



BACTERIOLOGICAL QUALITY OF FERMENTED MILK-CEREALS MIXTURE (FURA DA NONO) HAWKED IN TUDUN WADA, KADUNA METROPOLIS

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ABSTRACT

Milk cereal mixture (fura da nono) is the Fulani name for a Nigerian locally made food drink which translates to millet and milk. Dairy products have frequently been implicated in transmission of food-borne pathogens. This study was conducted to determine the bacteriological quality of fermented milk-cereal mixture “fura da nono” hawked in Tudun Wada. The study was conducted within Kaduna metropolis. A total of 60 samples of “fura da nono” sold around the different area of Tudun Wada Kaduna were collected. Out of the 60 samples collected, Zango road had the highest mean colony count of 16.7×10^6 CFU/ml and the least mean colony count was found in Faskari road (4.1×10^6 CFU/ml) which was not statistically significant ($P < 0.05$). The mean counts were higher than the 1×10^5 CFU/ml standard that is set for dairy food drinks or beverages under regulations (FAO, 2018). The highest coliform count was found in Zango road (54.0×10^6 CFU/ml) while the least coliform count was found in Kasuwan Barci (3.6×10^6 CFU/ml). The other types of bacteria isolated from the milk cereal mixture ‘fura da nono’ were identified as *Pseudomonas* spp, *Enterococcus* spp, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Escherichia coli*, *Lactobacillus* spp and *Corynebacteria*. The results obtained here could be important in ascertaining the overall safeness of locally prepared fura da nono, packaged Fura and yoghurt sold in Kaduna, Nigeria, by analyzing their microbial and nutritional contents.

Keywords: Bacteriological food analysis, fura da nona mixture, milk, nono

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INTRODUCTION

Milk is often described as nature’s most nearly preferred single food. It is that fresh, clean and normal mammary secretion obtained by milking of one or more dairy cows that is properly feed and kept. The cow milk is a good source of animal proteins, fats, vitamins and minerals to the human body [1]. Nutritionally, less useful substances like enzymes are also present in normal milk. Some of these enzymes are used as indices in screening or quality control test [1]. The nono is local uncontrolled fermented cow milk which forms a major part of the staple food in Northern Nigeria. They are produced mainly by the nomadic Fulani [1]. The fresh milk is directly obtained from a cow into a properly washed semi- dried calabash and kept wide open in the sun for approximately two hours to facilitate isolation of the fat layer. Some quantity of overnight fermented milk is added thereafter to serve as a starter culture and the inoculated fresh milk is left overnight at room temperature for fermentation to get sour milk known as “kindirmo”. The addition of large volume of water to the curdled sour milk which is then stirred with a T shaped stick to a liquid of fine consistency gives rise to “nono” [2]. The most commonly product often mixed with “nono” is called “Fura” (a dumping made of millet or maize) to make a preparation called “fura da nono” [2]. The mixture of fermented milk cereal (fura da nono) is highly nutritious beverage which is a two in one product consisting of a cereal “fura” made from millet and “nono” a fermented milk product similar to yoghurt.

“Fura da nono” is stored in a calabash covered with decorative mat and measured out using scopes made from the hard shell of a fruit in the gourd family known as *Lagerina siceraria*. Depending on the consistency, the product is used as food, refreshing drink and a weaning food for infants. The product is in high demand especially in the months of November in July [3]. The poor handling of “fura da nono” during processes exposes it to microbial contamination. “Fura” is usually molded into balls by hand during its production, and the hands of the producers could be a source of contamination. Houseflies are always found in large numbers of the production sites and at sales outlet [4]. A work cited by Mohamed *et al.* [4] reported that in order to increase the volume and improve colour of “nono”, the female use stream water while the milky white supernatant water was sourced from baobab tree seeds. This act could further lead to the contamination and spoilage of this product [3].

