Original Research Article

ASSESSMENT OF THE MICROBIOLOGICAL STATUS OF YOGHURT SOLD IN OWERRI, IMO STATE, NIGERIA

ABSTRACT

The assessment of the microbiological status of samples of yoghurt sold in Owerri, Imo state, Nigeria, was carried out to ascertain the microbiological fitness of the yoghurt samples for consumption. The yoghurt samples were collected from areas spanning three local governments in Owerri. Ten samples of commercial brands of yoghurt drinks was collected and analyzed bacteriologically by pour plate method using Nutrient Agar for heterotrophic bacteria, MacConkey Agar for total coliform and MacConkey Broth for fecal and thermo-tolerant coliform bacteria by Most Probable Number (MPN) technique and mycologically on Sabouraud dextrose Agar for fungi. Data from analysis were analyzed using ANOVA. The pH of the yoghurt samples were determined and the results showed that the pH values ranged from 4.28 to 4.79, while the total heterotrophic bacteria count ranged from 5.0 ± 7.1^{bc} to $9.0\pm7.1^{a}\times10^{5}$CFU/ml, while the total coliform bacteria ranged from 1.7 ± 0.5^{ab} to $3.6\pm1.2^{ab}\times10^{4}$CFU/ml and the thermo-tolerant coliform bacteria ranged from 11 to 120(MPN) 100^{-1}. The total fungal count ranged from 2.9 ± 1.6^{b} to $10.3\pm3.6^{a}\times10^{4}$CFU/ml. All the yoghurt samples had an acidic pH, this shows that the isolates are acidophiles. Statistical analysis using ANOVA showed that there is a significant difference at P >0.05 and the difference were separated using the least significant difference (LSD). Five bacterial isolates were identified included: *Escherichia coli, Staphylococcus aureus, Streptococcus* spp., *Lactobacillus bulgaricus* and *Serratia marcesecens.* The fungal genera identified were *Candida albicans, Aspergillus niger, Mucor spp.* and *Saccharomyces cerevisiae.* The presence of these bacteria and fungi, especially enteric organisms and indicators of fecal contamination such as *E. coli* is of public health concern, as they pose serious health hazards to the consumers. The result of this study therefore indicated poor Microbiological qualities of commercial yoghurts sold in Owerri at the time of this research. Therefore, the attention of the appropriate government agencies is needed to ensure that adequate hygiene is maintained during preparation, processing, storage and distribution of high quality yoghurt products to avert public health challenges.

KEY WORDS: Yoghurt, Milk, *Streptococcus thermophilus, Aspergillus niger,* MPN, *Saccharomyces cerevisiae*.

INTRODUCTION

Yoghurt is a cultured dairy product that can be made from whole low fat or skim milk, including reconstituted non fatty dried milk powder. The Food and Drug Administration (FDA) describes yoghurt as a food produced by culturing one or more of the basic ingredients (cream, milk, partially skimmed milk, skim milk, or the reconstituted versions of these ingredients may be used along or in combination) and any of the optional dairy ingredients with a characterizing bacteria (live and active) culture that contain the lactic acid-producing bacteria (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*).

Yoghurt is a very versatile product that suits all palates and meal occasions [1]. It is one of the most popular fermented milk products [2]. Its attractiveness has grown and is at this time used in most parts of the world [3]. Yoghurt is a soured milk product known for ages. It is a custard-like food with a tartflavor prepared from milk curdled by bacteria especially *Lactobacillus bulgaricus and Streptococcus thermophilus* and often sweetened or with fruit [4]. 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 milks [5].

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 [6]. 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 [7, 8]. There is a little difference between milk and yoghurt in terms of energy content, but sweetened yoghurt is richer in energy sources than milk [9].

