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Microbiological Quality of a Locally Fermented Milk-Cereals Mixture (*fura de nunu*) Sold in Owerri Metropolis, Imo State, Nigeria

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Abstract. The microbiological quality of two batches of *fura de nunu* sample was studied. The samples were obtained from six different hawkers at one-week interval in Mami Market, Obirinze, Owerri Metropolis. The same *fura de nunu* hawker sampled in week 1 and week 2. All the samples collected were analyzed microbiologically. The data obtained were subjected to One-way ANOVA to test for significant difference. The viable count showed variation in the colony count in Week 1 and week 2 from the same hawker. The coliform count showed variation from the same hawker. The gram reaction of bacteria obtained from different hawkers shows that gram positive and gram-negative were more predominant. The result ranged from 3.8×10^7 cfu/ml to 7.1×10^7 cfu/ml. The microbial limit for the total viable colony count is 1.0×10^2 cfu/ml. *E. coli* was confirmed to be present in almost all the samples except sample A, B and F. The presence of *E. coli* indicates the possibility of other pathogenic microorganisms present in the samples. The presence of coliform in *Fura de nunu* samples indicates probable fecal contamination and the presence of *E. coli* bacteria in all most all the samples are the major biotype of the family. It is recommended that a standard processing method that will ensure *Faru de nunu* of the highest microbial and nutritional quality be developed and the technology transferred to the local producers.

Keywords: *Fura de nunu*, pathogenic bacteria, *coliform bacteria*, *E. coli, viable count, hawker.*

1 Introduction

Milk is an opaque white liquid produce or secreted by the mammary glands of female mammals. Milk provides the primary source of nutrition for new borns before they are able to digest other types of food. The early lactation milk contains colestrum which carries the mother's antibodies to the baby and it can reduce the risk of many diseases in the baby. Milk is an excellent culture medium for many kinds of microorganisms [1].

Fermented milk cereal mix (*Fura de nunu*) is a beverage which is a two-in-one product, consisting of a cereal "*Fura*" made from millet and "*nunu*" a fermented milk product similar to yoghurt. *Nunu* pronounced *Ronino* is the Hausa word for fresh milk. *Nunu* as it is called by Igbo is a liquid food drink got from fermented raw milk. It is a healthful food whose consumption transverse the Saharan tribes of West Africa. Sub-region extending to the inhabitants of the Mediterranean region also the Middle East. In the Middle East, it is called *dahi* or *lassi* [2].

It contains good qualities of amino acids, calcium, phosphorus and vitamins A, C, E and the B complex. The fresh milk is usually collected early in the morning in calabash bowls before being pasteurized locally. This is done by boiling to a certain level to kill whatever contaminants. It's then left to cool and once this happens, the curd separates from the whey. The curd is skimmed off and used in the preparation of local cheese or butter while the whey (milk) is left to ferment with its own natural bacteria for some few hours, thereby converting it to yoghurt while *Fura* is usually prepared by mixing the dry flour ingredient (millet and spices together) followed by addition of a little water just enough to act as a binding agent for the dry ingredients. It is then mold into medium size balls and put into a pot of water and then heat for about 7minutes and when it starts to boil, the molded balls is dropped into

the water and leave to boil for about 20 minutes after which it is transferred into a mortar and pound. Thoroughly remold once more into small balls and sprinkle corn flour to keep the balls moist [3].

Fura de Nunu is served by pouring the *Nunu* into a bowl followed by addition of *Fura* (molded millet) which then mashed into paste, sugar is added to taste and it is then eaten with spoon. Predominantly, *Nunu* is being prepared and hawked by the nomadic Hausa/Fulani cattle herdsmen, who controls over soil of Nigerian's cattle production and consumption of *Nunu* was limited to Fulani/Hausa indigenes [4]. Since most non indigenes see their preparation as apparently unhygienic and since it has poor shelf life. The poor handling of *Fura de Nunu* during processing and marketing exposes it to microbial contamination. Uzuegbu and Eke [5] reported that unhygienic environment and handling of food product by the retailers could be another reason to be given for the post-processing contamination. Houseflies are always found in large numbers at the production sites and sale outlets.

