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Some locally fermented milk products sold in Onitsha main market and their bacteriological analysis

Abstract

This research was done to unveil the bacteria associated with three locally sourced fermented milk product sold at Onitsha main market in Anambra state in the South-East of Nigeria. The three locally sourced samples include; Nono, Soybean and Yoghurt. They were obtained aseptically from three different hawkers one for each. Ten-fold serial dilution was done while spread plate technique was adopted. After the inoculation of the second, fourth and six diluents followed by 24 hours incubation. The some of the results obtained include; 6.0 x 10^4, 2.0 x 10^4 and 1.0 10^4 for NONO corresponding to nutrient agar, MaConkey and SS agar plate. For the Soybean sample, 4.0 x 10^4, 1.2 x 10^4 and 1.0 x 10^4 corresponding to nutrient agar, MaConkey and SS agar plate. Finally, 5.0 x 10^4, 1,5 x 10^4 corresponding to nutrient agar, MaConkey agar plate. No growth was observed for SS agar. Streaking method was used for purification and isolates were stored in agar slants. Biochemical identification tests done revealed possible organisms like; *Kebsiella spp, Bacillus spp, Salmonella spp, Escherichia coli, Shigella sp, Lactobacillussp, Vibrio sp.* Further visit to the production and processing place of NONO by the research team revealed why it was more contaminated than other samples. Government should assist the locals to maintain good sanitary condition of the place or outright closure.

Keywords: Nono, soybean, yoghurt, sanitary, contaminations, incubation, inoculation

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Introduction

The fermented dairy products consumed today are generated through controlled microbial culturing and enzymatic conversions of major and minor milk components.[1] Fermentation improves shelf life, increases microbiological safety, adds flavor, and enhances palatability and organoleptic qualities. The fermentation process involves a series of complex reactions carried out by microorganisms, which transform milk constituents rendering new molecules of enhanced nutritive value and digestibility. Moreover, fermentation generates metabolites that can be major contributors of a daily healthful diet.[2] The contributions of milk components and dairy products to human health have been comprehensively reviewed.[3]

Exploration into the microbiology of nono, soya milk and yoghurt exposed that the fermented product is a culture of Lactobacillus bulgaricus growing with Streptococcus thermophiles. These organisms are symbiotic in their relationship as they are involved in conversion of almost all the sugar to lactic-acid and yielding small amount of bye-product. S. thermophiles produce diacetyl while acetaldehyde is produced L. bulgaricus.[4] They are also known as starter in milk fermentation that should be ample and feasible in the end fermented milk where aside from production of acid, similarly improves the product's flavour significantly.[5] Species of Lactobacillus and Streptococcus as well as Saccharomyces isolated from 'nono' has been used to develop starter cultures for controlled fermentation.[6] Other bacteria belonging to the general Lactococcus, Bacillus, Leuconostoc, Propionibacterium, ediococcus, and Bifidobacterium have been linked with fermented dairy products.[7] Species of Klebsiella, Enterococcus, Staphylococcus. Pseudomonas, Citrobacter, Micrococcus, Proteus, and Vibrio[8,9] has also been isolated. Fungal species that has been isolated include Aspergillus sp, Rhizopus sp, Trichophyton sp and Mucor.[10] While fermentation process is ongoing by lactic acid bacteria, other bacteria such as Listeria monocytogenes, Salmonella paratyphi, Brucella melitensis, Clostridium botulinum and Escherichia coli may create possible threat as they produce toxins that cause intoxication of the products.[11] The coliform count may be less in some studies[8,12] but their presence is an indication of fecal contamination and this can pose a health risk to the consumers. Enhancements in approaches (culture dependent and culture independent) used to identify microorganisms has greatly improved our knowledge on the micro biota of fresh milk.[13,14]

Materials and method

Study area

Onitsha Main Market, located in Anambra State, Nigeria, is one of the largest and busiest markets in West Africa. It spans a vast area and features thousands of stalls specializing in a wide range of goods, from electronics to textiles. The market is a major economic hub, providing employment for thousands and generating significant revenue for the state. Despite its bustling activity, the market faces challenges such as congestion, poor infrastructure, and security issues. Nonetheless, it remains a crucial center for commerce and trade, with ongoing plans for modernization and expansion. It has a geographic coordinate of Approximately 6.1454° N latitude and 6.7850° E longitude, the market is close to the famous River Niger Bridge, which connects Onitsha to Asaba in Delta State. These landmarks and the strategic location of the Onitsha Main Market contribute significantly to its status as a major commercial hub in the region.

Sample collection and processing

Samples (Nono, Soybean and Yoghurt) were collected from three difference vendors, hawkers and retail outlets in Onitsha main market, Anambra State. The samples were collected under aseptic conditions into sterile airtight sampling jars. They were further conveyed to Microbiology Department laboratory of Legacy University Okija, in

an insulated icebox immediately for bacteria analyses.

