MICROORGANISMS ASSOCIATED WITH STREET VENDED YOGHURT IN MILE 1 DIOBU AREA OF PORT HARCOURT, NIGERIA

Obire, Omokaro* and Berembo, Beremboba Telema

Department of Applied and Environmental Biology, Rivers State University Of Science and Technology, P.M.B. 5080, Port Harcourt, Nigeria,
*Correspondence Author E–Mail: omokaro515@yahoo.com

ABSTRACT

The microbiology of three different yoghurt samples from Mile I Diobu area of Port Harcourt was evaluated weekly for three weeks using standard plate count and most probable number (MPN) technique. This was carried out by analyzing for total aerobic heterotropic bacteria, total coliform bacteria, Thermotolerant coliform bacteria and fungi. The total aerobic heterotrophic bacteria count ranged from 4.0×10^5cfuml^{-1} to 1.13×10^6cfuml^{-1} of yoghurt, the total coliform bacteria ranged from 11 to 140 coliform (MPN) 100ml^{-1} while the thermotolerant coliform bacteria ranged from 17 to 90 coliform (MPN) 100ml^{-1}. The fungal count ranged from 1.0×10^2 spore forming unit (sfu) ml^{-1} to 5.0×10^2 sfuml^{-1}. The results of the mean values of pH of the samples were Green field yoghurt (pH 7.0), Home victory yoghurt (pH 7.5), and Mary gold natural yoghurt (pH 5.0). Generally, the bacterial, fungal and thermotolerant coliform counts were highest in the Mary gold samples which had an acidic pH. This shows that the isolates are acidophiles. On the other hand, the bacterial and fungal counts were lowest in Green field samples with a neutral pH which however, recorded the highest total coliform count. While the total coliform and thermotolerant coliform counts were lowest in the Home victory yoghurt samples. Generally, the incidence (%) of bacteria was; *Bacillus cereus* (22.5%), *Bifidobacterium* sp (7.5%), *Escherichia coli* (7.5%), *Lactobacillus acidophilus* (15%), *Lactobacillus bulgaricus* (12.5%), *Pseudomonas aeruginosa* (15%), and *Staphylococcus aureus* (20.0%). Incidence of fungi was; *Aspergillus niger* (10%), *Fusarium solani* (15%), *Mucor* sp (20%), *Penicillium italicum* and *Penicillium* spp (35%), and *Saccharomyces cerevisae* (20%). Statistical analysis using ANOVA showed that there is no significant difference at $P = 0.05$ in the microbial counts and in the incidence of the bacterial isolates between the three yoghurt samples. The presence of these bacteria and fungi especially enteric organisms and indicators of faecal contamination such as *E. coli* and *Enterobacter* is of public health concern as they pose serious health hazards to the unsuspecting consumers.

Key words: Yoghurt, bacteria, fungi, faecal coliform. *E. coli*

INTRODUCTION

Yoghurt is a soured milk product known for ages. It is a custard-like food with a tart flavor prepared from milk curdled by bacteria especially *Lactobacillus bulgaricus and Streptococcus thermophilus* and often sweetened or flavoured with fruit (American heritage, 2000). The *L. bulgaricus* produces amino acids which stimulate *S. thermophilus* to produce formic acid which is essential for the growth and survival of

the *L. bulgaricus*. The *S. thermophilus* turns the milk sour while *L. bulgaricus* produces the typical yoghurt aroma. Yoghurt can be made from the milk of goat, cow, ewe and buffalo or a combination of these milk (Alderton *et al*., 2000).

