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Full Length Research Paper

The effects of different preservation methods on the quality of nunu, a locally fermented Nigerian dairy product

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Biochemical and microbial analyses were carried out on fresh and preserved nunu for seven days. Fresh nunu contained protein, carbohydrates, lipids, calcium and other nutrients. Fresh samples had an initial pH of 4.4, microbial load of 4.2 x 10^3 cfu/ml and free fatty acid level of 2.21 mg/g of sample. Unpreserved sample showed appreciable degree of lipolysis, proteolysis and lactose fermentation. Preservation was by physical and chemical means. Physical methods included pasteurization and refrigeration. Pasteurization proved to be the least effective method of preservation. There was an appreciable level of increase in microbial count and biochemical changes. The chemical preservatives used were sodium benzoate and benzoic acid (1% concentration, w/v of sample). Sodium benzoate proved to be the most effective of all methods of preservation. The microbial count was significantly reduced ($p < 0.05$) and there were very slight biochemical changes. Benzoic acid was also effective, but less than sodium benzoate. Nunu, if well preserved, can thus be a very refreshing and nourishing beverage that is affordable to many

Key words: Nunu, fresh, biochemical, microbial, changes, preservation, probiotics.

INTRODUCTION

Nunu (noo-noo) is a locally fermented Nigerian milk product used as a staple food amongst the Saharan tribes of West African Sub-region, and is also popular amongst the inhabitants of the Mediterranean region and the Middle East where it is known as dahi or lassi (Davidson et al., 1975). Milk, if left untreated, spoils within a short time due to microbial activity; thus processing milk improves its storage and diversifies its use. Traditionally, nunu is prepared by inoculating freshly drawn cow milk with a little of the leftover as a starter and then is allowed to ferment for about 24 h at room temperature. During fermentation, some of the lactose is converted to lactic acid. At the end of fermentation period, the milk butter is removed by churning for further use, and the remaining sour milk, nunu, is a delicious and refreshing beverage (Olalokun, 1976). Most of the organisms involved in the fermentation process are usually of three main groups; bacteria, yeast and mould. Of these, *Lactobacilli* (*L. acidophilus* and *L. bulgaris*), *Lactococci* spp. (*L. cremoni* and *L. lactis*), *Streptococcus thermophilus*, *Leuconostoc* spp. and *Saccharomyces* species seem to be the most prominent, each giving products with characteristic flavours. Sour milk is thus not a uniform product. Variations in milk composition, bacterial flora and ambient temperatures can give rise to products of varying qualities (Gaman and Sherrington, 1965; O'Connor and Tripachi, 1995).

Nunu is yoghurt-like in taste (a sharp acid taste), and is therefore usually taken with sugar and "fura" which is made up of millet flour compressed in balls and cooked for about 20 - 40 min. The cooked "fura" is crumbled in a bowl of nunu (now called "fura de nunu"). Nunu is an excellent source of protein, rich in essential amino acids and a good source of calcium, phosphorous and vitamins A, C, E and the B complex. However, like other milk products, it is poor in ascorbic acid and iron. Nunu, if well prepared and well preserved, could serve as an equally good alternate but cheaper source of dairy product. It is

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Table 1. Quantitative analysis of fresh nunu.

Nutrient	Composition (mg/ml)
Protein	3.26
Lipid	4.4
Lactose	3.78
Calcium	0.128

Table 2. Rate of liberation of free fatty acid per day in preserved and unpreserved nunu.

Sample	Liberation of free fatty acid (mg/day)
Unpreserved	0.27
Sodium benzoate	0.074
Benzoic acid	0.11
Pasteurized	0.15
Refrigerated	0.09

at present being prepared and hawked mostly by the nomadic Hausa/Fulani cattle rearers, who invariably control over 80% of the country's cattle production and only available within walking distance of their settlements. Nunu is thus more available in the Northern part of Nigeria than in the South, and as such only a small percenttage of non-Fulanis has acquired the taste for it. It, however, does not appeal to majority of the people because of the apparent unhygienic conditions in which it is prepared, and also its poor shelf life (Wallander and Samson, 1967; Yahuza, 2001).

