

Some Locally Fermented Milk Products Sold in Onitsha Main Market and their Bacteriological Analysis

Osuji MI^{1*} and Nkwonta RC²

¹Department of Microbiology, Faculty of Natural and Applied Sciences, Spiritan University, Nneochi, Abia State, Nigeria

²Department of Microbiology, Faculty of natural and Applied Sciences, Legacy University, Okija, Anambra State, Nigeria

***Corresponding Author:** Osuji MI, Department of Microbiology, Faculty of Natural and Applied Sciences, Spiritan University, Nneochi, Abia State, Nigeria.

Received: May 19, 2025; **Published:** July 30, 2025

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, forth and six diluents followed by 24 hours incubation. The some of the results obtained include; 6.0×10^4 , 2.0×10^4 and 1.0×10^4 for NONO corresponding to nutrient agar, MacConkey and SS agar plate. For the Soybean sample, 4.0×10^4 , 1.2×10^4 and 1.0×10^4 corresponding to nutrient agar, MacConkey and SS agar plate. Finally, 5.0×10^4 , 1.5×10^4 corresponding to nutrient agar, MacConkey 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; *Klebsiella* spp, *Bacillus* spp, *Salmonella* spp, *Escherichia coli*, *Shigella* spp, *Lactobacillus* spp, *Vibrio* spp. 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

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* (Chanos., *et al.* 2020). 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 products 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*, *Edioccocus*, 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 Methods

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 milliliter (10 mL) of each sample was added aseptically to sterilized beaker tube containing 10 mL 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 0.1 mL of the sample was inoculated into well-labeled plate of Nutrient, MacConkey and *Salmonella/Shigella* agar using a pipette from the 10^{-2} , 10^{-4} and 10^{-6} dilution tubes (dilutents). 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, grams 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 24 hrs of incubation are as represented in the table.

	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 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	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

Table 1b: Results of total heterotrophic and coliform count (standard representation).

Cfu/ml: Coliform Forming Unit; NG: No Growth.

GR	CA	OX	CT	IN	MR	COA	M	Possible Organism
-R	+	-	+	-	-		-	<i>Klebsiella spp</i>
+R	+	-	+	-	-		+	<i>Bacillus subtilis</i>
+	-	+	-	+	-	+		<i>Salmonella spp</i>
-R	+	-	-	+	+	-	+	<i>Escherichia coli</i>
-R	+	-	-	-	+		+	<i>Citrobacter spp</i>
+C	+	-	+	-	+	+	-	<i>Staphylococcus spp</i>
+R	-	-	-	-	-		-	<i>Lactobacillus sp</i>
+C	-	-					-	<i>Streptococcus sp</i>
-R	-	+	-	-	-		-	<i>Vibrio sp</i>
+C	-	-	+	+	+		+	<i>Lactococcus sp</i>
-R	+	-	-	-	+	-	-	<i>Shigella sp</i>
-R	+	-	+	-	-		+	<i>Proteus sp</i>

Table 2: Morphological, cultural and biochemical, characteristics of bacterial isolates during the study.

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

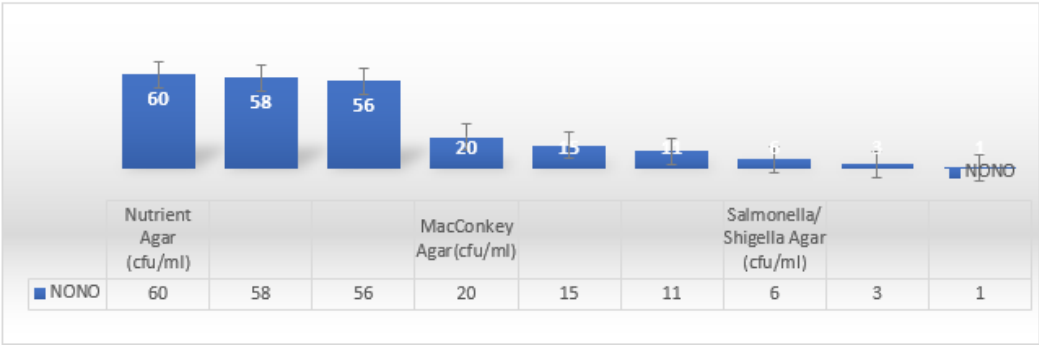


Figure 1: Colony count for NONO on the three media.

