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BACTERIOLOGICAL EXAMINATION OF FURA DA NONO (FERMENTED MILK; CEREALS MIX) SOLD IN SOME SELECTED AREAS OF BIRNIN KEBBI METROPOLIS

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ABSTRACT

Five locally prepared Fura Da Nono were obtained from five different areas of Birninkebbi. New Market, Old Market, Bello Way, Gesse and MakeranGandu. Bacteriological analysis were conducted on the sample for the isolation and identification of pathogenic and spoilage bacteria. Viable colony counts and coliform test were determined and the result reveal that the colony count ranged from 4.0x104 to 7.0x104 Cfu/ml. Samples from Old market, Bello way and MakeranGandu were found to positive to coliform test. Biochemical tests were conducted on the isolates. The bacteria isolated were Pseudomonas aeruginosa, Serratiamercesens, Bacillus pumillus, Staphylococcusaureus, Salmonella spp and Bacillus alvies. With Staphylococcus aureus having the highest level of occurrence, having been isolated from all the samples analyzed. The high microbial colony counts and the presence of the above isolated pathogenic bacteria may very well be related with free prevalence of some urinary tract infection and typhoid fever experiencing by some people consuming locally prepared Fura Da Nono.

INTRODUCTION

Fura da Nono is very popular in the northern state especially in SokotoState where it can be taken any time of the day. Nono is the Fulani word for cow's milk sold by the Fulani women. However, many people preferred to use plain Yoghurt to mix with Fura and drink. This is a desert of yoghurt when millet or ground millet where nono is the Hausa word for the cow's milk that traditionally Fulani women sold. Today this is more often than not made with yoghurt (Shehu and Adesiyun, 1990).

Fura da Nono (fermented milk cereal mix) 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 sold from calabash converted with mat using scopes made from calabash. In the market, fura is mix with nono in a bowl for customers. Usually one bowl is used in mixing for all customers without cleaning. 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 (Umoh, *etal* 1988).

The poor handling of fura da nono during processes and making 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. Shehu and Adesiyuh (1990) reported that in order to increase the volume and improve colour of nono, the female stream water and milky white supernatant of water sourced baoba tree seeds. This act could further lead to the contamination and spoilage of this product. Umoh, etal (1988) isolated staphylococcus SP from fura da nono while Shehu and Adesiyun (1990) isolated *escherichiacoli* from fura da nono. Their reports indicate the possibility of these product serving as source of microbial food poisoning, fura da nono

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offered for sale is usually poorly handled and presented to consumers mostly in unhygienic manner.

Biological monitoring of indicator species and monitoring of 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 can be measured. 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 concentration of bacteria (Sangodoyin and Osuji, 1990).

The presence of bacteria in food constitute health risks when consumed by man, thus, study was designed to determine the type of bacteria in fura da nono with the hope that finding will help in assessing the quality of the fura da nono for human consumption

PREPARATION OF FURA

Ingredients

1 ½ cups of millet or gunea corn flour

½ cup of soya beans flour

1 Tsp dried pepper (ground)

½ Tea spoon clones (ground)

¼Tea spoon African black pepper (ground)

1 Tea spoon corn flour

2 litre water

1 litre yoghurt or (nono)

Sugar to taste

Mix the flours with all the species thoroughly. Add a little water and mix to form a thick paste mold into medium size balls. Boil the water for about 5 minutes. Add the balls to the boiling water and continue boiling for about 20 minutes, remove the balls from the boiling pot and pound thoroughly mold into small balls and sprinkle corn flour to keep the balls moist serve mashed with nono or yoghurt and sugar to taste (Frazier and Westhoff, 1998).

PREPARATION OF NONO

Milk is a normal product of mammary gland secretion. It is produce from the female animals during lactation. The animals secrets their milk in the under (breast) during gestation and after parturition the milk is then flowing out which serve as food to field their younger ones (Frazier and Westhoff, 2008).

MATERIALS AND METHODS

SAMPLE COLLECTION

Five (5) different samples of fura da nono were collected from five different selling points in Birnin Kebbi.

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Old market

New market

Gesse phase

Bello Way

Makeran Gandu

They were collected and transported in a sterile specimen bottle. They were clearly labeled and stored in refrigerator before analysis

MEDIA PREPARATION

- **Nutrient of Agar Medium:** 28g of nutrient agar powder was weighed and dissolved in 1000ml and of distilled water. It was stirred vigorouslyand dissolved using hot plate after which was sterilized in autoclave for 15minutes at 121^0c. It was then allowed to cool after which it was dispensed in Petri dishes and allowed to solidify
- **Eosin Methylene Blue Medium (EMB):** 5.1g of the EMB agar powder was dissolved in wound at distilled water. It was stirred and dissolved. It was then sterilized in an autoclave for 15minutes at 121^0c it was allowed to cool after which was dispensed in to petri dishes (cheesebrough, 2000).

