BACTERIOLOGICAL, MYCOLOGICAL QUALITY ASSESSMENT AND PROXIMATE COMPOSITIONOF SELECTED BRANDS OF YOGHURT SOLD WITHIN KADUNA METROPOLIS, NIGERIA

Mohammed, Sani Sambo Datsugwai[1], **Aliyu**, Gaga Saddiya[2]

[1]Department of Microbiology and Biotechnology, Faculty of Natural and Applied and Sciences, Nile University of Nigeria, FCT, Abuja.
[2]Department of Microbiology, Faculty of Science, Kaduna State University, Kaduna, Nigeria
Email: mosada78@gmail.com.,sani.mohammed@nileuniversity.edu.ng

Abstract
The bacteriological, mycological quality assessment and proximate composition of selected brands of yoghurt within Kaduna metropolis was investigated. A total of fifteen (15) samples were collected; three (3) samples from five (5) different brands were purchased and analyzed in the laboratory for proximate composition using standard techniques, total bacterial and fungal counts using pour plate methods. Identification and characterization of the bacterial isolates were done using cultural, morphological and biochemical tests and mycological identification using macroscopic and microscopic technique after staining with lactophenol cotton blue. Antibiogram of selected antibiotics/antifungal agents against the bacteria and fungi isolates were investigated. The result from the proximate composition revealed that sample A had a percentage moisture, ash,protein,fat,fiberand carbohydrate contents of 89.90%,0.42%,3.40%,2.29%, 0.26% and 3.73% respectively. Sample B had a moisture content of 89.12%, ash content (0.40%), protein content (3.44%), fat content (3.11%), fiber content (0.24%) and carbohydrate content of 3.69%, while Sample C had a moisture content of 90.01%, ash content (0.39%), protein content (3.10%), fat content (3.02%), fiber content (0.23%) and carbohydrate content of 3.25%. Out of the fifteen (15) yoghurt samples analyzed, thirteen (13) of the samples recorded bacteria growth in the range of $1.36x10^3$ – $9.6x10^3$ CFU/mL and $1.04x10^4$ – $9.6x10^4$ CFU/mL for aerobic mesophilic count while nine (9) of the yoghurt samples recorded growth in the range of $1.0x10^3$ – $6.0x10^3$ CFU/mL for total fungal count. The bacterial isolates were identified asBacillussp., Staphylococcus aureus, Lactobacillus bulgaricus and Streptococcus thermophilus. Bacillus sp. was the predominant bacteria isolate in the yoghurt samples analyzed.Enterobacter aerogenes was the predominant coliform bacteria isolated. The species of fungi isolates were Aspergillus sp., Mucor sp., Rhizopus sp. and Trichoderma sp. The results of the antibiogram revealed that Streptococcus thermophilus was susceptible to all the antibiotics used and Bacillus sp. was susceptible to all but with exception of Rocephin at (25µg), Lactobacillus bulgaricus was susceptible to Pefloxacin (10µg), Gentamicin (10µg), Zinnacef (20µg), Rocephin (25µg), Streptomycin (30µg) and Erythromycin (10µg) while staphylococcus aureus was susceptible to Pefloxacin (10µg), Ampiclox (30µg), Ciprofloxacin (10µg), Streptomycin (30µg), Septirin (30µg) and Erythromycin (10µg). Aspergillus sp. and, Mucor sp. were susceptible to all the antifungal drugs tested and Rhizopus sp., was resistant to only Fluconazole (25µg) while Trichoderma sp. was susceptible to only Amphotericin B (20µg). The presence of coliforms is an indication of faecal contamination of the yoghurt samples analyzed. Proper care should be taken in storage and handling of yogurt products. The application of Hazard Analysis and Critical Control Points (HACCPs) systems/concepts in the process of yoghurt production will help in eradication of contaminations and guarantee safety of the products.

