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EFFECT OF SOME PRESERVATION TECHNIQUES ON THE MICROBIOLOGICAL CHARACTERISTICS OF *FURA DE NUNU* DURING STORAGE

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ABSTRACT

The study focused on the effect of pasteurisation, with or without addition of sodium benzoate or sorbic acid on the microbiological characteristics of *fura de nunu* samples stored at ambient and refrigeration temperatures. This was carried out with the view of extending the shelf-life of this locally prepared dairy based drinkFreshly prepared *fura* was added to fresh *nunu* at ratio 1: 3, different batches were each subjected to pasteurisation, with or without sodium benzoate at two concentrations (300 mg/l and 200 mg/l) and sorbic acid at two concentrations (1000 mg/l and 750 mg/l). Samples were stored at refrigeration (4 ±1 °C) and ambient temperatures (28 ±2 °C) over a period of four weeks. The Total Viable Count (TVC), Lactic Acid Bacteria (LAB) and Yeast counts of the *fura de nunu* during storage were enumerated and identified. Results showed that the TVC and LAB counts of untreated sample ranged from 6.76 - 14.90, and 6.64 - 13.72 (log cfu ml^{-1}), respectively while pasteurised samples treated with 300mg L^{-1} sodium benzoate were found to have TVC and LAB of 0 - 4.88, and 0 - 3.84 ((log cfu ml^{-1}), respectively over the four weeks storage. The microorganisms were identified as *Lactobacillus delbrueckii*, *Lactobacillus brevis*, *Lactobacillus plantarum*, *Lactobacillus helveliticus*, *Leuconostoc mensenteroides*, *Streptococcus thermophilus*, *Saccharomyces cerevisiae*, *Saccharomyces lactic*, *Rhodotorula glutinis*, *Hansenula anomala*, *Torulopsis versatalis*, *and Candida mycoderma.* In conclusion, the combination of pasteurisation and 300mg L^{-1} sodium benzoate effectively lowered the microbial load during the period of storage. Sorbic acid was also effective, but it had minimal effect on the growth of lactic acid bacteria.

Key words: *fura de nunu*, *microbial load*, *preservation*, *microbiological characteristics*, *probiotics.*

1. INTRODUCTION

Fura de nunu (fermented milk and millet mix) is a highly nutritious beverage which is a two-in-one product, consisting of cereal, "*Fura*" made from millet grains and '*nunu*' a fermented milk product (with acid taste) similar to yoghurt. Depending on the consistency, the product is used as food, refreshing drink and a weaning food for infants (Umoh *et al*., 1988). 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 cooked spiced millet provides energy. The sour taste is known to be particularly suited for quenching thirst (Owusu-kwanteng *et al*., 2010). It, however, does not appeal to majority of the people because of the apparent unhygienic conditions in which it is prepared, and its poor shelf life (Wallander and Samson, 1967; Yahuza, 2001).

Nunu is a delicious and refreshing beverage (Olalokun, 1976) that evolved empirically some centuries ago by allowing milk to sour at warm temperature (Adebesin *et al*., 2001). The fermentation occurs spontaneously without starter cultures at ambient temperature. 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* (Owuzu-kwateng *et al*., 2010).

Fermented milk contains probiotics which are live microbial food supplements that benefits the health of consumers by maintaining or improving their intestinal microbial balance. Lactic acid bacteria in fermented milk break down lactose into glucose and galactose in the intestine by synthesizing the enzyme lactase. People with lactose intolerance can consume fermented milk product because of its lower lactose content and availability of live probiotics (Adolfsson *et al*., 2004).

Most foods deteriorate in quality following harvest, slaughter or manufacture, in a manner that is dependent on food types, its composition and storage conditions. The principal cause of deterioration of foods may be microbiological, enzymatic, chemical and physical. Methods by which the microbial decomposition of foods can be delayed or prevented include; restriction of access of microorganisms to foods by aseptic packaging, removal of microorganisms by filtration or centrifugation, slowing or preventing the growth and activity of microorganisms by reducing the temperature, water activity and pH, removal of oxygen, modified atmosphere packaging and addition of preservatives, and inactivation of microorganisms by heat (Gupta, 2007).

Pasteurisation conditions are designed to give maximum protection from milk-borne diseases with minimum reduction in nutritional properties, and at the same time to retain as fully as possible the appearance and flavour of raw fresh milk (Ihekoronye and Ngoddy, 1985). Organic acids such as sorbic acid and sodium benzoates also help in killing or inactivating target organisms in food. Preservatives may be microbicidal and kill the target organism or they may be microbiostatic in which case they simply prevent them from growing, thus improving the shelf-life of the product (Fawole and Osho, 2002). Preservatives may also inhibit microorganism by interfering with the cell membrane, enzyme activity or genetic mechanism of microorganisms (William and Westhoff, 1995). Since food spoilage is usually a result of chemical reactions mediated by

microbial and endogenous enzymes, the useful life of foods can be increased at low temperatures (Adam and Moss, 1999). Nebedum and Obiakor (2007) reported the use of pasteurisation and application of sorbic acid and sodium benzoate on fermented milk (*nunu*) for seven days at refrigeration and ambient temperatures. Abiose and Adeniran (2010) reported the use of pasteurization and application of sodium benzoate/ sodium metabisulphite in extending the shelf-life of *Hibiscus sabdariffa* extract for six weeks. This study investigated the effect of pasteurisation with or without sodium benzoate or sorbic acid on the microbial population and microbial types in *fura de nunu* during storage at refrigeration and ambient temperatures.

