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Bacteriological Quality of Locally Fermented Milk (Nono) and Commercial Yoghurts Marketted in Ilorin.

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Abstract

Eleven samples comprising of six branded yoghurts and five unbranded 'nono' purchased from seven different locations randomly selected in Ilorin city were evaluated for their bacteriological quality. The physicochemical properties were determined using standard methods. Results for the total bacterial count for yoghurt ranged from 3.08×10^2 cfu/mL to 9.67×10^2cfu/mL while that of 'nono' ranged from 1.056×10^3cfu/mL to 3.152×10^3cfu/mL. Eleven bacteria species were identified in this study. A total of forty-five isolates were obtained with *Staphylococcus aureus* (17.8%) and *Pasturella multocida* (17.8%) having the highest frequency. Others were *Aeromonas hydrophilia* (15.6%) *Klebsiella oxytoca*, *Burkholderia pseudomallei*, *Actinobacillus spp* (8.9%), *Xanthomonas maltophilia* (6.7%) *Klebsiella pneumoniae*, *Escherichia coli* and *Salmonella* (4.4%) and *Pseudomonas aeruginosa* (2.2%). Marketed yoghurts and locally produced 'Nono' in Ilorin were found to be contaminated with mostly Gram negative bacteria belonging majorly to the Enterobacteriaceae family and a Gram positive organism (*Staphylococcus aureus*). The yoghurt and 'nono' samples were found to be of unacceptable microbiological quality. The presence of these pathogenic organisms in these commonly consumed dairy products could constitute public health hazards and possibly food poisoining especially if they are stored under inappropriate conditions for longer periods of time.

Keywords: bacteriological evaluation, nono, total bacterial count, yoghurt.

INTRODUCTION

The consumption of fermented milk by man dates from the beginning of civilization[1]. Yoghurt is one of the oldest fermented milk products consumed tremendously all over the world. The natural yoghurt is characterized by a smooth and viscous gel like texture and has a delicate walnutty flavour[2]. Yoghurt is a food produced by bacterial fermentation of milk, it is made by the fermentation or the addition of healthy bacteria and live cultures to milk. Yoghurt is obtained by lactic acid fermentation of milk by the action of a starter culture containing *Streptococcus thermophiles* and *Lactobacillus delbrueckii* sub-spp, *Bulgaricus* and some additional bacteria

having mutual complementing metabolism[3] with pH range of 4.25-6.50,[4]. The role of these two genera in yoghurt manufacture can be summarized as milk acidification and synthesis of aromatic compounds[5].

In Nigeria, locally processed cow milk products are prepared mainly by Fulanis, where raw milk is processed into 'Nono', Kindrimo and Manshanu[6]. 'Nono' is the Fulani word for cow's milk sold by Fulani women[7] and it is produced from non-pasteurized cow milk collected in a container called calabash and allowed to ferment naturally for 24 hours [6,8]. The pH of normal cow milk has been reported to range from 6.4- 6.6 and this is known to favour the growth of pathogenic microorganisms[9].

Raw milk is obtained from cows at farm homes in the Fulani hamlets and villages where shelf-life and safety of the products are not considered. The raw milk is then processed into its constituents products and sold to the public as food because milk and milk products provide a wealth of nutrition benefits. This raw, unpasteurized milk has been reported to carry dangerous bacteria such as *Salmonella*, *E. coli*, and *Listeria*, which are responsible for causing numerous foodborne illnesses[7]. These harmful bacteria which have been reported to seriously affect the health of anyone who drinks raw milk, or eats foods made from raw milk and can be especially dangerous to people with weakened immune systems, older adults, pregnant women, and children. Centre for Disease Control (CDC) reported that foodborne illness from raw milk especially affected children and teenagers[10]. In addition, it reported that unpasteurized milk is 150 times more likely to cause foodborne illness and results in 13 times more hospitalizations than illnesses involving pasteurized dairy products. Milk being a rich medium for spoilage and growth of pathogenic microorganisms, it is capable of being a source of illness/sickness for the large population of consumers[11].With the frequent consumption and increase demand for dairy products (yoghurt and nono), there is a need for the continuous assessment of the microbiological quality of these products. More so, microbial quality of yoghurt and nono reflects towards the quality and acceptability of the products. Therefore, the need for this study.

