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Full Length Research Paper

Effect of pasteurization and selected chemical preservatives on *Fura de nunu* during storage

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ABSTRACT

The study focused on effects of pasteurization, with or without sodium benzoate or sorbic acid on *fura de nunu* samples during storage. The products were assessed for proximate composition, total reducing sugar, individual sugars and titratable acidity. The results of the proximate composition were, 11.00 - 14.31 % (crude protein); 2.27 - 2.74 % (crude fat); 0.36 - 0.74 % (crude fibre); 1.31 - 1.46 % (total ash) and 84. 20 - 81.49 % (carbohydrate). Titratable acidity increased from 0.10 to 0.43 % and 0.51 % at refrigeration and ambient storage respectively. Total reducing sugar was within the range of 12.04 and 17.36 mg glucose/ml in samples stored at ambient temperature and 12.04 and 17.03 glucose mg/ml in samples stored at refrigeration temperature. The R_f value showed that lactose was present in all *fura de nunu* samples. Rate of biochemical changes were higher in *fura de nunu* samples stored at ambient temperature than refrigeration temperature. Pasteurization and chemical preservatives increased the stability of *fura de nunu* samples during storage. Least changes were observed in pasteurized *fura de nunu* treated with 300 mg/l sodium benzoate. The synergistic effect of pasteurization and chemical preservative improved the keeping quality of *fura de nunu* samples.

Keywords: Fermentation, chemical preservatives, pasteurization, proximate composition, sugar, refrigeration

INTRODUCTION

Fura de nunu is a fermented drink of West Africa origin. It is a nutritious beverage consisting of "*fura*'' made from millet grains and *nunu* a fermented milk product similar to yoghurt. *Fura de nunu* may be consumed as main food, refreshing drink and weaning food for infants (Umoh *et al*., 1988; Adebesin et al., 2001; Jideani et al., 2001).

Nunu is produced by allowing milk to undergo spontaneous fermentation at room temperature (Adebesin et al., 2001; Owuzu-Kwarteng and Akabanda, 2010). The nutritional composition of *nunu* depends on the source of milk, type of fermenting microorganisms and temperature of fermentation. *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 B complex (Nebedum and Obiakor, 2007).

Fura de nunu is produced in limited daily quantity because of its poor keeping quality. Pasteurization of milk is necessary to prevent the transmission of disease and to prevent microbial and enzymatic spoilage. It is designed to give maximum protection from milk-borne disease with minimum reduction in nutritional properties (Ihekoronye and Ngoddy, 1985). Preservatives are also added to fermented milk to inhibit spoilage microorganisms by interfering with their cell membrane, enzymatic activity or genetic mechanism (William and Westhoff, 1995). Low temperature reduces the metabolic activity of microorganisms and this has negative effect on their growth and reproduction (Jay, 1996; Frank, 1997). The aim of this study is to assess effect of pasteurization with or without preservatives on the chemical composition, sugars and titratable acidity of *fura de nunu* samples during storage at ambient and refrigeration temperatures with a view to extending the shelf life of the product.

MATERIALS AND METHODS

Milk and millet grains used for this study were obtained from Obafemi Awolowo Teaching and Research farm, Ile-Ife, Nigeria. The chemicals used were of analytical grade.

