

Microbial Contamination of Regulated and Unregulated Yoghurt Sold in Abraka, Delta State, Nigeria

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ABSTRACT

The quality of regulated and unregulated yoghurt sold in Abraka, Delta State, was determined using standard microbiological procedures. The physical parameters of the brands at time of purchase were also determined. Bacterial counts for branded yoghurt ranged from 0.0×10^3 CFU/ml (sample A) to 3.8×10^3 CFU/ml (sample E) and a total coliform counts ranged from 0.0×10^3 CFU/ml (sample A) to 3.60×10^3 CFU/ml (sample B). However, the bacterial counts for unbranded yoghurt ranged from 1.4×10^3 CFU/ml (sample D) to 3.0×10^3 (sample C) and total coliform counts ranged from 1.0×10^3 CFU/ml (sample A) to 2.5×10^3 CFU/ml (sample C). The occurrence of potential pathogenic microorganisms from both regulated and unregulated yoghurt sample is alarming as compared with Codex Alimentarius Standards for yoghurt products. The isolated bacteria identified include *Staphylococcus aureus*, *Lactobacillus* spp, and *Escherichia. coli* and *Streptococcus* spp. The result obtained from this study shows poor microbiological standards of unregulated yoghurts during the research period. This result underlines the need for improved hygienic measures in the processing and distribution of these products to prevent harmful public health issues.

Keywords: Quality, sensory, sweeteners, preservatives, emulsifier.

Introduction

Yoghurts are dairy products consumed throughout the world [1]. Yoghurt is rich in calcium and protein which makes it easy to digest. Fermentation during yoghurt production gives its sensory characteristics [2]. Fermentation of lactose by these bacteria acts on milk protein to give yoghurt its texture and characteristics. There is a reduction in pH which is characteristic of yoghurt [3]. *Streptococcus thermophiles* and *Lactobacillus bulgaricus* are two common species that finds application in the commercial production of yoghurt, and act as starter cultures which produces amino acid from milk proteins [4].

A minimum range of active cultures of (10^7 CFU) per gram has been proposed by the National Yoghurt Association, which requires an acidity of pH 4.6 or lower, and requires a total dairy ingredient of 51 percent, provides for pre-culture analogous and pasteurization, allows milk reconstituted and protein concentrates as optional milk ingredients, allows the use of suitable and safe sweeteners, preservatives and emulsifiers [5]. Yeast and molds in yoghurt utilise acid thereby reducing its acidity, this favors bacterial growth that are putrefactive changing its consistency, colour of food, tenderness, flavor, and may render it unsafe to consumers. To ensure a good yoghurt quality control, measures should be put in place [5].

Poor handling and personal hygiene practice are major cause of contamination of the yoghurts that are deemed below standard [6]. Pathogenic microorganisms in yoghurt is alarm. Contamination of yoghurt by coliform bacteria occurs through water and poor hygiene [7]. Further microbial contamination can occur during milking, handling, storage, processing and clearing [8].

Furthermore, species gotten from yoghurt products may

include *Escherichia coli*, *Staphylococcus aureus*, *Campylobacter jejuni*, *Salmonella*, *Yersinia enterocolitica* [8].

Poor handling is accountable for contamination with fungi, bacteria, and protozoa resulting in diseases condition [9].

Therefore, ensuring a good quality yoghurt by microbial quality assessment becomes important considering the implications linked to diseases such as brucellosis, tuberculosis, scarlet fever, gastroenteritis and diphtheria that are transmitted via milk product.

This study aimed at evaluating the bacterial quality of both regulated and unregulated yoghurts brands, and the dangers common to consumption of imperfectly pasteurized dairy products of both regulated and unregulated with a view to determine the bacterial species associated with yoghurts in Abraka, Delta State, Nigeria.

Materials and Methods

Study Area

The study was conducted in Abraka, Etiope East Local Government area of Delta State, Nigeria.

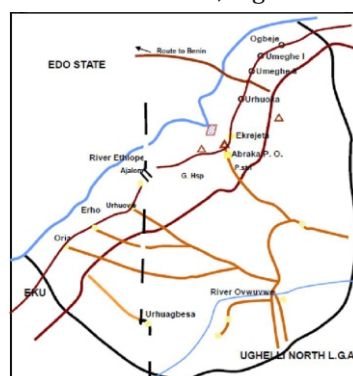


Figure 1: (Retrieved from researchgate.net on 23/11/2021). Showing the map of Abraka, Delta State, Nigeria.

Collection of sample

Five (5) samples branded and local brands of sachet yoghurt were obtained from supermarkets and yoghurt hawkers within the Ethiopia East Local government area. Samples were wrapped properly to avoid contact with air. All possible efforts for time lag reduction between collection and analysis were made so that no significant change in yoghurt quality would occur. Samples were then conveyed in an insulated foam box with ice to the laboratory to maintain a temperature range between 4°C to 6°C for analysis.