Shehu and Adesiyuh [6] reported that in order to increase the volume and improve the colour of *nunu*, the female hawkers, and prior to sale, engage in the fraudulent act of adding stream water and a milky white supernant of water-soaked baobab tree seeds. This act could further lead to the contamination and spoilage of this product. *Fura de nunu* offered for sale is usually poorly handled and presented to consumers mostly in unhygienic manner. In addition, raw milk has low keeping quality and at room temperature, spontaneous microbial spoilage occurs turning the product sour for some days. This is brought about by the activity of lactic bacteria [7]. Depending on its preservation and process-line, microorganisms other than lactic acid bacteria could be found in the *nunu*. It is noticed that raw milk often contains microorganisms which may likely cause foodborne diseases [8,9]. Even when the milk is fermented, the fermentation process with the attendant drop in pH may not rid the product of these organisms and may be carried to consumers.

This study was undertaken to determine the microbiological quality of *fura da nunu* hawked in Owerri metropolis and determine how different food handlers contribute in the transmission of food borne diseases to the food samples by testing for the presence and absence of *E. coli* in the food sample as well as to determine the level of sanitary and hygienic measures adopted by different hawkers of the food sample. It hoped that the findings will help in assessing its quality and safety for human consumption.

2 Materials and Methods

2.1 Collection of Material

Two batches of food samples (*fura de nunu*) (handmade local yoghurt) were collected at the same time from six different hawkers at Mami market Owerri metropolis in a sterilized bottle, they were clearly labelled and stored in refrigerator before analysis.

2.2 Preparation of Media

Peptone water, nutrient agar, MacConkey agar, EMB agar were prepared according to methods described by Uzuegbu *et al.* [10].

Peptone Water: This was used as diluents 35.5g of 0.1%. Peptone water was weighed and poured into a 500ml of distilled water in a conical flask and stirred thoroughly to dissolve [11].

Nutrient Agar: Fourteen gram (14g) of nutrient agar was weighed and poured into 500ml of distilled water in a conical flask and allowed to soak for 15minutes. It was stirred to obtain a homogeneous mixture and thereafter sealed with an aluminum foil and left on the table at room temperature [11].

MacConkey Agar: This media was used for coliform counts. About 26g of MacConkey agar was weighed and poured into 500ml of distilled water in a conical flask and allowed to soak for 15minutes. Thereafter it was stirred to obtain a uniform mixture and later covered with an aluminum foil and allowed on the table at room temperature [11].

Eosin methylene Blue Agar Medium (EMB): About 5.1g of the EMB agar powder was dissolved in 100ml of distilled water. It was stirred and dissolved, sterilized in an autoclave for 15 minutes at 121°C. It was allowed to cool after which it was dispersed into petri dishes [11].

2.3 Serial Dilution

About 9ml of the solution was introduced into the universal/MacConkey bottle. It was then autoclaved for 15minutes at 121°C and allowed to cool down after which 1ml of food sample (*fura de nunu)* was introduced into the first universal/MacConkey bottle, also 1ml was taken from the first bottle and introduced into the second bottle up to the seventh dilution [12].

2.4 Total Viable Counts

2.4.1 Inoculation of the medium (nutrient agar)

1ml of diluted samples were aseptically plated in duplicates directly onto the nutrient agar plates. The plates were incubated at room temperature (27°C for 24hours. Colonies which developed on the plates were counted using the colony counter (Model 6399/ Staurt Scientific Co. Ltd, Great Britain) and expressed as colony forming units per millimeter (cfu/ml) of samples. The colonies differing in size, shape and colour were selected from the different plates on nutrient agar and sub-cultured repeatedly to obtain pure isolates [13].

2.4.2 Inoculation of the medium (MacConkey agar) for total counts

Serially diluted 1ml of sample were aseptically platted on MacConkey agar for the enumeration of total coliform counts. The plate was incubated at room temperature (27°C) for 24hours. Colonies which developed on the plates were counted using the colony counter and expressed as colony forming units per millimeter (cfu/ml) of samples [13].

2.5 Gram Staining

The loop is place on top of the colony were transferred to the drop, and emulsify. The film was allowed to air dry. The film was fixed by passing it briefly through the bursen flame two or three times without exposing the dry film directly to the flame. The slide is flood washed with crystal violet solution for one minute, and later washed off briefly with tap water. Drain flood washing off iodine solution and allow to act as (mordant) for about one minute, wash with tap water and drain. Excess water is removed and 95% alcohol applied for 30 seconds, the slide is washed up with tap water and drain flood again with safranin solution with bibulous paper and finally examined under the oil immersion microscope [10].