Materials and Methods

Collection of samples

Three samples from eleven different products comprising of six yoghurts and five 'Nono' products were collected. Branded yoghurts from Tipper garage, Yoruba road, Sawmill park, Oja-oba, Zango and the main campus were obtained and unbranded 'Nono' (from Zango, Campus, Oja-oba, Sawmill Park and Post office) were collected within Ilorin metropolis from seven randomly selected locations. The samples were all collected in August, 2015. Samples were labelled according to the location of collection and transported to the laboratory and stored in a refrigerator at a temperature of 4^{o}C until they were needed for analysis.

Preparation of culture media

Different media were used for the isolation of different microorganisms from the samples and they are Nutrient Agar (NA), MacConkey Agar, Mannitol Salt Agar, Deoxycholate Citrate Agar (DCA). Some media were also employed to identify and characterize the bacterial isolates which included Eosin Methylene Blue (EMB), Xylose Lysine Deoxycholate (XLD) and Simmons citrate agar. Each medium was

prepared using the manufacturer's instructions.

Determination of physicochemical parameter

Physical Observation

Organoleptic test was performed on the samples in accordance to the procedure described by[12]. This involved observation and sensory analysis of products in terms of colour, taste, smell, texture (consistency) and packaging appearance.

pH Determination

The pH of the samples were determined using a pH meter, 30 mL of each sample was transferred into a beaker and the probe of the pH meter rinsed with distilled water and dipped into the samples, the probe was rinsed with distilled water after each sample. The pH of each samples was displayed on the meter and recorded.

Isolation and identification of microorganisms

Total bacterial count

A modification of the methods reported by[13,14] was done. Five-fold serial dilutions of the samples was made up to 10^{-5} as follows; 1mL of each yoghurt sample was withdrawn aseptically using a sterile pipette and transferred into a test tube containing 9 mL of peptone water. After shaking, 1 mL of the first diluted sample (10^{-1}) was aseptically withdrawn and transferred into another 9mL of sterile peptone water contained in a test tube and shaken again, this represents 10^{-2}. The dilution was done up to 10^{-5}. Subsequently 1 mL each from dilutions 10^{-1}, 10^{-3} and 10^{-5} was aseptically taken and plated on nutrient agar (NA) using the pour plate method. Each of the plates were incubated at 37^{o}C for 24 hours. Distinct colonies found on the plate after incubation were observed and counted using a colony counter and records were taken accordingly. Pure cultures were obtained by streaking distinct colonies on nutrient agar. The overnight cultures obtained were then subcultured on a nutrient agar slant in MacCartney bottles and incubated at 37^{o}C for 24 hours and these were stored in the refrigerator as stock culture.

Isolation of *Staphylococcus aureus*

A loopful of the 10^{-1} dilutions of the samples were streaked on Mannitol Salt Agar and incubated at 37^{o}C for 24 hours. The samples that showed positive growth were then sub-cultured on nutrient agar slants until required[14].

Isolation of lactose fermenting gram negative bacteria

The 10^{-1} dilutions of the samples were inoculated on single strength MacConkey Agar and incubated at 37^{o}C at 24 hours. Lactose fermenting colonies from MacConkey agar were subjected to microscopic examination to determine cell morphology and Gram stain reactions. Cultural characteristics and colonial morphology of the isolates were interpreted using standard microbiological procedure[15].

Isolation of Salmonella-Shigella bacteria

The 10^{-1} dilutions of the samples were inoculated in DCA and incubated at 37^{0}C for 24 hours. Cultural characteristics and colonial morphology of the isolates were interpreted using standard microbiological procedure[14,15].

Isolation of coliform bacteria

After the serial dilution of the yoghurt drinks, 1 mL each from dilutions 10^{-1}, 10^{-3} and 10^{-5} were aseptically taken and plated on MacConkey agar using pour plate method. This were used for the enteric bacteria coliform count. After the 24 hours incubation, plates that showed growth of organisms and colour change indicating presence of enteric bacteria with distinct colonies on the agar were sub-cultured on nutrient agar plates and incubated at 45^{o}C for 24 hours to test for presence of thermo-tolerant coliforms. Thereafter, indole tests were carried out on the isolates.

Identification and Biochemical Tests

Gram Staining

A sterile wire loop was used to pick a bacterial colony and a smear of the bacterial isolate was made on a slide with a drop of distilled water. This was air dried and then heat fixed. The slides were then flooded with crystal violet (primary stain) for 60 seconds, the stain was drained off, washed and flooded with Lugol's iodine (mordant) for 60 seconds. It was then flooded with 95 % alcohol for 15 seconds after which it was then rinsed with distilled water and finally counterstained with safranin for 30 seconds. The slide was then gently washed with distilled water and air dried. The slide was observed under oil immersion lens (× 100). Gram positive cells stained purple while Gram negative ones stained pink or red.