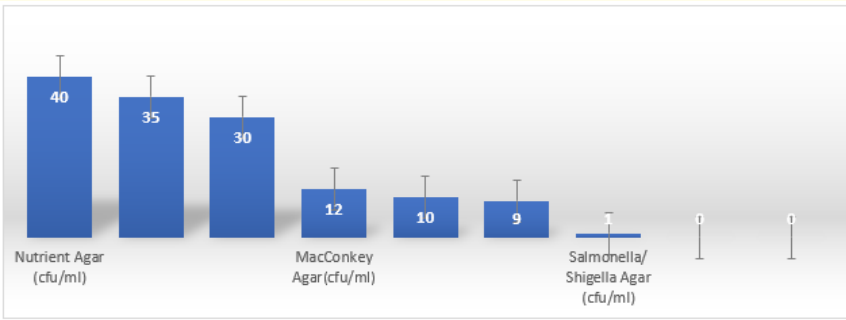


Figure 2: Colony count for Soybean on the three media.

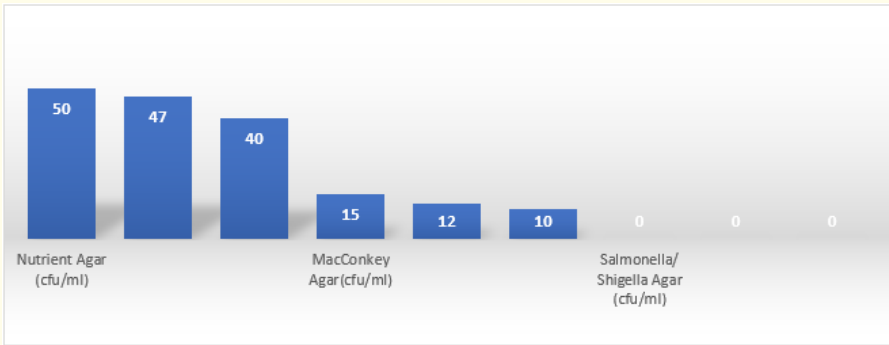


Figure 3: Colony count for Yoghurt on the three media.



Figure 4: Place where the Nono was purchased.

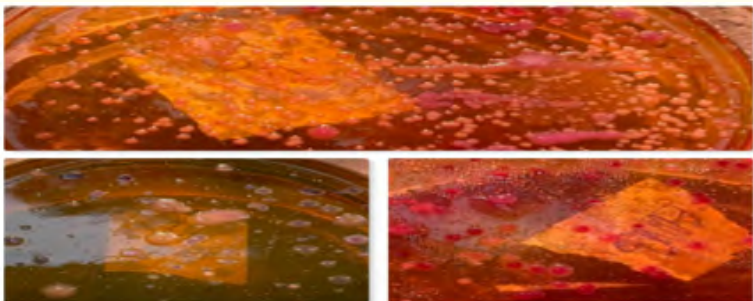


Figure 5: Colonies on some of the plate viewed on magnifying glass.



Figure 6: Media used and other materials.

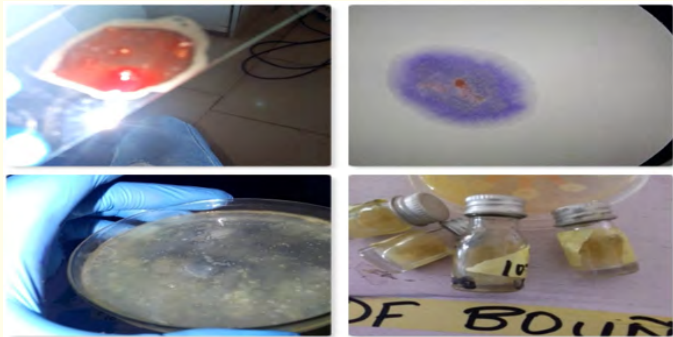


Figure 7: Agar slant, slide for gram staining and oxidase test.

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.

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:

1. The entrance/route to the place where the NONO was prepared was waterlogged.
2. 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.
3. The youth used in the mass production of the NONO do not observe routine hand washing.
4. Their dressing and outfit are not clean.
5. As shown in the photo, they use pit latrine.
6. Those preparing the NONO do not wear hand glove.
7. The milk for NONO production are not pretreated (pasteurized or sterilized) used use.
8. There is no sterilization technique adopted for the raw material or finished products.
9. The machine used in blending the soybean was not cleaned thoroughly before using it for another.
10. It was also observed that the farmers that bring the soybean do not was the seed very well.
11. There is a well proven case of carriage container contamination of the soybean.
12. 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:

1. Clean water should be provided for them
2. Access to the production site should be made clean
3. The water should be checked for fecal contamination before use
4. The NONO should be kept in the fridge and preparation to avoid contamination [17-19].