Keywords: Proximate,yoghurt, bacteria, fungi, antibiogram

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INTRODUCTION

Yoghurt is one of the oldest dairy products in the world. Food historians believe that people have been consuming yoghurt for more than 5,400 years. Yoghurt has also proved to be a very popular food with health-conscious consumers. Yoghurt is a semi fluid fermented milk having a smooth texture and mildly sour flavour because of its lactic acid content. In simple terms, yoghurt is a form of curdled milk. The bacteria contained in the milk ferments and coagulates. This causes the milk to thicken which creates yoghurts signature creamy texture and slightly tangy, astringent taste. Added to the yoghurt are flavoring and colours to give it a unique appearance and taste. The main processing steps involved in yoghurt manufacture include the standardization of milk (fat and protein content), homogenization, milk heat treatment, incubation/fermentation, cooling, and storage. In many of these countries, yoghurt is still manufactured using traditional procedures. Since the last world war, yoghurt consumption has been steadily increasing not only in European countries, but also in the United States, enhancing its industrial-scale production. It is accepted that the initial consumption of fermented or cultured milk products, such as yoghurt, butter and cheese, occurred around the time as they were recognized as effective means of prolonging the shelf-life of milk (Tamine and Robinson, 2008). The French called it *'la lait de la vie eternelle'* - the milk of eternity as it was believed to have therapeutic powers and gave long life to those who consumed it. The main reasons pointed out for yoghurt consumption is the cultural and the increasing search for healthy foods (Cueva and Aryana, 2008). It is a means of protein intake for an improved healthy living (Cueva and Aryana 2008). It also serves as a medium for microbial growth due to its high nutritional value and plays an important role in human nutrition, health maintaining, therapeutic and dietetic functions (Khan *et al.* 2008). Yoghurt is one of the most traditional cultured milk, which is a product of the lactic acid fermentation of milk by addition of a starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Tamine and Robinson, 2008). The role of these two bacteria genera in yogurt manufacture can be summarized as milk acidification and synthesis of aromatic compounds (Serra *et al*., 2009). These organisms produce organic acids and other flavor components and can grow in such numbers that a gram of yoghurt regularly contains 100 million bacteria. The natural yoghurt is characterized by a smooth and viscous gel-like texture and has a delicate Wal nutty flavour (Fuquay *et al*., 2011). Yoghurt is nutritionally rich in protein, riboflavin, vitamin B12. It has nutritional benefit beyond those of milk. Men and women who are lactose intolerant can sometimes tolerate yoghurt better than dairy products, because the lactose in milk is converted to glucose, galactose and partially fermented to lactic acid by bacterial culture. (Belewu *et al*., 2010).Yoghurts as other dairy products are frequently contaminated by bacteria and fungi which often led to food intoxication/poisoning. Moulds and yeasts are the primary contaminants in yoghurt produced commercially in Nigeria. Health complications associated with consumption of inadequately pasteurized milk products include serious infections that are hard to treat with antibiotics. There is a misnomer in the condition surrounding the sales of yogurt within Kaduna metropolis. Research in the field of quality assessment of yoghurt sold is the basic need to create awareness among common people about the existing situation and protect the consumers' health and rights (Yabaya and Idris, 2012). The occurrence of Enterococci, coliform, mould and Yeast, *Streptococcus* sp., *Salmonella* sp.,C*lostridium* sp. and *Bacillus* sp. is a sign of re-infection of yoghurt (Speck, 2011). Contamination can occur as a result of improper processing or handling. This is due to unclean equipment, contaminated milk or poor hygiene of the production staff. Pasteurization should ensure that fresh milk is not contaminated, but do not use old milk. This research was aimed at assessing the bacteriological and mycological quality of

some selected brands of yoghurt sold within Kaduna metropolis.

MATERIALS AND METHODS

The Study Area

The study was conducted in Kaduna State, Nigeria. This study area has been selected due to the high concentration of yoghurt production activities within the state, and because the close proximity to the researcher. Kaduna is located in the North-west geo political zone of Nigeria. The capital city of the state is Kaduna. Kaduna has a total area of 1,190 square meter (3,080 km^2) and its coordinates are 10°31'23'N'7°26'25''E. According to the National Population Commission, the 2006 census puts the population of the state at 6,113,503 people. The annual population growth rate is 2.47% (The National Bureau of Statistics, NBS, 2011).

Collection of Samples

A total of Fifteen (15) packaged yoghurt samples, three (3) samples from five (5) different brands each were purchased from different retail outlets within Kaduna Metropolis, their NAFDAC registration number, expiry date, manufacturing date and batch number were recorded. All samples purchased were properly labeled and transported and stored in a refrigerator in the Microbiology laboratory of Kaduna State University for analysis.

Proximate Analysis of the Yoghurt Samples

Proximate analysis of yoghurt samples were carried out and it includes the percentage moisture content, ash, crude protein, crude fibre, crude fat and carbohydrate. These were determined using Association of Official Analytical Chemists (AOAC) (2009) as described by Ammara and Imran (2010); Oladipo and Jadesimi (2012).

Media Preparation

All media used were prepared according to manufacturer's instruction (s).

Physical Observation of the Yoghurt Samples

The different brands of yoghurt on arrival to the laboratory were visually observed for packaging conditions, colour and texture and these were recorded accordingly before proximate /microbiological investigations were carried out on them.

Isolation of Bacteria and Total Bacterial Counts from Yoghurt

The bacteriological analysis of samples were carried out for total viable count and coliform count using the method described by Kawo *et al.* (2008). Ten (10) fold serial dilutions of the yoghurt samples were prepared up to 10^{-5} as follows; 25ml of each yoghurt sample were drawn aseptically and transferred into a test tube containing 225ml of sterile distilled water for each respectively. After shaking, exactly 1ml of the first diluted sample (10^{-1}) was aseptically withdrawn and transferred into another 9ml of sterile distilled water contained in a test tube and shaken again, this represents 10^{-2}. The dilution was done up to 10^{-5}. Subsequently 1ml each from dilutions 10^{-3} and 10^{-4} were aseptically pipette and poured correspondingly into duplicate of appropriately labeled Petri-dishes containing nutrient agar (NA) using the pour plate method. This was used for the total bacterial count as described by Kawo *et al.*(2008). Each of the plates were incubated at 37°C for 24 hours. Colonies that developed on the plate after incubation were observed and counted and records were made accordingly. Pure cultures were made by sub-culturing distinct colonies using the streak plate technique on nutrient agar until a clear distinct colon were obtained. The pure colonies obtained were then inoculated on a nutrient agar slant in a McCartney bottle and incubated at 37°C for 24 hours and these were stored in the refrigerator as stock culture.

