

## MICROBIOLOGICAL ANALYSES OF SOME PROCESSED YOGHURT SOLD IN KANO METROPOLIS, KANO-NIGERIA

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### ABSTRACT

*Yoghurt brands sold in Kano metropolis were analyzed for microbiological quality and consumer preference. A total of Two hundred samples, Twenty (20) each of ten brands of yoghurt namely: Fan, Bimco, Jamil, San, Farm fresh, Farm pride, Amico, Annashuwa, Sunrise and Ultimate were purchased randomly from vendors, provision stores, depots and supermarkets during the months of August and September, 2006. Microbiological analysis revealed that the total mesophilic aerobic bacteria count of all the yoghurt samples ranged between  $3.40 \times 10^3$  cfu/ml in Bimco yoghurt to  $3.85 \times 10^5$  cfu/ml in Amico yoghurt, the total psychrophilic count ranged between 12.2cfu/ml in Annashuwa yoghurt to 552 cfu/ml in farm fresh yoghurt, and thermophilic counts ranged between 100 cfu/ml in Ultimate yoghurt to  $8.56 \times 10^5$  cfu/ml in Farm fresh yoghurt. The best yoghurt according to this study whose coliform counts were found to be 0 MPN/ml include; Farm pride, Ultimate and Annashuwa yoghurt, while the worst yoghurt with high coliform counts was Farm fresh with 350 MPN/ml. The best yoghurt with lowest yeast count was Bimco (100 cfu/ml) while the worst yoghurt was Jamil with highest yeast count ( $5.13 \times 10^5$  cfu/ml). Characterization and identification tests showed the isolation of ten bacterial genera and nine yeast species. The bacterial isolates includes *S. thermophilus* 28 (14.0%); *L. bulgaricus* 17 (13.5%); *Bacillus* sp 16 (8.0%); *Proteus* sp 14 (7.0%); *Pseudomonas* sp 15 (7.5%); *E. coli* 11 (5.5%); *Klebsiella* sp 27 (13.5%); *S. aureus* 26 (13.0%) and *Salmonella* sp 8 (4.0%). The yeast isolates were *C. albicans* 26 (13.0%), *Debaryomyces* sp 23 (11.5%), *Hansenula* sp 18 (9.0%), *Kloeckera* sp 20 (10.0%), *Pichia* sp 15 (7.5%), *Rhodotorula* sp 24 (12.0%), *Saccharomyces* sp 26 (13.0%), *Schizosaccharomyces* sp 18 (9.0%) and *Torulopsis* sp 30 (15.0%). Method of Taura et al (2005) was adopted in the survey of consumer preference of yoghurt. Enumeration of mesophilic aerobic bacteria, psychrophilic and thermophilic bacteria; pour plate method was used as described by APHA (1978) and Oyeyi and Nkwewe (2005). The MPN method was adopted by Bryan et al (1962) and Akinyele et al (2000) for coliform; while for the enumeration of yeast and mold, serial dilution was used as described by APHA (1972).*

**KEYWORDS:** Yoghurt, Microbiological quality, Consumer, Kano.

### INTRODUCTION

Milk is defined as the secretion of mammary glands of animals to suckle their young one (Ihekoronye and Ngoddy, 1985). It is an excellent source of nutrients such as proteins, essential amino acids, minerals, vitamins, water, carbohydrate and fat. This also makes it an excellent medium for microbial proliferation (Braddley, 1982). Milk is produced in Nigeria mainly from cow by the Fulanis. Excess fresh cow milk is processed into different products such as "Wara" (West African soft cheese), "Nono" (fermented milk) and Yoghurt for their preservation (Braddley, 1982).

Yoghurt is defined microbiologically as the end product of a controlled fermentation of high solids, whole, skimmed or fortified milk by a symbiotic mixture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in the ratio of 1:1 (Adams and Moss, 1999). It is the most widely available fermented milk in the world and it has a mild acidic flavour, custard texture and high nutritive value makes it to be the most, commonly relished foods throughout the history

of mankind (Adams and Moss, 1995). Beside the starter organisms yoghurt is not expected to contain other microorganisms beyond certain tolerable limits (FAO, 1979).