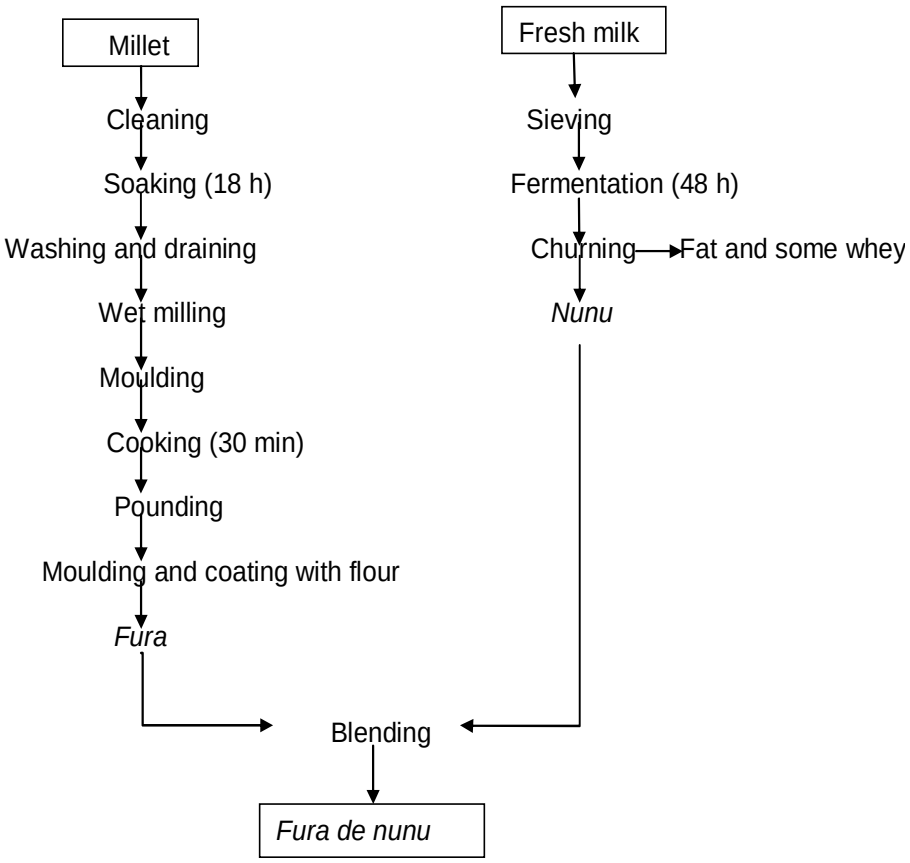

Figure 1. Process Flow Chart for *Fura de Nunu* Production

METHODS

Preparation of *fura de nunu*

Fura de nunu was produced using modified method of Owuzu-Kwarteng and Akabanda (2010) as shown in Figure 1. Cow milk was collected in a clean container; screened and left to ferment for 48 h at room temperature. The fermented milk was churned using a wooden ladle. Fat was removed and excess whey was drained off to obtain a product with a thick consistency (*nunu*). Millet grains were sorted, cleaned, steeped in water for 18 h at room temperature and wet milled. The paste was moulded into balls of about 10 cm in diameter and then cooked for 30 minutes. The cooked millet balls were pounded with mortar and pestle and moulded into smaller balls of about 4cm known as *fura*. *Nunu* was mixed with *fura* at ratio 3:1 and the mixture was packaged in sterile plastic bottles. The mixture was divided into six portions. Sample A was unpasteurized and B - F were pasteurized at 75 ^{o}C for 15 min in a water bath. Sample B was only pasteurized, sodium benzoate (300 mg/l) was added to sample C, sodium benzoates (200 mg/l) was added to sample D, sorbic acid (1000 mg/l) was added to sample E and sorbic acid (750 mg/l) was added to sample F. Samples were stored for a period of four weeks at ambient (28 ^{o}C) and refrigerated temperature (4 ^{o}C).

Proximate Composition of *fura de nunu*

The proximate composition was carried out in triplicate for each sample using AOAC (2000) and the mean result was recorded.

Determination of crude protein

Each sample (2.0 g) was measured into digestion flask. Kjedhal catalyst (0.8 g) was put in each flask with 15 ml of concentrated sulphuric acid added. Each flask was heated on pre heated digester for about 30 min in fume cupboard. This was digested until a clear homogenous mixture was obtained. After digestion, the flask was removed from the heater, cooled and the content was diluted with about 50 ml of distilled water. The flask was then placed in micro-kjedahl analyzer (distillation unit) where it received 5 ml of 40 % NaOH automatically. The mixture was subsequently heated up to release ammonia which was distilled into a conical flask containing 25 ml of

2 % boric acid for about 15 min. During the distillation process, the ammonia combined with boric acid to form ammonium borate solution which was titrated against 0.1 M HCl until a purplish- grey end point was attained (AOAC, 2000). The % protein was calculated as:

$$\% \text{ Protein} = \frac{A \times 0.0014 \times 6.25 \times 100}{\text{Weight of sample}}$$