Total Coliform Count (TCC) from Yoghurt

Presumptive Test for TCC

Lactose broth was prepared according to manufacturer's instruction. Peptone water was also prepared using 25g in 225ml of sterile water. Series of lactose broth primary fermentation tube was inoculated with the serially diluted yoghurt sample in triplicate test tubes, each diluent 10^{-1},10^{-2} and 10^{-3} having three test tubes each, Durham tubes were inserted inversely into the tubes and the test tube was swirled vigorously to remove

bubbles. The inoculated tubes were incubated at 35^0C for 24 hours. After 24 hours when there was no bubble or gas formation in the tubes the incubation was continued and examined for gas formation at the end of 48 hours (Huck, 2008).
Formation of bubble or gas in any amount within 48hours indicates a positive presumptive test.

Confirmation Test for TCC

The confirmation test was used on all primary fermentation tubes showing gas or bubble formation during 24hours and 48hours period. Fermentation tubes containing brilliant green bile lactose bile broth was inoculated with medium from the tubes showing a positive result in the presumptive test. Inoculation was performed as soon as possible after gas formation occurs and the inoculated tubes were incubated at 35^0C for 48 hours (Huck, 2008). Gas or bubble formation were observed and recorded.

Completed Test for TCC

The fermented test tubes were used in conducting the completed test. A loop full of the sample was taken from each test tube and streaked on Eosin methylene blue agar (EMB) separately. Growth of colonies was observed macroscopically and further identification and characterization were carried out (Huck, 2008).

Characterization and Identification of Bacteria from Yoghurt

Bacterial isolates were characterized and identified using cultural morphology, Gram staining technique and Biochemical tests as described by Fawole and Oso (2008) and Oyeleke and Manga (2008).

Antibiogram of Bacteria Isolates from Yoghurt Samples

The Kirby-Bauer Disc Method

A filter disk impregnated with the antibiotics: Pefloxacin (10µg), Gentamicin (10µg), Ampiclox (30µg), Zinnacef (20µg), Amoxacillin (30µg), Rocephin (25µg), Ciprofloxacin (10µg), Streptomycin (30µg), Septirin (30µg) and Erythromycin (10µg) were applied to the surface of an agar plate containing solid Mueller Hinton Agar which has been spread with a bit of the colony of the organism (s) to be tested and the plate was incubated at 37°C for 24 hours for each bacteria isolate respectively. The diameter of the ZOIs were measured with a venire caliper and the results of this experiment constitute an antibiogram (Bassiri, 2010).

Isolation of Fungi and Total Fungal Count

Following the serial dilution of the yoghurt, 25ml each of the diluted yoghurt sample were taken from 10^{-3} and 10^{-4} dilutions and were aseptically plated on Potato Dextrose Agar (PDA) using the pour plate method. This is used for the isolation of fungi and fungal count. The plates were incubated at 26 $\pm1^0$C for 5 days. Colonies that developed on the plate after incubation were carefully observed and counted and records were made accordingly. Pure cultures were made by sub-culturing distinct colonies using the streak plate technique on Potato Dextrose Agar until a clear distinct colony was obtained. The pure colonies obtained were picked with a sterile inoculating needle and inoculated into sterile PDA slant in McCartney bottle and incubated at 26 $\pm1^0$C for 5 days and were stored in the refrigerator as stock culture (Yabaya and Idris, 2012).

Identification of Fungal Isolates from Yoghurt Samples

Fungal isolates were characterized based on colonial morphology and microscopic appearance after staining with lactophenol cotton blue and viewed under 40X magnification and comparing their characteristics with those of known taxa as described by Oyeleke and Manga (2008).

Antibiogram of Selected Antifungal against Fungal Isolates from Yoghurt Samples

Fungi were sub -cultured on potato dextrose agar (PDA) and were incubated at26 $\pm1^0$C for 4 days. The conidia were harvested in sterile saline using a Hama cytometer, the conidial suspension was adjusted to 1.0x 10^6 conidia/mL. Muller Hinton agar (MHA) agar plates were spread evenly with a swab dipped into the standardized inoculum suspension. Lids were left ajar for 30 minutes in a laminar flow cabinet to allow any excess surface moisture to be absorbed into the agar before the drug impregnated such as fluconazole, amphotericin B, Itraconazole and voriconazole

at different concentrations were applied (Ana *et al*., 2017). The drugs were applied to the surface of the inoculated plates using sterile forceps. Plates were inverted and incubated for 26 $\pm 1^0$C for 4 to allow fungal growth. Inhibition Zone diameter (IZD) was measured in millimeters (Nweze, 2010).

Statistical Analysis of Data

Data generated were subjected to one way Analysis of variance (ANOVA) to test the level of significance within and between variables.