Enumeration and isolation of microorganisms from yoghurt, soybean milk and NONO: The microbial enumeration was done by determining the total viable counts (TVC) and total coliform count (TCC) using the techniques of Ogbonna.[15]

Total bacterial count: total viable counts and total coliform counts

Ten mililitre (10mL) of each sample was added aseptically to sterilized beaker tube containing 10mL of distilled water and mixed thoroughly to achieve a stock solution (sample) for 10-folds serial dilution. The aforementioned dilution was used to make subsequent dilutions. About 0.1mL of the sample was inoculated into well-labeled plate of Nutrient, MaConkey and Salmonella/Shigella agar using a pipette from the 10^{-2}, 10^{-4} and 10^{-6} dilution tubes (diluents). The inoculated plates were incubated at 37°C for 24 hours after they were well labelled. The colonies on the plates were then counted and recorded as (cfu/mL) using a colony counter.

Purification of the isolates

Colonies of various colours, shapes and sizes were picked from various plates and sub-cultured by streaking repetitively to acquire pure isolated. The pure isolates were stored on agar slant for further characterization and identification.

Identification of isolates from yoghurt, soybean milk and NONO: Bacterial isolates were identified by cultural, morphological and biochemical methods as described by Mubarack et al.,[16]and Ogbonna.[15] Bacterial identification was done using the appearance of colonies on culture media, Gram's reaction, biochemical tests (coagulase, indole, catalase, methyl red, Vogues-proskauer, citrate, carbohydrate fermentation test). Cultural identification was done by observing the appearance of isolates on the culture media and taking notes of the colour, sizes and shapes of colonies.[15,16]

Results

The results obtained after 24hrs of incubation are as represented in the Tables 1&2, Figures 1-7.

Table 1A Results of Total heterotrophic and coliform count

	Nutrient Agar (cfu/ml)			MacConkey Agar(cfu/ml)			Salmonella/ Shigella Agar (cfu/ml)		
	10^{-2}	10^{-4}	10^{-6}	10^{-2}	10^{-4}	10^{-6}	10^{-2}	10^{-4}	10^{-6}
NONO	60	58	56	20	15	11	6	3	1
SOYBEAN	40	35	30	12	10	9	1	NG	NG
YOGHURT	50	47	40	15	12	10	NG	NG	NG

Table 1B Results of Total heterotrophic and coliform count (standard representation)

	Nutrient Agar (cfu/ml)			MacConkey Agar(cfu/ml)			Salmonella/ Shigella Agar (cfu/ml)		
	10^{-2}	10^{-4}	10^{-6}	10^{-2}	10^{-4}	10^{-6}	10^{-2}	10^{-4}	10^{-6}
NONO	6.0×10^{-4}	5.8×10^{-6}	5.6×10^{-8}	2.0×10^{-4}	1.5×10^{-6}	1.1×10^{-8}	6×10^{-3}	3×10^{-5}	1×10^{-7}
SOYBEAN	4.0×10^{-4}	3.5×10^{-6}	3.0×10^{-8}	1.2×10^{-4}	1.0×10^{-6}	0.9×10^{-7}	1×10^{-3}	NG	NG
YOGHURT	5.0×10^{-4}	4.7×10^{-6}	4.0×10^{-8}	1.5×10^{-4}	1.2×10^{-6}	1.0×10^{-7}	NG	NG	NG

Cfu/ml : coliform forming unit, NG : No growth

Table 2 Morphological, cultural and BIOCHEMICAL, characteristics of bacterial isolates during the study

GR	CA	OX	CT	IN	MR	COA	M	Possible Organism
-R	+	-	+	-	-		-	*Kebsiella spp*
+R	+	-	+	-	-		+	*Bacillus subtilis*
+	-	+	-	+	-	+		*Salmonella spp*
-R	+	-	-	+	+	-	+	*Escherichia coli*
-R	+	-	-	-	+		+	*Citrobacter spp*
+C	+	-	+	-	+	+	-	*Staphylococcus spp*
+R	-	-	-	-	-		-	*Lactobacillus sp*
+C	-	-					-	*Streptococcus sp*
-R	-	+	-	-	-		-	*Vibrio sp*
+C	-	-	+	+	+		+	*Lctococcus sp*
-R	+	-	-	-	+	-	-	*Shigella sp*
-R	+	-	+	-	-		+	*Proteus sp*

LEGEND:

NG: No growth, GR = Gram reaction, CA = Catalase test OX = Oxidase test, CT = citrate test,IN = Indole test, MR = Methylred test,COA = Cozigulase test, M= motility test.

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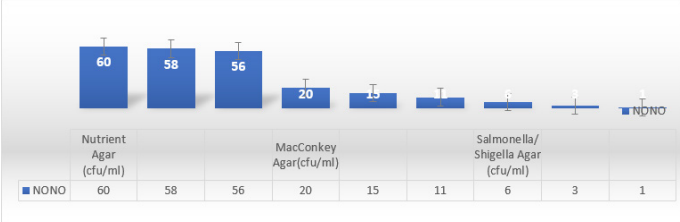

Figure 1 Colony count for NONO on the three media.