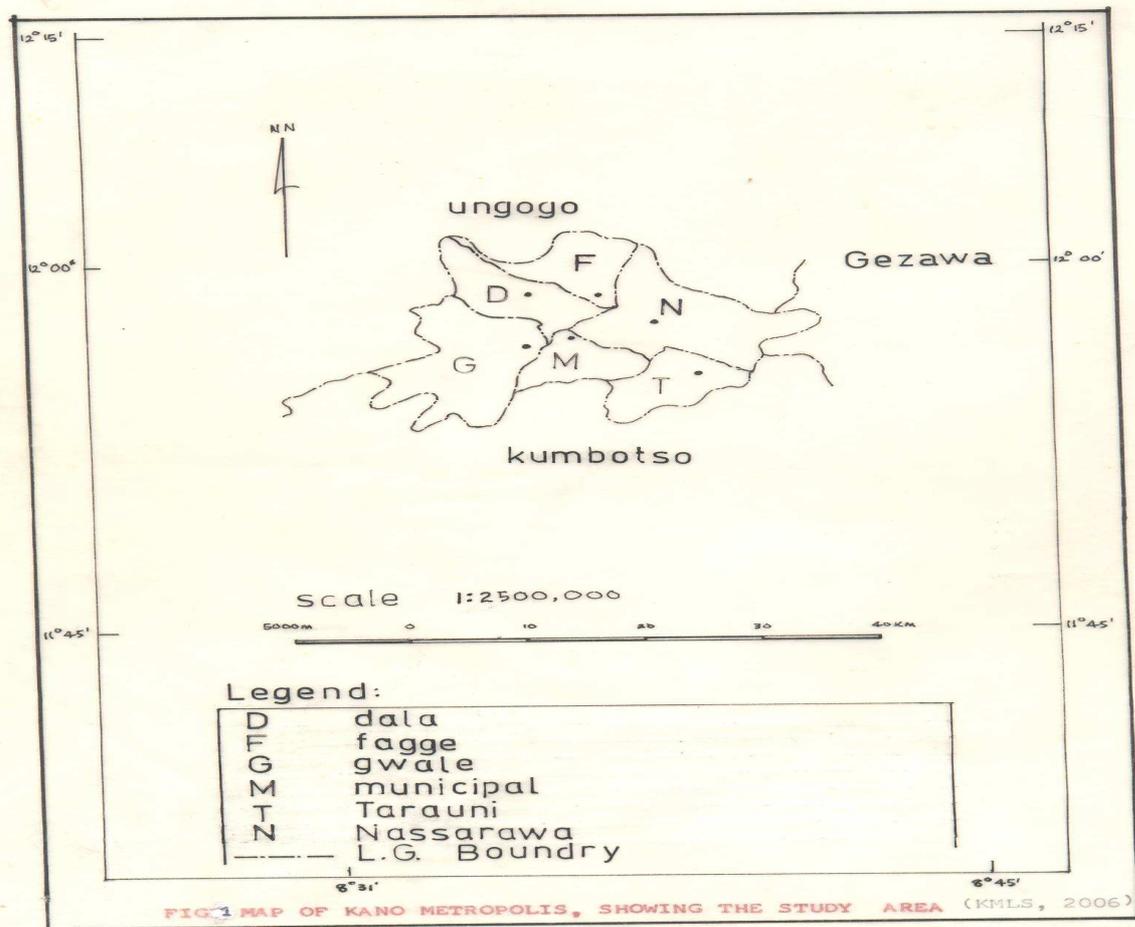
There is a need to provide baseline data on the quality of yoghurt sold within Kano metropolis. The data/information would be used to the yoghurt producers to improve and maintain the quality of the products. The data would also guide the consumers on the choice of yoghurt brand to buy. The aim of the present study is to determine the microbiological qualities of some processed yoghurts sold in Kano metropolis, Nigeria with a view to provide some information on the safety of such products for human consumption.

### MATERIALS AND METHODS

#### The Study Area

Kano metropolis is located between latitude  $11^{\circ} 49'$  and  $12^{\circ} 4'$  North of the equator and longitude  $8^{\circ} 31'$  and  $8^{\circ} 40'$  East of the Greenwich meridian.

Kano metropolis is comprised of six local governments namely Kano municipal, Gwale, Fagge, Nassarawa, Tarauni and Dala (Fig. 1) as obtained in Kano Ministry of Land and Survey (KMLS, 2006)



### Survey of Consumer Preference of Yoghurt Brand

#### Questionnaire Administration

Questionnaire on yoghurt preference were prepared and validated by two senior staff of Biological Sciences programme, Abubakar Tafawa Balewa University, Bauchi and randomly distributed to 300 respondents living within Kano metropolis.

#### Sample Collection

The method of Taura *et al* (2005) was adopted with little modification. Twenty samples of each of ten (10) preferred different brands of yoghurt were purchased randomly from supermarkets, vendors and provisions stores outlet in Kano metropolis. The samples were transported to the laboratory under aseptic condition in pre-cleaned ice box for analysis.

#### Microbiological Analysis

##### Sample preparation

One millilitre of the yoghurt samples was pipetted into a sterile test tube containing 9 ml of distilled water and the content mixed to give  $10^{-1}$  dilution. Pour plate method as described by APHA (1978) and Oyeyi and Nkwewe (2005) were used for the enumeration.

#### Enumeration of mesophilic aerobic bacteria

One millilitre of each  $10^{-1}$  dilution of the yoghurt homogenate was pipetted into each of the appropriately marked petri-dishes.

Fifteen millilitres of the molten plate count agar (oxoid) at  $45^{\circ}\text{C}$  was poured into each petridish. The plates were rotated to mix the diluted samples and the agar medium. This was allowed to solidify and incubated at  $32^{\circ}\text{C}$  for 48 hours. After the incubation period, the colonies were counted and the results were recorded and expressed as colony forming unit per ml (cfu/ml). The isolates were Gram stained and examined microscopically. Biochemical tests were also carried out.