Yoghurt has an antimicrobial activity to some bacteria. The lactic acid found in yoghurt also helps to protect the human gum and hinder protein putrefaction in the intestine [10]. 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 [11]. 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 [12]. Fermented milk, like the fresh milk from which they are produced, is liable to contamination. Knowledge of the behavior of yoghurt during storage is important, because its shelf life is based on whether the product displays any of the physical, chemical, or sensory characteristics that are unacceptable for consumption [13]. Changes in the chemical, physical and microbiological composition of yoghurt determine the storage and shelf life of the product [14]. The pH value of yoghurt immediately after production ranges between 4.5 and 4.2 [15]. The microbiological quality assessment of yoghurt is mainly concerned with protection of the consumers against exposure to any health hazard and ensuring that the material is not suffering microbiological deterioration during its anticipated shelf-life [16]. In addition to deterioration in sensory quality, microbiological counts have been used as indices for the end of shelf life of dairy products [17]. Coliform detection or enumeration is often used as parameters for evaluating the yoghurt quality indifferent countries [18]. Presence of coliforms in dairy

products is an indication of fecal contamination when the hygiene is poor [19]. Some members of coliforms are responsible for the development of objectionable taints in milk and its products rendering them of inferior quality or even unmarketable [20]. *Escherichia coli* (*E. coli*) frequently contaminate food and it considered a good indicator of fecal pollution, its presence in milk products indicates presence of other enteropathogenic microorganisms which constitute public health hazard [21]. *Enterococci* may have a distinctive role as indicators of poor factory sanitation owing to their relatively high resistance to drying, detergents, as well as freezing temperature. Moreover, these organisms are also implicated in food poisoning outbreaks [20]. *Enterococci* organisms have been proposed for hygienic condition inspections in process lines of fermented products [22].

The presence of *enterococci* in dairy products has long been considered an indication of inadequate sanitary conditions during the production and processing of milk [23]. *Staphylococcus aureus* in food article is an index of its contamination from personnel sharing in production and handling. Moreover, enterotoxigenic *Staphylococcus aureus* strains may find opportunity to grow and multiply in the food leading to food poisoning among consumers [24]. Yeasts are a major cause of spoilage of yoghurt and fermented milks in which the low pH provides a selective environment for their growth [25]. Yeasts and moulds are the major contaminants in yoghurt Micotoxigenic fungi and pathogenic bacteria are able to grow at refrigeration temperature to numbers, which can result in an infection [26]. Presence of yeasts and moulds in milk and dairy products are undesirable even when found in few numbers as they resulting in objectionable changes that render the products of inferior quality. Moulds and yeasts growing in yoghurt utilize some of the

acid and produce a corresponding decrease in the acidity, which may favour the growth of putrefactive bacteria [27].

There is a misnomer in the condition surrounding the sales of yoghurt in many parts of Nigeria. Vendors carry the products from manufacturers without making provisions for maintenance of appropriate storage temperature and sanitary control. This predisposes the yoghurt to post-production contamination. This post-production contamination leads to food poisoning like diarrhea which poses health risk to the public or consumers. Thus, it is necessary for this study to be carried out in order to assess the wholesomeness of yoghurt drink and use the information obtained in educating stakeholders on necessary precautions to safeguard public health.

2.0. MATERIALS AND METHODS

Collection of Yoghurt Samples

Ten samples of different brands of yoghurt drink in Owerri, Imo State, for the current study. The yoghurt samples were purchased from different yoghurt vendors, supermarkets and open markets at different locations in Owerri. The samples were immediately taken to the laboratory in ice containers, under aseptic conditions, where analysis was carried out immediately. These samples were labelled A to J.

Determination of Physical Parameters

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. Other packaging information including expiry dates, nature of packs, volume, colour of the contents were also noted.