2. MATERIALS AND METHODS

Fresh cow milk was obtained from Fulani settlement at Alakowe, Ile-Ife, Nigeria. Millet grains were obtained from a local market in Ile-Ife, Nigeria. The media and chemicals were of reagent grade.

2.1. Preparation of *fura*

Millet grains (700 g) were sorted, cleaned, steeped for 18 *h* and then wet milled. The paste was hand- moulded into balls of about 10 cm in diameter and then cooked for about 30 minutes. The cooked millet balls were pounded with mortar and pestle. The balls were finally moulded into much smaller balls of about 4 cm diameter known as *fura* (Owuzu-Kwanteng *et al*., 2010).

2.2. Preparation of *nunu*

Cow milk was collected, sieved, and left to ferment for 48 *h* in a covered container at ambient temperature. The fermented milk was churned using a wooden ladle. Fat and excess whey were drained off to obtain a product with a thick consistency (*nunu*) (Akabanda *et al*., 2010).

2.3. Preparation of *fura de nunu*

Nunu was added to *fura* at ratio 3:1 by blending and the mixture was then packaged in sterile plastic bottles. Sodium benzoate and sorbic acid were added at different. The mixture was split into six portions A-F; A was unpasteurised, B-F were pasteurised at 70 ^{o}C for 15 *min* in sterile plastic bottles. Sample B was only pasteurised, sample C was treated with 300 mg L^{-1} sodium benzoate, sample D was treated with 1000 mg L^{-1} sorbic acid, sample E was treated with 200 mg L^{-1} sodium benzoates, and sample F was treated with 750 mg L^{-1} sorbic acid. Samples were kept at ambient (28 ±2^{o}C) and refrigeration temperatures (4 ±1) ^{o}C for a period of four weeks.

2.4. Microbiological evaluation of *fura de nunu*

2.4.1. Enumeration of microbes

Serial dilution was carried out by first mixing 1 ml of *fura de nunu* sample with 9 ml of peptone water to obtain 10^{-1}dilution. From this subsequent dilutions were made serially until the desired level of dilution was achieved. From each dilution, 1.0ml was plated on sterile petridish before 20ml each of molten Nutrient Agar (NA), de Man Rogosa and Sharpe (MRS) and Potato Dextrose Agar (PDA) for total viable count, Lactic acid bacteria count, and yeast count respectively, using pour plate technique (Harrigan and McCance, 1976; McLandsborough, 2005).

Plates were incubated in an inverted position in the incubators set at 37 ^{o}C for total viable microorganism and lactic acid bacteria for 24 and 72 *h*, respectively. Plates were incubated at 28 ^{o}C ± 2 ^{o}C for yeast for 3 to 5 days. The microbial load was determined by counting distinct colonies with the aid of a colony counter. Plates with 25 -250 colonies were reckoned with and the number of colonies on each plate was multiplied with the reciprocal of the dilution factor to obtain the count (Harrigan and McCance, 1976; Harrigan, 1998).

2.4.2. Characterization and identification of microbes

Pure cultures were obtained from distinct colonies by repeated streaking on fresh agar plates and subjected to microscopic examination and biochemical tests (such as catalase test, gram staining, oxidase test, sugar fermentation test and production of carbon dioxide). Relevant bacterial and yeast identification schemes of Harrigan and McCance (1976), and Holt (1996) were employed for identification.

3. RESULTS AND DISCUSSIONS

3.1. Total Viable Count

There was a general increase in the total viable count of *fura de nunu* with increase in the period of storage (Tables 1a and 1b). All the samples except the unpasteurised *fura de nunu* had no count at week zero. Unpasteurised *fura de nunu* had the highest count at refrigeration and ambient temperatures. A range of total viable count reported for fresh *fura de nunu* has been put at 10^{-4} to 10^{-7} (Adebesin *et al*., 2001; Owuzu-kwateng *et al*., 2010). At refrigeration temperature the total viable count of pasteurised *fura de nunu* treated with 300 mg/l sodium benzoate increased from zero count at week 0 to 3.66 at week 1 and 4.69 (cfu ml^{-1}) at the end of week 4 of storage. In the pasteurised samples treated with 200 mg/l sodium benzoate, there was no count at week zero, the count increased to 3.82 at the first week and 4.81 (cfu ml^{-1}) at week 4 of refrigeration storage. At ambient temperature, a similar trend was observed. There was no significant difference ($p > 0.05$) in the total viable count of pasteurised sample treated with sodium benzoate at refrigeration temperature and ambient temperature. There was significant increase ($p > 0.05$) in the total viable count of unpasteurised and pasteurised *fura de nunu* at refrigeration and ambient temperatures.

Total viable count of pasteurised samples treated with sorbic acid increased significantly from week one to week four but the counts were significantly lower than the count in pasteurised sample. The result showed that pasteurisation destroyed the microorganisms in *fura de nunu* at the initial stage but some microorganisms that were able to survive the process later grew, but the growth was lower at refrigeration temperature than at ambient temperature. The best result was obtained in pasteurised sample treated with 300 mg L^{-1} sodium benzoate at refrigeration temperature. Pasteurisation of raw milk was reported to be effective in eliminating all but the thermoduric microorganisms and occasionally some Gram-negative rods (Jay, 1996). The use of preservatives such as sodium benzoate and sorbic acid further reduced the total viable count of *fura de nunu.* Psychrotrophs can grow at refrigeration temperatures below 7 °C, produce enzymes, toxins and other metabolites (Jay, 1996) and contribute to high TVC in both raw and pasteurised milk. Microorganisms also hinder the effort of increasing the shelf life of pasteurised milk (Frank, 1997).