2.6 Streak Plate Method

The EMB agar was measured out at the required amount, dissolved in peptone water and autoclaved. The EMB agar was allowed to cool at a temperature of 45°C. It is poured into the sterile petri dish and allowed to solidify. The cells or young growth were transferred to the edge of the EMB agar plate with a sterile inoculating loop or swab. Then, streaked out over the surface in one of the several patterns. After the first sector was streaked, the inoculating loop was sterilized by burning it on the furnace and an inoculum for the second sector was obtained from the first sector. A similar process was followed for streaking the third sector, except that the inoculum was from the second sector. After that, it was left under room temperature for 24 h for it to develop into separate colonies [13].

Statistical Analysis: The data obtained from the experiment were analyzed using One-Way Analysis of Variance (ANOVA) and the data were evaluated for significant difference ($P<0.05$) in their means. Differences between means were separated using the Fisher's Test-Least Significant Difference (LSD) [14,15].

3 Results and Discussion

3.1 Total Viable Count

The average viable bacteria count of the sample (*fura de nunu)* was obtained in Figure 1 and Table 1. The result showed that HKW5 has the highest microbial load compared to other hawkers while HKW4 has the lowest microbial count. The result obtained ranged from 3.0×10^7 cfu/ml to 7.0×10^7 cfu/ml in

the first week interval. During the second week interval, the result obtained shows that HKW5 has the highest microbial load followed by HKW2 when compared to other samples and HKW4 has the lowest microbial load. The result ranged from 3.8×10^7 cfu/ml to 7.1×10^7 cfu/ml. The result obtained were out of standard since the microbial limit for the total viable colony count is 1.0×10^2 cfu/ml (Figure 2 and Table 2). The variation obtained from the viable count could be as a result of uncontrolled fermentation procedure during the preparation of *fura de nunu* from the same hawker. The preparation procedure of *fura de nunu* is still traditional arts and the fermentation is uncontrolled, starter cultures are not normally used therefore variation in the quality and stability of the product is often observed. The technological and hygienic problems of traditional fermented food (*fura de nunu)* need to be addressed in other to reduce losses due to wasteful and in subsequent fermentation pathways, poor quality and un stable shelf life of the product [16].

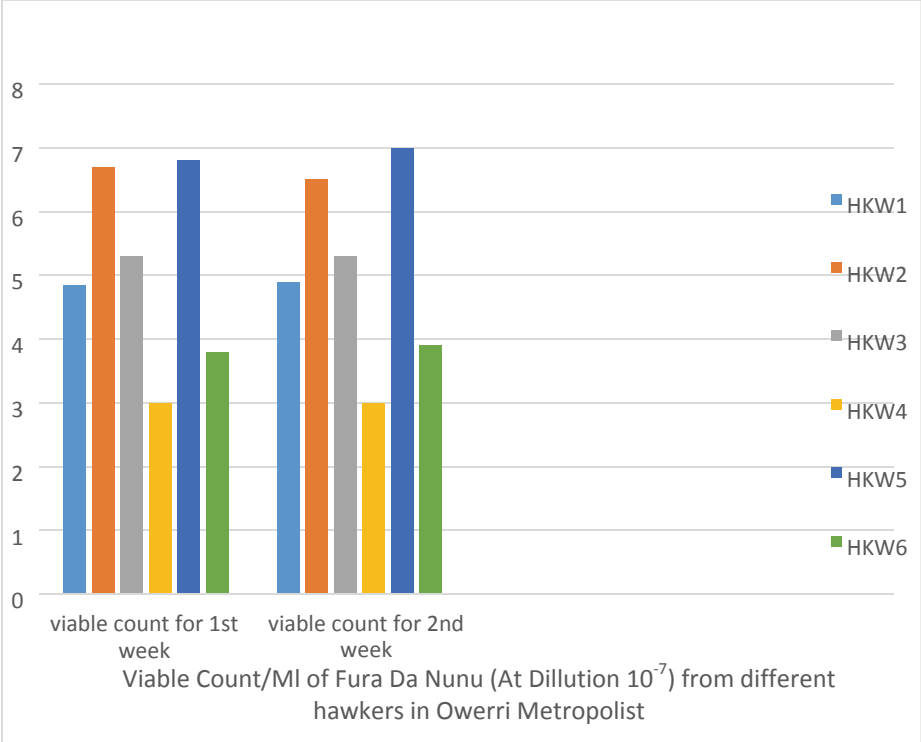

Figure 1. Column chart representing total viable count of two batches of food samples (*fura de nunu*) from six different hawkers at 1st and 2nd week interval after 24 hours