RESULTS

Table 1 shows the proximate composition of the yoghurt brands. The result obtained revealed that sample A had moisture content of 89.90%, Ash content (0.42%), protein content (3.40%), fat content (2.29%), fiber content (0.26%) and carbohydrate content (3.73%). Sample B had moisture content of 89.12%, Ash content (0.40%), protein content (3.44%), fat content (3.11%), fiber content (0.24%) and carbohydrate content (3.69%). While Sample C had moisture content of (90.01%), Ash content (0.39%), protein content (3.10%), fat content (3.02%), fiber content (0.23%) and carbohydrate content (3.25%). There was no significant difference ($p>0.05$) between all the samples in their percentage moisture, ash, protein, fat, fiber and carbohydrate contents. Table 2 shows the total aerobic mesophilic bacterial count of the analyzed yoghurt samples. The bacterial count ranged from $1.36x10^3$ -$9.6x10^3$ CFU/mL and 1.04 $x10^4$-$9.6x10^4$ CFU/mL with sample D2 having the highest bacterial count and samples E1 and E3 had the lowest bacterial count with dilution10^3. Sample C2 had the highest bacterial count and sample D3 had the lowest bacterial count in dilution 10^4.

Table 1: Proximate Composition of Yoghurt Samples

	Sample			*F*	*P*
Parameters (%)	**A**	**B**	**C**		
Moisture content	89.90 ±3.10[a]	89.12 ±5.10[a]	90.01 ±6.54[a]	*14.822*	*0.005*
Ash content	0.42 ±0.09[a]	0.40 ±0.04[a]	0.39 ±0.07[a]	*0.146*	*0.867*
Protein content	3.40 ±1.10[a]	3.44 ±0.82[a]	3.10 ±0.56[a]	*0.141*	*0.871*
Crude fat content	2.29 ±0.27[a]	3.11 ±0.67[a]	3.02 ±0.67[a]	*1.875*	*0.233*
Crude fiber content	0.26 ±0.05[a]	0.24 ±0.05[a]	0.23 ±0.05[a]	*0.290*	*0.758*
Carbohydrate content	3.73 ±0.39[a]	3.69 ±0.32[a]	3.25 ±0.50[a]	*1.253*	*0.351*

A, B and C: selected yoghurt samples, *P value:* level of significance*Values are mean ±Standard deviation. Results with the same superscripts on the same row are not significantly different at ($p>0.05$).

Table 2: Total Aerobic Mesophilic Bacterial Count from Yoghurt Samples

Sample Codes	10^3CFU/mL	10^4CFU/mL
A1	1.48	1.28
A2	Too numerous	7.6
A3	NG	NG
B1	NG	NG
B2	Too numerous	NG
B3	Too numerous	7.2
C1	2.8	3.2
C2	8.4	9.6
C3	10.0	1.3
D1	1.80	1.96
D2	9.6	3.0
D3	2.20	1.04
E1	1.36	6.9
E2	1.64	2.24
E3	1.36	2.10

NG = No growth, CFU/mL = Colony forming unit/mL.

Table 3 shows that there was no significant difference (p>0.05) in the mean aerobic mesophilic bacterial count of the samples at dilution 10^3 CFU/mL. There were also no significant difference (p>0.05) in the mean aerobic mesophilic bacterial count of the samples at dilution 10^4 CFU/mL. Table 4 shows the total coliform count of the analyzed yoghurt samples. The result obtained revealed the presumptive and the confirmatory tests of the samples. Sample E1 had the highest total coliform count of 3:3:3. Growth was not observed in sample A2 and D3. The completed test revealed the organisms to be coliform bacteria. Table 5 a shows the cultural, morphological and biochemical characterization and identification of the bacterial isolates from the analyzed yoghurt samples. The bacterial isolates were identified as *Bacillus* sp. Which was isolated from all the samples analyzed, *Staphylococcus aureus* was isolated from only eight (8) yoghurt samples (A1, B3, C1, C2, D1, D3, E2, E3), *Lactobacillus bulgaricus* was isolated from only three (3) yoghurt samples (A2, B2, B3) and *Streptococcus thermophilus* was isolated from five(5) yoghurt samples (A2, B2, B3, D1, D3). Table 5b shows the occurrence of bacteria in the yoghurt samples analyzed. *Bacillus* sp. had frequency and percentage 13(31%), *Staphylococcus aureus* had frequency and percentage of 8 (19%), *Lactobacillus bulgaricus* had frequency and percentage of 3 (7.2%) and *Streptococcus thermophilus* had frequency and percentage of 8 (19%).

Table 3: Mean Total Aerobic Mesophilic Bacterial Count from Yoghurt Samples

Sample	10^3 CFU/mL	10^4 CFU/mL
A	10.49 ±16.9	2.96 ±4.07
B	20.00 ±17.3	2.40 ±4.16
C	7.07 ±3.78	4.70 ±4.35
D	4.53 ±4.39	2.00 ±0.98
E	1.45 ±0.16	3.75 ±2.73
F-value	*1.231*	*0.287*
p-value	*0.358*	*0.880*

Values are mean ±Standard deviation

Table 4: Total Coliform Count from Yoghurt

Sample Codes	**Presumptive Test**			**Confirmatory Test**			**Completed Test**
	1:10	1:100	1:1000				
A1	2	3	2	2	3	2	+
A2	0	0	0	-	-	-	-
A3	0	2	0	-	2	-	+
B1	3	2	3	3	2	3	+
B2	2	3	1	2	3	1	+
B3	1	0	0	1	-	-	+
C1	1	1	3	1	1	3	+
C2	0	2	0	-	2	-	+
C3	1	3	0	1	3	-	+
D1	2	2	1	2	2	1	+
D2	0	1	0	-	1	-	+
D3	0	0	0	-	-	-	-
E1	3	3	3	3	3	3	+
E2	3	0	2	3	-	2	+
E3	3	0	0	3	-	-	+