Yoghurt is low in saturated fat and cholesterol but nutritionally rich in Protein, vitamins including Pantothenic acid, and Riboflavin. It is also a very good source of calcium, iron, potassium, other minerals and phosphorus which maintains the Red blood cells and helps keep your nervous system functioning well (Korlar and Aowi, 1994). Yoghurt may prevent high blood pressure. The potassium in yoghurt almost 600mg per eight ounce may help flush some of the excess sodium out of our body. The protein, carbohydrate and vitamin content are higher in yoghurt than in milk (Porter and Dryden, 1998; Parnel *et al*., 2006). There is a little different between milk and yoghurt in terms of energy content, but sweetened yoghurt is richer in energy sources than milk (Dryden, 1999).
Yoghurt has an antimicrobial activity to some bacteria (Hingst, 2000). The lactic acid found in yoghurt also helps to protect your gum and hinder protein putrefaction in the intestine (Schulz and Hingst, 2000). Yoghurt also has a nutritional benefit beyond that of milk, because lactose intolerant individual sometimes tolerate yoghurt better than other dairy products. The starter culture produces a lactose enzyme that aids the digestion (Shukla and Leifson, 2002). Consumption of yoghurt helps to alter microbial flora of the intestine. Yoghurt contains probiotics, beneficial bugs that helps crowd out harmful micro-organisms that can cause intestinal infections (Amanda *et al*., 2013).

Types of commercially made yoghurts are powdered yoghurt, soft or liquid yoghurt and firm yoghurt. The most popular type commonly produced is firm yoghurt (Hardman and Milliken, 1998). Microorganisms can contaminate yoghurt through different steps associated with its production. Fresh milk used in preparation may contain resistant spores of *Bacillus* and *Clostridium* species (Jay *et al.,* 1999). The addition of fruit, flavour, and sugar into yoghurt may act as a means to introduce yeast and moulds into the product. Yeast contaminant gives off flavours, loss of texture quality and eventually swelling and blowing of the container (Alderton, 2000). *C*ontaminants may get into the yoghurt during dispensation if proper good manufacturing practice (GMP) is not put into place during the process of production.
The aim and scope of this study is to determine the standard plate count of total bacteria and fungi of yoghurt samples, to estimate the total coliform and thermotolerant coliform bacteria using the most probable number technique (MPN technique); to isolate, characterize and determine the incidence of bacteria and fungi in samples of yoghurt as to ascertain the microbial load, pathogenic microorganisms present if any and to ascertain the sanitary level of the yoghurt producers or handlers.

MATERIALS AND METHOD

Collection of Yoghurt Samples

Samples of three different brands of yoghurt packaged in plastic bottles were bought from a distributor at Emenike Street in Mile 1 Area of Port Harcourt. The brands were Green Field yoghurt, Home Victory yoghurt, and Mary Gold natural yoghurt. Green field yoghurt is produced in Eleme; ingredients are Skimmed Milk, Sugar, yogflex starter culture and treated water. Home victory yoghurt is produced in Amadi-Ama; ingredients are Full cream milk, sugar, yogflex starter culture and treated water

while Mary Gold natural yoghurt is produced at Elelenwo; ingredient are Fresh milk, premium water, sugar, and flavour.
The samples were bought in frozen state and put into ice packed containers and immediately transported to the laboratory for analysis. The microbiological analyses were conducted after the frozen yoghurt samples were allowed to thaw and before the expiry dates of the products in July, August and September, 2013.

Determination of the pH

The pH of each yoghurt sample was determined by using Jenway pH meter. The sterilized pH rod of the meter was inserted into a beaker of distilled water for standardization. Each thawed yoghurt sample was thoroughly mixed and poured into sterile beaker after which the pH rod was inserted into the sample and reading was recorded after the readings have stabilized on the screen of the meter. This process was repeated for each yoghurt sample used during this study.