Where A = volume (ml) of 0.1M HCl

Determination of crude fat content

Crude fat content was determined using soxhlet extractor with a reflux condenser and a distillation flask (previously dried and weighed). Each sample (2.0 g) was weighed into fat free extraction thimble plugged with cotton wool and placed in the appropriate chamber of the extractor. The distillation flask was filtered to two-third capacities with n-hexane and boiled on heating mantle, the distillate was collected until the extractor siphoned over for 4 h. Thereafter, n-hexane was recovered into a clean container and the remaining solvent in the distillation flask was evaporated in oven at 70 ^{o}C. The flask was allowed to cool in desiccator after which the final weight of the flask was determined. The difference in the final and the initial weights of the distillation flask represented the oil extracted from the sample (AOAC, 2000).

$$\% \text{ Crude fat} = \frac{\text{final weight of the flask} - \text{Initial weight of flask}}{\text{Weight of flask}} \times 100$$

Determination of crude fibre content

Each sample (5 g) was transferred into 600 ml beaker and 700 ml of 1.25% sulphuric acid added. Each beaker was heated for 30 min while being rotated periodically to prevent adherence of solids to the side of the beakers. Thereafter, each solution was filtered and rinsed with 50 ml boiling water. This was repeated with three 50 ml portions of water and subsequently sucked dry. The entire residue was reduced and remained in the beaker with boiling 1.25 % NaOH added and boiled with each residue after which the content of each beaker was removed and filtered as described above. This was then washed with 25 ml of 1% sulphuric acid, three portions of 50 ml water and 25 ml ethanol. The residue was later transferred into ashing dish and dried at 103 ^{o}C. This was followed by cooling in desiccator and weighing. The residue was thereafter ignited at 600 ^{o}C for 30 min in muffle furnace, cooled in the desiccator and reweighed (AOAC, 2000). The percentage crude fibre was calculated as:

$$\% \text{ Crude Fibre} = \frac{\text{Loss in weight on ignition}}{\text{Weight of sample}} \times 100$$

Determination of total ash content

Each sample (5 g) was measured accurately into a previously ignited, cooled and weighed crucible. A few drops of glycerol was added, mixed thoroughly and heated until the sample charred. The crucible was transferred into a muffle furnace set at 550 ^{o}C until a white grey ash was obtained. The crucible was cooled in desiccator and reweighed (AOAC, 2000). The percentage ash was calculated as:

$$\% \text{ Total ash} = \frac{\text{Weight of Ash}}{\text{Weight of sample}} \times 100$$

Determination of carbohydrate content

The carbohydrate content of each *fura de nunu* sample was calculated by difference. The total of all the previously determined proximate parameters subtracted from 100 represent the carbohydrate content.

$$\% \text{ Carbohydrate} = 100 - (\% \text{ Moisture} + \% \text{ Crude protei}$$

$$\% \text{ Crude fibre} + \% \text{ Total}$$

ash)

Determination of total reducing sugar content of *fura de nunu*

The stock solution was prepared by dissolving 0.1 g of glucose in 100 ml (1mg/ml) of distilled water. The solution was dispensed into the test tubes as follows: 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 ml. The volume of sugar solution in each test tube was made up to 1ml and 2 ml of Dinitrosalicyclic acid reagent was added. The solution was boiled for 5 min in Gallenkamp water bath and test tubes were cooled under running water. Distilled water (7 ml) was added to each tube and absorbance was read at 540 nm in a UV Spectrophotometer. Each sample was diluted five folds with distilled water and filtered through Whatman No 1 filter paper. The filtrate (1 ml) was dispensed into test tube, 2 ml of Dinitrosalicyclic acid reagent was added and the mixture was boiled in Gallenkamp water bath for 5 min. After boiling, the tubes were thoroughly cooled under running water and 7 ml of distilled water was added. The absorbance was read against reagent blank at 540 nm in a UV Spectrophotometer. Amount of reducing sugars in each sample was extrapolated from the glucose calibration curve (Abiose and Adedeji, 1994).

