



MICROBIAL COUNT ASSESSMENT OF SOME COMMERCIAL YOGHURTS SOLD IN SAMARU, ZARIA METROPOLIS, KADUNA STATE

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ABSTRACT

In view of the importance attached to microbiological safety and identification of contamination associated with yoghurt production and distributions, this study titled "microbial counts assessment of commercial yoghurts sold in Samaru, Zaria metropolis, Kaduna state was conducted. Seven different brands of NAFDAC certified yoghurts from different retailers were purchased in Samaru of Sabon Gari Local Government Area of Zaria, Kaduna State. They were labelled and conveyed to the Department of Microbiology at Ahmadu Bello University, Zaria, in ice packs for microbiological analysis. Morphological Characterization and Microscopic Observation of the Bacteria, which consist of Gram Staining and Biochemical Characterization where carried out. The result revealed that, bacterial counts in the seven sampled brands of yogurts ranges from 1.20×10^6 (SB) to 3.70×10^6 (SC), *E. coli* counts ranged from 1.00×10^4 (SG) - 6.00×10^4 (SD), Fungal counts fall to a range of 1.00×10^4 (SE) - 8.00×10^4 (SB), Staphylococcal ranged between 6.00×10^4 (SB) - 1.10×10^5 (SA and SG) while Coliform viable count was recorded only in sample SB with value of 1.00×10^4 . Of all obtainable outcomes, only total bacterial count falls within regulatory limits specified by International Organization for Standardization, ISO, 2017 and 2018. E-Coli, Fungal, Staphylococcal and Coliform counts all yield outputs at many folds above their respective ISO regulatory limits. These findings suggest that, despite bearing NAFDAC label by each of these products, the yogurt being sold in Samaru cannot be said to be hygienically suitable for consumption and thus may pose risk to human health. Further studies of microbial counts of certified yogurts sold in Samaru is recommended to be able to verify observations made in this study.

Keywords: Microbial, Count, Commercial, Yoghurt, Contamination

INTRODUCTION

Yoghurt is the oldest fermented milk product obtained from the lactic acid fermentation of milk. This involves the addition of starter cultures which are known as *Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus*). The two genera play important roles in the manufacture of yogurt from milk product through acidification and synthesis of aromatic compounds (Serra *et al.*, 2009). Yoghurt is a balanced food containing nutrients such as protein, vitamins and calcium. People who are moderately lactose intolerant can consume yoghurt without ill-effect because much of the lactose in the milk precursor is converted to lactic acid by the bacterial culture (Alayande, 2008). The current handling and distribution methods employed by vendors, from manufacturers to consumers, can lead to post-production contamination due to inadequate temperature control and unhygienic transportation, posing potential public health risks. The study aims to determine the microbial content in yoghurt samples, isolate and characterize microorganisms, and identify bacterial isolates using biochemical tests. This is significant because vendors/marketers may not maintain healthy measures and proper temperatures after purchasing yoghurts, making them susceptible to post-production contamination, which endangers public health.

Previous research has highlighted concerns regarding the microbial content and potential contamination of yoghurt products. Güllü *et al.* (2023) found that strained yoghurt samples were subject to contamination from raw materials to consumption, which could negatively affect public