+: Present. –: Absent

Table 5a: Cultural, Morphological and Biochemical Characteristics of the Bacterial Isolates from the Yoghurt Samples

Isolate Codes	Colonial Characteristics	Cell Shape	Gram Stain	Catalase	Coagulase	Citrate	Sugar fermentation			Probable Organisms
							Glucose	Lactose	Sucrose	
A1a, A2a, B2a, B3a, C1a, C2a, C3a, D1a, D2a, D3a, E1a, E2a, E3a.	Creamy, circular, smooth, slightly raised colonies	Rod	+	+	-	-	A	A	A	*Bacillus* sp.
A1b, B3b, C1b, C2b, D1b, D3b, E2b, E3b.	Light yellow colonies with raised elevation	Cocci in clusters	+	+	+	-	A	AG	AG	*Staphylococcus aureus.*
A2c, B2c, B3c.	White round slight raised colonies.	Rod	+	-	-	-	A	A	A	*Lactobacillus bulgaricus*
A2d, B2d, B3d, D1d, D3d.	Creamy convex colonies with ciliated edges.	Cocci in chains	+	-	-	-	A	A	A	*Streptococcus thermophilus*
B1e.	Metallic green sheen with faecal odour on EMB.	Single rod	-	+	-	-	AG	AG	AG	*Escherichia coli.*
A1f, A3f, B2f, B3f, C1f, C2f, C3f, D1f, D2f, E1f, E2f, E3f.	Purple colonies on EMB	Rod	-	+	-	+	AG	AG	AG	*Enterobacter aerogenes.*

+ = Positive. – = Negative. A = Acid. AG = Acid and gas.

Table 5b: Percentage Occurrence of Bacterial Isolates from Yoghurt Samples

Sample														Freq/percentage (%)	Probable Organisms
A1	**A2**	**A3**	**B1**	**B2**	**B3**	**C1**	**C2**	**C3**	**D1**	**D2**	**D3**	**E1**	**E2**	**E3**	
+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	13/ 31%	*Bacillus* sp.
-	+	-	-	+	+	-	-	-	+	-	+	-	-	-	5/ 11.9%	*Streptococcus thermophilus*
-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	3/7.2%	*Lactobacillus bulgaricus*
+	-	-	-	-	+	+	+	-	+	-	+	-	+	+	8/19%	*Staphylococcus aureus*
-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	1/ 2.3%	*Escherichia coli*
+	-	+	-	+	+	+	+	+	+	+	-	+	+	+	12/28.6%	*Enterobacter aerogenes*

\+ = Presence of bacteria in the sample, - = Absence of bacteria in the sample

Table 6: Fungal Counts from Yoghurt Samples

Sample Codes	10^3 CFU/ml
A1	6.0
A2	1.0
A3	NG
B1	1.9
B2	1.8
B3	3,9
C1	NG
C2	NG
C3	NG
D1	NG
D2	4.3
D3	NG
E1	3.0
E2	4.3
E3	1.0

KEY: NG = No growth,
CFU/mL = Colony forming unit/mL

Table 6 shows the total fungal count of the analyzed yoghurt samples. The fungal count ranged from $1.0x10^3$ -$6.0x10^3$ CFU/mL with sample A1 having the highest fungal count and sample A2 had the lowest fungal count. Table 7 shows that there was no significant difference ($p>0.05$) in the mean colony forming of fungal isolates of the samples at dilution10^3 CFU/mL.

Table 7: Mean Fungal Count from Yoghurt Samples

Sample	$\times10^3$ CFU/mL
A	2.33±3.21
B	2.53±1.18
C	0.00±0.00
D	1.43±2.48
E	2.77±1.66
F-value	*0.931*
p-value	*0.484*

Values are mean ±Standard deviation

Table 8a shows the cultural, morphological and microscopic characteristics of the fungal isolates from the analyzed yoghurt samples. The result showed that the fungi isolates were identified using pigmentation and forms of the colonial appearance. The fungal isolates were *Aspergillus* sp., which was isolatedfrom8 yoghurt samples namely A1, A2, B1, B2, B3, E1, E2, E3. *Mucor* sp. were isolated from 5 yoghurt samples namely (B1, B2, B3, D2, E1), *Rhizopus* sp. was isolated from sample B3 and *Trichoderma* sp. was isolated from 4 samples namely A1, B1, B2, E2. Table 8b shows the occurrence of moulds in the yoghurt sample. *Aspergillus* sp. had occurrence and percentage frequency of 8(44.4%), *Mucor* sp. 5(27.8%), *Rhizopus* sp. 1(5.6%) and *Trichoderma* sp.4 (22.2%). Table 9 shows the antibiogram of selected antibiotics against the bacterial isolates from the analyzed yoghurt samples. The result revealed that *Streptococcus thermophilus* was susceptible to all the antibiotics used which were Pefloxacin (10µg), Gentamicin (10µg), Ampiclox (30µg), Zinnacef (20µg), Amoxacillin (30µg), Rocephin (25µg), Ciprofloxacin (10µg), Streptomycin (30µg), Septirin (30µg) and Erythromycin (10µg). *Bacillus*sp. was susceptible to all except Rocephin (25µg), *Lactobacillus bulgaricus* was susceptible to Pefloxacin (10µg), Gentamicin (10µg), Zinnacef (20µg), Rocephin (25µg), Streptomycin (30µg) and Erythromycin (10µg) while *Staphylococcus aureus* was susceptible to Pefloxacin (10µg), Ampiclox (30µg), Ciprofloxacin (10µg), Streptomycin (30µg), Septirin (30µg) and Erythromycin (10µg). Table 10 shows the antibiogram of selected antifungal drugs against the fungal isolatesfrom the analyzed yoghurt samples. The result revealed that *Aspergillus* sp. and *Mucor* sp. were susceptible to all the antifungal drugs which are Amphotericin B (20µg), Fluconazole (25µg), Voriconazole (10µg) and Ketoconazole (10µg) and *Rhizopus* sp., was resistant to only Fluconazole (25µg) while *Trichoderma* sp. was susceptible to only Amphotericin B at concentration of 20µg.