Table 1. Viable count of two batches of food samples (*fura de nunu*) from six different hawkers at 1st and 2nd week interval after 24 hours

Samples	Mean (cfu/g)
HWK1	$4.8 \times 10^{7\,a}$
HWK2	$6.575 \times 10^{7\,a}$
HWK3	$5.25 \times 10^{7\,a}$
HWK4	$3.225 \times 10^{7\,a}$
HWK5	$5.65 \times 10^{7\,a}$
HWK6	$3.80 \times 10^{7\,a}$

Means with the same superscript for viable count are not significant different from six different hawkers of food sample *fura de nunu* for week 1 and week 2

KEY: HWK1 = Hawker 1, HWK3 = Hawker 2, HWK3 = Hawker 3, HWK4 = Hawker 4, HWK5 = Hawker 5, HWK6 = Hawker 6, Cfu/ml: Colony foaming unit per milliliter

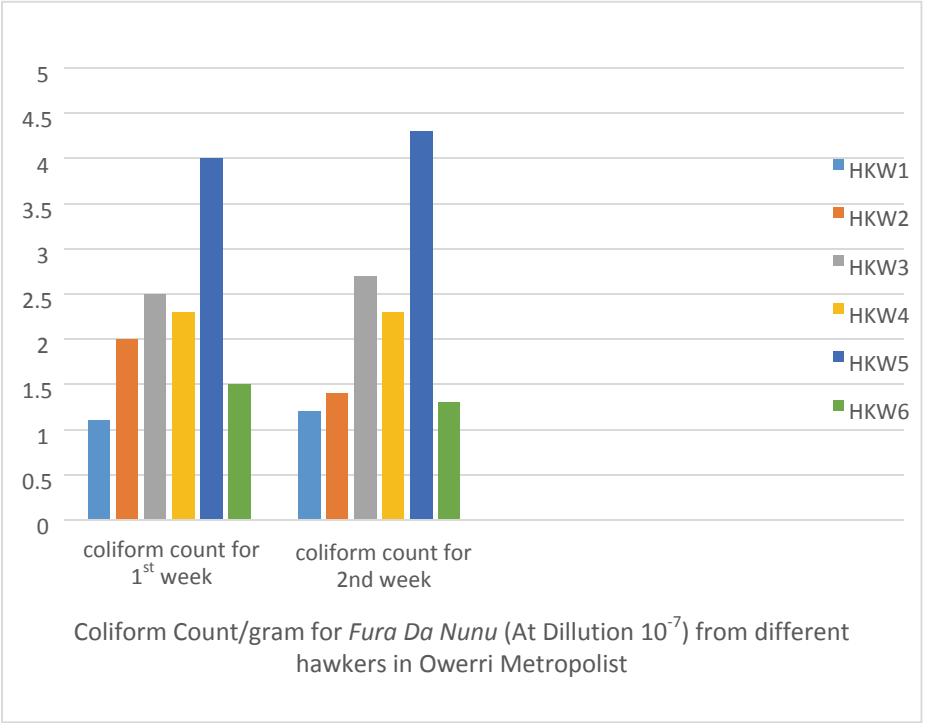

Figure 2. Column chart representing total coliform count of two batches of food samples (*fura de nunu*) from six different hawkers at 1st and 2nd week interval after 24 hours
KEY: HKW1= Hawker 1, HKW2= Hawker 2, HKW3= Hawker 3, HKW4= Hawker 4, HKW5= Hawker 5, HKW6= Hawker 6. Cfu/ml: Colony foaming unit per gram

Table 2. Coliform count of two batches of food samples (*fura de nunu*) from six different hawkers at 1st and 2nd week interval after 24hours