3.2. Lactic acid bacteria count

Lactic acid bacteria count increased as the period of storage increased (Tables 2a and 2b). There was no count in all the samples except in the unpasteurised sample at week zero. Unpasteurised sample had the highest lactic acid bacteria count at refrigeration and ambient temperatures at different stages of storage (6.64 -13.26 cfu ml^{-1}). There was significant difference ($p > 0.05$) in the lactic acid bacteria count of unpasteurised sample and pasteurised sample at refrigeration and ambient temperature.

The count in pasteurised *fura de nunu* treated with 300mg L^{-1} sodium benzoate increased from week zero to week one (0 – 3.38 cfu ml^{-1}) and also increased at the second week but reduced at the third week (4.72 – 3.40 cfu ml^{-1}) but slightly increased at the fourth week of storage (3.44 cfu ml^{-1}). There was no significant difference ($p > 0.05$) in the lactic acid bacteria count of pasteurised *fura de nunu* treated with sodium benzoate at refrigeration and ambient temperatures. Pasteurised *fura de nunu* treated with 300mg/l sodium benzoate had the

Table 1a: Total Viable Count of fura de nunu stored at Refrigeration Temperature (log cfu ml^{-1})

Weeks	Samples					
	A	B	C	D	E	F
0	6.76 ± 0.01^{b}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}
1	9.86 ± 0.09^{d}	5.92 ± 0.44^{c}	3.66 ± 0.17^{a}	3.82 ± 0.14^{a}	4.77± 0.17^{b}	4.78 ± 0.24^{b}
2	11.59 ± 0.59^{d}	7.40 ± 0.38^{c}	3.66 ± 0.13^{a}	4.72 ± 0.17^{b}	4.81 ± 0.14^{b}	4.82 ± 0.18^{b}
3	12.78 ± 0.38^{d}	9.53 ± 0.14^{c}	4.72 ± 0.16^{a}	4.76 ± 0.16^{a}	5.69 ± 0.30^{b}	5.80 ± 0.14^{b}
4	14.41 ± 0.07^{d}	10.54 ± 0.13^{c}	4.69 ± 0.20^{a}	4.81 ± 0.18^{a}	6.58 ± 0.20^{b}	6.71 ± 0.17^{b}

n=3, ±Standard Deviation. Means in the same row with the same superscript are not significantly different at (p › 0.05).

Table 1b: Total Viable Count of fura de nunu stored at Ambient Temperature (log cfu ml^{-1})

Weeks	Samples					
	A	B	C	D	E	F
0	6.76 ± 0.01^{b}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}
1	11.99 ± 0.18^{d}	6.62 ± 0.54^{c}	3.79 ± 0.24^{a}	3.86 ± 0.11^{a}	4.81± 0.16^{b}	4.84 ± 0.02^{b}
2	13.98 ± 0.25^{d}	9.73 ± 0.17^{c}	4.83 ± 0.17^{a}	4.78 ± 0.20^{a}	5.69 ± 0.28^{b}	5.72 ± 0.21^{b}
3	14.94 ± 0.33^{d}	11.86 ± 0.08^{c}	4.78 ± 0.08^{a}	4.91 ± 0.10^{a}	6.68 ± 0.11^{b}	6.78 ± 0.11^{b}
4	14.90 ± 0.18^{d}	12.98 ± 0.14^{c}	4.88 ± 0.21^{a}	4.96 ± 0.24^{a}	6.87 ± 0.14^{b}	6.88 ± 0.23^{b}

n=3, ±Standard Deviation. Means in the same row with the same superscript are not significantly different at (p › 0.05). A: Unpasteurised *fura de nunu*, B: Pasteurised *fura de nunu*, C: Pasteurised *fura de nunu* treated with 300 mg/l sodium benzoate, D: Pasteurised *fura de nunu* treated with 200 mg/l sodium benzoate, E: Pasteurised *fura de nunu* treated with 1000 mg/l sorbic acid. F: Pasteurised *fura de nunu* treated with 750 mg/l sorbic acid.

Table 2a: Lactic Acid Bacteria Count of fura de nunu stored at Refrigeration Temperature (log cfu ml^{-1})

Weeks	A	B	C	D	E	F
0	6.64 ± 0.14^{b}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}
1	9.68 ± 0.17^{c}	5.49 ± 0.14^{b}	3.38 ± 0.30^{a}	3.58 ± 0.13^{a}	3.64 ± 0.14^{a}	3.80 ± 0.33^{a}
2	11.38 ± 0.17^{c}	7.89 ± 0.13^{b}	4.72 ± 0.16^{a}	4.75 ± 0.10^{a}	4.75 ± 0.28^{a}	4.89 ± 0.20^{a}
3	12.91± 0.14^{d}	9.46 ± 0.16^{c}	3.40 ± 0.27^{a}	3.77 ± 0.33^{a}	5.38 ± 0.13^{b}	5.85 ± 0.14^{b}
4	13.26 ± 0.25^{d}	9.68 ± 0.16^{c}	3.44 ± 0.21^{a}	3.58 ± 0.24^{a}	6.40 ± 0.23^{b}	6.83 ± 0.20^{b}

n=3, ±Standard Deviation. Means in the same row with the same superscript are not significantly different at (p › 0.05).