Table 1.Proximate Composition of Fresh *Fura de Nunu*

***Fura de nunu* samples**	**Crude protein**	**Crude fat**	**Crude fibre**	**Total ash**	**Carbohydrate**
Unpasteurized *fura de nunu*	11.00^{f}	2.74^{a}	0.71^{b}	1.35^{ab}	84.20^{a}
Pasteurized *fura de nunu*	11.37^{e}	2.57^{c}	0.74^{a}	1.46^{a}	83.86^{b}
Pasteurized *fura de nunu* with 300 mg/l sodium benzoate	12.47^{a}	2.48^{d}	0.58^{e}	1.36^{b}	83.11^{f}
Pasteurized *fura de nunu* with 200 mg/l sodium benzoate	12.36^{b}	2.48^{d}	0.57^{e}	1.33^{bc}	83.26^{d}
Pasteurized *fura de nunu* with 1000 mg/l sorbic acid	12.19^{c}	2.64^{b}	0.64^{d}	1.33^{bc}	83.20^{e}
Pasteurized *fura de nunu* with 750 mg/l sorbic acid	12.14^{d}	2.57^{c}	0.67^{c}	1.31^{d}	83.31^{c}

Means followed by different superscript in the same column are significantly different at $p < 0.05$

Table 2. Proximate Composition of *Fura de Nunu* at End of Refrigerated Storage

***Fura de nunu* samples**	**Crude protein**	**Crude fat**	**Crude fibre**	**Total ash**	**Carbohydrate**
Unpasteurized *fura de nunu*	13.20^{c}	2.52^{a}	0.36^{d}	1.54^{b}	82.38^{a}
Pasteurized *fura de nunu*	13.06^{d}	2.40^{c}	0.50^{a}	1.64^{a}	82.40^{a}
Pasteurized *fura de nunu* with 300 mg/l sodium benzoate	13.76^{a}	2.38^{cd}	0.40^{c}	1.46^{c}	82.00^{e}
Pasteurized *fura de nunu* with 200 mg/l sodium benzoate	13.48^{b}	2.37^{d}	0.36^{d}	1.46^{c}	82.33^{b}
Pasteurized *fura de nunu* with 1000 mg/l sorbic acid	13.43^{b}	2.50^{a}	0.45^{b}	1.47^{c}	82.15^{d}
Pasteurized *fura de nunu* with 750 mg/l sorbic acid	13.40^{b}	2.46^{b}	0.45^{b}	1.48^{c}	82.21^{c}

Means followed by different superscript in the same column are significantly different at $p < 0.05$

Table 3. Proximate Composition of *Fura de Nunu* at the End of Storage at Ambient Temperature

***Fura de nunu* samples**	**Crude protein**	**Crude fat**	**Crude fibre**	**Total ash**	**Carbohydrate**
Unpasteurized *fura de nunu*	14.31^{a}	2.30^{c}	0.24^{b}	1.66^{b}	81.49^{e}
Pasteurized *fura de nunu*	13.80^{c}	2.27^{d}	0.38^{a}	1.75^{a}	81.80^{d}
Pasteurized *fura de nunu* with 300 mg/l sodium benzoate	13.90^{b}	2.33^{c}	0.36^{a}	1.49^{d}	81.92^{c}
Pasteurized *fura de nunu* with 200 mg/l sodium benzoate	13.71^{d}	2.30^{c}	0.32^{a}	1.50^{d}	82.17^{a}
Pasteurized *fura de nunu* with 1000 mg/l sorbic acid	13.60^{f}	2.44^{a}	0.40^{a}	1.51^{cd}	82.05^{b}
Pasteurized *fura de nunu* with 750 mg/l sorbic acid	13.67^{e}	2.38^{b}	0.39^{a}	1.53^{c}	82.03^{b}

Means followed by different superscript in the same column are significantly different at $p < 0.05$

Identification of individual sugar content of *fura de nunu*

Thin layer chromatography was employed in the identification of sugar. Sugar standards containing 0.5 % w/v maltose, lactose, glucose and galactose was prepared. The solvent system was prepared by mixing 60 % ethyl acetate, 15 % glacier acetic acid, 15 % ethanol and 10 % distilled water. *Fura de nunu* samples were diluted five folds with distilled water. Standard sugar solutions (10 µg) and *fura de nunu* samples (10 µg) were spotted on the chromatograph plate with the aid of micro pipette. Plate was dried and placed in chromatograph tank containing the solvent system for 1 h. The plate was

removed and dried at room temperature, sprayed with detection agent containing 0.2 ml of concentrated sulphuric acid, 20 mg of naphthol recorsinol and 10 ml of 90 % ethanol and dried in the oven at 100 ^{o}C for 10 min (Adeniran and Abiose, 2012).

$$R_f = \frac{\text{Distance travelled by substance}}{\text{Distance travelled by solvent}}$$

Determination of titratable acidity

Titratable acidity was determined by measuring 1 ml aliquot portion of each *fura de nunu* sample into 250 ml Erlenmeyer flask, 10 ml of distilled water and 3 drops of phenolphthalein indicator were added and titrated against 0.1 M sodium hydroxide (NaOH) solution (AOAC, 2000).