Biological monitoring of indicator species and basic physiochemical parameters provides preliminary information about matter quality. These quality criteria are well suited to public involvement because of the relative ease with which it can be measured [4]. Bacteriological food analysis is a method of analyzing food to estimates the numbers of bacteria present and if needed to find out what sort of bacteria they are. It is the microbiological and analytical procedure which uses samples of food and from this sample determines the bacterial load and associated health risks when consumed by man [5].

This study aims at determining the bacteriological quality of fermented milk-cereal

mixture “fura da nono” hawked in Tudun Wada, Kaduna for the purpose of assessing the safety of consuming “fura da nono”.

MATERIALS AND METHODS

Study area/ design

The study was conducted at Tudun Wada, situated in Kaduna South Local Government Area of Kaduna state. It was prospectively designed to determine the bacteriological quality of fermented milk-cereal mixture “Fura da nono” hawked in Tudun Wada Kaduna metropolis.

Sample size

Using the Cochran’s formula,

$$n = \frac{Z^2 p (1-p)}{e^2}$$

Where Z is the confidence interval,

P is the prevalence rate; e is the level of precision and n is the sample size.

n =? Z = 1.96, p = 17%, e = 5%

$$n = \frac{1.96^2 \times 0.041(1-0.041)}{0.05^2}$$

$$n = \frac{3.8416 \times 0.039319}{0.0025}$$

n = 60.42

There for sample size n is approximately = 60

Bacterial enumeration

The spread method of inoculation after serial dilution of the sample by 10⁻⁵ dilution factor was conducted as describe by Manga and Oyeleke [7]. One gram of milk cereal mixture was dissolved in 9 ml of distilled water. The sample suspension was diluted using a tenfold serial dilution. Then 0.1 ml of each sample was pipetted from 10⁻⁴ and 10⁻⁵ dilutions into respective petri dishes. The respective media were poured on the samples in the petri dishes at a molten temperature of about 40-50°C. The plates were incubated at 35°C for 24 hours. Plates that showed discrete colonies were counted and total numbers of bacteria count were calculated using the formula and expressed in CFU/m [3].

$$\frac{\text{No of colonies counted} \times \text{dilution factor}}{\text{Volume of inoculum used}}$$

Detection and enumeration of coliforms

In this method, 9 test tubes were used. The first 3 test tubes contained 10 ml of lactose broth for the double strands, the second 3 test tubes contained 1ml of lactose while the last 3 test tubes contained 0.1 ml of the lactose broth for the single strength. The Durham tubes were inverted and autoclaved to expel air and to sterilize them. Inoculation where made from the samples as follows: 1 ml of inoculum was transferred to each of the 9 test tubes containing a lactose broth, all the 9 tubes were incubated at 37°C for 24 hours in the absence of gas (Presumptive test). After 24 hours of incubation, the tubes where observed for gas

production and the numbers of gas positive tubes was compared with the most probable numbers (MPN) table to estimate the most probable numbers of coliforms per ml of the samples. A loopful of inoculums from gas positive tubes was streaked on to Eosine Methylene Blue (EMB) agar plate and incubated at 37°C for 24 hours. Following incubation, colonies which formed bluish black colour with green metallic sheen, and reddish colonies were noted and isolated on agar slants. This is called the confirmatory test. Also, colonies showing metallic sheen on EMB where sub-cultured in to tubes of lactose broth and incubate at 37°C. The tubes were observed after 24 hours of incubation for gas production. This is the completed test for fecal coliforms. Colonies that formed green metallic sheen on EMB were stored on nutrient agar slant for further analysis [3].