health and product quality. Jaafar *et al.* (2024) showed that drying bio-yoghurt at 40-60 °C decreased the number of probiotic starter bacteria, while Dushkova *et al.* (2023) examined the microbiological, physicochemical, and organoleptic properties of yoghurts produced by ultrafiltered goat's milk. Delgado-Fernández *et al.* (2019) investigated the impact of prebiotics on yoghurt fermentation and cold storage. Niamah (2023) explored the use of mixed starter cultures to improve yoghurt flavour. Hoxha *et al.* (2023) studied lactic acid bacterial strains with probiotic potential and bioprotective properties as starter culture additions. Sert *et al.* (2023) examined the effects of high-pressure homogenization on yoghurt samples from sheep milk. Paszczyk & Tońska (2022) analyzed the composition of fatty acids, minerals, and lipid quality indices in milk and yoghurts produced with selected starter cultures enriched with *Bifidobacterium bifidum*. Wijesekara *et al.* (2022) evaluated the properties of probiotic stirred yogurts with plant pigments. Jańczuk *et al.* (2023) evaluated the nutritional value, microbiological quality, organoleptic properties, and structure of natural yoghurts made with the addition of lactoferrin during refrigerated storage. Luzzi *et al.* (2020) investigated the microbial ecology of reformulated yoghurt produced using bi-enzymatic modification of lactose. Nasr & Abd-Alhalim (2023) focused on the identification of probiotic properties of lactic acid bacteria isolated from traditional fermented dairy products from Egypt. Gürkan *et al.* (2022) studies, 54 yeast strains were isolated from dairy products. Benkirane *et al.* (2023) focused studies on was the characterization and assessment of F21 strain, isolated from Lben (a traditional Moroccan fermented milk), as food bio-preservative.

These studies collectively highlight the importance of monitoring and controlling microbial content in yoghurt to ensure food safety and quality. This underscores the significance of the present study, which seeks to evaluate the microbial quality of commercial yoghurts in Samaru, Zaria, and provide recommendations for improving hygiene practices in yoghurt production and handling.

MATERIALS AND METHODS

Study Area

This study assessed the microbial quality of commercial yoghurts sold in Samaru, Zaria metropolis, Kaduna State. Seven different brands of NAFDAC-certified yoghurts were purchased from various retailers in Samaru, Sabon Gari Local Government Area of Zaria, Kaduna State. The samples were labelled and transported in ice packs to the Department of Microbiology laboratory at Ahmadu Bello University, Zaria, for microbiological analysis.

Data Collection

Sample Collection: Seven different brands of yoghurts were purchased from different retailers in Sabon Gari Local Government Area of Kaduna State.

Microbiological Analysis: Morphological characterization and microscopic observation of bacteria, including Gram staining and biochemical characterization, were performed.

Morphological Characterization of Bacteria: The nutrient agar was prepared, sterilized, and poured into sterile Petri dishes to solidify. Using aseptic technique, a loop full of a 24-hour pure bacterial culture was streaked on the agar using the three-quadrant streak method to obtain isolated colonies. The plates were incubated at 37°C for 24 hours, after which bacterial growth was assessed for size, pigmentation, form, margin, elevation, and texture (Cappuccino & Sherman, 2005). **Microscopic Observation of Bacteria:** Potential bacteria were observed under a microscope to study their visual properties. **Gram Staining:** A thin film of each of the isolate was prepared by picking a bit of growth from the plate into a drop of sterile distilled water on a clean grease-free glass slide using a sterile wire loop and then smeared and allowed to air-dry then heat fixed over a Bunsen burner flame. The fixed smear was then flooded with crystal violet stain for 60 seconds (basic dye). It was rinsed off gently with water and stained with iodine solution for 60 seconds (Mordant). The Smear was rinsed with water and decolorized by flooding with acetone for 10 seconds. The smear was finally counterstained with safranin. After which it was rinsed off with water after 60 seconds and allowed to air dry and viewed under the microscope using a x40 objective (oil immersion objective). (Cheesebrough, 2004).

Biochemical Characterization: Several biochemical tests were done according to the microbiology laboratory manual (Cappuccino and Sherman, 2005), including Indole production test, Methyl red, Voges-Proskauer, Citrate utilization, and Catalase tests, which were conducted for presumptive identification of bacteria chosen before.

Sampling Techniques

Serial dilution:

Samples were serially diluted using peptone water to create dilutions from 1:10 to 10⁻⁵.