#### Enumeration of psychrophilic and thermophilic bacteria

One millilitre of appropriate dilution of all samples was inoculated into petridishes of plate count agar (oxoid). Plates for total psychrophilic bacteria were incubated at  $7^{\circ}\text{C}$  for 10 days in an incubator while those for total thermophilic bacteria were incubated at  $55^{\circ}\text{C}$  for 48 hours.

### Enumeration of coliform

The most probable number (MPN) method as described by Bryan *et al.* (1962) and Akinyele *et al.* (2000) was adopted for the coliform enumeration and identification.

For the presumptive test, dilutions of the yoghurt samples were made using peptone water. One millilitre of each dilution ( $10^{-1}$ ,  $10^{-2}$  and  $10^{-3}$ ) was transferred into already prepared MacConkey broth tubes (triplicate) with inverted sterile Durham tubes. The tubes were incubated at 37°C for 24 hours and observed for gas production. The most probable number (MPN) values are recorded and expressed in MPN/ml.

For the confirmatory test, a loopful each from the gas positive test tubes is inoculated onto the surface of an Eosin Methylene Blue (EMB) agar and then incubated at 37°C for 24 hours.

For the completed test, Gram staining and standard biochemical tests (which include motility, citrate, oxidase, indole, catalase, coagulase, glucose, lactose, sucrose, and mannitol utilization) were carried out as described in Bergey's manual of determinative bacteriology and Monica (2000) were used.

### Enumeration and identification of yeast and moulds

The enumeration of mould colonies was carried out by the method described by APHA (1972). A serially diluted aliquot of yoghurt (0.2 ml) samples was inoculated on potato dextrose agar (oxid) containing 250 mg/l chloramphenicol. The plates were incubated at 37°C for 2-3 days. The colonies of yeast and moulds were observed and counted with the aid of Gallenkamp digital colony counter and recorded as colony forming units per millilitre (cfu/ml) of the test samples.

### Identification of Yeast

The methods of Mackie and McCartney (1989) were adopted as described below:

#### Direct Mounts:

A wet suspension of the yeast sample was prepared on a clean dried slide using wire loop

The slide was covered with cover slip

The slide was then examined microscopically to study yeast morphology and to determine purity of the isolates.

#### Yeast Staining:

An elongated drop of lactophenol cotton blue was placed on a clean glass slide

A piece of clear vinyl adhesive tape (sellotape) 6-7cm long was held loosely between the thumb and forefinger of each hand

The center of the adhesive side of the tape was allowed to touch the colony lightly under its own weight

It was transferred to the slide, placing the area with adhered mycelium on to the lactophenol cotton blue

The whole set-up was examined under low and high power objective for the presence of hyphae and spores.

#### Germ Tube Test:

A single colony from pure culture was lightly touched with a Pasteur pipette

Excess inoculums were removed and then the yeast cells were emulsified in 0.5ml of horse serum in a small test tube with a loose cotton wool plug.

It was incubated at 37°C in a water bath for 2-4 hours.

The presence of parent cell and germ tube (tube-like outgrowth) was finally examined for *Candida albicans*.

#### Sucrose Assimilation:

The test organism was inoculated using wire loop into a test tube containing 0.1g/ml of sucrose

It was incubated at 30°C for 48 hours

The medium changed from red to yellow (sucrose positive).

#### Dalmeida Culture Plate:

A loop full of the test organism was placed in a well at the top of a plate of cornmeal agar.

A dilution strokes were made by cutting into the agar with the edge of the sterile loop.

A glass cover slip was dipped in alcohol; it was sterilized by flaming, and left to cool

It was then placed on the agar to cover part of the well and some of the dilution strokes.

It was incubated at 22°C for 48 hours

It was finally examined by placing the plate on a microscope from which the slide carrier has been removed.

The presence of parent cell and germ tube (tube-like outgrowth) indicates *Candida albicans*.

#### Urease Test:

A slant of Christensen urea agar was seeded with the test organism and incubated at 30°C for 48 hours.

The non-conversion of the phenolphthalein indicator in the medium from yellow to pink indicates *Torulopsis* species.

#### Statistical Analysis

Analysis of Variance (ANOVA) and Chi-square test were employed to determine whether there is a significant difference in the microbial load among the yoghurt brands.

## RESULTS

### Questionnaire Analysis

The results of Yoghurt brand preference and disease outbreak associated with yoghurt consumption in Kano is presented in Table 1. A total of ten (10) different brands of yoghurt were preferred with a degree of preference ranging from a maximum of 38 (21.0%) in Fan yoghurt to a minimum of 10 (5.6%) in Sunrise. There was no reported disease outbreak associated with any of the brands. Close examination of the 10 brands revealed that only eight of them have NAFDAC registration and expiry dates on their pack.