BACTERIAL COUNT

The spread method of inoculation after serial dilution of the sample by 10^{-4} dilution factor was conducted as described by Manga and Oyeleke, (2008) .Microscopy was conducted as described by Cheesbrough (2000).

GRAM STAINING

Colonies that were grow on nutrient agar where gram stained in accordance with standard gram staining procedure described by Totora *et'al*, (2003).

BIOCHEMICAL TESTS

- **Indole test** One percent tryptophan broth in a test tube was inoculated with bacteria colony. After incubation period of 37^oc for 48 hours, then one militre (1ml) of chloroform was added to the broth. The test tube was shaken gently, then 2.1 of Kovac's reagent were added and this was also shaken gently and allowed to stand for twenty (20) minutes. The formation of red colouration at the top layer indicated positive and yellow colouration indicates negative.
- **Catalase Test:** This was carried out by putting a drop of hydrogen peroxide on a clean slide. With the edge of another slide, a colony of the organism was picked and allowed to be in contact with the hydrogen peroxide. Presence of bubbles indicates positive reaction while absence of bubble indicates negative reaction.
- **Citrate Utilization Test:** This was carried out by inoculating the test organism in test tube containing Simon's citrate medium and this was inoculated for 24 hours to 72 hours. The development of deep blue colour after incubation indicates a positive result.
- **MR-VP Test:** Five millilitres (5ml) or MRVP broth was inoculated with the test organism and incubated for 48-72 hour at 37^oc after which, one milliliter (1ml) of the broth was transferred into a small tube. Some small quantity (2.3 drops) of methyl red test was added. A red colour one the addition of the indicator signified a positive methyl

red test while yellow colour signified a negative test. To the rest of the broth in the original tube some drops (five) of 4% Potassium hydroxide (KHO) were added followed by some (fifteen) drops of 5% α-naphtol in ethanol. The test tube (sealed with cotton plug) was shaken and place in a sloping position. The development of a red colour starting from the liquid- air interface within 1 hour indicated a VP positive test while no color change indicated a VP negative test.

- **Triple-sugar iron Agar Test (TSI):** The medium contains three (3) sugars namely: glucose, lactose and sucrose. The PH indicator is phenol red, and detection system for hydrogen suphide (H_2S) is included. This medium was prepared as agar slope and the test organism was inoculated by stabbing the medium with the aid of sterilized straight wire loop, and the surface of slope is inoculated by streaking and then incubated at 37°c for 24 hours, after which observation was made. Gas production was determined by cracking of the medium, formation of H_2S was determine by the blackening of the whole buffer or a streak of ring of blackening at the slant butt junction, glucose fermentation was determine by the yellowing of the butt. The fermentation of lactose or sucrose or both was determined by the yellowing of both the butt and the slant and the motility was determined by observing the line inoculation; sharply defined line of inoculation indicating positive motility (Manga and Oyeleke, 2008).

RESULTS

The result of the total viable bacteria count of the samples (OM, MM, GE, BW and MG) was shown in Table 1. The results obtained raged from 4.0 x 10^4cfu/ml to 7.0 x 10^4cfu/ml with GE having the highest microbial load. Biochemical characterization for bacterial isolates from the samples was presented in table 2. The bacteria were identified as genus of *Bacillus, Pseudomonas, Staphlococcus and Serratia.*Percentage of occurrence was presented in table 3, *Staphylococcus aureus* were more frequently isolated and constituted the highest number of the total bacteria isolated. *Escherichia coli* (BAC 7)were isolated on the EMB agar presented in table 4. *E. coli* showed a characteristic green metallic sheen on EMB agar. The bacteria were isolated in accordance with the findings of Cowan and Steel, (1992).

Table 1; Total viable bacterial count

Samples	Viable count (cfu/ml)		
	Plate 1	Plate 2	Average
OM	$4.0x10^4$	$4.0 x10^4$	$4.0x10^4$
MM	$4.2x10^4$	$4.3x10^4$	$4.25x10^4$
GE	$7 .0x10^4$	$7.0x 10^4$	$7.0x10^4$
BW	$5.1x10^4$	$5.1x 10^4$	$5.1x10^4$
MG	$5.0x10^4$	$5.4x10^4$	$5.2x10^4$

Cfu/m1: Colony Forming Unit per millilitre.

KEY: OM; Old Market, MM; Main Market, GE; Gesse, BW; Bello Way, MG; MakeranGwandu

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TABLE 2: MORPHOLOGY AND BIOCHEMICAL CHARACTERIZATION OF THE BACTERIA ISOLATES.