Spread plate method:

Aliquots of diluted samples were labeled as raw, 10⁻¹, 10⁻², 10⁻³ and 10⁻⁴. Three Mannitol salt agar plates, three MacConkey agar plates and three XLD agar plates were labeled as raw, 10, 10, 10, and 10. From each of the diluted sample test tubes 0.2 ml of sample from the test tubes labelled raw, 10⁻¹, 10⁻², 10⁻³ and 10⁻⁴ were added to the respective plates and the drops were spread using the spread plate technique with a sterile bend glass rod. All plates were then incubated at 37 °C for 24-48 hours (Cheesebrough, 2004). Bacterial colonies were then counted and recorded as colony-forming units per gram (CFU/g).

Catalase test: A drop of 3% hydrogen peroxide (H₂O₂) was placed on a clean glass slide and a sterile wire loop was used to pick a bit of the isolate from the slant unto the 3% hydrogen peroxide solution on the glass slide and emulsified. A positive catalase reaction shows bubbles and frothing while negative reveals an absence of bubbles (Cheesebrough, 2004).

Citrate Utilization test: The isolates were inoculated on Simmon's citrate agar slant and incubated at 37°C for 24 – 48 hours. The development of a deep blue color indicates a positive reaction and no change in color indicates negative test (Cheesebrough, 2004).

Indole Production test: The isolates were grown in 5ml peptone water in bijoux bottles for 24hours. After this, three drops of Kovac's reagent was added, about 3 to 4 drops of the reagent are usually sufficient. It was then shaken gently. A positive reaction was indicated by the development of a red color in the reagent layer above the broth within a few minutes. In a negative reaction, the indole reagent retains its yellow color (Cheesebrough, 2004).

Methyl red-Voges-proskauer test (MRVP): The isolates were grown in 5ml MR-VP broth and incubated for 24-48 hours at 35-37°C. After this, 1ml of the broth was transferred into sterile test tubes. To this small quantity, 2 drops of methyl red indicator were added and mixed properly. A positive test is indicated by the development of an intense red color and a yellow color signifies a negative test. To the other tubes, 5 drops of 40% potassium hydroxide (KOH) was added followed by 5 drops of 5% alpha- naphthol. The tubes were shaken and placed in a sloping position. The development of red color starting from the liquid air interface within 1 hour indicates a Voges Proskauer positive test. Absence/no red color change indicates a negative result (Cheesebrough, 2004).

RESULTS

Total Bacterial Counts

The study revealed that bacterial counts in the seven sampled brands of yogurts ranged from

Table 1: Total Viable Microbial Counts

S/N	Sample Code	Bacterial Count	E.coli Count	Fungal Count	Staphyccocal Count	Coliform Count
1	SA	2.10E+06	-	3.00E+04	1.10E+05	-
2	SB	1.20E+06	4.00+04	8.00E+04	6.00E+04	1.00E+04
3	SC	3.70+06	-	6.00E+06	1.30E+05	-
4	SD	2.30E+06	6.00+04	4.00E+04	1.80E+05	-
5	SE	1.60+06	2.00E+04	1.00E+04	-	-
6	SF	1.90+06	-	-	8.00E+04	-
7	SG	3.10+06	1.00E+04	4.00E+04	1.10E+05	-
	Microbiological Standard	1.0E+07 cfu/ml	1.0E+01cfu/ml	1.0E+02cfu/ml	1.0E+01cfu/ml	1.0E+01cfu/ml
	Source	ISO, 1987	ISO, 2018	ISO, 2017	ISO, 2017	ISO, 2018



1.20E+06(SB) - 3.70E+06 (SC), *E. Coli* counts ranged from 1.00E+04 (SG) - 6.00E+04(SD), Fungal counts fall to range 1.00E+04 (SE) - 8.00E+04 (SB), Staphylococcal ranged between 6.00E+04 (SB) 1.10E+05(SA and SG) while coliform viable count was recorded only in sample SB with value of 1.00E+04. Meanwhile, no viable count registered for Salmonella across all seven samples.