### Bacteriological Profile of Some Yoghurt Brands Sold in Kano Metropolis

Bacteriological assessment of some yoghurt brands sold in Kano metropolis revealed the presence of ten (10) different bacterial genera: *Streptococcus thermophilus* 28 (14.0%), *Lactobacillus bulgaricus* 27 (13.5%), *Bacillus* sp 28 (14.0%), *Proteus* sp 14 (7.0%), *Pseudomonas* sp 15 (7.5%), *E coli* 11 (5.5%), *Staphylococcus aureus* 26 (13.0%), *Micrococcus* sp 28 (14.0%), *Klebsiella* and *Salmonella* sp 8 (4.0%) each (Table 3).

The frequency of occurrence of bacteria genera and biochemical results are presented in Table 3. These include: *S thermophilus*. These are Gram-positive cocci, non-motile, citrate and coagulase negative. It fermented all the sugars producing acid and gas in lactose and glucose while *L bulgaricus* showed similar reactions but differed in being Gram-positive rod, motile and was not fermenting sucrose and mannitol. *Bacillus* sp differed from *L bulgaricus* by being citrate and catalase positive and fermented sucrose. *Proteus* sp is Gram-negative and motile and differed from *Bacillus* sp by being citrate and catalase negative, and Indole-methyl red and oxidase positive. *Pseudomonas* sp showed similar reactions with *Proteus* sp but differed being catalase and mannitol positive and showed negative reactions in indole and methyl red. *E coli* also showed similar reactions with *Pseudomonas* sp but differed being catalase and oxidase negative. *S aureus* was Gram-positive, non-

motile, citrate, mannitol, Indole and oxidase negative. It was catalase and coagulase positive. In addition, it fermented lactose and sucrose; it was methyl red and Voges Proskauer positive. *Micrococcus* sp are Gram-positive, non motile and citrate, catalase, lactose, Indole, methyl red and oxidase positive. It was coagulase, sucrose, mannitol and Voges Proskauer negative.

*Klebsiella* sp was Gram-positive rods, motile, citrate, and lactose positive. It fermented glucose, sucrose and mannitol with production of acid and gas. In addition, it was indole, methyl red and oxidase negative. It had similar reactions with *Staphylococcus aureus* but differed by being motile, citrate positive and fermented mannitol with formation of acid and gas. *Salmonella* sp was Gram negative, motile, they are lactose and methyl red positive and fermented glucose and mannitol with the production of acid and gas. It was sucrose, Indole and Voges Proskauer negative.

The prevalence of bacteria isolated from various yoghurt brands sold in Kano metropolis showed that Jamil and Farm fresh yoghurt brands had the highest microbial load 24 (12.0%) while Bimco yoghurt had the least microbial load 16 (8.0%). No coliform was isolated in Farm pride, Annashuwa and Ultimate yoghurt brands analysed. *Streptococcus thermophilus* and *Micrococcus* sp are the highest genera isolated 28 (14.0%). They occurred in almost all the yoghurt brands analysed while *E coli* is the least isolated genus 11 (5.5%) (Table3).

**Table 1: Consumer Opinion on Yoghurt Preference and Associated Disease Outbreak in Kano Metropolis**

S/N	Yoghurt Brand	No. of respondents	Percentage Preference	NAFDAC Number	Reported cases of diseases	Expiry date
1	Fan	38	21.0	01-5076	-	24-10-2006
2	Bimco	28	15.6	01-4359	-	13-10-2006
3	Jamil	18	10.0	01-3036	-	-
4	San	18	10.0	01-1297L	-	-
5	Farm Fresh	16	8.8	-	-	31-10-2006
6	Farm Pride	16	8.8	01-5249	-	16-10-2006
7	Amico	12	6.7	01-7466	-	15-10-2006
8	Annashuwa	12	6.7	01-5597	-	20-10-2006
9	Ultimate	12	6.7	01-2122L	-	20-10-2006
10	Sunrise	10	5.6	-	-	-