This work was aimed at analyzing the proximate nutriational value of nunu, monitoring the biochemical and microbial changes associated with its spoilage, and the effects of various methods of preservation on its quality.

MATERIALS AND METHODS

The fresh nunu used for this study was obtained from Awka Garki market (i.e. the Hausa/Fulani section of the market). The samples were divided into 5 portions and treated as follows:

sample 1, unpreserved; sample 2, preserved with sodium benzoate (1%, w/v); sample 3, preserved with benzoic acid (1%, w/v); sample 4, pasteurized at 62.8°C for 30 min; and sample 5, stored at 4°C. Quantitative analyses for protein, carbohydrates and lipids were first carried out on the fresh samples, then subsequently on a daily basis to determine the biochemical changes associated with the spoilage for 7 days. The rate of liberation of free fatty acid was used as an index for the measurement of the rate of lipolysis in the sample. Culture, isolation, characterization and count of viable microbial flora of nunu were done at 48-h intervals for 7 days. All samples were stored at room temperature with the exception of sample 5.

Quantitative analyses

Carbohydrate was quantified using the anthrone method (Shields and Burnett, 1960). Protein was measured by the Biuret method (Reinhold, 1953). Quantitative assay for calcium was done using the method of Clark and Collip (1925). Assay of lipase activity by the method of Chandan et al. (1978). Rate of liberation of free fatty acid was measured using the method of Adoga (1985). The pH of the samples was taken using a pH meter (Corning).

Isolation and count of microorganisms associated with nunu

Various microorganisms associated with nunu were isolated using the pour plate method of Collins and Lyne (2004). 1.0 ml of sample was serially diluted using 9.0mls of sterile distilled water. 0.5mls of 10-6 dilution were mixed with agar and poured into petri dishes and incubated at room temperature for 24 h. The media used for growth and development of colonies was Rogosa, nutrient and MRS agar. Duplicate plates of each were incubated aerobically and anaerobically. Total viable count was obtained. The biochemical tests conducted for the confirmation of their identities included spore stain, motility test, catalase, oxidase, starch hydrolysis, sugar fermentation, proteolytic activity and lipolytic activity tests described by Breed et al. (1957) and Collins and Lyne (2004).

RESULTS AND DISCUSSION

Quantitative analysis of fresh nunu was done prior to preservation and storage (Table 1). The nunu samples were then subjected to different methods of preservation. Some of these and other parameters generally decreesed with time. There was marked decrease in lactose content of the unpreserved sample from 3.78 – 2.70 mg/100 ml during the 7 days of shelf-life study. The level of decrease was not significant ($p<0.05$) in the preserved samples (Figure 1). Protein levels decreased in both the preserved and unpreserved nunu, indicating proteolysis. This was highest in the unpreserved sample, ranging from 3.26 - 2.20 mg/ml by the 7^{th} day. Changes were least in the sample preserved with sodium benzoate (Figure 2). The pH decreased in the unpreserved sample from 4.4 – 3.6. pH changes were also observed in all other samples, with the sodium benzoate preserved sample being the most stable (Figure 3). The rate of lipolysis was determined by the rate of liberation of free fatty acid. Free fatty acid level (determined as oleic acid) increased in the unpreserved sample as expected at the rate of 0.27 mg/day (Table 2). The sample preserved with benzoic acid had the lowest rate of free fatty acid liberation (Figure 4). Four different strains of microorganisms were isolated from nunu. They belong to *Lactobacillus, Streptococcus, Leuconostoc* and *Pseudomonas* (Table 3). The initial microbial flora count was found to be 4.2×10^3 cfu/ml. This increased to 3.4×10^8 cfu/ml by the 5^{th} day and fell to 7.2×10^6 cfu/ml by the 7^{th} day in the unpreserved. On the contrary, there were decreases in microbial flora in preserved samples except in the pasteurized sample. Table 4 shows the viable microbial count in the samples.

