



Assessment of Microbiological Quality of Yogurt Sold By Street Vendors in Onitsha Metropolis, Anambra State, Nigeria

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Authors' contributions

This work was designed and carried out in collaboration between all authors. Author VOI supervised the study and performed the statistical analysis. Authors EOI, OMM and CI wrote the protocol, managed the literature searches and wrote the first draft of the manuscript. All Authors collaborated during the analyses of the study, read and approved the final manuscript.

Research Paper

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ABSTRACT

Four brands of yogurt sold by street vendors in Onitsha Metropolis, Anambra State, Eastern Nigeria were sampled, the pH was determined and microbiological assessments were conducted in order to ascertain the total heterotrophic bacteria, coliforms and yeast in the samples (A – D) during a seven day period. The results revealed that values of pH monitored varied from 3.69 – 4.50 while a total of five bacteria species belonging to *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus*, *Lactobacillus* and *Bacillus* species, and three fungi species belonging to *Aspergillus*, *Rhizopus* and *Saccharomyces* were isolated from the samples. Sample B had the highest mean heterotrophic bacteria count with a value of 6.1×10^5 cfu/ml. Statistical analysis of heterotrophic bacteria count among the 4 sample groups had p-value = 0.000374. There is a significant difference in the heterotrophic bacteria count among the groups. Low titre values of starter cultures were recorded in the control samples. *Escherichia coli*, an indicator of coliform was detected in all the samples and the value of 4.4×10^5 cfu/ml was observed in sample B. Coliforms, *S. aureus*, *Bacillus* species and fungi were not detected from control samples purchased directly from the producing companies. Statistical analysis of coliform count in the four groups had p-value = 0.529296. There was no significant difference in the coliform count among the 4 sample groups at $\alpha = 0.05$ and p-value = 0.529296. The findings suggest that

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the yogurt traded by street vendors in Onitsha Metropolis has poor microbiological quality control. This poses danger to public health. Therefore, attention of the appropriate government agencies and manufacturers is needed to ensure that sale of yogurt by vendors is done in most appropriate condition and in a mobile refrigerator to maintain adequate temperature, thereby, reduce contamination.

Keywords: Yogurt; bacteria; yeast; heterotrophic count; coliform; pH; contamination.

1. INTRODUCTION

The consumption of fermented milk by man dates back to the advent of civilization [1]. These fermented and / or cultured milk prolong the shelf-life of some food and milk related preparations [2]. Indeed, yogurt arising from lactic acid fermentation is increasing according to [2], by the addition of a starter culture that contains *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus*. These yogurt product blends are consumed worldwide and much more in Indian sub-continent [3]. It is a means of protein intake for an improved healthy living [4]. Following the higher demand for yogurt foods, an automated equipment to facilitate industrial production in order to meet not only the quantity, but, also to ensure high and safety quality products was put in place [5]. Also, the improved industrial process has not only improved the storage, transportation and commercialization of the product, the microbiological parameters according to [2] has maintained coliform free products. Microbiological parameters are generally used to verify these conditions, especially by coliforms [2]. At commercial points, yogurts produced in Brazil must contain at least 10^7 colony formers unit per gram (CFU/g) of lactic acid bacteria and less than 10 most probable number per gram (MPN/g) of thermotolerant coliforms for indicative samples [6]. Coliforms quantification allows the verification of raw milk quality and the efficiency of the processing, In addition, the measurement of acidity is also observed in order to evaluate the preservation of yogurt status, varying from 0.6 to 1.5 g of lactic acid per 100 g of product, and the temperature of preservation in dairy industries and markets must not be higher than 10°C [6].

Fermented milk, like the fresh milk from which they are produced, is liable to contamination. Moulds and yeast are the primary contaminants in yogurt produced commercially in Nigeria [7]. Moulds and yeasts growing in yogurt utilize some of the acid and produce a corresponding decrease in the acidity, which may favour the growth of putrefactive bacteria [7].

As a test case presently, there are no published studies on microbiological quality of yogurts produced and sold in Onitsha Metropolis Eastern State of Nigeria. Onitsha is characterized by low level of environmental sanitation, lack of potable water and poor waste disposal. Contamination by total coliforms, other bacteria and yeast is probable. Quality indicators in yogurt include microbial evaluation and pH determination [2]. Therefore, this study as a test case is aimed at the assessment of the microbiological quality of yogurt product blends marketed in Onitsha Metropolis of Anambra State, Nigeria.