Key: - = Nil

**Table 2: Microbial Load of Some Processed Yoghurt Brands Sold In Kano Metropolis**

S/N	Sample	Collection Center	Mesophilic (cfu/ml) <sup>a</sup>	Psychrophilic (cfu/ml) <sup>b</sup>	Thermophilic (cfu/ml) <sup>b</sup>	Coliforms (MPN/ml) <sup>c</sup>	Yeasts (cfu/ml) <sup>b</sup>	Molds (cfu/ml)	Total
1	Fan	Depot	$9.17 \times 10^3$	15.5	$2.82 \times 10^3$	11.1	150	NG	$9.35 \times 10^4$
2	Bimco	Depot	$3.40 \times 10^3$	16.5	$2.29 \times 10^3$	13.5	100	NG	$5.82 \times 10^3$
3	Jamil	Vendor	$2.60 \times 10^4$	321	$5.31 \times 10^4$	30	$5.13 \times 10^5$	NG	$5.69 \times 10^5$
4	San	Prov. Store	$5.40 \times 10^4$	15	$4.00 \times 10^3$	15	$6.50 \times 10^4$	NG	$1.23 \times 10^5$
5	Farm fresh	Prov. Store	$4.37 \times 10^4$	552	$8.56 \times 10^5$	350	$2.76 \times 10^5$	NG	$1.17 \times 10^6$
6	Farm pride	Sup. Markt.	$9.17 \times 10^3$	36	$3.12 \times 10^4$	0.00	$2.00 \times 10^4$	NG	$6.04 \times 10^4$
7	Amico	Vendor	$3.85 \times 10^5$	29.6	$3.36 \times 10^4$	20	$7.33 \times 10^4$	NG	$4.92 \times 10^5$
8	Annashuwa	Sup. Markt.	$8.10 \times 10^4$	12.2	$1.50 \times 10^3$	0.00	$5.67 \times 10^4$	NG	$1.39 \times 10^5$
9	Sunrise	Prov. Store	$3.33 \times 10^4$	70	$2.25 \times 10^3$	170	$6.67 \times 10^4$	NG	$1.02 \times 10^5$
10	Ultimate	Vendor	$7.27 \times 10^4$	12.8	$1.00 \times 10^2$	0.00	$1.75 \times 10^4$	NG	$9.03 \times 10^4$
	Total	-	$5.70 \times 10^5$	$1.08 \times 10^3$	$9.87 \times 10^5$	610	$1.08 \times 10^6$	-	$2.77 \times 10^6$

**Key:** Prov. Store = Provision store, Sup. Markt = Super Market, NG = No Growth, - = Nil.

There is a significant difference among all the yoghurt samples ( $X^2$ ,  $P < 0.05$ )

- 9 cells (100%) have expected frequencies less than 5. The minimum expected cell frequency is 1.1
- 10 cells (100%) have expected frequencies less than 5. The minimum expected cell frequency is 1.0
- 8 cells (100%) have expected frequencies less than 5. The minimum expected cell frequency is 1.3

Table 3: Characteristics of Bacteria Isolated from various Yoghurt Brands

Inference	No. of Isolates	GR	Mot	Cit	Cat	Coag	Lac	Glu	Suc	Man	Ind	Met	VP.	Oxi	Identity of isolates
Convex, translucent & Pinpoint in size	28(14%)	G+ cocci	N	N	N	N	A/G	A/G	P	P	N	N	N	ND	<i>S thermophilus</i>
Small, white milky, rough surface with entire edge colonies	27(13.5%)	G+ rods	P	N	N	N	A/G	A/G	N	N	ND	ND	ND	ND	<i>L bulgaricus</i>
Creamy big spreading finely wrinkled slimmy colonies	16(8%)	G+ rods	P	P	P	N	P	ND	P	N	N	N	N	N	<i>Bacillus sp</i>
Translucent milkish colonies, has a fishy smell and swarming appearance	14(7%)	g- rods	P	N	N	N	P	ND	P	N	P	P	N	P	<i>Proteus sp</i>
Small greenish raised smooth circular colonies	15(7.5%)	g- rods	P	N	P	N	P	ND	N	P	N	N	N	P	<i>Pseudomonas sp</i>
Small, opaque circular smooth colonies	11(5.5%)	g- rods	P	N	N	N	P	ND	P	P	P	ND	N	N	<i>E coli</i>
Light yellow, entire elevated colonies 0.1mm in diameter	26(13%)	G+ rods	N	N	P	P	P	ND	P	N	N	P	P	N	<i>Staph aureus</i>
Deep yellow colonies, about 2mm raised smooth entire edge.	28(14%)	G+ cocci	N	P	P	N	P	ND	N	N	P	P	N	P	<i>Micrococcus sp</i>
The organisms are straight rods in shape	27(13.5%)	G+ rods	P	P	ND	ND	P	A/G	A/G	A/G	N	N	P	N	<i>Klebsiella sp</i>
Colonies are colourless, translucent to opaque with surrounding medium pink	8(4%)	g-rods	P	ND	ND	ND	P	A/G	N	A/G	N	P	N	ND	<i>Salmonella sp</i>
Total	200														

**Key:** A/G = Acid and gas, ND = Not done, N = Negative, P = Positive, G+ cocci = Gram positive cocci, G+ rods = Gram positive rods, G- cocci = Gram negative cocci, G-rods = Gram negative rods. GR = Gram reaction, Mot. = motility, Cit. = citrate, Cat. = catalase, Coag. = coagulase,

Table 4. Prevalence of Bacteria Isolated from Various Yoghurt Brands

S/ N	Organisms	Frequency of Isolates in Yoghurt Brands										Tot. no.of Isolates
		Fan	Bimco	Jamil	San	Farm fresh	Farm pride	Amico	Annashuw	Sunris	Ultimate	
1	<i>S thermophilus</i>	2	2	3	2	3	4	3	3	2	4	28
2	<i>L bulgaricus</i>	2	3	2	3	2	4	2	3	2	4	27
3	<i>Bacillus</i> sp	2	2	2	NIL	3	NIL	2	3	2	NIL	16
4	<i>Proteus</i> sp	3	2	3	2	2	NIL	1	NIL	1	NIL	14
5	<i>Pseudomonas</i> sp	2	2	2	3	3	NIL	1	NIL	2	NIL	15
6	<i>E coli</i>	NIL	NIL	2	3	3	NIL	2	NIL	1	NIL	11
7	<i>Staph aureus</i>	1	1	2	3	2	5	2	4	2	4	26
8	<i>Micrococcus</i> sp	3	2	2	2	NIL	5	3	4	3	4	28
9	<i>Klebsiella</i> sp	2	2	3	2	3	5	3	NIL	3	4	27
10	<i>Salmonella</i> sp	NIL	NIL	3	NIL	3	NIL	NIL	NIL	2	NIL	08
Total no. of isolates		17	16	24	20	24	23	19	17	20	20	200