Table 8a: Morphology and Microscopic Characteristics of Fungal Isolates from Yoghurt

Isolate Codes	Colony Morphology	Microscope Characteristics	Probable Fungal
A1a, A2a, B1a, B2a, B3a, E1a, E2a, E3a.	Black flaggy colonies	Conidia branches resembling bushes, conidia head splits into columns.	*Aspergillus* sp.
B3b	Brown cotton candy like in texture.	Non-septate, some scaly septate with hyphae that has rhizoids and sporangia.	*Rhizopus* sp.
B1c, B2c, B3c, D2c, E2c.	Wooly growth resembling cotton candy, milk, yellowish, milk fish.	Branched hyphae, non-septate, many spores, some are branched and some terminate in a round spore filled with sporangia	*Mucor* sp.
A1d, B1d, B2d, E2d.	Green cushions	Septate, sporulating filaments	*Trichoderma* sp.

Table 8b: Percentage Occurrence of Fungal Isolates from Yoghurt Samples

Sample codes															Frequency/percentage (%)	Probable Organisms
A1	A2	A3	B1	B2	B3	C1	C2	C3	D1	D2	D3	E1	E2	E3		
+	+	-	+	+	+	-	-	-	-	-	-	+	+	+	8/44.4%	*Aspergillus* sp.
-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	1/5.6%	*Rhizopus* sp.
-	-	-	+	+	+	-	-	-	-	+	-	-	+	-	5/27.8%	*Mucor* sp.
+	-	-	+	+	-	-	-	-	-	-	-	-	+	-	4/22.2%	*Trichoderma* sp.

Key + = Presence of fungi in the sample, - = Absence of fungi in the sample, A1-E3: sample codes

Table 9: Antibiogram of the Bacterial Isolates from Yoghurt Samples

Bacterial isolate	Antibiotics(mm)									
	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E
Bacillus sp.	+	+	+	+	+	-	+	+	+	+
Staphylococcus aureus.	+	-	-	-	+	-	+	+	+	+
Lactobacillus bulgaricus	+	+	-	+	-	+	-	+	-	+
Streptococcus thermophilus	+	+	+	+	+	+	+	+	+	+

– = No zone of inhibition
+ = Zone of inhibition
PEF = Pefloxacin (10µg)
CN = Gentamicin (10µg)
APX = Ampiclox (30µg)
Z = Zinnacef (20µg)
AM = Amoxacillin (30µg)
R = Rocephin(25µg)
CPX = Ciprofloxacin(10µg)
S = Streptomycin(30µg)
SXT = Septirin(30µg)
E = Erythromycin (10µg)

Table 10: Antibiogram of the Fungal Isolates from Yoghurt Samples

Fungal Isolates	Antifungal Drugs			
	Amphotericin B (20µg)	Fluconazole (25µg)	Voriconazole (10µg)	Ketoconazole (10µg)
Aspergillus sp.	+	+	+	+
Rhizopus sp.	+	–	+	+
Mucor sp.	+	+	+	+
Trichoderma sp.	+	–	–	–

KEY – = No zone of inhibition
+ = Zone of inhibition

DISCUSSION

The proximate composition recorded showed that moisture content of the samples ranged from 89.12% in sample A to 90.01% in sample C. All the samples were not significantly different ($p>0.05$) for the moisture content. This could be attributed to the method used for the preparations of the yoghurts. The moisture content was relatively high as the value did not correspond with the report by Ahmad (2010) who reported that the maximum moisture content of yoghurt should be 84%, as much water in yoghurt makes it less viscous there by affecting texture and mouth feel. The ash content recorded ranged from 0.39% in sample C to 0.42% in sample A. The results indicated that there was no significant difference ($p>0.05$) between all the samples in their ash content. The ash value is an index of mineral content, which is needed for bone development, teeth formation and body functions as reported by Trachoo and Mistry (2011). This therefore indicates that sample A is the better source of minerals among the yoghurt samples analyzed. The protein content recorded ranged from 3.10% in sample C to