Milk from its natural source is a sterile product (Robert and Bradley, 1977), but its rich nutritional composition makes it a fertile ground for microbial inhabitation. Contamination may come from external sources like air, soil,

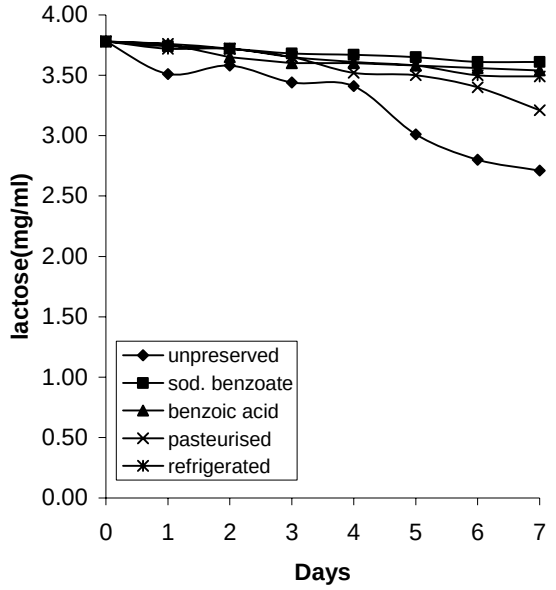

Figure 1. Changes in lactose concentration (mg/ml) in preserved and unpreserved nunu with time (days).

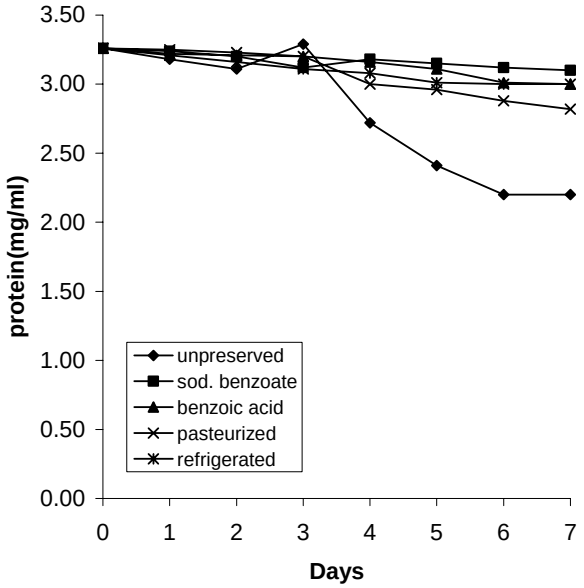

Figure 2. Decrease in protein content with time (days) in preserved and unpreserved nunu.

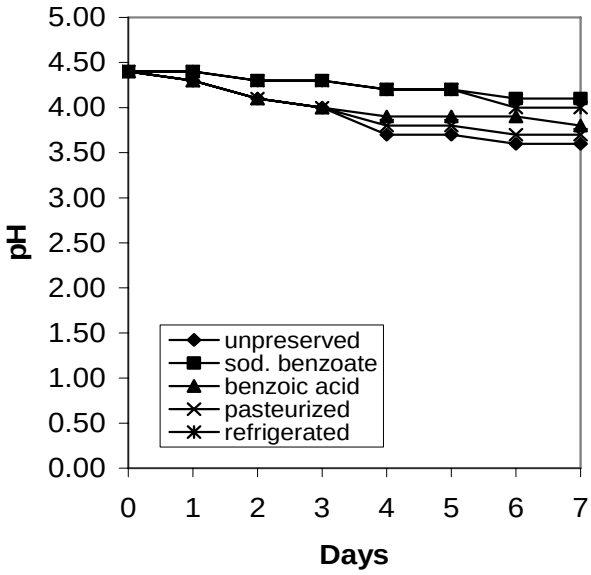

Figure 3. Changes in pH with time (days) in preserved and unpreserved nunu.