Gram Reaction, Morphology and Biochemical Characteristics of Bacterial Isolates

Gram reaction, morphology of different isolates and their Biochemical Characteristics were shown in Table 2.

During the study, after all the biochemical tests has been conducted, it was found that *Staphylococcus aureus* was present in samples SB. SC. SF and SG, *Bacillus* species isolates was found in 3 different samples of labels SA, SB and SD. while *Klebsiella* species isolates was found in 2 different samples (SD and SG).

Table 2: Grams reaction and Biochemical Characteristics of the Bacterial Isolates

S/N	Sample code	Grams reaction	Cell morphology	Indole	MR	VP	Citrate	Cat	Coag	Probable Bacteria
1	SA(MSA)	+	rod	-	+	-	-	-	-	<i>Bacillus</i> species
2	SB(MSA)	+	rod	-	+	-	-	-	-	<i>Bacillus</i> species
3	SB(EMB)	+	cocci	-	+	+	+	+	+	<i>Staphylococcus</i> sp
4	SC(MSA)	+	cocci	-	+	+	+	+	+	<i>Staphylococcus</i> sp
5	SD(MSA)	+	rod	-	+	-	-	-	-	<i>Bacillus</i> species
6	SD(EMB)	-	short rod	-	-	+	+	-	-	<i>Klebsiella</i> species
7	SF(MSA)	+	cocci	-	+	+	+	+	+	<i>Staphylococcus</i> sp
8	SG(MAC)	-	short rod	-	-	+	+	-	-	<i>Klebsiella</i> species
9	SG(MSA)	+	rod	-	+	+	+	+	+	<i>Staphylococcus</i> sp

+ = present, - = absent

DISCUSSIONS

The effectiveness of hygienic practice and hygienic status in the production of dairy food products is reflected by their total bacterial count, fungus count and coliform organisms present. These are commonly used methods for evaluation of quality control of food products. A variety of diseases can potentially be transmitted through milk and other milk products. The pathogenic agent source can be cow or humans associated with the production and handling of the foods and it can be transmitted into other milk products (Ariful, 2017).

There was high microbial count in the samples. The total bacterial counts of 1.20E+06(SB)-3.70E+06 (SC) cfu/mL. The high counts, although lower than the Codex standard of 10^7 cfu/mL, is an indication of contamination, which may have resulted from unhygienic practices such as the use of non-potable water and unsterilized utensils during production (Ilyasu, 2018).

The presence of coliforms in milk indicates secondary infections and is a sign of it being unsafe for human consumption. Coliforms bacteria are destroyed very rapidly during the pasteurization Process. Based on current standards, the number of coliforms must be less than 100 CFU/mL in raw cow's milk and less than 10 CFU/mL in pasteurized milk (Fateme and Maryam, 2018). The coliform viable count recorded only in sample SB with value of 1.00E+04 cfu/mL is greater than the codex standard of 10 cfu/mL (FAO/WHO, 2018). Coliforms have been proven and endorsed by public health authorities worldwide as a pointer of post handling contagion in manufacture (Rojas *et al.*, 2020). Coliforms are considered as normal flora of the intestinal tract of human and animals and their presence indicates direct faecal contamination. They have been used as indicator organisms for bacteriological quality of milk and its products (ICMSF, 1986).

Klebsiella sp, is a coliform, it is susceptible to pasteurization but its presence in post pasteurized yogurt may be as a result of faulty heat process or water used in manufacture, unhygienic hawking habits, handlers with poor sanitary practices and unhygienic storage environment. The level of presence of coliform and indicator organisms has been described as index of food hygiene. *Klebsiella* sp, has been related to bacterial pneumonia cases more severe than those produced by *Streptococcus pneumonia* and urinary tract infection (Agu *et al.*, 2014).