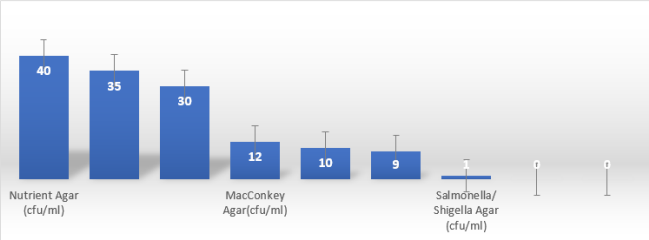

Figure 2 Colony count for Soybean on the three media.

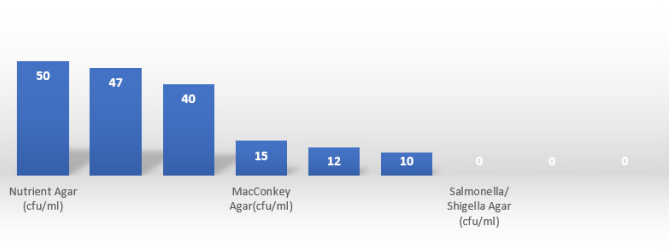

Figure 3 Colony count for Yoghurt on the three media.

Figure 4 Place where the Nono was purchased.

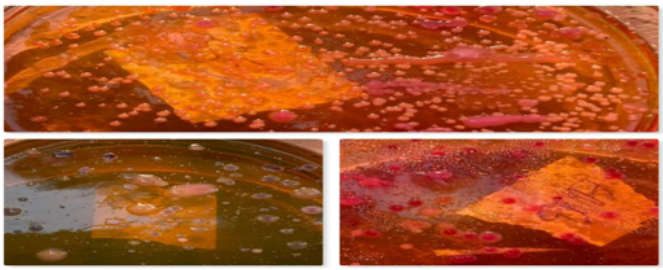

Figure 5 Colonies on some of the plate viewed on magnifying glass.

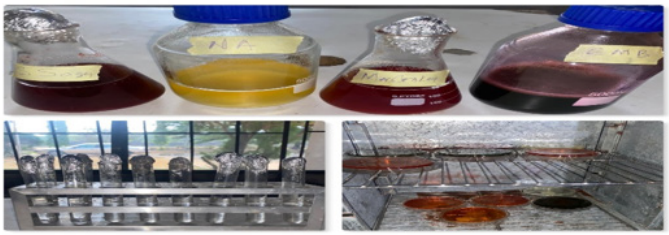

Figure 6 Media used and other materials.

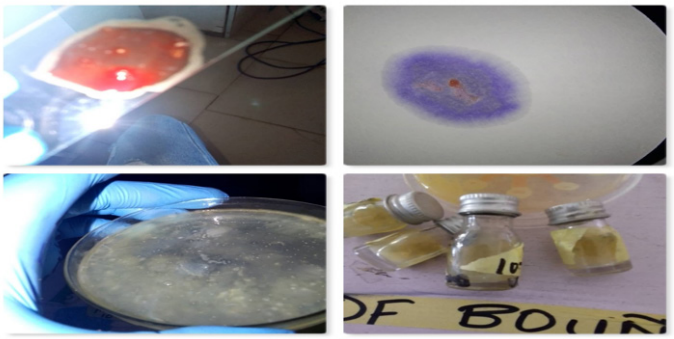

Figure 7 Agar slant, Slide for Gram staining and oxidase test.

Discussion

From the results as shown in both the tables and charts revealed that all the locally sourced products are contaminated. Of the three locally sourced samples, Nono has high bacterial load followed by the soybean. The source of the contamination may not be unconnected with poor handling, preparation and storage condition. The soybean may be contaminated right from the farm. Another way of contamination may be through the processing machine. From Table 1, NONO revealed that there was a growth on the Salmonella/Shigella agar. This could be from the water used in its final preparation. There is also the possibility of fecal contamination of the water used. At stage 1 during collection of samples directly from the udder, the presence of coliform in this stage may be due to contamination by fecal matter attached to the teats of the animal or the hand of the milking man.[17-19]

Results of further investigation on the source of the samples

Research team visited the place where these samples were prepared and processed. The following were observed.

a. The entrance/ route to the place where the NONO was prepared was waterlogged.

b. The water used by the locals to mix the powders in the preparation showed clear evidence of fecal contamination which was evidenced in the presence of growth on the SS agar.

c. The youth used in the mass production of the NONO do not observe routine hand washing.

d. Their dressing and outfit are not clean.

e. As shown in the photo, they use pit latrine.

f. Those preparing the NONO do not wear hand glove.

g. The milk for NONO production is not pretreated (pasteurized or sterilized) used use.

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h. There is no sterilization technique adopted for the raw material or finished products.

i. The machine used in blending the soybean was not cleaned thoroughly before using it for another.

j. It was also observed that the farmers that bring the soybean do not was the seed very well.

k. There is a well proven case of carriage container contamination of the soybean.

l. The hawkers of NONO move around leaving the prepared NONO open while on their head.

Recommendation by the research team

At the end of the research and visitation, the research team recommend as follows.

a. Clean water should be provided for them

b. Access to the production site should be made clean

c. The water should be checked for fecal contamination before use

d. The NONO should be kept in the fridge and preparation to avoid contamination.

Acknowledgement

None

Conflict of interests

Authors declare no conflict of interest.

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