Key: NIL = No organism isolated

There is a significant difference among all the yoghurt samples analyzed for bacteria (P < 0.05)

**Yeasts Isolates from yoghurt brands.**

The results of this study showed that the yeast isolates belonged to nine (9) genera namely: *Candida albicans* 26 (13.0%), *Debaryomyces* sp 23 (11.5%), *Hansenula* sp 18

(9.0%), *Kloeckera* sp 20 (10.0%), *Pichia* sp 15 (7.5%), *Rhodotorula* sp 24 (12.0%), *Saccharomyces* sp 26 (13.0%), *Schizosaccharomyces* sp 18 (9.0%), and *Torulopsis* sp 30 (15.0%)(Table 5).

**Table 5: Yeasts Isolates from Yoghurt Brands**

S/N	Genus	Number of Isolates
1	<i>Candida</i> sp	26 (13.0%)
2	<i>Debaryomyces</i> sp	23 (11.5%)
3	<i>Hansenula</i> sp	18 (9.0%)
4	<i>Kloeckera</i> sp	20 (10.0%)
5	<i>Pichia</i> sp	15 (7.5%)
6	<i>Rhodotorula</i> sp	24 (12.0%)
7	<i>Saccharomyces</i> sp	26 (13.0%)
8	<i>Schizosaccharomyces</i> sp	18 (9.0%)
9	<i>Torulopsis</i> sp	30 (15.0%)
<b>Total</b>		<b>200</b>

The prevalence of yeasts isolated from various yoghurt brands sold in Kano metropolis revealed that Farm pride yoghurt had the highest yeast load of 24 (12.0%) followed by San yoghurt 23 (11.5%), Fan and Farm fresh yoghurt had 22

(11.0%) each, Annashuwa 20 (10.0%), Jamil yoghurt 19 (9.5%), Bimco and Amico yoghurt 18 (9.0%) each, while Sunrise and Ultimate yoghurt had the lowest 17 (8.5%) (Table 6).

**Table 6: Prevalence of Yeasts Isolated from Various Yoghurt Brands Sold in Kano Metropolis**

Frequency of Yeast Isolates from Yoghurt Brands												
S/N	Organisms	Fan	Bim	Jam	San	FF	FP	Ami	Ann	Sun	Ult	Tot
1	<i>Candida</i> sp	3	2	3	2	3	4	3	3	2	2	26
2	<i>Debaryomyces</i> sp	NIL	3	2	3	2	4	2	3	2	2	23
3	<i>Hansenula</i> sp	2	2	2	NIL	3	3	2	2	2	NIL	18
4	<i>Kloeckera</i> sp	3	2	3	2	2	2	2	NIL	2	2	20
5	<i>Pichia</i> sp	2	2	NIL	3	3	1	NIL	1	2	1	15
6	<i>Rhodotorula</i> sp	4	3	2	3	4	NIL	2	3	1	2	24
7	<i>Saccharomyces</i> sp	2	2	2	3	2	4	2	4	22	3	26
8	<i>Schizo-saccharomyces</i> sp	3	2	2	2	NIL	2	2	1	2	2	18
9	<i>Torulopsis</i> sp	3	NIL	3	5	3	4	3	3	2	3	30
<b>Total</b>		22	18	19	23	22	24	18	20	17	17	200

**Key:** NIL = No organism isolated

There is a significant different among all the yoghurt samples analyzed for yeasts ( $P < 0.05$ ).

Bim = Bimco, Jam = Jamil, FF = Farm fresh, FP = Farm pride, Ami = Aminco, Ann = Annashuwa, Sun = Sunrise, Ult = Ultimatum, Tot = Total Number of isolates

**DISCUSSION**

**Questionnaire Analysis of Yoghurt Samples of some brands**

From the study with respect to questionnaire the results obtained showed consumer preference of yoghurt brands sold in Kano metropolis revealed that some of the yoghurt brands were noted not having a legal NAFDAC permission to sell their

products as well as expiry dates on their pack. This showed that some of the products were of doubtful quality according to the guidelines of Nduka (2004) and FAO (1979). This means that there is a need to expedite action towards cautioning the defaulting manufacturers as well as their vendors in line with the requirement of the national agency for food, drug administration

and control policy. The good manufacturing practice should also be the main area of concern in the industries so that better quality products can be sold to consumers. It is also a challenge to the environmental and public health superintendents to ensure compliance at all levels. There was no reported disease outbreak associated with any of the brands; this finding supported the earlier work conducted by Taura *et al.*, (2005) who also reported that there was no disease outbreak as a result of yoghurt consumption in the study area.