Catalase Test

The test was carried out by adding 2mL of 3% H_2O_2 solution into a test tube. A glass rod was then used to remove colonies of the test organisms and immersed into the hydrogen peroxide solution. A positive test catalase was indicated by the presence of effervescence due to the liberation of oxygen by the bacteria. This test differentiated catalase producing *Staphylocoocus aureus* from *Streptococci*[15].

Coagulase Test

This is done to identify *Staphylococcus aureus* which produces the enzyme coagulase. Two drops of physiological saline about 2cm apart were dropped separately by a mark on the slide. A colony of the suspension was emulsified in each drop to make two thick suspensions then a loop full of little citrated human plasma was added to one of the suspension slightly rocked for about 10 seconds. Clumping of the organism after rocking indicates a positive result[14,15].

Oxidase Test

This test indicates the presence of cytochrome c oxidase that has the ability to reduce oxygen electron acceptors[16]. An oxidase strip was used for this test, a sterile loop was used to take a loop full of the organism and rubbed on the oxidase paper strip and the colour change within 10 seconds was noted and recorded.

Identification of Isolates using Microbact™ GNB 24E

Microbact™ set D 24E was used for the final biochemical identification of the isolates. Overnight cultures were emulsified in 5 mL of sterile normal saline in universal bottles to match 0.5 McFarland turbidity standards and 100 µl each was transferred into 24 wells of different biochemical tests for each isolate. Mineral oil was then used to overlay wells 1, 2, 3, 20 and 24 and incubated at 37^{0}C for 24 hours. After incubation, the colour changes were

observed after the addition of reagents which included Indole which was added to well 8 and evaluated after 2 minutes, VPI and VPII which was added to well 10 and evaluated after 15 to 30 minutes, TDA which was added to well 12 and evaluated immediately, one drop of Nitrate reagent A and Nitrate reagent B were added to well 7 and the colour change was observed. The colour change for each well was then observed and compared with the colour chart provided and later interpreted as either positive or negative. The values of the positive tests were summed up to obtain Octa codes which was then computed into the Microbact Computer Aided Identification software which identified the bacteria and their percentage yields.

RESULTS AND DISCUSSION

Physicochemical parameter of samples

The organoleptic properties of the samples used in this study were observed to have white colouration as expected due to milk as the primary constituent. However, a sample showed a plain colouration which was just a bit opaque than water hence raising concerns for its primary constituents. Another sample had a yellow colouration but was due to the banana flavouring of the yoghurt. The pH of the samples ranged between 4.01 and 4.51.

Total Bacterial Count (TBC)

The total bacterial count (TBC) is shown in Table 2. The mean TBC of the " nono" was about 3.152×10^3 cfu/mL, higher than the mean TBC for the branded yoghurts (9.67×10^2 cfu/mL). **Identification of microorganisms isolated based on location of collection**

Data presented in Table 3 shows the distribution of isolates based on the collection centres. All the samples from the collection centres yielded bacterial species. *Staphylococcus aureus* and *Pasturella multocida* had the highest frequency of 20% and the lowest frequency was from *Pseudomonas aeruginosa* (2.2%). Sample products purchased from Sawmill, Oja Oba and Zango were observed to be more contaminated with nine (9) bacteria species isolated from each location while samples from Tipper garage and Yoruba road were observed to show the least contamination with three (3) bacteria species isolated from each location.

Table 1: Organoleptic properties of the yoghurt and 'Nono' samples.

Sample	Colour	Taste	Consistency	Packaging	Smell	Production Date	Expiry Date
Yg1	White	Sweet	Thick	Satisfactory	Good	-	-
Yg2	Yellow	Sweet	Thick	Satisfactory	Good	24/07/2015	24/08/2015
Yg3	Plain	Sour	Watery	Poor	Fair	-	-
Yg4	White	Sweet	Thick	Satisfactory	Good	21/07/2015	20/08/2015
Yg5	White	Sweet	Watery	Fair	Good	15/07/2015	14/09/2015
Yg6	White	Sour	Watery	Satisfactory	Stale	25/06/2015	25/08/2015
Nn7	White	Sour	Watery	-	Stale	-	-
Nn8	White	Sour	Watery	-	Stale	-	-
Nn9	White	Sour	Watery	-	Stale	-	-
Nn10	White	Sour	Watery	-	Stale	-	-
Nn11	White	Sour	Watery	-	Stale	-	-