Statistical Analysis

Data obtained were subjected to descriptive and inferential statistics (Analysis of Variance) using SPSS (version 17 incorporation, Chicago, Illinois, USA). Means of samples were separated using Duncan Multiple range Test.

RESULTS AND DISCUSSION

Proximate composition of *fura de nunu*

The proximate composition of *fura de nunu* is shown in Tables 1, 2 and 3. There was a general increase in the crude protein and total ash and decrease in crude fat, crude fibre and carbohydrate contents. Crude protein content was highest in pasteurized sample treated with 300 mg/l sodium benzoate (12.47 %) and lowest in unpasteurized sample (11.00 %) at week zero. The increase was generally higher at ambient storage than refrigeration storage and the highest increase was recorded in unpasteurized *fura de nunu* while the lowest was observed in pasteurized sample treated with sorbic acid followed by pasteurized sample treated with sodium benzoate. Increase in crude protein content could be due to increase in the number of microbes since they are single cell protein or increasing density of microorganism since they have ability to synthesis new protein from amino acid. Nebedum and Obiakor (2007) reported that protein levels decreased in the preserved and unpreserved *nunu*, indicating proteolysis. Tsaio et al. (1975) stated that the increase in protein could be due to mobilization of storage nitrogen producing the nutritionally high quality proteins.

Crude fat content was highest in unpasteurized *fura de nunu* (2.74 %) and lowest in pasteurized *fura de nunu* treated with sodium benzoate (2.48 %) at week zero. There was no significant difference ($p > 0.05$) in the fat content of pasteurized samples treated with sodium benzoate at week zero. The highest decrease in crude fat content was observed in unpasteurized *fura de nunu*, where the crude fat content decreased from 2.74 % to 2.52 % at refrigeration temperature and 2.30 % at ambient temperature while the lowest decrease was recorded in pasteurized samples treated with 300 mg/l sodium benzoate. The observed decrease in crude fat content might be due to the increased activities of the lipolytic enzymes (Raham and Aal, 1986), which hydrolyse fats to fatty acids and glycerol (Inyang and Zakari, 2008). Heat resistant extracellular lipases produced by psychrotrophic bacteria before processing represent a major spoilage factor of stored milk (Sorhaug and Stepaniak, 1997).

Crude fibre content was highest in pasteurized sample (0.74 %) and lowest in pasteurized sample treated with 200 mg/l sodium benzoate (0.57 %) at week zero. The decrease in crude fibre was higher in samples stored at ambient temperature than refrigeration temperature. Highest decrease was recorded in unpasteurized sample followed by pasteurized sample while the lowest decrease was recorded in pasteurized sample treated with 300 mg/l sodium benzoate. Liberation of cellulolytic enzyme by fermenting microbes could have caused a reduction in the crude fibre content over the period of storage.

Total ash content was highest in unpasteurized *fura de nunu* (1.46 %) while the lowest value was recorded in pasteurized samples treated with 1000 mg/l sorbic acid (1.31 %) at week zero (Table 1). Increase was generally higher at ambient temperature than refrigeration temperature. The increase was highest in unpasteurized sample and lowest in pasteurized sample treated with 300 mg/l sodium benzoate. Eissa et al. (2011) also reported increase in total ash content of yoghurt with increase in storage period.

Pasteurized *fura de nunu* had the highest carbohydrate content (84.20 %) while the pasteurized sample treated with 300mg/l sodium benzoate (83.11 %) had the lowest carbohydrate content at week zero. The increase was highest in the unpasteurized sample and lowest in pasteurized sample treated with 200 mg/l sodium benzoate followed by pasteurized sample treated with 1000 mg/l sorbic acid. The increase was higher at ambient storage temperature than at refrigeration storage temperature. Kazanas and Field (1981) attributed the decreased in carbohydrate content to increase activities of amylolytic enzyme which hydrolyse starch and other carbohydrates to simple sugars or other compounds which were utilized as carbon sources by the fermenting microorganisms. Changes in proximate composition of *fura de nunu* was generally higher in unpasteurized sample than pasteurized sample, however, addition of sodium benzoate or sorbic acid further decreased the changes and the least changes was recorded in