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 pour 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 was10^2. This was done so as to obtain discrete colonies 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 aseptically taken and plated on nutrient agar (NA) using the pour plate method for the isolation of bacteria and onto Sabouraud dextrose agar plates for the isolation of fungi. Cultures were prepared in duplicates. Cultured Nutrient agar plates were incubated at 37^{o}C for 24 hours while the cultured SDA plates were incubated on the laboratory bench at 22^{o}C for 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^{o}C overnight. Pure colonies were later stored in MacCartney bottles containing nutrient agar slants and put into the fridge as stock 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 sub-cultured 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 thermo-tolerant 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 steps: presumptive, confirmatory and completed test. It was performed as described by Verma *et al*., [28]. This involved the observation of the broth as lactose sugar fermentation change the medium color from pink to yellow & for gas production, bubbles collected in the invertid Durhum tubes inside the broth medium.

Enumeration of Fecal Coliform Test

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 37^0C for 24 hours. The isolates which developed were further sub cultured onto agar slopes/slants and incubated at 37^0C 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 and indole test were carried out as described by Cappuccino and Macfaddin [29] and Kirk *et al*., [30]. The pure cultures were identified on the basis of their cultural, morphological and physiological characteristics in accordance with methods described by Cruikshank *et al.,* [31].

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 sub-cultured 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 bubbles. The slides were observed under low and high power objectives, and observation recorded as the cultural characteristics, sporangia, conidia, arthrospores, and vegetative mycelium, septate and non-septate hyphae according to Barnett and Hunter, [32].

3.0 RESULT

A total of ten (10) different brands of yoghurt samples obtained from different markets and vendors within Owerri were used in this study. The result of the microbiological status assessment of the yoghurt samples are shown in the tables below.

Table 1: pH Values of the ten samples of yoghurt

Samples	pH Values
A	4.39
B	4.70
C	4.29
D	4.46
E	4.65
F	4.28
G	4.62
H	4.56
I	4.79
J	4.35

Table 1 Shows the pH readings of ten different yoghurt samples which ranged between 4.28 and 4.79. Yoghurt sample F had the lowest pH value of 4.28 whereas yoghurt sample I recorded the highest pH value of 4.79. These pH values portrayed the acidic status of the yoghurt samples.

Table 2: Mean± standard deviation of total viablemicrobial counts of the yoghurt samples

Samples	THBC x 10^5(CFU/ml)	TCC ×10^4(CFU/ml)	TFC x 10^4 (CFU/ml)
A	7.5 ± 2.7^{ab}	3.6 ± 1.2^{ab}	8.0 ± 5.7^{b}
B	8.8 ± 5.1^{ab}	2.7 ± 1.7^{a}	5.5 ± 3.5^{ab}
C	6.0 ± 1.4^{b}	2.0 ± 2.2^{b}	2.9 ± 1.6^{b}
D	6.7 ± 0.9^{a}	1.7 ± 0.5^{ab}	10.3 ± 3.6^{a}
E	8.6 ± 0.6^{a}	3.0 ± 1.6^{a}	9.3 ± 6.1^{a}
F	6.0 ± 2.8^{ab}	2.0 ± 0.8^{bc}	6.8 ± 4.6^{a}
G	5.0 ± 7.1^{bc}	3.3 ± 1.7^{a}	10.0 ± 1.4^{bc}
H	8.0 ± 1.4^{a}	2.0 ± 1.6^{bc}	4.8 ± 2.5^{ab}
I	9.0 ± 7.1^{a}	3.3 ± 1.3^{a}	9.5 ± 4.9^{a}
J	7.5 ± 3.5^{ab}	2.3 ± 2.1^{bc}	5.0 ± 7.1^{b}

Key: THBC: Total heterotrophic bacteria Count; TCC: Total coliform Count,TCF:Total count of fungi. *Means on the row with the same letters (s) are not significant different (at P> 0.05), according to least significant difference (LSD)
Source: Field Survey Data, (2017)

Table 2 shows the result of the microbial load of the ten samples of yoghurt. Total heterotrophic bacteria count ranges from5.0 ± 7.1^{bc} to 9.0 ± 7.1^{a}x 10^5CFU/ml, Total coliform count ranges from $1.7\pm0.5^{ab}$to 3.6 ± 1.2^{ab}× 10^4CFU/ml, Total count for fungi ranges from 2.9 ± 1.6^{b} to 10.3 ± 3.6^{a} x 10^4CPFU/ml as shown in figure 1.