1.1 Justification of the Study

There is a misnomer in the condition surrounding the sales of yogurt in many parts of Nigeria. Vendors carry the products from manufacturers without making provisions for

maintenance of appropriate temperature and sanitary control. This predisposes the yogurt to post production contamination which poses danger to public health.

2. MATERIALS AND METHODS

2.1 Sample Collection

Various blends of yogurt products in plastic bottle were purchased from vendors in Onitsha Metropolis, Anambra State, South-Eastern Nigeria. The samples A to D trade names were Kings, Cynthesis, Daddy and Jiroh yogurts respectively and their registration numbers were 013267, 019869L, 016908L and 011869L respectively. Each day four bottled packs of four different blends of yogurt were purchased from vendors in duplicates; four in the morning soon after the vendors begin business for the day and four in the evening after the yogurt has stayed out of the keeping temperature in a refrigerator which might affect the quality and expose the yoghurt to contamination. This was repeated for a period of seven days during which time a total of fifty six samples were analyzed. The information on their labels was recorded. Each set of samples were transported under aseptic conditions in an ice packed container to the laboratory and evaluation commenced immediately. Control samples were collected directly from the four companies and the total heterotrophic bacteria, coliform and yeast counts were determined.

2.2 Microbiological Analysis

The microbiological evaluation was conducted according to [8]. Total count of heterotrophic bacteria was taken after making a decimal serial dilution of each sample. Plating was carried out using 0.1 ml of 10^{-3} and 10^{-4} dilutions on separate nutrient agar plates, Growth on the 10^{-3} dilution was used for calculating the cfu. by spread technique onto MacConkey and Sabouraud's dextrose (SDA) agar. The plates were incubated at 28°C for fungi and 37°C for bacteria both incubated for 24-48 hours. The presumptive test for coliform was conducted with lactose broth at 35°C for 48 hour followed by the confirmation of total coliforms using brilliant green bile broth at 35°C for 48 hours and completed test using Eison Methylene Blue (EMB) agar followed by Gram staining and endospore test. The other bacterial isolates were characterized and identified based on morphological features and biochemical tests [9]. Fungal isolates were characterized based on colonial morphology and microscopic appearance comparing their characteristics with those of known taxa as described by [10].

2.3 pH Analyses

The pH of the samples was measured at 12-hourly intervals using Knick-digital pH meter, model 646 after calibrating with pH 7.0.

2.4 Statistical Analysis

Mean of the pH and analysis of variance (ANOVA) of the heterotrophic bacteria, coliform and yeast were calculated to determine if significance existed among the groups. The Scheffe/Tukey test were used to show which samples are different.

3. RESULTS

The labels on the yogurt blend products provide little information including registration numbers of the products. The mean total heterotrophic bacteria, total coliform and yeast count is provided (Fig. 1). Comparatively, sample B maintained a higher range of heterotrophic count, 4.1×10^5 CFU/ml to 6.1×10^5 CFU/ml than the other three brands of yogurt. Sample A contained the least with a range of 1.9×10^5 CFU/ml to 3.6×10^5 CFU/ml. The fluctuation may imply inconsistency in the titre of starter cultures during the production of the yogurt. The titre values of the starter cultures in the control samples from the four companies were 3.8×10^5 CFU/ml, 4.8×10^5 CFU/ml, 4.0×10^5 CFU/ml and 4.3×10^5 CFU/ml respectively. The low titre values of starter cultures in the control samples suggested addition of weak titre starter cultures during processing. *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus*, *Lactobacillus* and *Bacillus* species were the bacteria isolated from the samples (Table 1).

The coliform count in the samples from vendors was high; 4.4×10^5 CFU/ml on the first day for sample B but subsequent samples had reduced counts, of which, the mean count on the day seven was 0.6×10^5 CFU/ml. The mean value of coliform for sample A on the seventh day was 0.1×10^5 CFU/ml as against the mean value of 1.8×10^5 CFU/ml on the first day. The control samples were negative for coliform tests. Same observation was made in respect of *S. aureus*, *Bacillus* species and fungi.

Comparatively, sample B maintained a higher range of mean yeast count than the other three brands. The highest value of mean count was 4.5×10^5 CFU/ml on the fourth day in sample B. *Streptococcus* and *Lactobacillus* species were the starter cultures. *Aspergillus*, *Rhizopus*, *Saccharomyces* were the fungi isolated (Table 2). The mean values of total yeast count fluctuated in the four brands of yogurt collected from vendors. *Escherichia coli*, *S. aureus*, *Bacillus* species, and the fungi species appeared as contaminants.

The pH monitored for a period of seven days had different acidic values. The mean values for the seven days ranged from 3.69 – 4.09 (Fig. 2). There was an increase in the mean values of the C samples on the third day.