Table 4. Total Reducing Sugar of *Fura de Nunu* during Storage (mg glucose /ml)

Fura de nunu sample	Fresh *Fura de Nunu*	*Fura de Nunu* Stored at Refrigerated Temperature	*Fura de Nunu* Stored at Ambient Temperature
Unpasteurized *fura de nunu*	12.04[f]	16.86[b]	17.36[a]
Pasteurized *fura de nunu*	12.73[d]	17.03[a]	16.97[b]
Pasteurized *fura de nunu* with 300 mg/l sodium benzoate	13.22[b]	16.42[d]	16.47[e]
Pasteurized *fura de nunu* with 200 mg/l sodium benzoate	13.42[a]	16.68[c]	16.84[c]
Pasteurized *fura de nunu* with 1000 mg/l sorbic acid	13.11[c]	16.35[e]	16.64[d]
Pasteurized *fura de nunu* with 750 mg/l sorbic acid	12.26[e]	16.30[f]	16.48[e]

Means followed by different superscript in the same column are significantly different at $p < 0.05$

Table 5: R_f Value of Sugars in *Fura de Nunu*

Fura de nunu sample	R_f Values	Sugar Standards	R_f Values
Unpasteurized *fura de nunu*	0.091	Glucose	0.179
Pasteurized *fura de nunu*	0.091	Galactose	0.161
Pasteurized *fura de nunu* with 300 mg/l sodium benzoate	0.091	Maltose	0.110
Pasteurized *fura de nunu* with 200 mg/l sodium benzoate	0.091	Lactose	0.091
Pasteurized *fura de nunu* with 1000 mg/l sorbic acid	0.091		
Pasteurized *fura de nunu* with 750 mg/l sorbic acid	0.091		

pasteurized sample treated with 300 mg/l sodium benzoate.

Total reducing sugar content and individual sugar content of *fura de nunu*

There was general increase in the total reducing sugar content of *fura de nunu* samples during storage (Table 4). It was within the range of 12.04 and 17.36 mg glucose /ml. It was highest in unpasteurized sample and lowest in pasteurized sample treated with 200 mg/l sodium benzoate at the beginning of storage. It significantly increased ($p < 0.05$) in all samples from the beginning of storage to the fourth week of both ambient and refrigeration temperatures. It was lower at refrigeration temperature than ambient temperature except in pasteurized sample. Changes in total reducing sugar may be attributed to breakdown of carbohydrate into fermentable sugars by the fermenting microorganisms and their enzymes. It has been reported that amylase activity increases the level of fermentable and reducing sugars in foods (Goesaert et al., 2006). The R_f value of *fura de nunu* samples were in the same range with R_f value of lactose as shown in Table 5. This is expected because lactose is the principal carbohydrate in milk (McGee, 1984).

Titratable acidity of *fura de nunu*

The titratable acidity of *fura de nunu* samples increased with increase in period of storage. It increased from 0.10 % to 0.28 % and 0.10 % to 0.41 % during four weeks of refrigerated and ambient storage respectively. The increase was higher at ambient temperature than refrigeration temperature as shown in Figures 2 and 3. The increase was highest in unpasteurized sample followed by pasteurized sample at both ambient and refrigeration storage. The least increase was observed in pasteurized sample treated with 300 mg/l sodium benzoate. Increase in titratable acidity has been attributed to production of organic acids by fermenting microorganisms (Oyeyiola, 1990; Vergas et al., 2008; Owusu-Kwarteng and Akabanda, 2010). Higher titratable acidity at ambient storage temperature was due to higher rate of metabolism of sugar, hence, higher rate of acid production by the microorganisms. It has been reported that weak acids may have bacteriocidal or bacteriostatic effect on microorganisms and storage at low temperature