Microbiological Analysis

Bacterial enumeration: The isolation of the microorganism in each dilution sample was done by pour plate technique. Freshly prepared media was separately inoculated with an aliquot of 0.1ml of the serial dilutions. Cool, molten media were aseptically poured onto the inocula (obtained from 10^{-2} and 10^{-3} dilutions) and were properly mixed to allow uniform mixing of the inoculum, and were incubated at 37°C for 24 - 48 hours. The media used were nutrient agar, blood agar (Oxoid limited), Salmonella-Shigella agar (Oxoid Limited), mannitol salt agar (Oxoid Limited), MacConkey agar (Oxoid Limited) and eosin methylene blue agar (Oxoid Limited). The bacterial isolates were characterized based on phenotypic and biochemical characteristics using standard methods following Clinical Laboratory Standards Institute (CLSI) [10] guidelines.

Determination of pH: pH of samples were determined with pH meter (H.Jurgons Co).

Results

pH of branded yoghurts is presented as Table 1. All branded yoghurt brands had pH which ranged from 2.82 ± 0.01 - 3.0 ± 0.21 with sample C with a pH value (2.82) and B (3.13)

Table 1: pH of branded yoghurt

Branded Yoghurt	pH CXS standards 243
A	3.0 ± 0.21 4.25 - 4.5.
B	3.13 ± 0.25
C	2.82 ± 0.21
D	3.12 ± 0.16
E	3.10 ± 0.07

(CXS 243-2021) Codex Alimentarius Standards.

pH of five different unbranded yoghurts is presented as Table 2. All unbranded yoghurt brands had pH which ranged from 3.23 ± 0.21 - 6.13 ± 0.42 with sample C with lowest pH value (3.23) and B (6.13)

Table 5: Morphological and Biochemical reactions of bacteria isolated from branded yoghurt sample

Sample	Gram Stain	Shape	Catalase	Indole	Citrate	Nitrate reduction	Urease	H ₂ S	VP	Motility	Methyl Red	Coagulase	Oxidase	Inference
A	+	Rods	+	-	-	-	-	-	-	-	-	-	-	<i>Lactobacillus Spp</i>
B	+	Rods	-	-	-	-	-	-	-	-	-	-	-	<i>Lactobacillus Spp</i>
B	+	Rods	+	-	-	+	-	-	-	+	+	-	-	<i>E.coli</i>
C	+	Rods	+	-	-	-	-	-	-	-	-	-	-	<i>Lactobacillus Spp</i>
D	+	Rods	+	-	-	-	-	-	-	-	-	-	-	<i>Lactobacillus Spp</i>
E	-	Rods	+	+	-	+	-	-	-	+	+	-	-	<i>E.coli</i>
E	+	Cocci	+	-	-	-	+	-	+	-	-	+	+	<i>Staphylococcus aureus</i>

Biochemical and Morphological reactions of bacterial isolate from the unbranded yoghurt sample is presented as Table 6. Gram-positive *Streptococcus Spp* were catalase, methyl red, VP and nitrate reduction positive, urease, H₂S, motility, coagulase oxidase negative, in sample C and E Gram positive *Staphylococcus aureus* were catalase, coagulase, methyl red, VP, Urease and oxidase positive, and indole, citrate, H₂S production and motility negative, while *E. coli* were Gram-negative rod and catalase, MR, indole, nitrate reduction and motility positive, and oxidase, VP, citrate, urease, coagulase and H₂S negative

Table 2: pH of unbranded yoghurt

Branded Yoghurt	pH CXS standards 243
A	5.0 ± 0.25 4.25 - 4.5.
B	6.13 ± 0.42
C	3.23 ± 0.21
D	3.45 ± 0.26
E	4.10 ± 0.37

(CXS 243-2021) Codex Alimentarius Standards.

Bacteria and coliform of branded yoghurt is presented in Table 3. Bacterial count of sample B was 3.4×10^3 , C (3.6×10^3) and E (3.8×10^3 cfu/ml) while coliform counts for samples B, C and E were 3.60×10^3 cfu/ml, 2.75×10^3 cfu/ml and 3.35×10^3 cfu/ml.

Table 3 Bacteria and coliform (cfu/ml) of branded yoghurt

A	0.0×10^3	$0.0 \times 10^3 < 1$ cfu/ml
B	3.4×10^3	3.60×10^3
C	3.6×10^3	2.75×10^3
D	0.0×10^3	2.35×10^3
E	3.8×10^3	3.35×10^3

Branded yoghurt Bacterial Coliform (CXS 243)
(CXS 243-2021) Codex Alimentarius Standards

Bacteria and coliform of unbranded yoghurt is presented as Table 4. Yoghurts samples (A) having 2.0×10^3 , (B) 2.4×10^3 , (C) 3.0×10^3 , (D) 1.4×10^3 and (E) 2.8×10^3 cfu/ml and coliform of A (1.0×10^3), B (1.60×10^3) C (2.5×10^3), D (2.2×10^3) and E (2.3×10^3 cfu/ml).