Samples	Mean
HWK1	$1.1\times 10^{7\,a}$
HWK2	$1.72\times 10^{7\,d}$
HWK3	$2.625\times 10^{7\,b}$
HWK4	$2.25\times 10^{7\,c}$
HWK5	$4.125\times 10^{7\,a}$
HWK6	$1.34\times 10^{7\,e}$
LSD	0.086

Mean with different superscript for coliform count are not significant different from six different hawkers of food sample *fura de nunu* for week 1 and week 2
KEY: HWK1 = Hawker 1, HWK3 = Hawker 2, HWK3 = Hawker 3, HWK4 = Hawker 4, HWK5 = Hawker 5, HWK6 = Hawker 6

3.2 Gram Staining Reaction

From Table 3, the result obtained shows that Gram-Positive and Gram-Negative clusters of cocci and rod shapes are predominant, they exist in all food sample (*fura de nunu*) and microorganisms obtained varies in Gram reaction from different hawkers for both first- and second-week intervals. The Gram-positive shows blue and purple colour and they are cluster of Bacilli rod in shape. The Gram-Negative shows pink and red colour and are cluster of cocci or round and some of the gram-negative rod observed could be *E. coli* and or coliforms. The variation in the morphology of microorganism obtained in weekly basis of the same hawker of the food sample (*fura de nunu*) indicate differences in their weekly sanitary procedure adopted during the production process.

Table 3. Gram staining reaction of two batches of food samples (*fura de nunu*) from six different hawkers at 1st and 2nd week interval

Samples	Hwk1	Hwk2	Hwk3	Hwk4	Hwk5	Hwk6
1st week interval	Cluster gram +ve bacilli (rod) (blue purple)	Cluster Gram +ve bacilli (rod) (blue purple)	Cluster of Gram –ve cocci pink/red	Cluster Gram +ve rod blue/purple	Cluster of Gram +ve. Cocci blue/purple	Cluster of Gram –ve bacilli (pink/red)
2nd week interval	Cluster of Gram +ve cocci	Cluster of Gram +ve rod	Cluster of Gram +ve rod	Cluster Gram +ve rod blue/purple	Cluster of Gram +ve cocci blue/purple	Cluster of Gram –ve bacilli (rod) (pink/red)

KEY: Gram +ve= blue/ purple, Gram –ve= pink/red, HWK1 = Hawker 1, HWK3 = Hawker 2, HWK3 = Hawker 3, HWK4 = Hawker 4, HWK5 = Hawker 5, HWK6 = Hawker 6

3.2.1 The result of E. coli in two batches of Fura de nunu from six different hawkers at 1st and 2nd week interval after 24hours

The result obtained from the test of *E. coli* as shown in Table 4 indicates the presence of *E. coli* in almost all the samples except, HWK 1 and HWK 2. *E. coli* showed a characteristics green metallic sheen on EMB agar in HWK 2, 3, 4, 5. In the first week interval with no colour observation in HWK 1 and HWK 6 samples. Also, in the second week interval *E. coli* showed a characteristics green metallic sheen on EMB agar in HWK 3, 4 and 5 samples with no colour observation in HWK 1, 2 and 6 samples.

Table 4. Eosin methylene blue agar (emb) test for the presence/absence of *E. coli* in two batches of food samples (*fura de nunu*) from six different hawkers at 1st and 2nd week interval after 24hours

Samples	Colour of EMB for 1 week	Present/Absent of E.coli	Colour of Present/Absent EMB for 2 of E.coli weeks	
HKW1	No colour observed	-ve	No colour observed	-ve
HKW2	Green metallic sheen	+ve	No colour observed	-ve
HKW3	Green metallic sheen	+ve	Green metallic sheen	+ve
HKW4	Green metallic sheen	+ve	Green metallic sheen	+ve
HKW5	Green metallic sheen	+ve	Green metallic sheen	+ve
HKW6	No colour observed	-ve	No colour observed	-ve

KEY: -ve= Negative; +ve= Positive; Hwk1= hawker 1; Hwk2= hawker 2; Hwk3= hawker 3; Hwk4= hawker 4; Hwk5= hawker 5; Hwk6= hawker 6