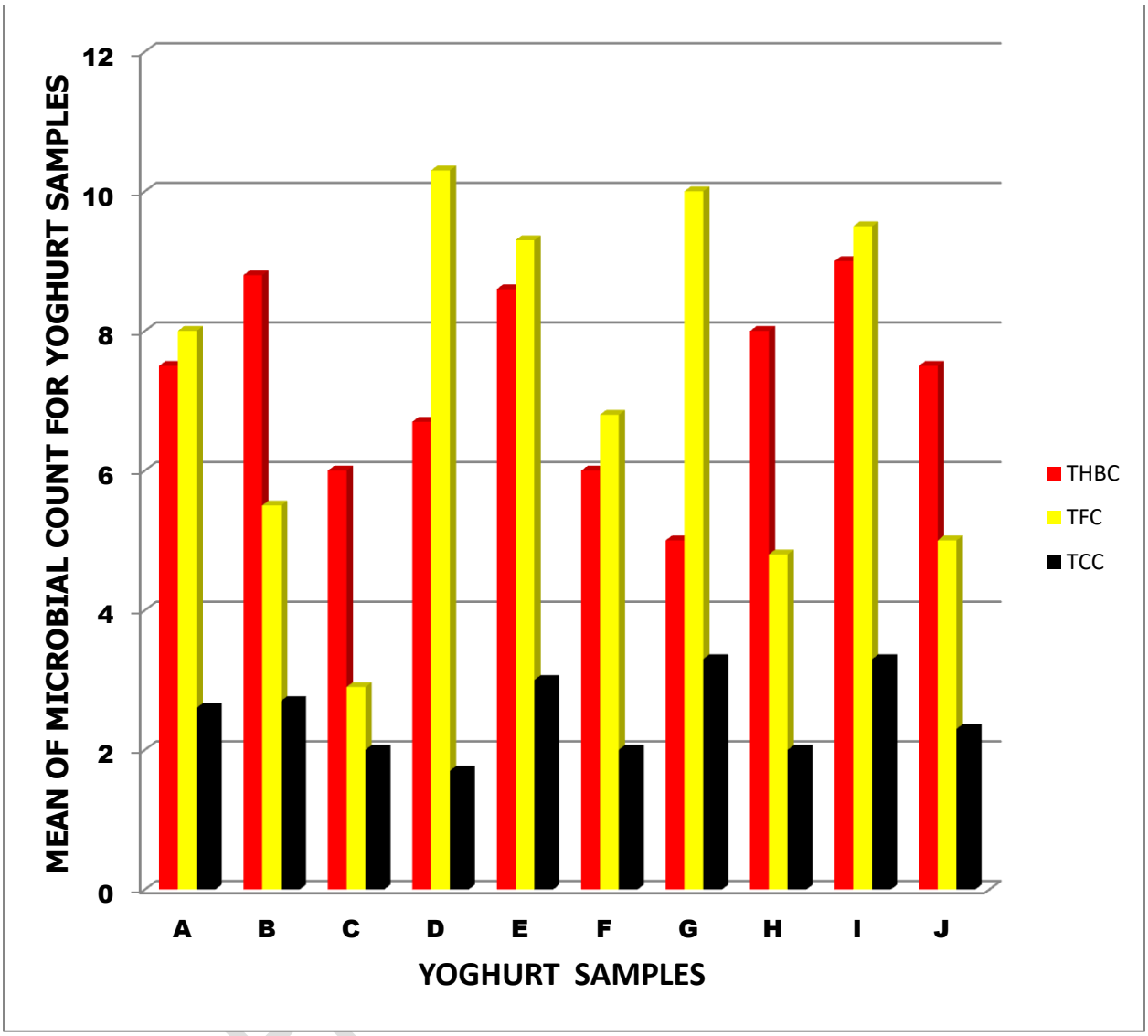

Figure 1: Bar Chart of Mean Samples of Yoghurt

TABLE 3: Thermotolerant Coliform and Fecal Coliform Count Of Various Yoghurt Samples