3.44% in sample B. The result indicated that there were no significant difference ($p>0.05$) between all the samples in their protein content. The protein content of the yoghurt samples is relatively normal as compared to the 3.5% protein content of yoghurt reported by Igbabul *et al.* (2014).The crude fat content recorded ranged from 2.29% in sample A to 3.11% in sample B. The result indicated that there were no significant difference ($p>0.05$) between all the samples. This showed that sample B had the highest fat content. Fat play an important role in improving the consistency of yoghurt and also provide twice as much energy as same quantity of carbohydrate and protein as reported by Ehirim and Onyeneke (2013). The crude fiber content recorded ranged from 0.23% in sample C to 0.26% in sample A. The result indicated that there were no significant difference ($p>0.05$) between all the samples. According to Igbabul *et al*. (2014) the crude fiber contributes to the health of the gastrointestinal system and metabolic system in man. The carbohydrate content recorded ranged from 3.25% in sample C to 3.73% in sample A. The low carbohydrate value could attributed to the process of fermentation which converts carbohydrate basically lactose to lactic acid. This makes yoghurt an ideal food for lactose intolerance individuals (Ehirim and Onyeneke, 2013). The proximate composition of the yoghurt samples had a significant role to play in relation to the microbiological contaminations of the samples, because during the fermentation processes, the carbohydrate are broken down which could serves as source of carbon/energy for microbial growth and nourishment.

The bacterial aerobic mesophilic count recorded a lowest count of $1.36x\ 10^3$ CFU/mL in sample E1 and $1.04\ x10^4$ in sample D3 and a highest count of $9.6x10^3$ CFU/mL in sample D2 and $9.6x10^4$ CFU/mL in sample C2 with no growth recorded in sample A3, B1 and B2. Highaerobic bacterial load in yoghurt was attributed toinadequate hygienic measures in production orinadequate processing recontamination as reported by El-Diastyand El -Kaseh(2009). The samples of yoghurt analyzed recorded counts that ranged from $1.36x\ 10^3$ - $9.6x10^4$ CFU/mL which fell within the acceptable limit of 10^3 to 10^4as in the guidelines for the microbiological quality of ready to eat food sampled at point of sale(El-Diasty and El Kaseh, 2009), but the count recorded was an indication of contamination of the product either during packaging or at the preparatory stage or during handling. Highest total coliform was recorded in sample E1 and lowest in sample A2 and D3. 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 as stated by Kawo *et al*. (2008). The presence of coliforms from this yoghurt samples pose great danger to the health of the consumers and suggest neglect on the part of the processors or the vendors. The tolerable limit for coliform presence in yoghurt is less than 10 CFU/mLbut a higher count of 4000 is of serious concern. This contamination might be from contaminated water source or equipment used or probably as reported by Younus *et al.* (2009) due to contamination at storage and display/sale outlet. 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 (Yabaya and Idris, 2012). The bacterial isolates were *Bacillus* sp., *Streptococcus*, *Lactobacillus* and species of *Escherichia coli, Enterobacter aerogenes* and that of *Staphylococcus aureus* were identified from the yoghurt samples. The two lactic acid bacteria which comprise the starter cultures isolated from the yoghurt samples were *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. These starter cultures were not in the right proportion and only three (3) of the yoghurt drinks contained both organisms; others contained only one of the two in minute quantity due to the fact that many local yoghurt manufacturers don't inoculate adequate amounts of starter cultures needed to attain about 10 million cells into the

pasteurized milk for fermentation. This unequal proportion of starter cultures was against the report of Tamime and Robinson (2008) that successful preparation of yoghurt depends upon the proper symbiotic relationship between the two organisms at equal proportion. However, the isolation of the two organisms: *Streptococcus* and *Lactobacillus* sp. agreed with the claims that they are the most commonly employed starter cultures in the fermentation of milk into yoghurt as reported by Tamime and Robinson (2008). *Bacillus* sp was isolated from all the samples, the presence of *Bacillus* sp in all the yogurt samples implies post pasteurization contamination (Huck *et al*.,2008).The *Staphylococcus aureus* was isolated from 19% of the fifteen (15) yoghurt samples analyzed. El-Malt *et al.* (2012) also detected *S. aureus* in 72% of the 100 yoghurt samples they analyzed in Qena City, Egypt. Presence of *S. aureus* usually indicates contamination from food handlers (Abdel Hameed *et al*., 2009). The presence of *Escherichia coli* has been incriminated as a potential food poisoning agent and are associated with infantile diarrhea and gastroenteritis in adults as reported by Okpalago *et al*.(2008) and El-Diasty and El Kaseh (2009). In most foods, the total bacterial count is often an indication for the sanitary quality, safety and utility of foods. It may reflect the conditions under which the product is manufactured such as contamination of raw materials and ingredients, the effectiveness of processing and the sanitary conditions of equipment and utensils at the processing plants (El-Diasty and El Kaseh 2009). The total fungal plate count ranged from 1.0 -6.0x10^3 CFU/mL, similar report was made by Oyeleke (2009) in the microbial assessment of commercially prepared yoghurt. The fungal population obtained from the samples were above the acceptable limit that is recommended. The fungal population of the samples may be attributed to several factors which include the initial contamination of raw materials, non-aseptic milking, poor cleaning of equipment, water used in production, the sanitary conditions of the pressing environment and poor handling of finished products. The total effect of such a contaminating factor determine the quality of the yoghurt, its probable shelf life and the potential public health risk (Yabaya and Idris, 2012). The fungal isolates were *Aspergillus* sp., *Mucor* sp., *Rhizopus* sp., *Trichoderma* sp., *Aspergillus* sp. especially *A. flavus* and *A. parasiticus* produces mycotoxins which includes metabolic by products produced by a number of different fungi that may or may not be toxic. The type of diseases caused by *Aspergillus*sp are varied, ranging from an "Allergy" type of illness to life threatening generalized infections as reported by Iloh and Ilodu (2008). *Mucor* sp. have been linked with allergies and mold sensitivity. In some cases, they can cause severe pulmonary distress including immunocompromised individuals. The Fungi can cause opportunistic infections, this occurs when the spores are ingested or inhaled and causes a variety of problem. The presence of *Mucor* sp., in the yoghurt analyzed may be due to their rapid colonization and utilization of food substances. *Mucor* sp., is one of the fungus involved in the decay of dairy products during storage, as reported by Frazier and Westhoff (2009). *Rhizopus* sp., is a genus of common saprobic fungi on plants and special parasites on animals. They are found on wide variety of organic substrates including fruits, syrups, breads, milk etc. Most *Rhizopus* species are opportunistic agents of human zygomycosis (fungal infection) and can be fatal, as also seen by many authors. Some *Rhizopus* infections are also in associated complications of diabetic ketoacidosis. *Trichoderma* sp. are very common in soil, and cellulolytic materials including stored dairy products and plants food stuffs. Many species are strongly cellulolytic (i.e. they are capable of degrading cellulose since they produce large quantities of the enzymes cellulose) for this reason, they are important spoilage organism. Human infection by species of *Trichoderma* is limited to individuals with severely weakened immune systems, and other species are able to produce mycotoxins. Antibiotics susceptibility assessment of bacteria isolates from the

