Microbiological quality assessment of bottled yogurt of different brands sold in Central Market, Kaduna Metropolis, Kaduna, Nigeria

*Nandita, De., Goodluck, T. Ma'aji., and Bobai, Matthew.

Department of Microbiology Kaduna State University Kaduna, Nigeria
*manadina93@gmail.com

Abstract: An investigation on microbiological quality of twenty commercial samples of ten different brands of bottled plain yogurt sold in Central market, Kaduna was carried out using standard microbiological procedures. Out of 10 different brands, five were registered by NAFDAC and the other five were not registered by NAFDAC. The pH of the registered yogurt samples were in the range of 4.01-4.79 while the pH of the non-registered samples were in the range of 5.28-5.63. The total bacterial counts (TBC) of registered and nonregistered samples were in the range of $3.0x10^3$-$10.5x10^4$ and $8.2x10^4$-$28.4x10^5$ respectively. Out of registered samples, sample $A2_1$ had the highest count whereas sample $A5_{11}$ had the lowest count. In case of non- registered samples, sample $a4_{11}$ had the highest count and sample $a3_{11}$had the lowest count. Statistical test (t=-2.28 and F= 9.78) revealed that there is significant difference between the colony counts of registered and non-registered samples at $\alpha_{0.05}$ level. *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, the bacteria present in the starter culture used for manufacturing yogurt, were present in all the samples as the manufacturers claimed on their labels. *Bacillus* sp. were obtained from all the samples whereas *Staphylococcus aureus* was isolated from $A4_1$, $A4_{11}$, $a2_1$ and $a2_{11}$. *Aspergillus* sp. was isolated from all the samples tested. *Mucor* sp. was isolated from $A1_1$, $A1_{11}$, $a1_1$, $a1_{11}$, $a4_1$and $a4_{11}$. *Penicillium* sp. was isolated from samples $A2_1$, $A2_{11}$, $A3_1$, $A3_{11}$, $a2_1$, $a2_{11}$, $a3_1$and$a3_{11}$. *Acremonium sp.* was isolated from $A4_1$, $A4_{11}$, $a1_1$ and $a1_{11}$. Proper care should be taken for storage and handling of yogurt due to the fact that fungi like *Aspergillus* and bacteria like *S. aureus* were isolated from some NAFDAC registered samples along with some non-registered samples.
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1. Introduction

Yogurt is one of the most popular fermented dairy products widely consumed all over the world. It is obtained by lactic acid fermentation of milk by the action of a starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbruckii* spp. *bulgaricus*. The role of these two genera in yogurt manufacture can be summarized as milk acidification and synthesis of aromatic compounds (Serra *et al*., 2009). In Nigeria, it is a popular drink due to its nutritional, probiotic and organoleptic characteristics (Hassan *et al*., 2010; Sanful, 2009). But yogurt can be contaminated by fungi able to modify organoleptic characteristics and be a risk of human health. Yogurt can be easily subjected to microbial contamination, as especially by fungi which grow and reproduce in acid environment with feasible oxygen. Some of the molds can produce secondary toxic metabolites resisting to common reclamation treatments. Aflatoxins are carcinogenic and toxic, which is a secondary metabolic product of some *Aspergillus sp*. Issazadeh *et al*. (2012) worked on 60 yogurt samples and out of these 60 samples, 59 samples were found to be contaminated with AFMI. Ahmad *et al*. (2013) worked on quality assessment of yogurt produced at industrial and small scale and were commercially available in Faisalabad, Pakistan. They observed that the coliform count of yogurt produced at small scale was much higher than that of branded samples (produced at large scale). Montagna *et al*. (1998) studied on microbiological quality of 166 samples and observed that 7.2% of the contaminated samples were positive for fungi. El-Malt *et al*. (2012) worked on microbiological evaluation of yogurt products in Qena city, Egypt. They showed that out of 100 random samples purchased from various dairy shops, street vendors and supermarkets located in Qena city, Egypt, *S. aureus* were detected in 72% and 35% of small and large scale yogurt samples.

Though bottled yogurt is a popular drink in Kaduna Metropolis, Kaduna, Nigeria, very little research has done on microbiological quality of yogurt sold in different markets by hawkers. An attempt was made here to investigate the microbiological quality of yogurt of different brands approved by NAFDAC and also of some yogurts not approved by NAFDAC but readily available in the market for consumption in Kaduna. NAFDAC is a governmental organization which is in charge of maintaining the quality of food products made in Nigeria.