Table 2b: Lactic Acid Bacteria Count of fura de nunu stored at Ambient Temperature (log cfu ml^{-1})

Weeks	A	B	C	D	E	F
0	6.64 ± 0.14^{b}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}
1	10.80 ± 0.28^{c}	6.66 ± 0.17^{b}	3.68 ± 0.20^{a}	3.79 ± 0.16^{a}	3.81± 0.24^{a}	3.88 ± 0.18^{a}
2	12.71 ± 0.35^{c}	8.83 ± 0.17^{b}	4.83 ± 0.20^{a}	4.83 ± 0.20^{a}	4.83 ± 0.20^{a}	4.89 ± 0.20^{a}
3	13.52 ± 0.30^{d}	10.61 ± 0.23^{c}	4.55 ± 0.16^{a}	4.56 ± 0.16^{a}	5.76 ± 0.20^{b}	5.85 ± 0.14^{b}
4	13.72 ± 0.30^{d}	9.97 ± 0.23^{c}	3.76 ± 0.28^{a}	3.84 ± 0.20^{a}	6.62 ± 0.32^{b}	6.83 ± 0.20^{b}

n=3, ±Standard Deviation. Means in the same row with the same superscript are not significantly different at (p › 0.05). A: Unpasteurised *fura de nunu*, B: Pasteurised *fura de nunu*, C: Pasteurised *fura de nunu* treated with 300 mg/l sodium benzoate, D: Pasteurised *fura de nunu* treated with 200 mg/l sodium benzoate, E: Pasteurised *fura de nunu* treated with 1000 mg/l sorbic acid. F: Pasteurised *fura de nunu* treated with 750 mg/l sorbic acid.

lowest count at both refrigeration and ambient temperature. Lactic acid bacteria count in pasteurised *fura de nunu* treated with sorbic acid was higher than that of pasteurised *fura de nunu* treated with sodium benzoate but lower than the lactic acid bacteria count in pasteurised sample. According to Adam and Moss (1999) sorbic acid is active against yeast, moulds and catalase-positive bacteria but, less active against catalase-negative bacteria. The result showed that it was not effective against all the lactic acid bacteria in *fura de nunu.* Thus, it can be used in the preservation of probiotic food to keep the beneficial lactic acid bacteria viable during the period of storage.

3.3. Yeast count

Yeast counts in unpasteurised *fura de nunu* were higher than in pasteurised *fura de nunu* at refrigeration and ambient temperatures. There was significant increase (p › 0.05) in yeast count of pasteurised and unpasteurised *fura de nunu* at refrigeration and ambient temperatures.

There was a general increase in yeast count of *fura de nunu* at refrigeration and ambient temperatures except in pasteurised sample treated with sorbic acid. At refrigeration temperature, pasteurised *fura de nunu* treated with 1000 mg L $^{-1}$ sorbic acid increased from initial count 0 to 3.60 cfu ml^{-1} at the first week and then reduced to 2.46 cfu ml^{-1}at the fourth week.

There was no significant difference (p › 0.05) in yeast count of pasteurised *fura de nunu* treated with sorbic acid at refrigeration and ambient temperatures.

Pasteurised *fura de nunu* treated with sorbic acid had a lower yeast count than pasteurised *fura de nunu* treated with sodium benzoate. Yeast count of pasteurised sample treated with sodium benzoate was significantly lower than in pasteurised sample. Sorbic acid is more effective at lower pH levels because more will exist in the undissociation form, allowing entry into the cell (Foegeding and Busta, 1999). The highest count was recorded in the unpasteurised *fura de nunu* because the growth process was not controlled.

3.4. Identity of Microbial Isolates

3.4.1. Lactic acid bacteria

Lactic acid bacteria identified in fura de nunu were Lactobacillus plantarum, Leuconostoc mensenteroides, Streptococcus thermophilus, Lactobacillus delbrueckii, Lactobacillus brevis, and Lactobacillus helveliticus (Table 4). This high number of lactic acid bacteria, coupled with the low values of pH and high acidity may be responsible for the sour taste, flavour and unique aroma of fura de nunu. The production of lactic acid gives the fermented product a desired sour taste. In addition to this, various flavour compounds are

Table 3a: Yeast Count of fura de nunu stored at Refrigeration Temperature (log cfu ml^{-1})

Weeks	A	B	C	D	E	F
0	5.53 ± 0.30^{b}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}
1	8.94 ± 0.17^{c}	4.64 ± 0.14^{b}	3.49 ± 0.24^{a}	3.65 ± 0.28^{a}	3.60 ± 0.28^{a}	3.68 ± 0.18^{a}
2	10.51 ± 0.31	6.90 ± 0.14^{c}	2.84 ± 0.13^{a}	3.41 ± 0.16^{b}	3.56 ± 0.14^{b}	3.61 ± 0.17^{b}
3	11.98 ± 0.17^{d}	8.83 ± 0.16^{c}	3.26 ± 0.25^{b}	3.51 ± 0.08^{b}	2.51 ± 0.27^{a}	2.56 ± 0.16^{a}
4	13.75 ± 0.20	10.74 ± 0.35	4.00 ± 0.30^{b}	4.20 ± 0.14^{b}	2.46 ± 0.13^{a}	2.76 ± 0.24^{a}

n=3, ±Standard Deviation. Means in the same row with the same superscript are not significantly different at (p › 0.05).