The presence of *Staphylococcus aureus* in the samples could be as a result of poor processing, handling and packaging, since they are often found on the outer surface of the body Prescott *et al.*, (2004). *Staphylococcus aureus* is resistant to heat, drying and radiation and has been linked to gastro enteritis, skin infections, boils, pneumonia and meningitis in debilitated patients. The Occurrence of Streptococci is in line with the works of Bramley and Mckinnon (2004) who showed that organisms that contaminate the surface teat and udders of the cow include *Staphylococci*, Streptococci, spore formers, coliforms and gram negative bacteria which can survive pasteurization temperature and Streptococci which can grow under refrigeration. The detection of *Staphylococcus aureus* and *Bacillus subtilis* implied contamination from personnel and the environment respectively and this is of public health importance because of their ability to cause a wide range of infections especially food-borne intoxication. This is buttressed by the assertion made in several reports that *Staphylococcus aureus* has link with gastroenteritis by producing toxic chemical enterotoxins (Raji and Jiva. 2019). The detection of *Bacillus subtilis* could also be due to easy distribution of their spores in the environment. These spores can withstand harsh conditions including pasteurization which could be responsible for their proliferation and contamination of milk products (Umaru *et al.*, 2013). *Klebsiella aerogenes* has been related to severe bacterial pneumonia. *Escherichia coli* are indicators of faecal contamination. Their occurrence in yoghurt may be due to inadequate heat processing and post-pasteurization contamination from the environment and handlers (Samuel and Ifeany (2016).

The occurrence of fungi in milk and its products is unwanted even in a small number as they can lead to unpleasant change that reduce the quality of the product. Fungi constitute the major indicator of general essential quality (Huera-Lucero *et al.*, 2020). The range of total fungal count observed in the present study (1.00E+04 (SE) - 8.00E+04 (SB) cfu/mL is beyond the standard bound fixed for milk (FAO/WHO, 2018). The high fungi load in all the samples can be due to raw milk contamination, contamination of former yoghurt used as ferment, or wind that can transport spores into the process chamber. This can induce production of mycotoxin, off-odour and flavour (Garnier *et al.*, 2017). Fungi growing in fermented milk use some of the acid supporting the proliferation of putrefactive bacteria and other disease causing microbes such as *Staphylococcus aureus* and *Listeria monocytogenes* (Banwo *et al.*, 2020). Furthermore, fermented milk is a high acidic product naturally, which is a greatly discerning environment or medium supporting the progression of Fungi as spoilage microbes whose existence in fermented milk is an pointer of poor management practices (Bereda *et al.*, 2018). Fungi can produce poisonous metabolites (mycotoxins) and taint many foods, mycotoxins are not destroyed in the course of food processing (Bereda *et al.*, 2018) and could be potentially hazardous to human health. Contamination of milk and its products by fungi could be from air, feed, ineffectively washed milk apparatuses and poor personal cleanliness of milk handlers (Owusu-Kwarteng *et al.*, 2020).

CONCLUSION AND RECOMMENDATIONS

The study highlighted poor hygiene standards in milk and yoghurt processing in Samaru markets, where high bacterial and coliform contamination was detected, mainly from manufacturing and packaging. While some samples were pathogen-free, contamination can be prevented through strict hygienic practices, quality control, and proper storage. Preventing post-pasteurization contamination and maintaining aseptic conditions are essential for ensuring safe, long-lasting dairy products in Nigeria. Based on the result of this study, the following recommendations were made:

1. Every small and large scale yoghurt producer is suggested to maintain adequate hygienic condition to make the good quality and healthy yoghurt which will reduce the microorganisms load.
2. There is a need to control animal feeds, raw milk collection, and wind getting in the room where yogurt is being produced
3. Use of biopreservation such as antifungal bioprotective cultures or fermenters. If not possible, then use of safe antifungi like natamycin.
4. Yoghurt from local producers needs improvement in microbiological quality. This can be done by adjusting the pasteurization process.



5. Effective measures to ensure safe milk for human consumption should be routinely performed on each batch of milk processed by dairy companies.
6. Medical examination of milk handlers should also be done to reduce milk contamination by infected handlers.

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