The incidence of yeasts in all these samples, however, may suggest that yeasts are a common flora of the milking parlours, containers and fermentation vessels (Gadaga *et al*. 2000).

Enterobacteriaceae identified include *Enterobacter* (47.5%)), *Klebsiella* (17.5%), *Escherichia* (20%), *Proteus vulgaris* (12.5%) and *Shigella* (2.5%). Similar organisms were identified in informally marketed raw milk in Ghana (Donkor *et al*., 2007), and in Moroccan traditional fermented dairy products like *Lben* and *Jben* which showed a high number of indicator microorganisms (coliforms and *Enterococci)* and pathogens such as *Salmonella* spp., *Yersinia enterocolitica, Listeria monocytogenes* and enterotoxigenic *Staphylococcus aureus* (Hamama, 1992). The organisms identified are potential pathogens; however some of them including *Proteus* and the various *Klebsiella* spp. are rarely associated with foodborne infections. *Klebsiella pneumoniae*, the main *Klebsiella* pathogen causes pneumonia while *Proteus* is mainly associated with wound and urinary tract infections. Thus the occurrence of these organisms in milk may not pose a risk to consumers. Most of the other bacteria identified in the study have been implicated in milk and other food related infections (Donkor *et al*., 2007). Enterobacteriaceae were not detected at 48hours of fermentation. This may reflect the inhibitory effect of reduced pH values thereby contributing to the quality and safety of the fermented milk products. Their presence at the initial fermentations period suggests they were probably introduced from the external environment, i.e from the udder surface, bovine faeces, soil, bedding, feed with other microbes and they survived because of the high pH (Harrigan, 1998).

5.0 CONCLUSIONS

This study has enabled a process flow diagram for the traditional processing of *nunu* to be developed which is similar to other naturally fermented milk products. There are no standardized methods of processing *nunu* and this seems to result in a product of varying quality and stability. Due to the poor hygienic practices observed during processing, the quality of the final product appears to be compromised. *Nunu*, however, was found to be microbiologically safe as no Enterobacteria survived by 48hours of fermentation when pH decreased below 4.0. It is however recommended that a standard processing method that will ensure *nunu* of the highest microbial and nutritional quality be developed and the technology transferred to the local producers. Education of producers on good manufacturing practices including basic hygienic principles will equally be crucial in achieving a quality standard product.

The microorganisms isolated from *nunu* were diverse, including aerobic mesophiles, LAB, Yeasts and Enterobacteria. There is the need to characterize these organisms using modern molecular methods and the technological properties of the dominant types determined to facilitate selection and development of starter cultures from them for the production of *nunu*. In this way the fermentation process can be controlled, thereby enhancing the quality of the product.

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