Cultivation and Enumeration of Total Heterotrophic Bacteria and Fungi

Enumeration of Viable Microbial count of microorganisms, the total viable count of bacteria and fungi in the yoghurt samples were estimated using the spread plate method.
Serial dilution was carried out on each yoghurt sample. The dilution factor for the isolation of bacteria was 10^{-5} while the dilution factor for the isolation of fungi was 10^{-2}. This was done so as to obtain discrete colonics when plated on the medium. One milliliter (1.0ml) of each yoghurt sample was added to separate 9.0ml of normal saline (diluent) and further dilution was made up to 10^{-5} and 10^{-2}.
An aliquot (0.1ml) of the appropriately diluted sample was then inoculated onto nutrient agar plates for the isolation of bacteria and onto Sabouraud dextrose agar plates for the isolation of fungi. The spread plate method was done using sterile bent glass spreader to spread the sample evenly on the agar plates. Cultures were prepared in duplicates. Cultured Nutrient agar plates were incubated at 37^0C for 24 hours while the cultured SDA plates were incubated on the laboratory bench for 3 to 5 days. Discrete colonies that developed on the plates (overnight culture) were counted, the average taken and recorded as total heterotrophic counts of bacteria.
Discrete colonies were collected aseptically and streaked onto nutrient agar plates (for bacteria purification) and incubated at 37^0C overnight. Pure colonies were later stored in Mac Cartney bottles containing nutrient agar slants and put into the fridge as stocks cultures for further biochemical tests. A total of eleven (11) pure cultures were stored and regarded as the bacteria isolates. Colonies which developed after 5 days on SDA plates were counted and the average count for the duplicate cultures were recorded as total viable fungi of each sample. The colour and colonial morphologies or characteristics were also recorded. Discrete colonies were subcultured onto freshly prepared SDA to obtain pure cultures.

Estimation of Coliforms

Estimation of the coliform bacteria was done using the most probable number technique (MPN technique). Reaction to MPN technique and thermotolerant coliform bacteria MPN index 100ml of each yoghurt sample was done using double strength MacConkey broth for 10ml of sample and single strength MacConkey broth for 0.1ml and 1ml of the sample. The test for the estimation of coliforms involves the following

steps: presumptive, confirmatory and completed test. It was performed as described by Verma *et al*., (1999).

Enumeration of Faecal Coliform Test

The test for coliform does not distinguish coliform of animal origin and from others (Doyle and Erickson, 2006). In this test, the test tube with the production of gas in the presumptive test were streaked with the aid of a sterile wire loop onto MacConkey agar plates, and incubated at 37^0C for 24 hours.

Isolation, Characterization and Identification of Bacteria in Yoghurt Samples

Pure cultures of bacteria were obtained by aseptically streaking representative colonies of different morphological types which appeared on the cultured plates onto freshly prepared nutrient agar plates which were incubated at 28^0C for 24 hours. The isolates which developed were further sub cultured onto agar slopes/slants and incubated at 28^0C for 24 hours. These served as pure stock cultures used for subsequent characterization tests. The following characterization tests were performed in duplicates. Gram staining, catalase test, coagulase test, urease test sugar fermentation test, methyl red test, indole test and acid gas test were carried out as described by Cappuccino and Macfaddin (2005) and Kirk *et al*., (2005). The pure cultures were identified on the basis of their cultural, morphological and physiological characteristics in accordance with methods described by Cruikshank *et al*., (1975) and with reference to Holt (1977).

Isolation, Characterization and Identification of Fungi in Yoghurt Samples

Pure cultures of fungi were obtained by sub culturing discrete colonies onto freshly prepared Sabouraud dextrose agar plates and incubated at 28^0C for 5 to 7 days. The colonies which developed were further subcultured onto agar slopes or slants and incubated at 28^0C for 5 to 7 days. The following standard characterization tests were performed in duplicate; macroscopic examination of fungal growth was carried out by observing the colony morphology-diameter, colour (pigmentation), texture and surface appearance. Microscopic examination was done by needle mount or wet mount method and observing sexual and asexual reproductive structures.

Microscopic examination of fungi

A wet mount was carried out for the fungi isolated. A drop of sterile distilled water was aseptically dropped on a grease free clean slide. A piece of fungal hyphae under test was teased into it using two sterile needles. The teasing was done carefully and slowly so as to make good spread of the fungal hyphae. Each prepared slide was gently covered with a cover slip to avoid air bubble. The slides were observed under low and high power objective, and observation recorded as the cultural characteristics, sporangia, conidia, arthrospores, and vegetative mycelium, septate and non-septate hyphae according to Barnett and Hunter (1972).

RESULTS

Total Viable Count for bacteria and fungi of the different yoghurt samples

The results of the mean values of pH of the yoghurt samples were Green field yoghurt (pH 7.0), Home victory yoghurt (pH 7.5), and Mary Gold natural yoghurt (pH 5.0).