Statistical analysis using Anova showed that there exists a significant difference in the heterotrophic bacteria and yeast count among the 4 groups at $\alpha = 0.05$ and p-value = 0.0000374 and 0.020033 for bacteria and yeast respectively. There was no significant difference in the coliform count among the 4 groups at p-value = 0.529296 (Table 3). The Scheffe/Tukey test showed that group B in the heterotrophic bacteria count differed from A and C at p-value of 0.000 and group D differs from A and C at p-value of 0.005 and 0.030 respectively. Group B in the yeast count differed from group C at a p-value of 0.023 but does not differ from the other groups.

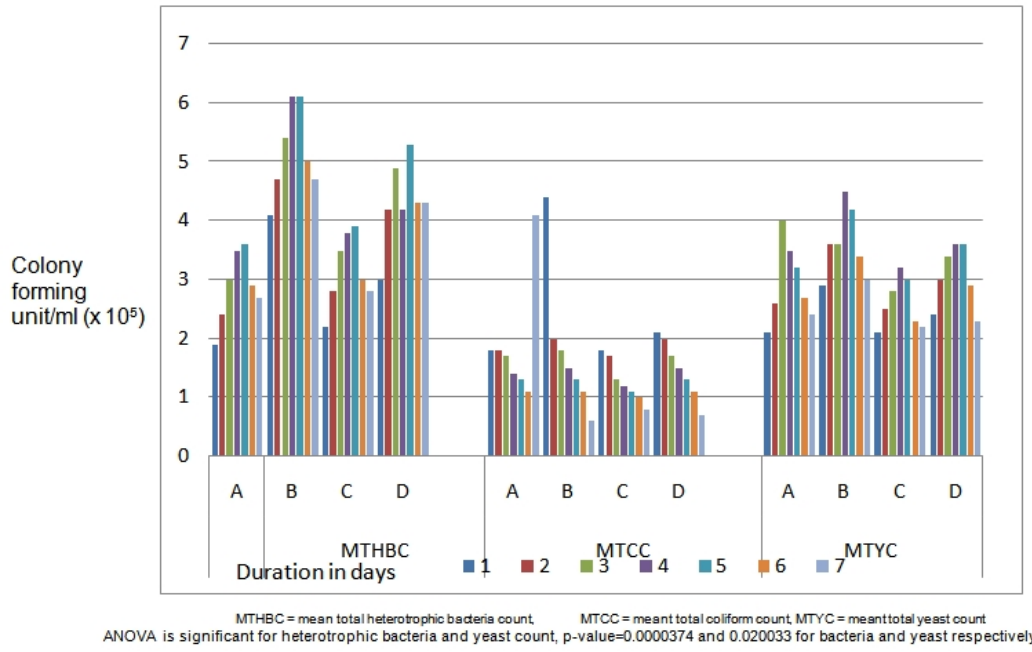


Fig. 1. Mean total heterotrophic bacteria/coliform/yeast count (cfu/ml)

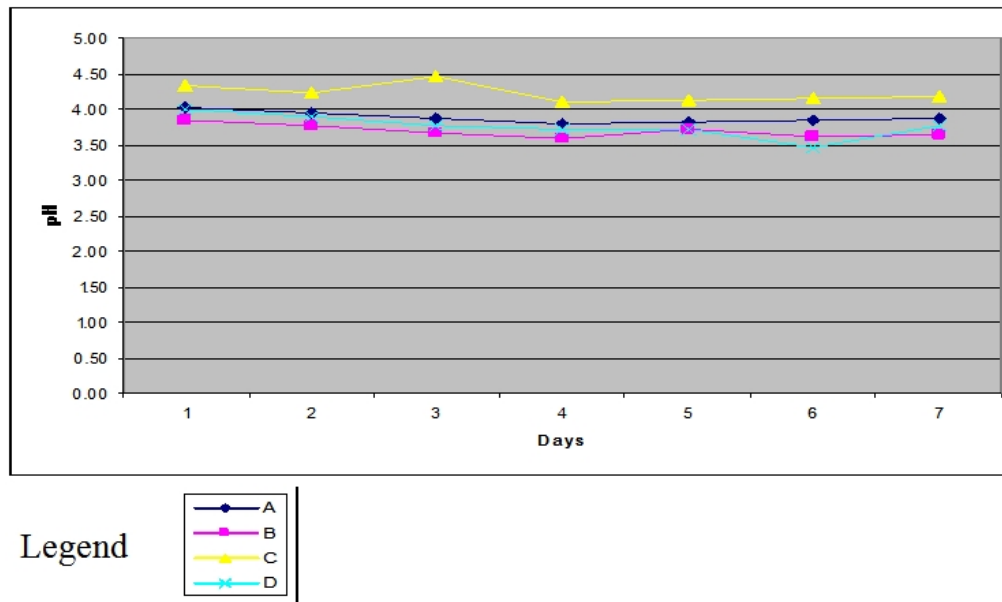


Fig. 2. Mean values of pH for morning and evening samples

Table 1. Cultural, morphological and biochemical characterisation of isolates from the yogurt sample