MEDIA	**MAcCONKEY BROTH**		**NUMBER OF POSITIVE TUBE**	**MPN INDEX/100ml**	**CONFIRMATION TEST**	**COMPLETED TEST**
STRENTH	DOUBLE STRENGTH	SINGLE STRENGTH				
QUANTITY OF YOGHURT SAMPLS (ml)	10	1 0.1	10 1 0.1			
NUMBER OF TUBES INNOCULATED	1 2 3 4 5	1 2 3 4 5	5 5 5			
A	+ - + - +	+ - + + -	4 2 5	50	-	+
B	+	+ - + + -	5 2 1	70	-	-
C	+ - + - +	+ + - - +	0 1 5	11	+	-
D	-	+ - + + +	2 1 2	12	+	+
E	- + + - +	- + + - -	5 1 3	84	+	-
F	+	+ - + - +	2 1 3	14	-	+
G	+ - + - +	+ + - + -	4 1 5	42	+	-
H	+	- + + - +	0 2 4	11	-	-
I	- - + - +	+ + - + +	5 2 3	120	-	+
J	+	- + - + +	5 0 2	43	+	+
	+ - + + +					
	- - - + +					
	- + + + +					
	+ - + + -					
	+ + - + -					

KEY: +=Positive (Acid and Gas production, Coliform or Fecal Coliform).
- = Negative.

Table 3 Shows the result of most probable number (MPN) of thermotolerant and fecal coliform bacteria which ranged from 11 to 120 (MPN) $100ml^{-1}$ of yoghurt sample.

Table 4: Microorganisms isolated from the different Yoghurt samples

Organism	SAMPLES									
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
E. coli	+	+	+	+	+	+	+	+	+	+
S. aureus	-	-	+	-	+	-	+	-	+	-
L. bulgaricus	+	+	+	-	+	+	+	-	+	+
Streptococcus spp	-	+	+	+	-	+	+	+	-	+
S. marcescens	-	-	+	-	+	+	+	-	+	+
A. niger	-	-	+	+	+	+	+	+	-	-
S. cerevisiae	+	+	+	-	+	+	+	-	-	-
C. albican	-	-	-	-	+	+	+	+	+	+
Mucor spp	-	+	+	+	-	-	-	+	+	+

Key: +, present; -, absent

Table 4 shows the different types of microorganisms isolated and identified from different yoghurt samples. Five bacterial genera included *Escherichia coli, Staphylococcus aureus, Serratia marcescens, Lactobacillus bulgaricus* and *Streptococcus spp* were identified. The first three bacteria were contaminants in the yoghurts and therefore undesirable while the last two are desirable microorganisms as they constitute the starter cultures used in the fermentation of milk to produce yoghurt. Also four fungal genera which included *Aspergillus niger, Candida albicans, Saccharomyces cerevisiae and Mucor spp* were identified.

Table 5: Cultural, morphological and biochemical characterization of isolates from the yogurt samples

Colonial Characteristics	Cell Shape	Gram Reaction	Catalase	Coagulase	Indole	Methyl Red	Urease	Sugar Fermentation	Probable Bacteria
Light pink colonies with smooth edge	Single rod	-	+	-	+	+	-	AG	*E. coli*
Smooth light yellow colonies with raised elevation	Cocci in clusters	+	+	+	-	+	-	A	*S. aureus*
Creamy convex colonies with ciliated edge	Cocci in short chains	+	-	-	-	-	+	A	*Streptococcus Spp.*
White round slight raised colonies	Rod	+	-	-	-	-	+	A	*L. bulgaricus*
Pink smooth irregular flat colonies	Cocci	-	+	-	-	+	-	AG	*S. marcescens*

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

The bacteria isolates were characterized and identified based on colonial and cellular morphological features as well as biochemical tests as presented in table 5 above. Table 5 shows the colonial morphology (macroscopic observation of colony on plates) and the cellular morphology (microscopic characteristics) of the bacteria isolated from different yoghurt samples. The bacteria were characterized based on their reaction to various biochemical tests. The reactions of the bacterial isolates to the various biochemical tests performed on them were recorded and the probable bacteria were reported as well.