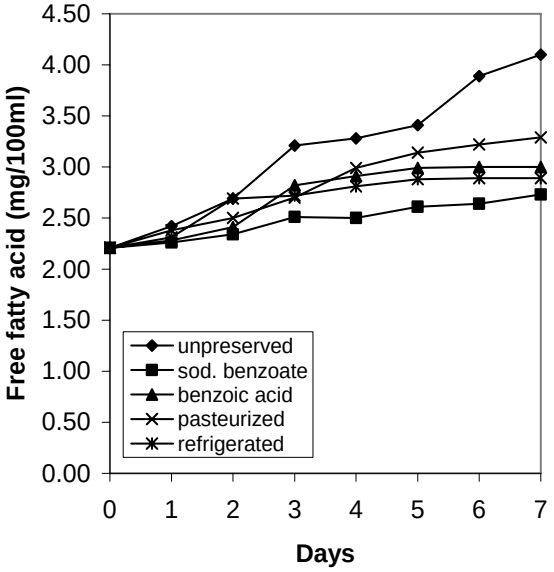

Figure 4. Free fatty acid profile in preserved and unpreserved nunu.

water, milking utensils and personnel, as well as the disease pathogens of the animals. Many pathogenic and saprophytic microorganisms are found in fresh milk. These microorganisms with the help of extracellular enzymes also found in milk cause such biochemical changes as lactose fermerntation, proteolysis and lipolysis (O'Connor and Tripachi, 1995; Livia, 1981b). As the fermentation takes place, there is marked decrease in pH thereby creating an unbearable environment for most of the microorganisms. Most bacteria in nunu such as the isolated *Leuconostoc* and *Lactobacillus* come from the inoculated leftover, but the isolated *Pseudomonas* and *Strepto-coccus* species were most likely contaminants from soil and water, respectively. These four strains of bacteria isolated from nunu have been demonstrated to be proteolytic, lipolytic and lactose fermenting (Chandan et al., 1978), thus accounting for the observed decrease in lactose and protein, and increase in free fatty acids in the samples. The increase in free fatty acids was as a result of structural breakdown of the lipids. The heat pasteurized sample contained strains of *Streptococcus* and

Table 3. Characterization of microorganisms isolated from nunu.

Strain	1	2	3	4
Colony morphology	Very small, flat, cream to yellow. Entire on rogosa	Spherical and ovoid cells in chain	Small, gray, entire And slightly raised	Complex, glistering, occasionally viscid, smooth
Cell shape	Rods in chain and clumps	Colci in pairs or chains	Spheres in pairs, in long or short chains	Rods in pairs and chains
Gram stain	+	+	+	–
Motility	–	–	–	+
Spore	–	–	–	–
Catalase	+	+	+	+
Oxidase	–	–	–	–
Proteolysis	–	+	+	+
Starch hydrolysis	–	–	–	–
Lipolysis	+	+	+	+
Sugar utilization	Ferments lac, glu, man, but not xyl	Ferments glu, lac, suc but not man	Ferments glu, fru, gal, lac	Ferments glu, gal, lac
Probable identification	Lactobacillus spp.	Streptococcus spp.	Leuconostoc spp.	Pseudomonas spp.

Lac = lactose
Glu = glucose
Suc = sucrose
Fru = fructose
Gal = galactose
Man = manitol
Xyl = xylose

Table 4. Viable microbial count in preserved and unpreserved nunu (cfu/ml).