The presence of *E. coli* in almost all the samples emphasized the importance of production hygiene during the manufacturing of *Fura da nunu* and other dairy products in small scale operations [17]. This also indicates the lower standards of hygiene in the preparation process of *Fura da nunu*. The result obtained shows that there is presence of pathogenic microorganisms in thee food product (*Fura de nunu*) that may be potential source of food borne infection and some related diseases for the consumers of this product in the sampling area. The total viable counts in all the samples were out of standard. The microbial limit for the total viable colony count is 1.0×10^7 *cfu*/g and *Escherichia coli* should not be present in any sample. *E. coli* was confirmed to be present in almost all the samples except sample A, B and F. The presence of *E. coli* indicates the possibility of other pathogenic microorganisms present in the samples such as *Haffinia, Entrobacter, Introbacter*, *Klebsella, Serratia, Salmonella specie and Staphylococcus aureus,* all of which are pathogenic bacteria. According to Karla and Blaker [18], the most important source of *Staphylococci* capable of causing food poisoning is the human body.

Even healthy person carries these bacteria in their mouths, throats and noses. Infected wounds are particularly rich in food poisoning *Staphylococcus aureus*, and so are pimples, furuncles, infected throats, eyes, ears, sinuses and others. The organisms get into food if the food handlers do not exercise care. *Staphylococcus aureus* survives mostly in protein food such as milk, meat, fish etc., because of their composition and lack of acidity. The presence of coliform in *Fura de nunu* samples indicates probable fecal contamination and the presence of *E. coli* bacteria in all most all the samples are the major biotype of the family. *Enterobacteriaceae* can be considered fecal in origin.

The presence of coliform in the samples could be as the result of cross contamination between the personnel and the milking equipment during the milking operation. Contamination occurs from many

external sources through which microorganisms can come into milk during milking as well as during subsequent handling of the milk. The milking equipment such as pails, cans coolers, pipeline, bulk tank and milking machine are the most serious sources of bacterial contamination. Again, if the milking personnel are not in good health or have infections on their skin and hands, pathogenic bacteria may be added to the milk. Milk may serve as a carrier of human pathogens from one person to another. The high microbial count obtained may be as a result of cow suffering from mastitis. Mastitis is an inflammatory disease of the mammary tissues. Microorganisms that causes mastitis include staphylococcus specie, *E. coli*, *Pseudomonas specie* and *Corynebacterium* specie count of up to 10^8 *cfu*/ml can be obtained in such milk.

The presence of coliform in the samples could also be as a result of post processing contamination since not all coliforms are of enteric origin [19]. Unhygienic environment and handlings of *Fura de nunu* by the post processing contamination [5]. The fact that *E. coli* was not isolated in some samples (HKW1, HKW2 and HKW6) did not mean that it could not be in the MacConkey broth positive tube. It could have been that *E. coli* and even pathogens may have been out grown by coliform and other organism thereby escaping isolation [5].

Moreover, most handlers or sellers of *Fura de nunu* are street peddlers. Often, not all *nunu* sent to the market by peddlers is sold the same day and most at time those left overs are being sent back to market with no special attention to preservation or safety. The possible sources of contaminating organisms associated with these food sample (*fura de nunu*) could be traced to the use of the old portion of previously fermented *Nunu* as starter and the use of well and stream water for processing. The contaminating organisms could also be through air micro flora which stick to the smoothening stick calabash spoons and bowls used for sale of the products. Moreover, normal human flora of the customers could also be served as contaminants especially when one bowl is used for mixing the product for all customers without cleaning between uses. Since the safety and keeping of consumables are relaxed to bacteria load of its content, bacteriological standard has been proposed for variety of food products. It is therefore necessary to investigate the bacteriological quality of food and food product exposed to unhygienic sanitary conditions during and after processing.

4 Conclusion and Recommendation

From the result of this research study, it can be concluded that the locally prepared *Fura de nunu* contains potential pathogenic and spoilage bacteria. Their presence indicates unhygienic handling during production. 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 hygiene practices observed during processing, the quality of the final product appears to be compromised. The microorganisms isolated from *Fura de nunu* were diverse. 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 *Fura de nunu.* In this way, the fermentation process can be controlled, thereby enhancing the quality of the product.

Therefore, it is recommended that a standard processing method that will ensure *Fura de 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 hygiene principles will equally be crucial in achieving a quality standard product.

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