Colonial Characteristics	cell shape	Gram stain	Catalase	Oxidase	Coagulase	Indole	Citrate	Glucose	Probable genera or species
Light pink colonies with smooth edge	single rod	-	+	-	-	+	-	AG	<i>E. coli</i>
Smooth light yellow colonies with raised elevation	cocci in cluster	+	+	-	-	-	-	A	<i>S. aureus</i>
Creamy convex colonies with ciliated edge	cocci in short chain	+	-	-	-	-	-	A	<i>Streptococcus</i>
White round slight raised colonies	rods	+	-	-	-	-	-	A	<i>Lactobacillus</i>
Creamy round and Fat colonies	rods	+	+	-	-	+	+	A	<i>Bacillus</i>

Key: AG = acid and gas, A= acid, + = positive, - = negative

Table 2. Cultural and Morphological Characterization of Fungal Isolates

Colony morphology	Microscopic characterization	Probable fungus
Pale green appearance at the center lining at the exterior with white fluffy	Map-like conidiospores	<i>Aspergillus</i> spp.
Black fluffy colouration with Powdery appearance	non-septate hyphae with sporangiophores and rhizoids	<i>Rhizopus</i> spp.
Cream colour circular colonies	Spherical to oval shape fungi	<i>Saccharomyces</i> spp.

Table 3. Statistical analysis of heterotrophic bacteria, coliform and yeast count

ANOVA	Result	Scheffe/Tukey
Single factor of heterotrophic bacteria count in the four groups	p-value = 0.0000374, there is a significant difference in the heterotrophic bacteria count among the four groups	Group B differs from A and C at p-value of 0.000 while group 4 differs from A and C at p-value of 0.005 and 0.030 Respectively. However, there, is no difference between B and D.
Single factor of the coliform count in the four groups	p-value = 0.529296, there is no significant difference in the coliform count among the four groups.	
Single factor of the yeast count in the four groups	p-value = 0.020033, there is a significant difference in the four groups	Group 2 differs from group 3 at a p-value of 0.023 but does not differ from the other groups.

4. DISCUSSION

All the yogurts manufactures gave information about their products, though, not adequate. The study of the microflora of some available yogurt in Onitsha Metropolis showed a total of five bacteria species and three fungi genera. The isolation of *Streptococcus* and *Lactobacillus* spp. agreed with the claims that they are the most commonly functional organisms in the fermentation of milk into yogurt [11]. The presence of *Saccharomyces* spp. is desirable because *Lactobacillus* and *Saccharomyces* spp. are responsible for flavour and aroma, and the latter is involved in the fermentation of milk for yogurt production, as reported by [12]. The isolation of *Aspergillus* and *Rhizopus* species agreed with [7] that moulds are the primary contaminants in yogurt produced in Nigeria. *Escherichia coli* and *Bacillus* species were contaminants and this was not surprising considering the low level of hygiene and development of Onitsha. The observations of coliforms agreed with the report of [13].

The samples showed microbiological parameters not in conformity with official standards. The starter cultures were lower than 10^7 CFU/ml standards as reported by [6]. The disagreement might be caused by inadequate heating treatment of milk and poor hygienic standards of processing and packaging that precluded recontamination of the product or post production contamination in the environment during sales. The detection of fungi, coliforms, *S. aureus* and *Bacillus* probably indicated post-production contamination. Post-production contamination was not impossible because of the environment in which the production and sales were carried out. This reasoning might be correct considering that the control samples had no growth of coliforms. Coliforms detection is often used as parameters for evaluating the yogurt quality in different countries [14,2]. The mean pH values were not significantly different. These findings were similar to the ones observed by [6] who studied viable lactic acid bacteria in yogurts.

5. CONCLUSIONS

The observations of this study suggest post-production contamination considering that the control samples had results free of contaminants. This poses danger to public health. Therefore, attention of the stake holders including manufactures is needed to reduce post-production contamination and check condition of yogurt on sale by vendors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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