2. Materials and Methods

2.1 *Collection of yogurt samples*:

Twenty samples of ten different brands of bottled plain yogurt were randomly purchased from different sellers hawking in the market. Out of 20 samples, ten were purchased with registration no. approved by NAFDAC and with manufacture date and expiration date on their labels. For registered samples, the samples were labeled as A1-A5. For brand A, two samples were collected from two different hawkers and were labeled as $A1_1$-$A1_{11}$. The other ten samples were collected without any registration no. from NAFDAC and the labeling on these samples did not have any manufacturing date and expiration date. For non-registered samples, the samples were labeled as a1-a5. For each brand, 2 samples were collected and labeled accordingly ($a1_1$-$a1_{11}$). After collection, the samples were put into a cooler packed with ice and then were transported to the Microbiology Laboratory of Kaduna State University within 3h for analysis purpose.

The study period was between the months of July '10, 2013 to August '27, 2013.

2.2 *Analysis of samples*:

2.2.1 Physio-chemical characteristics of yoghurt samples:

After collection of samples, the **color**, **organoleptic properties**, **consistency** of each sample were recorded. Then the **pH** value of each sample was also determined using the 3505 pH meter. For determination of pH, 80ml of each yoghurt sample was poured into 100ml of beaker and the pH of the sample was then determined using pH meter.

2.2.2 Total bacterial count of yogurt samples:

The total bacterial count was done using pour plate method as described in Benson, 2005. Serial dilutions for each sample were made up to 10^{-4}. One ml of each dilution was introduced onto sterile petridish containing plate count agar (PCA) and the plates were incubated at 37°C for 24 h. The total bacterial count was expressed as cfu/ml.

2.2.3 Isolation of bacteria associated with purchased yoghurt samples:

(i) Isolation and identification of Bacteria associated with yogurt samples:

For lactic acid bacteria, MRS Agar was used. Serial dilutions upto 10^{-4} were made and 1 ml of each suspension was introduced onto agar medium using pour plate method. The plates were incubated anaerobically at 37°C, 45°C and 50°C for 48 to 72 h to obtain lactic acid bacteria colonies. Discrete colonies were isolated and reinoculated onto appropriate medium in order to obtain pure isolates. These pure isolates were then kept at 4°C for identification purpose (Michaylova, 2007).

For isolation of bacteria other than lactic bacteria, Nutrient agar (NA) and MacConkey agar (MCA) were used. Serial dilutions upto 10^{-4} were made and 1 ml of each suspension was introduced onto NA.

agar medium using pour plate method. The plates were incubated at 37°C for 24 hrs.

Discrete colonies were isolated and reinoculated onto appropriate medium in order to obtain pure isolates. These pure isolates were then kept at 4°C for identification purpose (Benson, 2005).

2.2.4 Identification of Isolates obtained from MRS agar and NA agar.

For isolates obtained from MRS agar and NA agar, gram staining was done according to the procedure as described in Benson, 2005. Biochemical tests such as catalase test, coagulase test, indole test, urease test, Methyl red and other biochemical tests were also done as described in Benson, 2005 in order to identify the isolates.

2.3 Isolation and identification of fungi associated with the collected samples:

The isolation and identification of the fungi was done using Potato dextrose agar (PDA). Wet preparation was done using lactophenol cotton blue as mountant and viewed under the low power objective lens and then using ×40 high power objectives (Benson, 2005).

3.0 Results

3.1*Physiochemical characteristics of samples*:

All the twenty samples of yogurt purchased from Central Market were white in color. Both $A1_1$ and $A1_{11}$ were solid in nature, $A2_1$- $A5_{11}$ were semisolid in nature whereas $a1_1$- $a5_{11}$ were watery when the samples were purchased from the market. The pH values of samples were in the range of 4.01- 5.63. The results are expressed in Table 1.

3.2 *Total bacterial count of yoghurt samples*:

The values of total bacterial count of registered samples were in the range of 3.0×10^3-10.5×10^4whereas the values for nonregistered samples were in the range of 8.2×10^4- 28.4×10^5. The results are expressed in Table 1.

3.3 *Isolation of bacterial isolates from purchased yoghurt samples*

The cultural and morphological characteristics of isolates obtained from nutrient agar medium were listed in Table 2. The isolates were labeled as NS1-NS24. Biochemical characteristics revealed that NS1-NS20 were *Bacillus* sp. and NS21- NS24 were *Staphylococcusaureus*. *Bacillussp*. was isolated from all the samples whereas *Staphylococcusaureus* was isolated from $A4_1$, $A4_{11}$, $a2_1$ and $a2_{11}$.

Two types of colonies were isolated from all the samples in MRS agar medium. The colonies were

labeled as MS1-MS20. All the colonies were subjected to gram reaction and the size and shape of the cells were observed. The results are expressed in Table 2. The results of catalase activity, sugar utilization tests are also listed in Table 3.