Table 3b: Yeast Count of fura de nunu stored at Ambient Temperature (log cfu ml^{-1})

Weeks	A	B	C	D	E	F
0	5.53± 0.30^{b}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}
1	9.81± 0.27^{c}	5.95± 0.18^{b}	3.52 ± 0.28^{a}	3.69 ± 0.18^{a}	3.84 ± 0.18^{a}	3.85 ± 0.24^{a}
2	11.49 ± 0.13^{c}	7.99 ± 0.27^{b}	3.56 ± 0.14^{a}	3.58 ± 0.24^{a}	3.69 ± 0.33^{a}	3.78 ± 0.21^{a}
3	12.76 ± 0.14^{d}	10.72 ± 0.21^{c}	3.72 ± 0.20^{b}	3.81 ± 0.21^{b}	2.72 ± 0.16^{a}	2.76 ± 0.18^{a}
4	13.90 ± 0.30^{d}	12.52 ± 0.45^{c}	4.50 ± 0.16^{b}	4.72 ± 0.23^{b}	2.67 ± 0.20^{a}	2.75 ± 0.27^{a}

n=3, ±Standard Deviation. Means in the same row with the same superscript are not significantly different at (p › 0.05). A: Unpasteurised *fura de nunu*, B: Pasteurised *fura de nunu*, C: Pasteurised *fura de nunu* treated with 300 mg/l sodium benzoate, D: Pasteurised *fura de nunu* treated with 200 mg/l sodium benzoate, E: Pasteurised *fura de nunu* treated with 1000 mg/l sorbic acid. F: Pasteurised *fura de nunu* treated with 750 mg/l sorbic acid.

Table 4: Identification of Lactic Acid Bacteria in fura de nunu

Tests	Isolates					
	B1	B2	B3	B4	B5	B6
Morphology	Rods	Cocci	Cocci	Rods	Rods	Rods
Colour of colony	Cream	Cream	Cream	Cream	Cream	Cream
Gram reaction	+	+	+	+	+	+
Catalase test	-	-	-	-	-	-
Growth at:						
15°C	-	+	-	+	-	+
45°C	+	+	+	-	+	-
Growth in:						
4 % NaCl	-	-	-	-	-	-
6.5 % NaCl	-	-	-	-	-	-
Production of CO_2	-	+	-	-	-	+
Sugars fermentation:						
Glucose	+	+	+	+	+	+
Galactose	+	+	+	-	+	+
Lactose	+	+	+	+	+	+
Arabinose	-	+	-	+	+	+
Trehalose	+	+	-	-	+	-
Salicin	+	+	-	+	-	+
Sucrose	+	+	-	+	-	+
Maltose	+	+	-	-	+	+
Raffinose	-	+	+	+	-	+
Dextran production	-	+	-	-	-	-
Probable identity of Organism	*Lactobacillus Delbrueckii*	*Leuconostoc mesenteroides*	*Streptococcus thermophilus*	*Lactobacillus plantarum*	*Lactobacillus helveliticus*	*Lactobacillus Brevis*

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 (Akabanda et al., 2010). Many microorganisms in fermented dairy products stabilize the bowl micro flora, and some appear to have antimicrobial properties. Several lactobacilli have antitumor compounds in their cell walls. Such findings suggest that diets including lactic acid bacteria may contribute to the control of colon cancer (Prescott et al., 2002). Leuconostoc mesenteroides was isolated from unpasteurised fura de nunu at week zero (Table 2). Leuconostoc mesenteroides and Leuconostoc lactis are the dominant Leuconostoc in milk and fermented milk product (Marshal, 1987). Leuconostoc mesenteroides was only present in the fresh unpasteurised fura de nunu. Leuconostoc sp have also been shown to exhibit a weak competitive ability during the fermentation of milk (Wood and Hozapfel, 1995).

3.4.2. Yeasts

Yeast isolates identified in *fura de nunu* were *Saccharomyces cerevesiae*, *Hansenula anomala*, *Torulopsis versatalis*, *Rhodotorula glutinis*, and *Candida mycoderma* (Table 5). Yeasts have been reported to make a useful contribution to the improvement of flavour and acceptability of fermented cereal gruels (Owuzu-kwanteng et al., 2010). 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*., 1999; Beukes *et al*., 2001; Adebesin *et al*., 2001; Akabanda *et al*., 2010). Yeasts are common in several fermented foods and beverages including *ogi* , *fufu*, *kunu*, and *nunu* produced in the tropical part of the world (Odunfa, 1985, Adegoke and Babalola, 1988, Halm *et al*., 1993; Akabanda *et al*., 2010).

3.5. Occurrence Pattern of Lactic Acid Bacteria in during Storage

Leuconostoc mesenteroides was isolated from unpasteurised *fura de nunu* at week zero (Table 6). *Leuconostoc mesenteroides* and *Leuconostoc lactis* are the dominant *Leuconostoc* in milk and fermented milk product (Marshal, 1987). *Leuconostoc mesenteroides* was only present in the fresh unpasteurised *fura de nunu*. *Leuconostoc* have practical importance in the

role they play in changing the organoleptic quality and texture of fermented food products such as milk, butter, cheese and meat.

Lactobacillus delbrueckii was isolated from fresh unpasteurised *fura de nunu* and from pasteurised *fura de nunu* at the first week of storage (Table 6). *Streptococcus thermophilus* was isolated from all *fura de nunu* samples except from pasteurised *fura de nunu* treated with sodium benzoate (Table 6). This showed that *Streptococcus thermophilus* can grow at high temperature. Wood and Holzaphel, (1995) reported that *Streptococcus thermophilus* can be found in heated or pasteurised milk. *Lactobacillus plantarum* was isolated from all the samples from the first week to the fourth week of storage, except in unpasteurised *fura de nunu* which contain *Lactobacillus plantarum* at week zero. *L. plantarum* has been identified as the dominant organism at the end of several natural lactic acid fermentations (Nout, 1980; Mbugua, 1984; Braunman *et al*., 1996; Olasupo *et al*., 1997; Kunene *et al*., 2000; Mugula *et al*., 2003), probably due to its acid tolerance (Fleming and McFeters, 1981) and superior ability to utilize the substrate including dextrins (Akinrele, 1970). *Lactobacillus helveliticus* was isolated from freshly prepared unpasteurised milk, from pasteurised *fura de nunu*, pasteurised *fura de nunu* treated with sodium benzoate and sorbic acid at the second week of storage. *Lactobacillus brevis* was isolated from freshly prepared unpasteurised *fura de nunu*. *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).