Isolation of bacteria from milk cereal mixture (fura da nono)

Standard microbiological methods were used for the isolation and identification of isolates. Milk cereal mixture samples were serially diluted by homogenizing them. Ten (10) Millimeters of each of the ‘fura da nono’ was weighed and diluted in 90 ml of distilled water and then mixed thoroughly to make a homogenate. Serial dilution was done by pipetting 1 ml of the ‘fura’ homogenate in to a test tube with 9 ml of distilled water. From the first dilution 1 ml was transferred to the second dilution tube containing 9 ml of diluents. This was repeated using a third, fourth, fifth and sixth as the maximum desired serial dilution as described by Lee and Kim [3] and cultured on Nutrient agar, MacConkey Agar, Cystine Lactose Electrolyte Deficient (CLED) Agar and Eosin Methylene Blue (EMB) plates. A standard loop full was streaked onto the prepared media and incubated for 24 hours at 37°C. Prolonged incubation was done for further 24 hours for plates with no growth. Bacteria that developed colonies were sub cultured on EMB and MacConkey Agar and later stored on sterile nutrient agar slants bottles and incubated for 24 hours at 37°C for characterization and identification. The colonies that showed green metallic sheen on EMB or pink on MacConkey Agar were further subjected to biochemical tests for confirmation [6]. The identified bacterial isolates were maintained on nutrient agar slants, incubated at 37°C for 24 hours and sub-cultured periodically and stored in the refrigerator at 4°C until further analysis.

Identification of bacterial isolated from milk cereal mixture (fura da nono)

The pure colonies that were obtained in the slant bottles were subjected to series of biochemical tests in order to identify the bacteria isolates yielded by observing their cultural characteristics, morphology by Gram’s staining and biochemical tests. The biochemical test that were carried out on the bacterial isolates includes Catalase, Coagulase, Triple sugar iron test (TSI), Indole, Methyl-red, Voges-Proskauer, Citrate

utilization tests as described by Manga and Oyeleke [7].

RESULTS AND DISCUSSION

A total of 60 samples of “fura da nono” were collected (Table 1); Zango Road had the highest mean count of 16.7×10^6 followed by Unguwan Muazu (15.2×10^6), Kabala Road (9.7×10^6), Kinkinau (5.1×10^6) and Kasuwan Barci (4.8×10^6) CFU/ml. The least mean count was found in Faskari Road (4.1×10^6) CFU/ml which was not statistically significant ($P < 0.05$). The high mean count observed in “fura da nono” could be due to the presence of *Lactobacillus* and *Staphylococcus* spp. which are always associated with milk cereal mixture [4]. Furthermore, contamination of “fura da nono” could also be due to the general habit of unhygienic handling and preparation of the product as well as sharing of containers among the consumers by the vendors. More so, instability of vendors in one location could lead to cross contamination within the different environments [9].

Table 1: Total aerobic count of “fura da nono”

Location	Total Aerobic Count ($\times 10^6$) CFU/ml	P value
Kasuwan Bacci	4.95 ± 0.21	0.0012
Kabala Road	7.55 ± 3.04	
Zango Road	18.35 ± 2.33	
Unguwan	17.5 ± 3.25	
Mu'azu		
Faskari	4.5 ± 0.57	
Kinkinau	5.35 ± 0.35	

Acceptable limit is 1.0×10^5 cfu/ml according to FAO

The total coliform count of “fura da nono” hawked in Tudun Wada is presented in (Table 2). The highest coliform count was found in Zango road 54.0×10^6 followed by Unguwan Muazu (25.4×10^6), Kinkinau (5.6×10^6), Kabala Road (5.4×10^6) and Faskari Road (4.9×10^6) CFU/ml. The least coliform count was found in Kasuwan Barci (3.6×10^6) CFU/ml which was not statistically significant ($P < 0.05$). Halablab *et al.*, [10] reported that the total coliform can be considered as a hygiene indicator especially for fecal contamination. In this study the coliform observed in “fura da nono” could suggest a high level of background fecal contamination of milk cereal mixture. A potential source of the contamination could be from water used in processing the milk product. The presence of coliforms especially *E. coli* could also be

attributed to human and animal fecal contamination as these coliforms are usually present in large numbers of fecal matter [10].