	BAC 1	**BAC 2**	**BAC 3**	**BAC 4**	**BAC 5**	**BAC 6**
Gram reaction	-ve	+ve	+ve	+ve	-ve	+ve
Shape	Bacilli	Bacilli	Bacilli	Cocci	Bacilli	Bacilli
Arrangement	Single	Single	Chain	Cluster	Single	Chain
Endospore test	-	-	+	-	-	+
Motility	+	+	+	-	+	+
Oxygen relationship	Aerobes	Facultative anaerobe	Facultative anaerobes	Facultative anaerobes	Facultative anaerobes	Facultative anaerobes
Catalase	-	+	+	+	+	+
Coagulase	-	-	-	+	+	-
Citrate	+	+	+	-	-	-
Indole test	-	+	-	+	+	+
Methyl Red	+	-	-	+	+	-
Glucose	-	+	+	+	-	+
Lactose	+	+	+	+	-	+
Sucrose	+	+	-	+	-	+
Mannitol	+	-	-	+	-	-
Probable identity	*Pseudomonads aeruginosa*	*Serratia mercescens*	*Bacillus pumillus*	*Staphlococcus aureus*	*Salmonella spp*	*Bacillus alvei*

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TABLE 3: THE OCCURRENCE OF THE ISOLATED ORGANISMS IN ALL THE SAMPLES OF FURA DA NONO ANALYZED.

35 BACTERIA WERE ISOLATED IN ALL THE SAMPLES.

DESIGNATION	BACTERIA ISOLATES	OM	MM	GE	BE	MA	TOTAL	%
BAC 1	*Psendomonads aeruginosa*	4	3	0	1	2	8	22.86%
BAC 2	*Serratia mercescens*	1	1	0	1	0	3	28.57%
BAC 3	*Bacillus pumillus*	2	2	1	0	1	6	17.14%
BAC 4	*Staphlococcus aureus*	2	1	1	2	3	9	25.71%
BAC 5	*Salmonella spp*	1	0	0	1	0	2	5.71%
BAC 6	*Bacillus alvei*	2	1	2	1	1	7	20%
							35	

TABLE 4: Eosin Methylene Blue Agar (EMB)

Samples	Colour On EMB	*E. coli (BAC 7).*
OM	Green metallic sheen	Positive
MM	No growth	Negetive
GE	No growth	Negative
BE	Green metallic sheen	Positive
MA	Green metallic sheen	Positive

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DISCUSSION

The result obtain shows that there are presence of pathogenic microorganism that may be potential source of food borne infection and some related diseases for the consumers of this product in the sampling areas. The total viable bacteria counts in all samples were out of standard. According to NAFDAC 2009 the microbial limited for total liable colony count is 1.0x102 cfu/ml and *Escherichiacoli* should not be present in all samples. *E.coli* was confirmed to be present in almost all the samples except sample from main market and Gesse.

The presence of *Pseudomonas aeruginosa, Salmonella spp, Staphlococcus aureus, Escherichia coli, Serratia marcescens and Bacillus Pumillus.* All of which are pathogenic bacteria. *Staphlococcus aureus* causes disease in human, it is responsible for a long list of different disease such as follculitis, furuncles, carbuncles, erysipelas, , cellulites, scalded skin syndrome, impetigo, pneumonia, Osteoporosis, toxic shock syndrome (Toxemia) meningitis and staphylococcus food poisoning (Marjorie and Kathleen 2006).

*Salmonella spp*this is a strict pathogen and has no habitat other than human or animal body, the source of human infection is therefore human or animals (carrier) the organism been excreted in the faeces or urine and transmitted by food or water which is ingested by another subject. It can cause any one of these three (3) types of infections. Bacterial food poisoning, enteric fever and systemic fever (septicemia) (Stewart and Besorick, 1997).

The isolation of *Bacillus pumilus* can however be due to the fact that they are common in the environment in high population.It has been known as a common contaminant of food, vegetable and diary product (Bourdillon and Colebook 1996).

Pseudomonads aeruginosa is widely distributed in soil and water. It can therefore contaminate food and dairy product by the sellers (Pelczer *et al* 2007). However contaminating organism are the objectionable microorganism that can proliferates in the food adversely affecting the quality portability of the food (Sangodoyin and Osuji, 1990).

CONCLUSION

From the result of this research study it can be concluded that some locally prepared Fura da Nono contains potential pathogenic and spoilage bacteria. Their presence indicates unhygienic handling during production. Since the safety and keeping of consumables are related to bacteria load of its content, bacteriological standard have been proposed for variety of food products. It is therefore necessary to investigate the bacteriological quality of food and food products exposed to unhygienic sanitary conditions during and after processing.

RECOMMENDATION

Since Fura da Nono serve as one of the major source of beverages for the inhabitants of BirninKebbi metropolis, it is recommended that bacteriological examination of this food be carried out periodically so as to assess their suitability for consumption.

It is also recommended that the regulatory agencies should carry out surveillance on hawkers of the food and public enlightment campaign should be embarked on.

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