3.6. Occurrence Pattern of Yeasts in during Storage

Yeast isolates identified in *fura de nunu* were *Saccharomyces cerevesiae*, *Hansenula anomala*, *Torulopsis versatalis*, *Rhodotorula glutinis*, and *Candida mycoderma* (Table 5). *Torulopsis versatilis* was isolated from unpasteurised *fura de nunu* at the third week of storage while *Candida mycoderma* was isolated from unpasteurised *fura de nunu* at week zero,

Table 5: Identification of Yeast in Fura de nunu

Test	Isolates					
	Y1	Y2	Y3	Y4	Y5	Y6
Morphology:						
Pellicle	-	+	-	-	-	+
Colour	White	Cream	Pink	Cream	Cream	Cream
Shape	Ovoid	Cylindrical	Ovoid	Ovoid	Ovoid	Ovoid
Reproduction	Budding	Budding	Budding	Budding	Budding	Budding
Sugar fermentation:						
Glucose	+	+	-	+	+	-
Sucrose	+	+	-	+	+	-
Maltose	+	+	-	-	+	-
Galactose	+	+	-	+	+	-
Raffinose	+	+	-	+	+	-
Lactose	-	-	-	+	-	-
Sugar assimilation:						
Glucose	+	+	+	+	+	+
Sucrose	+	+	+	+	+	-
Maltose	+	+	+	+	+	-
Galactose	+	+	+	+	+	-
Raffinose	-	-	-	+	-	-
Lactose	-	-	-	+	+	-
Nitrate assimilation	-	+	-	-	+	-
Probably identity of organism	*Saccharomyces cerevisiae*	*Hansenula anomala*	*Rhodotorula glutinis*	*Saccharomyces lactis*	*Torulopsis versatalis*	*Candida mycoderma*

Table 6: Occurrence Pattern of Lactic Acid Bacteria in during Storage

Sample code	Lactic acid bacteria	Week 0	Week 1	Week 2	Week 3	Week 4
A	*Lactobacillus plantarum*	+	+	+	+	-
	Lactobacillus debrueckii	+	+	-	-	-
	Lactobacillus brevis	+	-	-	-	-
	Leuconostoc mesenteriodes	+	+	-	-	-
	Streptococcus thermophilus	+	+	-	-	-
	Lactobacillus helveliticus	+	+	+	-	-
B	*Lactobacillus plantarum*	-	+	+	+	+
	Streptococcus thermophilus	-	+	+	-	-
	Lactobacillus helveliticus	-	-	-	-	-
C	*Lactobacillus plantarum*	-	-	+	+	+
D	*Lactobacillus plantarum*	-	-	+	+	+
E	*Lactobacillus plantarum*	-	-	+	+	+
	Streptococcus thermophilus	-	-	+	+	+
	Lactobacillus brevis	-	-	+	+	+
F	*Lactobacillus plantarum*	-	-	+	+	+
	Streptococcus thermophilus	-	-	+	+	+
	Lactobacillus brevis	+	-	+	+	+

A: Unpasteurised *fura de nunu*, B: Pasteurised *fura de nunu*, C: Pasteurised *fura de nunu* treated with 300 mg/l sodium benzoate, D: Pasteurised *fura de nunu* treated with 200 mg/l sodium benzoate, E: Pasteurised *fura de nunu* treated with 1000 mg/l sorbic acid, F: Pasteurised *fura de nunu* treated with 750 mg/l sorbic acid.

+: Present, -: Absent

week one and week two (Table 7). Akinrele, (1970) reported the contribution of *Candida mycoderma* to the flavour acceptability of *ogi. Saccharomyces cerevesiae* was isolated from all *fura de nunu* samples. *Saccharomyces cerevisiae* is the most encountered yeast in fermented beverages and food based vegetables (Adam and Moss, 1999). *Saccharomyces lactis* was isolated from freshly prepared unpasteurised fura *de nunu* and pasteurised *fura de nunu* at the first week of storage.

It was also present in *fura de nunu* treated with sodium benzoate at the second and third week of storage (Table 7). *Hansenula anomala was* isolated from unpasteurised *fura de nunu* at the initial stage, from pasteurised *fura de nunu* at the second week of storage and from pasteurised *fura de nunu* treated with sodium benzoate at the fourth week of storage (Table 7). *Rhodotorula glutinis* was isolated from unpasteurised *fura de nunu* at the second week of storage. Yeasts appear to be commonly associated with traditionally fermented dairy products and have been reported in several studies (Beukes *et al*., 2001; Adebesin *et al*, 2001; Akabanda *et al*., 2001). Yeasts have been reported to make a useful contribution to the improvement of flavour and acceptability of fermented cereal gruels (Baningo *et al*., 1974; Odunfa and Adeyele, 1985).