The result of the mean value of total viable count for bacteria and fungi of the different yoghurt samples is shown in Table 1 and in Table 2 respectively.

The mean values of the total viable bacteria of Green field yoghurt, Home victory yoghurt, and Mary Gold natural yoghurt samples ranged from 4.0×10^5 to $5.2 \times 10^5 cfuml^{-1}$, 4.0×10^5 to $5.5 \times 10^5 cfuml^{-1}$, and 6.0×10^5 to $1.13 \times 10^6 cfuml^{-1}$ respectively. While the mean value of the total fungal count ranged from 1.0×10^2 to $3.0 \times 10^2 cfuml^{-1}$, from 2.0×10^2 to $5.0 \times 10^2 cfuml^{-1}$, and from 3.0×10^2 to $5.0 \times 10^2 cfuml^{-1}$ respectively. Generally, both bacterial and fungal counts were highest in Mary Gold natural yoghurt samples and lowest in Green field yoghurt.

The result of the total coliform and of the thermotolerant coliform and facecal coliform is shown in Table 1 and Table 2 respectively. The total coliform count ranged from 11 to 140 coliform (MPN) $100ml^{-1}$ while the thermotolerant coliform and facecal coliform ranged from 17 to 90 coliform (MPN) $100ml^{-1}$ of yoghurt sample.

The incidence (%) of bacteria isolated from each yoghurt sample is shown in Table 3. Generally, incidence of bacteria in all the samples of yoghurt were; *Bacillus cereus* (22.5%), *Bifidobacterium* sp (7.5%), *Escherichia coli* (7.5%), *Lactobacillus acidophilus* (15%), *Lactobacillus bulgaricus* (12.5%), *Pseudomonas aeruginosa* (15%), and *Staphylococcus aureus* (20.0%). However, *Bifidobacterium* sp, *Escherichia coli*, and *Lactobacillus bulgaricus* were not isolated from Green field yoghurt, Home victory yoghurt and Mary Gold natural yoghurt respectively.

The incidence fungi isolated from each yoghurt sample is shown in Table 4. Generally, the fungi isolated and incidence (%) was *Aspergillus niger* (10%), *Fusarium solani* (15%), *Mucor* sp (20%), *Penicillium italicum* and *Penicillium* sp (35%) and *Saccharomyces cerevisiae* (20%). All the fungi were isolated from Mary gold natural yoghurt while *Mucor* and *Penicillium* species were not isolated from Green field yoghurt and Home victory yoghurt respectively.

Statistical analysis using ANOVA showed that the Calculated F- value for the data obtained for the microbial counts and for the incidence of the bacterial isolates was 2.85 and 0.12 respectively. These F – values are lower than their respective tabular values at $P = 0.05$. This showed that, there is no significant difference at $P = 0.05$ in the microbial counts and in the incidence of the bacterial isolates between the three yoghurt samples.

Table 1: Total coliform bacteria Count of various yoghurt samples

Yoghurt sample	**Total Coliform Bacteria (MPN) INDEX/100ml of yoghurt**		
	Week 1	**Week 2**	**Week 3**
Green field	140	70	17
Home victory	17	11	26
Mary Gold Natural	70	26	70

Table 2: Thermotolerant Coliform Bacteria and Facecal Coliform Bacteria Count of the various yoghurt samples

Yoghurt sample	Thermotolerant Coliform Bacteria and Facecal Coliform Bacteria (MPN) INDEX/100ml of yoghurt		
	Week 1	Week 2	Week 3
Green field	33	33	33
Home victory	17	17	17
Mary Gold Natural	90	90	90

Table 3: Incidence (%) of Bacteria Isolated From Each Yoghurt Sample

Isolates	Green field yoghurt	Home Victory yoghurt	Mary Gold natural yoghurt
Bacillus cereus	16.67	21.43	28.57
Bifidobacterium sp	-	7.14	14.29
Escherichia coli	16.67	-	7.14
Lactobacillus acidophilus	16.67	14.29	14.29
Lactobacillus bulgaricus	16.67	21.43	-
Pseudomonas aeruginosa	25	14.29	7.14
Staphylococcus aureus	8.33	21.43	28.57