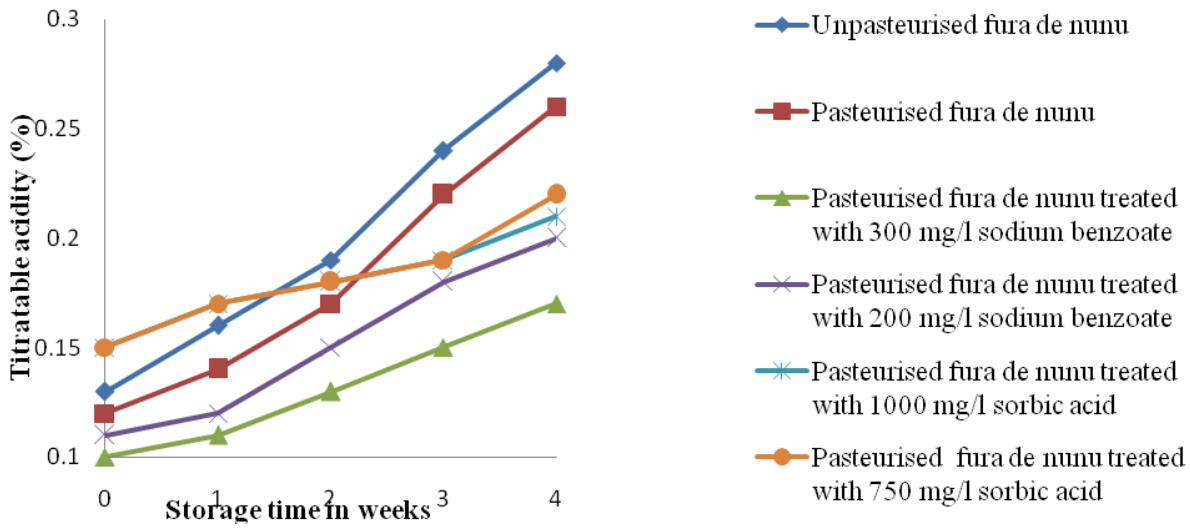

Figure 2. Titratable Acidity of *Fura de Nunu* at Refrigeration Temperature

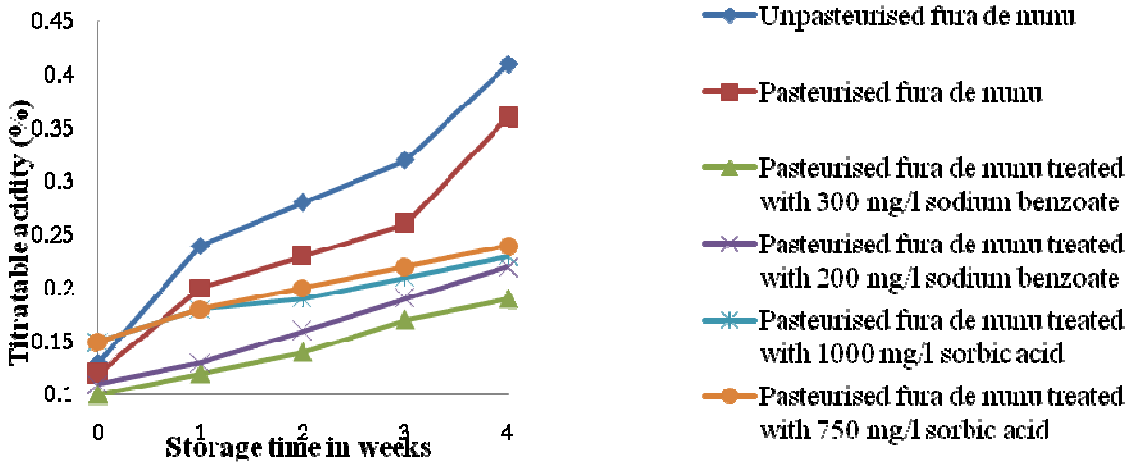

Figure 3. Titratable Acidity of *Fura de Nunu* at Ambient Temperature

also reduces the metabolic activity of microorganisms and their enzymes (Adams and Moss, 1999; Nebedum and Obiakor, 2007).

CONCLUSION

The result showed that samples were more stable at refrigeration temperature than ambient temperature. Sodium benzoate was considered to be better preservative in *fura de nunu* in terms of stability in chemical composition and sample preserved with 300 mg/l sodium benzoate was the most preferred. However, combined methods of pasteurization and chemical preservation, with refrigeration was more effective. Knowledge of biochemical changes that is associated with the spoilage of *fura de nunu* using different methods of preservation will enhance the production of safe products with extended shelf life and also encourage production on a larger scale.

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