yoghurt samples showed varying degrees of bacterial resistance as well as multiple antibiotics resistances in bacterial isolates. Result of antibiotic susceptibility tests on isolates recorded for most of the isolates as multi resistant to more than one of the antibiotics which was also reported by Ahmed *et al.* (2010), Okpalugo *etal.* (2008), Nováková*et al.* (2010). The order of antibacterial ineffectiveness of the studied antibiotics was Pefloxacin (10µg) > Streptomycin (30µg) > Erythromycin (10µg) > Gentamicin (10µg) > Zinnacef (20µg) > Amoxicillin(30µg) > Ciprofloxacin (10µg) > Septirin (30µg) > Ampiclox (30µg) > Rocephin (25µg). All the bacterial isolates obtained from the yoghurt samples were susceptible to Pefloxacin (10µg) and Streptomycin (30µg). The findings from this present study agreed with work reported by Okonko*et al.* (2009), who reported high bacterial isolates susceptible to Pefloxacin (10µg), Streptomycin (30µg) and Gentamicin (10µg). Antifungal Susceptibility assessment of fungal isolates from yoghurt samples showed varying degree of fungal resistance as well as more than two antifungal resistances in fungal isolates. The fungal isolates were more susceptible to Amphotericin B (20µg), Voriconazole (10µg), Ketoconazole (10µg) and less susceptible to Fluconazole (25µg). This finding is in agreement with previous research of Ahmed *et al.* (2010).From a microbiological point of view, most of the bacterial and fungal species isolated should not be present in carefully manufactured yoghurt. This indicates lack of good manufacturing practice (GMP) or inadequate storage. This can pose a serious health problem from a public health point of view, which will cause an exposure to high risk of food borne infection and intoxication. Although the levels of these contaminants were considerably low and safe at the time of analysis, if stored further for a longer period of time under inappropriate conditions, the contaminants would grow, multiply and attain a high level which could pose a health danger to the consumers.

CONCLUSIONS

From results of this research, it can be concluded that most of the yoghurt on sale within Kaduna metropolis is having low bacteriological and mycological 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 and direct health effects in consumers.

RECOMMENDATIONS

In order to ensure the availability of yoghurts with good microbiological quality for consumers in Kaduna as a state and in Nigeria as a whole, the following measures are recommended:

1.Local yoghurt manufacturers should try as much as possible to always ensure aseptic conditions in their production environment in order to prevent the contamination of their products during processing.
2.The manufacturing companies should also ensure that their production personnel always maintain good personal hygiene so as to reduce the risk of contaminating the yoghurts with members of the normal microbiota of their skin, mouth, nose, etc. Nose masks should be worn always in the production room.
3.Adequate pasteurization of raw milk must be ensured in order to eliminate all the pathogenic microorganisms and reduce to a substantial level other contaminating or spoilage microorganisms in the milk before it is used for yoghurt production. Attention of the stake holders including manufacturers and retailers is therefore needed to reduce postproduction contamination.
4.Periodical factory inspection must be done by regulators in the industry such as National Agency for Food, Drug and Administration and Control (NAFDAC), Standard Organization of Nigeria (SON) and Consumer protection Council (CPC) to checkmate the problem of poor hygiene using HACCP concept/systems and to apply sanctions where necessary. The manufacturers should make it a duty upon themselves to educate their staff on clean and

hygienic practice considering the high level of coliform contaminations recorded in the course of this study.

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