#### **Microbiological Analysis of Yoghurt Samples**

With respect to Microbiological analysis of the yoghurt brands indicated the absence of mould. This could be due to high temperature-short time (HT-ST) pasteurisation effect as well as good cleaning procedures during processing. It could also be due to the high pH recorded in most of the samples analysed since mould are known to proliferate at low pH (Pelczar and Reid, 1972).

Results obtained from this study showed that there is significant difference ( $P < 0.05$ ) for all the yoghurt brands analysed. Thus, the presence of yeasts is an indication of poor sanitary conditions of processing, handling and/or storage. However, in the present study, this observation might be linked to poor vendors' personnel and handling hygiene practices.

The brands examined for aerobic mesophilic bacteria had figures that were above the accepted limit of less than 10 cfu/ml recommended by FAO (1979; 1993). In addition, the presence of yeasts in the brands analysed poses a health threat to the consumers as these organisms have been reported as undesirable in foods. This is because yeasts being undesirable in foods have been reported in various studies as agents of food spoilage (Hobbs and Gilbert, 1982; FAO; 1977; 1979; 1993; Frazier and Westhoff, 1978; WHO, 2003). All the yoghurt samples analysed were contaminated with microorganisms. Similar results were reported by Anorth *et al.* (1979) and Jordano (1987).

There are many non-pathogenic bacteria normally found in yoghurt to a greater or lesser extent even after pasteurisation and these can bring about changes in yoghurt, some of which make it unpalatable for consumption. These may include *Pseudomonas* sp and *Klebsiella* sp which are pathogens of burns, wounds and abscesses (Rogers *et al.*, 1977), *E coli* is known to cause gastroenteritis and urinary tract infections, *Staphylococcus aureus* causes food poisoning and toxic shock syndrome. *Micrococcus* sp, *Streptococcus* sp, and *Proteus* sp are some of the microorganisms responsible for the spontaneous souring of milk (Rogers *et al.*, 1977). *Klebsiella* sp are opportunistic pathogens and therefore could cause pneumonia (Rogers *et al.*, 1977).

In general, according to Farret and Rose (1992), the type to gain ascendancy in the

yoghurt after pasteurisation will depend upon the temperature at which it is held. Derbinova (1994) supported this suggestion when he showed that cooler holding temperatures encourage the growth of fermentative varieties such as *Streptococcus* sp and *Micrococcus* sp that are responsible for the normal souring of milk, while warmer temperatures encourage the growth of the putrefactive type (*Pseudomonas* sp, *Klebsiella* sp, *E coli* and *Proteus* sp) which give rise to foul and disagreeable odours.

The possible sources of *Staphylococcus* and *Bacillus* sp in the yoghurt brands during transportation, storage and retailing, could be due to the nose, hands, skin and clothing of handlers as well as coughing, talking and sneezing (Duru *et al.*, 2006).

The presence of bacteria such as *Bacillus* sp, *Pseudomonas* sp and *Salmonella* sp could be attributed to their ability to withstand adverse conditions (Lovel, 1981).

Pasteurization is designed to heat and cool the product simultaneously without altering the flavour or texture of the product; yoghurt mixes are heated to 72°C for 15 seconds, cooled to the inoculation temperature of 42°C and flavour addition before cooling to 12°C for filling and packaging. The pre-pasteurization temperature is lethal to mesophilic bacteria but not the thermotolerants (Suriyarachchi and Fleet, 1981).

The high aerobic mesophilic count in some of the brands in this study even after pasteurisation might have resulted from the temperature at which it was held. This correlates with the reports of Farret and Rose (1992) and Derbinova (1994) who observed that after pasteurisation cooler and warmer temperatures encourage the growth of fermentative and proteolytic organisms respectively. In a very high aerobic mesophilic counts as found in some brands in the present study, Hobbs and Gilbert (1982) opined that there was the probability of human pathogens being present. They also noted that low counts do not always represent safe products because it is possible to have low counts in foods in which toxin-producing organisms have grown and produced toxins that remain stable to conditions, which may not favour the continued survival of the microbial cells. Results obtained from aerobic mesophilic, psychrophilic and thermophilic counts of all the yoghurt samples showed a significant difference ( $P < 0.05$ ) in all the yoghurt brands analysed. In a post pasteurisation contamination by psychrophilic Gram-negative rods, are the important factors that limits the shelf life of products at cold storage (Nunez *et al.*, 1984). Besides, incessant power failures would enhance the growth of thermotolerant bacteria as well as yeasts or moulds that may be derived from the environment particularly where there are leaks in the packaged yoghurt.

The abnormal fermentation occurring in dairy products as a result of microbial contaminants results in rancidity, bitter flavour, bad odour, discoloration, slime formation and accumulation of gas leading to reduced shelf life and eventual rejection of the product by consumers and hence huge economic loss of the dairy industry concerned (Oyeyi and Nkweke, 2005).