3.7. pH of fura de nunu

The pH of *fura de nunu* samples decreased as the storage period increased. At refrigeration temperature (Figure 1), the pH of pasteurised *fura de nunu* treated with 300 mg/l sodium benzoate was stable with the value of 5.00, 4.97, 4.95 and 4.93 from week 0 to the third week and the decrease was significant at the fourth week of refrigeration storage. At ambient temperature the pH was stable from week 0 to the first week and reduced significantly ($p > 0.05$) at the third week. There was significant difference ($p > 0.05$) in the pH of pasteurised *fura de nunu* treated with sodium benzoate at refrigeration and ambient temperatures (Figure 4). pH of the pasteurised sample treated with sorbic acid was stable at week zero and week one but there was significant decrease from week two to week four at both refrigeration and ambient temperatures. There was no significant difference ($p > 0.05$) in pH of pasteurised *fura de nunu* treated with sorbic acid at refrigeration and ambient the pH of pasteurised *fura de nunu* treated with 300 mg/l sodium benzoate was stable with the value of 5.00, 4.97, 4.95 and 4.93 from week 0 to the third week and the decrease was significant at the fourth week of refrigeration storage. At ambient temperature the pH was stable from week 0 to the first week and reduced significantly ($p > 0.05$) at the third week. There was significant difference ($p > 0.05$) in the pH of pasteurised *fura de nunu* treated with sodium benzoate at refrigeration and ambient temperatures. pH of the pasteurised sample treated with sorbic acid was stable at week zero and week one but there was significant decrease from week two to week four at both refrigeration and ambient temperatures. There was no significant difference ($p > 0.05$) in pH of pasteurised *fura de nunu* treated with sorbic acid at refrigeration and ambient temperature during the four weeks of storage. Decrease in pH of *fura de nunu* was significant ($p > 0.05$) at week 0 and week four in unpasteurised *fura de nunu* and pasteurised sample, and there was a significant difference in their pH at refrigeration and ambient temperatures. There was progressive decrease in pH of the unpasteurised *fura de nunu* and the decrease was similar to that of pasteurised sample from the first week to fourth week of storage. It was observed that while the pH decreases, the titratable acidity increases with weeks of storage. It appears however, that the higher the storage temperature, the more pronounced the decrease in pH. This is expected, since the higher the storage temperature, the higher the rate of metabolism of sugar, hence, the higher the rate of acid production by the relevant microorganisms. Oyeyiola (1990) reported that production of acid during fermentation led to decrease in pH. These observations are in agreement with those reported by Fraizer, (1978) and Adebayo *et al*., (2010) who reported the production of acid from sugar by various metabolic microorganisms such as lactic acid bacteria, acetic and butyric acid bacteria.

3.8. Microbiological stability of fura de nunu

The shelf life of *fura de nunu* was extended by the combination of pasteurisation and chemical preservatives. The unpasteurised *fura de nunu* sample had the highest total viable count with less than one week shelf life when compared with fresh *fura de nunu* sample. Sodium benzoate was considered to be the best preservative in terms of lower microbial count. It was effective against lactic acid bacteria and other bacteria but, it was less effective against yeast. Pasteurised *fura de nunu* treated with sorbic acid had a good keeping quality throughout the storage period of 4 weeks especially at refrigeration temperature. The inhibitory effect of sorbic acid against yeast was observed as their number decreased over the period of storage at both ambient and refrigeration temperature. The lower the pH of *fura de nunu* the higher the inhibitory effect of sorbic acid against yeast but lactic acid bacteria count increased progressively with the storage period. In terms of viability of lactic acid bacteria, sorbic acid was considered as the best because it had little effect on the activities and growth of lactic acid bacteria. The microbial counts of *fura de nunu* samples were lower at refrigeration temperature than ambient storage temperature. There was no evidence of coliform in all the *fura de nunu* samples at ambient and refrigeration temperatures during the four weeks period of storage.

Table 7: Occurrence Pattern of Yeast in fura de nunu during Storage

Sample code	Yeast and mould	Week 0	Week 1	Week 2	Week 3	Week 4
A	*Saccharomyces cerevisiae*	-	-	-	-	-
	Hansenula anomala	-	-	-	-	-
	Rhodotorula glutinis	-	-	+	-	-
	Saccharomyces lactis	-	-	-	-	-
	Torulopsis versatalis	-	-	-	+	+
B	*Hansenula anomala*	-	-	+	+	+
	Saccharomyces lactis	-	+	+	+	-
	Saccharomyces cerevisiae	-	+	+	+	+
C	*Saccharomyces cerevisiae*	-	+	+	+	+
	Hansenula anomala	-	-	-	-	+
D	*Saccharomyces cerevisiae*	-	+	+	+	+
	Hansenula anomala	-	-	-	-	+
E	*Saccharomyces cerevisiae*	-	+	+	+	+
F	*Saccharomyces cerevisiae*	-	+	+	+	+

A: Unpasteurised *fura de nunu*, B: Pasteurised *fura de nunu*, C: Pasteurised *fura de nunu* treated with 300 mg/l sodium benzoate, D: Pasteurised *fura de nunu* treated with 200 mg/l sodium benzoate, E: Pasteurised *fura de nunu* treated with 1000 mg/l sorbic acid, F: Pasteurised *fura de nunu* treated with 750 mg/l sorbic acid.