Conflicting of Interest

There is no conflicting of interest in this research.

Bibliography

1. Macori G and PD Cotter. "Novel insights into the microbiology of fermented dairy foods". *Current Opinion in Biotechnology* 49 (2018): 172-178.
2. Marco ML., *et al.* "Health benefits of fermented foods: Microbiota and beyond". *Current Opinion in Biotechnology* 44 (2017): 94-102.
3. Tunick MH and DL Van Hekken. "Dairy products and health: Recent insights". *Journal of Agricultural and Food Chemistry* 63.43 (2024): 9381-9388.
4. UmaMaheswari T., *et al.* "GTG5 fingerprinting of native *Streptococcus thermophilus* strains and its authentication by principal component analysis-a road to value added commercial yoghurt starter cultures". *International Dairy Journal* 122 (2021): 105161.
5. Petrova P., *et al.* "Traditional Bulgarian dairy products: ethnic foods with health benefits". *Microorganisms* 9.3 (2021): 480.
6. Fagbemigun O., *et al.* "Isolation and characterization of potential starter cultures from the Nigerian fermented milk product nono". *Microorganisms* 9.3 (2021): 640.
7. Ghosh T., *et al.* "Mechanistic insights into probiotic properties of lactic acid bacteria associated with ethnic fermented dairy products". *Frontiers in Microbiology* 10 (2019): 502.
8. Maikai BV and Madaki PD. "Enumeration of coliforms in fermented milk product (nono) sold in Samaru, Kaduna State, Nigeria". *Sokoto Journal of Veterinary Sciences* 16.4 (2021): 50-57.
9. Nassarawa SS and Sulaiman SA. "Microbiological and chemical evaluation of traditional fermented skimmed milk (nono) in Kano State Nigeria". *International Journal of Food Sciences* 3.1 (2020): 33-46.
10. Bazata AY., *et al.* "Assessment of microbiological quality of fura da nono produced in kebbi state, Nigeria". *Equity Journal of Science and Technology* 7.2 (2020): 45-48.
11. Ezeonu CS and Ezeonu NC. "Biological risks associated with fermented dairy products, fruits, vegetables and meat: a critical review". *Advanced Biotechnology and Microbiology* 2.1 (2024): 555577.
12. Yabaya A., *et al.* "Bacteriological quality of fermented milk sold locally in Samaru and Sabon-gari Market, Zaria-Nigeria". *Continental Journal of Microbiology* 6.1 (2023): 14-18.
13. Pang M., *et al.* "Insights into the bovine milk microbiota in dairy farms with different incidence rates of subclinical mastitis". *Frontiers in Microbiology* 9 (2023): 2379.
14. Fusco V., *et al.* "Microbial quality and safety of milk and milk products in the 21st century". *Comprehensive Reviews in Food Science and Food Safety* 19.4 (2020): 2013-2049.
15. Ogbonna IO. "Microbiological analyses and safety evaluation of nono: a fermented milk product consumed in most parts of Northern Nigeria". *International Journal of Dairy Science* 6.3 (2011): 181-189.
16. Mubarack HM., *et al.* "Microbial quality of raw milk samples collected from different villages of Coimbatore District, Tamilnadu, South India". *Indian Journal of Science and Technology* 3.1 (2010): 61-63.
17. Esonu DO., *et al.* "Occurrence and antimicrobial susceptibility patterns of *Staphylococcus aureus* and *Salmonella* species in fresh milk and milk products sold in Zaria and environs, Kaduna State, Nigeria". *Sahel Journal of Veterinary Sciences* 18.2 (2021): 1-8.

18. Mosiara S. "Prevalence and risk factors associated with Brucellosis; a critical literature review". *Animal Health Journal* 3.1 (2022): 16-26.
19. Rojas A., *et al.* "Coliform Petrifilm as an alternative method for detecting total gram-negative bacteria in fluid milk". *Journal of Dairy Science* 103.6 (2020): 5043-5046.

Volume 21 Issue 8 August 2025

©All rights reserved by Osuji MI and Nkwonta RC.