The total coliforms counts from some of the yoghurt brands analysed were lower than the  $10^9$ - $10^7$  cfu/g. According to Silliker (1985), in frozen foods such as yoghurt, total counts most effectively indicate the general sanitation and care that had been invested in the prior handling of the yoghurt. High counts of coliforms recorded in some of the yoghurt brands may be attributed to their ability to recover from heat stress (Lin and Flint, 1995) and to post pasteurisation contamination through handling and fixing of packaging materials (Nelson, 1981). Although, it was not possible to assess the contribution of personnel, contamination from these sources cannot be ruled out as most dairy workers are carriers of microorganisms such as *Staphylococcus aureus*; hence their presence in the finished product is blamed on the poor sanitation.

Generally, it is expected that all yoghurt most contain bacteria. Advisory guidelines for microbiological quality have suggested that satisfactory yoghurt should contain more than  $10^8$  cfu/g of the starter organisms < 1 coliform/g < 1 mould/g and < 10 yeast/g. Fruit containing yoghurt may contain up to 100 yeasts/g and remain satisfactory quality (Adams and Moss, 1999). Information on the microbiological quality of the yoghurt could be drawn from the high aerobic, coliform and yeast counts obtained in some of the yoghurt brands generally indicated poor sanitation and poor care invested in their production, procurement and distribution and, according to Hobbs and Gilbert (1982), in a very high aerobic counts like these, there is the probability that there are likely to be human pathogens present.

The results of the present research showed that few of the bacteria isolated are normal flora of the yoghurt, which includes *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. However, most of the bacteria isolated are contaminants; these include *Bacillus* sp, *Proteus* sp, *Pseudomonas* sp, *Staphylococcus aureus*, *Klebsiella* sp and *Micrococcus* sp. There are organisms which are of public health significance such as *Salmonella* sp and *Escherichia coli*, which are indicators of faecal contamination and cause diseases, such as typhoid fever and infantile diarrhoea respectively.

Yeast contaminants in the first set of ingredients are destroyed by the heating temperatures and times given above, and it is generally accepted that yeast contamination of the final product arises from added fruit material or poor hygiene practices during the packaging

operation (Davis, 1975 and Kroger, 1976). The association of yeast with fruits in general is well documented (Robinson and Temine, 1975).

Important criteria governing the growth of yeasts in yoghurt are the ability to grow well at refrigeration temperatures and to ferment sucrose, lactose or both. These criteria are consistent with the storage of yoghurt under refrigerated conditions and the presence of sucrose and lactose as the major carbohydrates. The lactose concentration of yoghurt is around 4.0% and the sucrose concentration for fruit and flavoured yoghurt may be between 5 and 10.0% (Davis, 1974). Davis (1976) pointed out that glucose and fructose may occur in yoghurt through the use of invertase sugar by some manufacturers and that small amount of galactose may arise from the bacterial metabolism of milk lactose. These three sugars, therefore, cooled also act as fermentable substrates yeast growth, and this could account for the growth in yoghurt of species such as *Debaryomyces*, *Pichia*, *Hansenula*, *Saccharomyces*, *Schizosaccharomyces*, *Candida albicans*, *Kloeckera*, *Torulopsis* and *Rhodotorula* as recorded in the present study. Few genera of the yeasts isolated from the present study are essential; these include *Saccharomyces* (e.g. *S. cerevisiae* is employed in many food industries used for the leavening of bread), *Candida* (e.g. *Candida utilis* is grown for food and feed); while majority of the yeasts isolated are contaminants. These include *Rhodotorula* causing discoloration of foods e.g. Colored spots on meats or pink areas in Sauerkraut. *Torulopsis* (e.g. *T. sphaerica* ferments lactose and may spoil milk products such as yoghurt).

## CONCLUSION AND RECOMMENDATIONS

Results obtained from this study showed that many different brands of yoghurt manufactured in Kano, Katsina, Kaduna, Zaria, Borno and Abuja were on sale in Kano metropolis. Mobile and stationary vendors ensured a seemingly illegal sale of nearly expired brands that were not registered with NAFDAC. Percentage level of preference as a measure of consumer acceptance varied with the brands and this may be related with the popularity of the products. However, there were no reported cases of disease outbreak specifically due to consumption of yoghurt in the area according to public view. Some yoghurt brands were of NAFDAC standard and therefore safe for human consumption, others were substandard and this calls for yoghurt manufacturers and the appropriate governmental agencies concerned with public health to consider this trend with concern as food poisoning by *Staphylococcus aureus*, *Bacillus* sp and some strains of *E. coli* and *Salmonella* sp is possible through consumption of contaminated yoghurt.

The monitoring of the sanitary conditions of production site as well as organizing seminars and workshops for mobile vendors and other retail agents specifically on environmental and

personal hygiene. Finally, quality control and food safety are a joint responsibility of all manufacturers to ensure that the yoghurt brands are safe for human consumption.

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