Key: Yg= Yoghurt No. = 'Nono' - = Not present

Table 2: The Total Bacterial Count and pH of the yoghurt and 'Nono' samples

Samples Codes	Mean Total Bacterial Count (cfu/mL)	Ph
Yg1	$9.05 \pm 0.29 \times 10^2$	4.01
Yg2	$6.51 \pm 0.30 \times 10^2$	4.51
Yg3	$5.13 \pm 0.15 \times 10^2$	3.56
Yg4	$3.28 \pm 0.10 \times 10^2$	4.10
Yg5	$4.17 \pm 0.15 \times 10^2$	4.06
Yg6	$9.46 \pm 0.13 \times 10^2$	4.13
No7	$1.09 \pm 0.007 \times 10^3$	4.00
No8	$1.32 \pm 0.132 \times 10^3$	4.33
No9	$3.20 \pm 0.026 \times 10^3$	4.33
No10	$2.26 \pm 0.085 \times 10^3$	4.39
No11	$1.37 \pm 0.027 \times 10^3$	4.27

Key: Yg = Yoghurt No = 'Nono'

Table 3: Distribution of microorganisms isolated based on the collection centres.

	Tipper Garage	Yoruba Road	Sawmill	Oja Oba	Zango	Campus	Post Office
S . aureus	1	-	2	2	1	1	1
P. multocida	-	-	2	2	2	1	1
K. pneumoniae	-	-	1	-	1	-	-
K. oxytoca	-	1	-	1	1	1	-
B. pseudomallei	1	-	-	2	-	1	-
X. maltophilia	-	1	1	-	1	-	-
E. coli	-	-	-	1	-	1	-
Actinobacillus spp	-	-	1	1	1	1	-
A. hydrophilia	1	1	1	-	2	1	1
Salmonella	-	-	1	-	-	-	1
Ps. Aeruginosa	-	-	-	-	-	-	1
Total	3	3	9	9	9	7	5

Nine (9) bacterial species were isolated from the branded yoghurt samples. This includes *Staphylococcus aureus*, *Klebsiella oxytoca*, *Aeromonas hydrophilia*, *Burkholderia pseudomallei*, Actinobacillus spp, *Escherichia coli*, *Pasturella multocida*, *Xanthomonas maltophilia* and *Klebsiella pneumoniae.* A total of twenty one isolates were obtained from the samples.

Eleven (11) different bacterial species were isolated from the "nono" samples. They included, *Staphylococcus aureus*, *Klebsiella oxytoca*, *Aeromonas hydrophilia*, *Burkholderia pseudomallei*, Actinobacillus spp, *Escherichia coli*, *Pasturella multocida*, *Xanthomonas maltophilia Salmonella*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* The total number of bacteria isolates in the 'nono samples were twenty-four. The test for thermo tolerant coliforms indicated the absence of faecal contaminants.

Table 4: Distribution of organisms isolated from different brands of Yoghurt samples.

	Brand 1	Brand 2	Brand 3	Brand 4	Brand 5	Brand 6	FREQ	% FREQ
Staphylococcus aureus	1	-	1	1	-	1	4	19.05
Klebsiella oxytoca	1	-	-	-	-	-	1	4.76
Aeromonas hydrophila	1	1	1	-	1	1	5	23.81
B. pseudomallei	-	1	-	1	-	-	2	9.52
X. maltophilia	-	1	1	-	-	-	2	9.52
Actinobacillus spp	-	-	1	-	-	1	2	9.52
Pasturella multocida	-	-	1	1	1	-	3	14.29
Escherichia coli	-	-	-	1	-	-	1	4.76
Klebsiella pneumoniaee	-	-	-	-	1	-	1	4.76
TOTAL	**3**	**3**	**5**	**4**	**3**	**3**	**21**	

Table 5: Distribution of organisms isolated from different brands of 'Nono' Samples.

ORGANISMS	Brand 7	Brand 8	Brand 9	Brand 10	Brand 11	FREQ	% FREQ
Staphylococcus aureus	1	-	1	1	1	4	16.6
Klebsiella oxytoca	-	1	1	-	1	3	12.50
Aeromonas hydrophila	-	-	1	1	-	2	8.33
B. pseudomallei	-	1	-	-	1	2	8.33
X. maltophilia	-	-	1	-	-	1	4.17
Actinobacillus spp	-	-	1	-	1	2	8.33
Pasturella multocida	1	1	1	1	1	5	20.83
Escherichia coli	-	1	-	-	-	1	4.17
Klebsiella pneumonia	1	-	-	-	-	1	4.17
Salmonella spp	1	-	-	1	-	2	8.33
Ps. Aeruginosa	-	-	-	1	-	1	4.17
TOTAL	**4**	**4**	**6**	**5**	**5**	**24**	

DISCUSSION

The organoleptic properties of the samples showed that most of the samples had white colouration as expected due to the fact that milk which is the primary constituent is whitish[17].