Sample	0	1 day	3 day	5 day	7 day
Unpreserved	4.2×10^3	6.7×10^5	7.4×10^7	3.4×10^8	7.2×10^6
Sodium benzoate	4.2×10^3	2.4×10^2	4.6 x 10	2.2 x 10	1.2 x 10
Benzoic acid	4.2×10^3	4.1×10^2	7.2 x 10	6.3 x 10	2.8 x 10
Pasteurized	4.2×10^3	2.0 x 10	4.9 x 10	2.2×10^2	7.2×10^4
Refrigerated	4.2×10^3	9.1×10^2	6.2×10^2	3.1×10^2	8.0 x 10

Pseudomonas. Heat treatment could not eliminate *Streptococcus* because of its thermoduric nature (Wallander et al., 1967). *Pseudomonas* species, which is gram negative, is endowed with a complex three-layer cell wall (Foster, 1957). This cell wall, made up of mucopeptide, lipopolysaccharide and lipoprotein, shields the organism from conventional heat and chemical treatments.

The decrease in microbial flora in the refrigerated sample could as well be attributed to the low temperature (4^oC), which is well below the optimal temperature for most lactose fermenters (35 – 50^oC). This naturally impeded normal metabolism in the organisms and could suggest the reason for low utilization of nutrients in the refrigerated sample. There were also marked decreases in the microbial flora in the chemically preserved samples. The microbial count decreased from 4.2×10^3 to 1.2 x 10 and 4.2 x 10 in the sodium benzoate and benzoic acid preserved samples, respectively. The resulting microbial populations were, however, below the Food and Agricultural Organization (FAO) recommended maximum non-pathogenic microbial population of 10^4 cells/ml for milk products (FAO, 1970). The inhibitory action of the preservatives was further enhanced by the low pH of the samples. These chemicals, being acids, were less dissociated at low pH and could therefore permeate the microbial cells by passive diffusion or active transport more readily than ions. Thus the decrease in microbial population tallied with the milder decrease in available nutrients (Mustapha and Collins, 1978; Collins, 1976).

The presence of certain strains of microorganisms such as *Lactobacilli* and *Streptococcus* species may be beneficial (Perdigon et al., 1987). Several of these have been found to have probiotic properties and immunomodulatory function. Several *Lactobacillus* strains have been reported to display stimulatory properties on cells of innate immunity *in vitro* and *in vivo* in both animal models and in humans given fermented milk products containing probiotics. Additionally, these effects also involve B-cell responses, characterized by immunoglobulin A secretion, which is involved in controlling bacterial adherence to the

mucosal epithelium or cause clumping of bacteria near the mucosa to enhance washout by peristalsis. On the other hand, secretory immunoglobulin A is also important for preventing translocation of resident bacteria into mucosal tissue (Ibnou-Zekni et al., 2003). These effects were however found to be species and strain specific, since not all strains of any particular microorganism have equal probiotic efficacy (Perdigon et al., 1987; Schiffrin et al, 1995). Probiotics also generally inhibit the growth of harmful bacteria and prevent the production of harmful metabolites (Bowley, 2005).

Nunu is an excellent refreshing and nourishing drink that is used by nomadic cattle rearers. The lactic acid content determines the sensory and rheological properties of the milk and also makes it more easily digestible. Lactic acid is also energy yielding (Livia, 1981a). Nunu, which is very much like yoghurt, is being produced in limited daily consumable quantities due to its poor keeping quality. Knowledge of the biochemical and microbial changes that are associated with its spoilage and the various methods of preservation will obviously enhance the production and proper utilization on a larger scale. Results from this work showed that sodium benzoate provided the best preservation option. However, combined methods of pasteurization and chemical treatment, with or without refrigeration would certainly be more effective, to give a product that is palatable, safe and affordable. Fermented sour milk products continue to receive worldwide popularity, particularly in Africa and North America. Many of these products are blended with sweeteners and fruit juices, which largely modify the characteristic acid taste. Nunu can thus be made to have a wider acceptability, if greater attention is made to improve its flavour and shelf-life.

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