+: Present, -: Absent

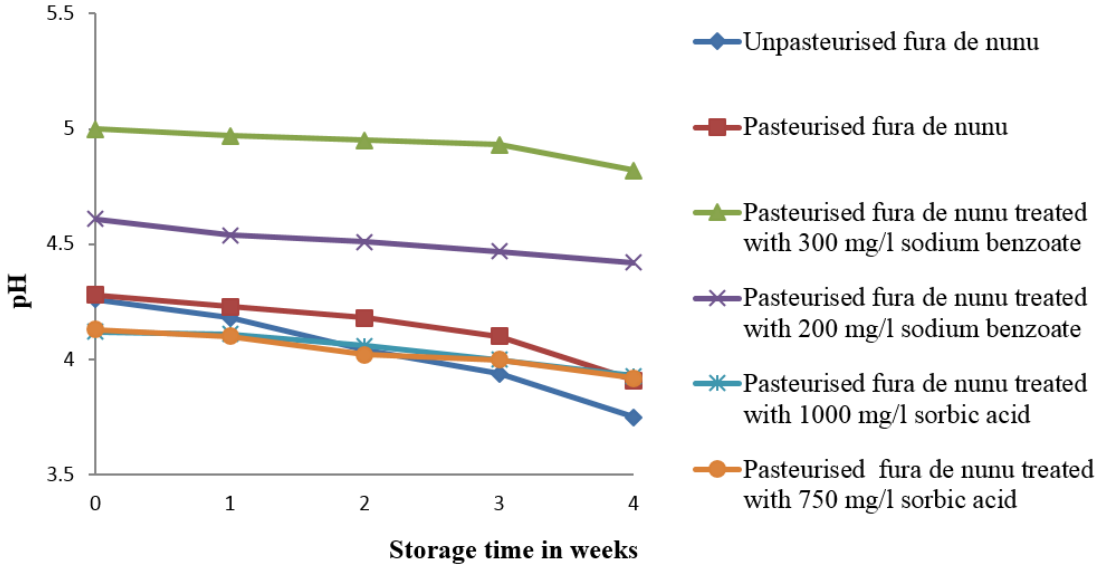

Figure 1. pH of Fura de nunu at Refrigeration Temperature

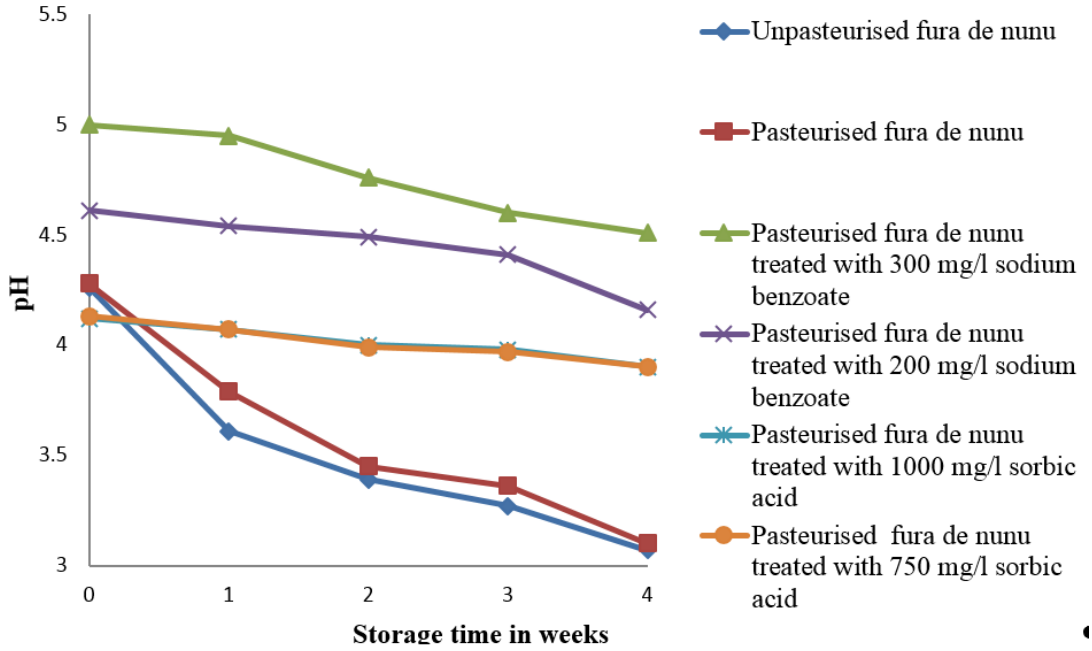

Figure 2. pH of Fura de nunu at Ambient Temperature

Akabanda *et al*., 2010 reported that *nunu*, however, was found to be microbiologically safe as no Enterobacteria survived by 48 h of fermentation when pH decreased below 4.0. The results showed that good quality *fura de nunu* with better shelf life can be produced by pasteurisation and addition of sodium benzoate and sorbic acid. Pasteurised *fura de nunu* sample treated with sodium benzoate had the lowest lactic acid bacteria population. The number of lactic acid bacteria in pasteurised *fura de nunu* treated with sorbic acid had higher population of lactic acid bacteria which are potentially probiotic organisms that help in maintaining intestinal balance.

4. CONCLUSION

The study has established that 200 and 300 mg L^{-1} of sodium benzoate when applied in combination with pasteurization at 70 °C for 15 minutes could effectively suppress Total Viable count, Lactic acid bacteria count and yeast count in *fura de nunu* for 4 weeks at ambient and refrigeration temperatures. It has also been found from this study that sorbic acid applied at 750 and 1000 mg L^{-1} together with pasteurization was also effective in extending the shelf-life of fura de nunu at both storage temperatures, though to a lesser extent. Also established in this study are the microbes associated with microbial deterioration of *fura de nunu* which are mainly LAB and yeasts.

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