Table 2: Total coliform count of “fura da nono”

Location	Total Coliform Count ($\times 10^6$) CFU/ml	P value
Kasuwan Bacci	4.35 ± 1.06	0.0766
Kabala Road	4.85 ± 0.78	
Zango Road	37.0 ± 24.04	
Unguwan	22.6 ± 3.96	
Mu'azu		
Faskari	6.0 ± 1.56	
Kinkinau	6.9 ± 1.84	

Acceptable Coliform limit is 7.5×10^2 (CFU/ml) according to FAO

The result of morphological characteristics and biochemical identification of bacteria isolated from milk cereal mixture “fura da nono” is presented in Table 3. *Escherichia coli* was Gram negative rod, citrate, Vogues-Proskauer, Oxidase, and Coagulase negative, but Indole, Methyl red, Hydrogen sulphide, and Catalase positive. *Staphylococcus aureus* was gram positive cocci, citrate, methyl red, vogues proskauer, catalase, and hydrogen sulphide positive but Indole, Oxidase, and coagulase negative. *Corynebacteria* were gram positive, methyle red, hydrogen sulphide, and catalase positive but citrate, Indole, Vogues-Proskuer, Oxidase and coagulase negative. *Enterococcus faecalis* was gram positive bacterium, Vogues-Proskuer, and hydrogen sulphide positive but citrate, indole, methyl red, oxidase, catalase and coagulase negative. *Lactobacillus* was Gram positive citrate, hydrogen sulphide, oxidase, catalase but indole, methyl red, vogues proskauer and coagulase negative. *Pseudomonas aeruginosa* was Gram negative indole, methyl red, Vogues-Proskuer, coagulase but citrate, hydrogen sulphide, oxidase and catalase positive. *Staphylococcus saprophyticus* was gram positive hydrogen sulphide, Catalase but Citrate, Indole, Methyl Red, Vogues-Proskuer, Oxidase and Coagulase negative. The different types of bacteria (*Pseudomonas spp*, *Enterococcus spp*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *E. coli*, *Lactobacillus spp* and *Corynebacteria*) isolated from the milk product were similar to the types reported by Mohamed *et al.* [4]; Zhu and Prince [9] who conducted similar research, where similar species associated with “fura da nono” were also isolated.

Table 3: Morphological characteristics and biochemical identification of bacteria isolated from milk cereal mixture of “fura da nono”

Morphological Characteristics	GM	CIT	IND	MR	VP	H ₂ S	OXI	CAT	COA	
Large, elevated, yellow opaque colonies with a center more intense yellowish medium.	-	-	+	+	-	A/A	-	+	-	<i>E. coli</i>
Deep yellow colonies about 0.75mm diameter, uniform in color. Yellow medium	+	+	-	+	+	K/A	-	+	-	<i>S. aureus</i>
Very small grey colonies	+	-	-	+	-	+	-	+	-	<i>Corynebacterium</i>
Yellow colonies about 0.5mm in diameter, yellow medium.	+	-	-	-	+	+	-	-	-	<i>E. faecalis</i>
Very small grey colonies with a rougher surface.	+	-	-	-	-	+	-	-	+	<i>Lactobacilli</i>
Green colonies with a typical matter surface and periphery “sweet” odor	-	+	-	-	-	+	+	+	-	<i>Pseudomonas</i>
Pale yellow or white more opaque than <i>E. faecalis</i> often with pale periphery.	-	-	-	-	-	+	-	+	-	<i>S. saprophyticus</i>

Keys: +: positive; -: Negative; Cat: Catalase; Coa: Coagulase; Cit: Citrate; MR: Methyl Red; VP: Vogues Proskauer; H₂S: hydrogen sulphide

CONCLUSION

Out of the 60 samples collected, Zango road had the highest mean count of 16.7×10^6 and the least mean count was found in Faskari road (4.1×10^6) CFU/ml which was not statistically significant ($P < 0.05$). The highest coliform count was found in Zango road 54.0×10^6 while the least coliform count was found in Kasuwan Barci (3.6×10^6) CFU/ml. The bacteria isolated from the milk cereal mixture ‘fura da nono’ were identified as *Pseudomonas* spp, *Enterococcus* spp, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Escherichia coli*, *Lactobacillus* spp and *Corynebacteria*.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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