Table 4: Incidence (%) of Fungi Isolated From Each Yoghurt Sample

Fungi	Green field yoghurt	Home Victory yoghurt	Mary Gold natural yoghurt
Aspergillus niger	16.67	-	14.29
Fusarium solani	16.67	14.29	14.29
Mucor sp	-	28.57	28.57
Penicillium italicum	33.33	14.29	14.29
Penicillium sp	16.67	14.29	14.29
Saccharomyces cerevisae	16.67	28.57	14.29

DISCUSSION

The present study has revealed the population and types of bacteria, fungi and of coliforms in the various samples of yoghurt. The results of the mean values of pH of the samples were Green field yoghurt was in the neutral range, Home victory yoghurt is slightly alkaline and Mary gold natural yoghurt is acidic. Generally, the bacterial,

fungal and thermotolerant coliform counts were highest in the Mary gold samples which had an acidic pH. This shows that the isolates are acidophiles. It has also been reported that yoghurt that has an acidic content seem to act as a selective media for yeasts and moulds using lacteal as their possible source of energy (Porter *et al.,* 2005). On the other hand, the bacterial and fungal counts were lowest in Green field samples with a neutral pH which however, recorded the highest total coliform count. While the total coliform and thermotolerant coliform counts were lowest in the Home victory yoghurt sample which is slightly alkaline.

The presence of various types of bacteria and fungi was also revealed. Statistical analysis showed that there was no significant difference at $P = 0.05$ in the microbial counts and in the incidence of the bacterial isolates between the three yoghurt samples. Among the bacteria isolates *Bacillus cereus* had the highest incidence of 22.5% while; *Bifidobacterium* sp and *Escherichia coli* recorded the lowest incidence of 7.5% each. Among the fungi isolates *Penicillium italicum* and *Penicillium* spp had the highest incidence of 35% while *Aspergillus niger* recorded the lowest incidence of 10%. Bacteria such as *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, and *Bifidobacterium bifidum* isolated in this present study has been reported by Suaze *et al*., (2000) as beneficial microorganisms found in yoghurt. These organisms which are the starter culture for the fermentation of milk to produce yoghurt have been termed legal milk bacteria (Eka and Ohaba, 1997). *Escherichia coli* and *Staphylococcus aureus* isolated in this study has been reported and proved to be potential contaminants of yoghurt (David and Carr, 2003). The incidence of *Staphylococcus aureus* in all the samples of yoghurt is a source of concern. Its presence in the diary products is undesirable and should be prevented because it can easily multiply in diary products if held between 10^{o}C and 45^{o}C (Atanda and Ikenebomeh, 1991). The presence of *E. coli* which is an indicator of faecal contamination and the presence of other pathogens such as *Bacillus*, *Staphylococcus*, and *Pseudomonas* species indicate that the yoghurt samples are highly contaminated.

Strains of some fungal genera such as *Aspergillus*, *Fusarium*, and *Penicillium* reported in this study produce toxins and carcinogenic agents (Uraih and Ogbadu, 2008). Aflatoxin contamination of milk and ice-cream was also reported by Atanda (2007). *Mucor* species causes necrosis and thrombosis. The presence of these fungi in the yoghurt samples also has serious health implications and is of public health concern as they pose serious health hazards to the unsuspecting consumers.

From the results obtained the microbiological quality of the various yoghurt samples showed contamination of the samples with different kinds of microorganisms including potential pathogens which are of public health concern. Proper hygiene and sanitation therefore should be put in place so as to eradicate these pathogens.

To improve the keeping quality of the yoghurts, the yoghurt should be refrigerated at about 5°C so as to prevent further production of acid by lactic acid bacteria used in the production of the yoghurt. It is important that these yoghurts are supplied in cooling vans other than buses and taxes. The relevant agencies should ensure that manufacturers of yoghurts follow good manufacturing practices (GMP) guidelines during and after the production of these products.

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