Table 4 Bacteria and coliform (cfu/ml) of unbranded yoghurt.

A	2.0×10^3	1.0×10^3	<1 cfu/ml
B	2.4×10^3	1.60×10^3	
C	3.0×10^3	2.5×10^3	
D	1.4×10^3	2.2×10^3	
E	2.8×10^3	2.3×10^3	

Branded yoghurt Bacterial Coliform (CXS 243)
(CXS 243-2021) Codex Alimentarius Standards

Biochemical and morphological reactions of isolates from the branded yoghurt sample is presented as Table 5. *Lactobacillus spp* were identified in sample A, and were Gram-positive rod, and were catalase positive, and indole, citrate, urease, H₂S, VP, motility, methyl red, coagulase and oxidase negative. *S. aureus* were obtained from sample E, and were positive for catalase, urease, coagulase and oxidase but negative for H₂S, motility, samples B and *E. coli* was gotten, these were Gram - rod and showed positive for catalase, MR, indole and nitrate reduction, motility, and negative for oxidase, VP, citrate, urease, coagulase and H₂S.

Table 6: Biochemical and Morphological reactions of bacterial isolate from unbranded yoghurt sample.

Sample	Gram Stain	Shape	Catalase	Indole	Citrate	Nitrate reduction	H ₂ S	VP	Urease	Motility	Methyl Red	Coagulase	Oxidase	Inference
A	+	Cocci	+	-	+	+	-	-	-	-	+	-	-	<i>Streptococcus Spp.</i>
A	-	Rod	+	+	-	+	-	-	-	+	+	-	-	<i>E.coli</i>
B	+	Cocci	+	-	-	+	-	-	-	-	+	-	-	<i>Streptococcus Spp</i>
C	+	Cocci	+	-	-	-	-	+	+	-	+	+	+	<i>Staphylococcus aureus</i>
C	-	Rod	+	+	-	+	-	-	-	+	+	-	-	<i>Escherichia.coli</i>
D	+	Rods	+	-	-	-	-	-	-	-	-	-	-	<i>Lactobacillus Spp</i>
E	+	Cocci	+	-	-	-	-	+	+	-	+	+	+	<i>Staphylococcus Aureus</i>
E	-	Rod	+	+	-	+	-	-	-	+	+	-	-	<i>E. coli</i>

Discussion

Batch number, manufacturer's address, and NAFDAC number were provided, but they do not provide information on microbial composition/contents. Studies vary largely due to intrinsic factors in yoghurt production. The occurrence of *Lactobacillus* spp in all samples showed it's an important yoghurt starter culture.

This statement agrees with earlier work Ifeanyi *et al.*, (2013) [11] where *Lactobacillus* spp, *Streptococcus* spp and *Staphylococcus* spp were recovered, and that bacteria other than *Lactobacillus* spp can cause food poisoning [12, 13].

Also, *Lactobacillus* spp fermentation also confer sensory characteristics in yoghurt. Additionally, some must have been contaminated with microorganisms, and this will create awareness about the existing situation of possible contamination. Isolated bacteria from both branded and unbranded yoghurt indicates post-production contamination. This observation agrees with studies where bacteria are known as yoghurt contaminants [14, 15].

Isolates from both branded and unbranded yoghurt indicate post-production contamination. This observation agrees with previous reports where bacteria are known as yoghurt contaminants [14, 15].

The microbiological and biochemical analyses revealed that identified organisms agree with previous report by Okonkwo *et al.* (2011) [16], who reported the possibility of contamination. In several fermented foods, *Lactobacilli* are often a relevant microbial component and can interact with gut microflora [17]. *Streptococcus* sp in regulated and unregulated yoghurt brands presents consumer risk. Report by Abdel-Hameed says bacteria in food particle suggested that personnel during handling are possible route of contamination [18]. These findings showed that these bacteria are indication of poor sanitary practice.

Therefore, strict factory and personnel hygiene practices during processing have been known as steps to ensuring safety of yoghurts in Nigeria.

Conclusion

This work revealed that these bacteria in both branded and unbranded yoghurts indicate post-production and cross-contamination. Cross-contamination of microorganisms is an ongoing risk in yoghurt production, and a route of disease infection. Therefore, consciously established systems to avert health challenges that may ensue is necessary. Awareness of staff through training, print and electronic media should be given priority. Stakeholders, yoghurt producers, vendors and customers be duty-bound to pay attention to the safety and standard of yoghurt.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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