4.0. DISCUSSION

The present study has revealed the types of heterotrophic bacteria, coliform and fungi in the various samples of yoghurt. The labels on the yoghurt brands provided little information about the products which included only production date, expiry date, batch number and NAFDAC Registration number.

The pH readings of between 4.28 and 4.79 are somewhat above the high acidity and low pH of between 3.8 and 4.2 expected for yoghurt storage. At this pH yoghurt is not a hospitable medium for pathogens which will not grow in acidic medium and will not survive well either. The pH values show that the bacteria isolates are acidophiles. It has also been reported that yoghurt that has an acidic content seems to act as a selective media for yeasts and moulds using lacteal as a possible source of energy [7].

The total heterotrophic count (THBC) ranged from 5.0 ± 7.1^{bc} to $9.0\pm7.1^{a}\times10^{5}$CFU/ml, average total coliform counts (TCC) ranged from 1.7 ± 0.5^{ab} to $3.6\pm1.2^{ab}\times10^{4}$CFU/ml and the thermo-tolerant coliform bacteria and fecal coliform ranged from 11 to 120(MPN) $100ml^{-1}$. The total fungal count (TFC) on the other hand varied between 2.9 ± 1.6^{b} to $10.3\pm3.6^{a}\times10^{4}$CFU/ml. Some of the samples showed microbiological parameters not in conformity with the official standards, since their total heterotrophic counts (THC), total coliform counts (TCC) and total fungal counts (TFC) had values far greater than the maximum tolerable limits of 5 x 10^{4}CFU/ml,10 CFU/ml and 1 mould /ml for THC, TCC and TFC respectively [33]. These results are similar with that of Taura *et al.* [34] whose analysis of 20 yoghurt brands in Kano, Nigeria showed 40%, 55% and 90% of the samples had counts higher than the acceptable standards for THC, TCC and TFC respectively. However, only 1% of his samples passed all three safety limits. Okpala and Jideani

[35] also reported poor microbiological standards of commercial yoghurts sold in Bauchi, Nigeria.

Five different bacterial genera were identified. These were *Escherichia coli, Staphylococcus aureus, Lactobacillus bulgaricus, Streptococcus spp* and *Serratia marcesecens.* The presence of *Streptococcus* spp. and *Lactobacillus* spp. inthe yoghurt samples agrees with the assertion that they are the most common bacterial species used in the fermentation of milk into yoghurt [36].

The occurrence of *Streptococci* in this study is in line with the works of Bramley *et al*. [37], 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.

Park *et al*. [38] reported the frequent contamination of dairy products by *Staphylococcus aureus.* The possible source of this organism may be from the nasal passage, skin and other mammals. Talking, coughing and talking produce droplets which settle on the yoghurt during production, transportation, storage and retailing. *Staphylococcus aureus* is resistant to heat, drying and radiation. The presence of *Staphylococcus aureus* in yoghurt may causes Staphylococcal food poisoning which is a major type of food intoxication caused by ingestion of improperly stored or cooked food in which *S. aureus* has grown [39].