The result of the physiochemical screening of the yoghurt samples in Ilorin metropolis agreed with the results obtained by other researchers in various parts of the country. The pH of the six (6) yoghurt drinks in this study ranged from 3.56- 4.51 (Mean of 4.06) which is similar to the range of 3.69-4.09 obtained in the evaluation of the microbiological quality of yoghurt sold by street vendors in Onitsha, South-eastern Nigeria and it is also in agreement with the pH of 3.57- 4.12 obtained in the quantification of the acidic content of commercial yoghurt drinks[18,19]. However, the mean was below and different from the mean pH of 4.28 obtained in a report of the study of microbiological quality of yoghurts in Brazil. Although the pH is not an official parameter to verify the quality of yoghurts, it can be measured in order to obtain additional information on the quality of milk and dairy product[20].

The pH of the 'nono' samples in this study ranged from 4.00-4.39 (mean of 4.26) which is in agreement with the report of[21] and of the evaluation of the microbiological quality of locally fermented milk ('Nono') in Bauchi, a geographical zone of the northern part of Nigeria[22].

The microbial load obtained in this study is an indication of the sanitary quality, safety and utility of the samples. It also reflects the conditions under which the product is manufactured such as contamination of raw materials and ingredients, the effectiveness of processing and the sanitary conditions of equipment and utensils at the processing plants.

Escherichia coli was isolated in two samples each of yoghurt and 'nono', and E. coli is a faecal organism. The coliform test carried out in this study was negative and this agrees with another work carried out[23]. This is an indication of absence of faecal contaminants.

Some of the organisms isolated in this study has also been reported by other workers. The results of the distribution of bacterial isolates in yoghurt samples agrees with other works who reported the presence of *Staphylococcus* spp, *Klebsiella* spp and *Pseudomonas* spp in yoghurts[24,25,26]. Results of the statistical analysis carried out for the two locations, Tipper garage and Oja oba which had the lowest and highest distribution of isolates respectively, showed that there was no significant difference in the organisms isolated irrespective of the different location at $P \leq 0.05$.

The organisms obtained from this study is similar to those obtained from work in which *Staphylococcus* spp, *Klebsiella* spp and *Pseudomonas* spp were isolated from the assessment of bacteria in yoghurt sold in Awka metropolis[26].

Three of the organisms isolated have been reported to cause food poisoning; *Salmonella*, *Staphylococcus aureus* and *E.coli*. The presence of these organisms has been explained that processed milk is a well-known good medium that supports the growth of several microorganisms with resultant spoilage of product or infections in consumers and that microorganisms found in 'nono' and other milk products mostly come from the water used in preparing the products or handling storage and processing

property activities[27]. Hence, the bacterial isolates obtained from this study must have been contaminated by any or all these three means. Some of the bacterial organisms that were isolated, for example *Aeromonas hydrophilia* are known to be found in the soil. This could indicate that the water used for the production of these samples were not sanitary.

Power supply is generally poor and epileptic in Nigeria and Ilorin is not exempted. The growth of the microorganisms could therefore have been encouraged by improper storage of the samples as refrigeration is needed to maintain the quality of the samples and inhibit the growth of spoilage organisms.

The 'nono' samples contained more microorganisms than the yoghurt samples. This is expected due to the procedure and environment in which 'nono' is being produced. The practice of adding stream water and milky white supernatants of soaked baobab seeds, to colour 'nono' as reported by could be a source of microbial contamination to the dairy products[28]. It has been reported that contamination might result from poor hygiene in processing, handling, preservation and storage by the Fulani women that prepare and hawk 'nono'[8]. This factor could also contribute to the high amount of pathogenic microorganisms that were found in the 'nono' samples in this studies.

Conclusion

The different brands of yoghurts and 'nono' marketed in Ilorin metropolis are of unacceptable microbiological quality due to the presence of pathogenic oganisms. Although, the contamination load of the yoghurt is not as high as values observed in "nono" samples, presence of these pathogenic organisms in the commonly consumed products could constitute public health hazards and possibly food poisoining especially if they are stored under inappropriate conditions for long periods of time. With the frequent consumption and increase demand for dairy products (yoghurt and nono), there is the need for the continuous assessment of the microbiological quality of these products, proper measures to maintain good manufacturing procedures and standard public health measures like monitoring in order to minimize infections or poisoning through these products.

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