The presence of coliform indicated contamination and the poor level of hygiene after processing. Coliforms are not supposed to be present in yoghurt because of high temperature, short time pasteurization and effective cleaning and good hygienic procedures [40], the presence of coliforms from this poses great danger to the health of the consumers and suggest neglect on the part of the processors or the yoghurt vendors. The tolerable limit for coliform presence in

yoghurt is less than 10CFU/ml, but a higher count of 4000 is of serious public health concern [41]. This contamination might be from contaminated water source or equipment used or probably as reported by Karagul-Yuceer *et al.* [41], due to contamination at storage and display/sale outlet. *Escherichia coli* and *Staphylococcus aureus* isolated in this study has been reported and proved to be potential contaminants of yoghurt [42]. The incidence of *Staphylococcus aureus* in yoghurt samples is a source of concern. Its presence in the dairy products is undesirable and should be prevented because it can easily multiply in dairy products if held between 10^0C and 45^oC [43]. The presence of *E. coli* which is an indicator of fecal contamination and the presence of pathogen such as *Staphylococcus aureus* indicates that the yoghurt samples were highly contaminated.

Four different fungal genera were identified and included *Aspergillus niger, Candida albicans, Mucor spp and Saccharomyces cerevisiae.* The isolation of fungi such as *Aspergillus and Mucor* species agreed with Oyeleke [27] that moulds are the primary contaminants of yoghurt produced in Nigeria.

According to Adams and Moss [44], yoghurts are spoiled by acidoduric organisms like yeasts and moulds. In fruit containing yoghurts, *S. cerevisiae* has been implicated in spoilage, as well as *Mucor, Rhizopus, Aspergillus, Penicillium and Alternaria*. According to Arnott *et al*., [1979], contamination of yoghurts by yeasts or moulds is generally related to the fruits added for flavour or poor hygienic practices during packaging. *Saccharomyces cerevisiae* was also isolated from yoghurt samples in Brazil (Moreira *et al*., 2001). Ifeanyi *et al.,* (2013) also isolated *E. coli, Aspergillus and Rhizopus* from yoghurt samples sold in Onitsha while De *et al.,* (2014) isolated *Staphylococcus* spp. from yoghurt samples sold in Kaduna metropolis.

Yoghurt is not expected to be sterile (free of microorganisms) as the heat treatment of the milk used for production only kills pathogenic microorganisms and substantially reduces the level of spoilage microorganisms. The presence of these contaminants therefore might be caused by inadequate heat treatment (Pasteurization) of milk and poor hygienic standards of processing and packaging that led to recontamination of the product. In addition, the microorganisms could have been introduced into the products from the skin microflora (e.g. *S. aureus* and *Micrococcus*) of personnel employed in the production or from the non-sterile production environment. The detection of fungi and other bacteria probably indicated post-production contamination. Furthermore, the detection of these contaminating microorganisms could also possibly indicate post-production contamination as a result of storage under inappropriate conditions (above 10^0C) during sales in the market environment. Post-production contamination was not impossible, considering the non-sterile environment in which production and sales were carried out.

According to Habibu and Mukhtar [49], many of the home-based local factories of food and drinks undertake the filling of the packs, polythene bags and bottles carelessly without observing any form of sanitation in the production and packaging of the yoghurt drinks. Frazier and Westhoff [50], pointed out that this may be another reason for the high counts of heterotrophic bacteria as well as coliform and fungal counts observed in yoghurt sample drinks.

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.

CONCLUSION

From the available result, it can be concluded that most of the yoghurt sold and consumed within Owerri do not present adequate microbiological quality. This suggests the need for strict hygienic measures to be applied during production, processing and distribution of yoghurts and its products to avoid contamination with unwanted materials and microorganisms.

Periodical factory inspection must be done by regulators in the industry such as NAFDAC to checkmate the problem of poor hygiene and to apply sanctions where necessary. The manufacturers should make it a duty upon themselves to educate their staffs on clean and hygienic practices considering the high level of coliform contamination.

NAFDAC registered samples are commonly products of high standard but in this case these products are not safe for people to consume. So there is need fora HACCP (Hazard Analysis Critical Control Points) program for transportation, packaging and storing yogurt in Nigeria.

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