3.4 *Isolation and identification of fungi from samples*

Aspergillus sp. was isolated from all the samples tested. *Mucor* sp. was isolated from $A1_1$, $A1_{11}$, $a1_1$, $a1_{11}$, $a4_1$anda4_{11}. *Penicillium* sp. was isolated from samples $A2_1$, $A2_{11}$, $A3_1$, $A3_{11}$, $a2_1$, $a2_{11}$, $a3_1$anda3_{11}. *Acremonium spp*. was isolated from $A4_1$, $A4_{11}$, $a1_1$ and $a1_{11}$. The results are shown in Table 4.

Table 1: **Total bacterial count (cfu/ml) and pH of different yogurt samples purchased from different hawkers in Central Market, Kaduna.**

S/n	Sample code	pH	TBC
1	$A1_1$	4.79	4.5×10^4
2	$A1_{11}$	4.63	4.9×10^4
3	$A2_1$	4.23	9.0×10^4
4	$A2_{11}$	4.30	8.9×10^4
5	$A3_1$	4.52	6.4×10^4
6	$A3_{11}$	4.50	6.6×10^4
7	$A4_1$	4.62	10.4×10^4
8	$A4_{11}$	4.57	10.5×10^4
9	$A5_1$	4.01	3.3×10^3
10	$A5_{11}$	4.06	3.0×10^3
11	$a1_1$	5.31	22.1×10^4
12	$a1_{11}$	5.28	22.6×10^4
13	$a2_1$	5.45	21.2×10^4
14	$a2_{11}$	5.42	21.0×10^4
15	$a3_1$	5.58	8.6×10^4
16	$a3_{11}$	5.60	8.2×10^4
17	$a4_1$	5.45	28.4×10^4
18	$a4_{11}$	5.48	28.2×10^4
19	a51	5.63	20.5×10^4
20	$a5_{11}$	5.59	20.5×10^4

Key: $A1_1$-$A5_{11}$are registered yogurt samples, $a1_1$-$a5_{11}$ are non-registered samples

Table 2: **Cultural and Biochemical Characteristics of Isolates on NA Medium.**

S/N	IN	MC	GR	CS	CA	CO	ID	UR	CI	MR	VP	MO	OR
1	NS1-NS20	Cream, circular, smooth, opaque and mucoid.	+	Rod	+	+	-	+	+	+	-	+	*Bacillus sp.*
2	NS21-NS24	Yellowish, circular, opaque and mucoid	+	Cocci	+	+	+	-	-	-	-	-	*Staphylococcus aureus*

Keys: IN- Isolate number; GR- Gram reaction; CS- cell shape; CA-catalase test; Co- coagulase test; ID-indole test; UR-urease test; CI- citrate utilization; MR- methyl red test; VP- Voges- Proskauer test; MO- motility test, OR-isolated organism.

Table 3: **Cultural and Biochemical Characteristics of Isolates on MRS agar.**

Isolate Number	Shape and color	Glucose utilization	GR	CS	CA	Organism
MS1-MS10	Yellowish, mucoid rounded colonies	+No gas Production	+	Growth at 45°C Rod	-	*L. bulgaricus*
MS11-MS20	”	,,	,,	Growth at 50°C Cocci	-	*S. thermophiles*

Keys: GR= gram reaction, CS= colony size, CA- catalase activity, += Positive, -= negative

Table 4: Cultural and morphological characteristics of fungi isolated from yogurt samples

Isolate number	Colony morphology	Microscopic morphology	Organism
PS1-PS20	Growth begins as yellow colonies that soon develop a black, dotted surface as conidia are produce within 2-6 days the colony becomes jet black and powdery and the reverse remains cream color.	Exhibits septate hyphae long conidiophores' that support spherical vesicle that give rise to mutulae and phalides from which conidia are produce.	*Aspergillus nigger*
PS21-PS26	Colonies characteristically produce a fluffy white growth that diffusely covers the surface of the agar within 24-48 hours.	The hyphae appear to be coarse and fills the entire culture dish rapidly with hyphae dotted with brown or black sporangia. sporangiosphores are branch and have at their tip a sporangium filled with sporangiophores, no rhizoids or stolons.	*Mucorsp*
PS27-PS38	Green colonies, surface of colonies becomes powdery due to presence of conidia.	Hyphae are septate and produce brush like conidiophores, conidiophores produce metulae from which phalides producing chains of conidia arise.	*Penicilluimsp*
PS39-PS42	White colonies.	Septate hyphae that produce single unbranched tube like phalides, phalides give rise to cluster of conidia at the tip of the phalide.	*Acremoniumsp*

4. Discussion:

The pH values of yogurt samples registered by NAFDAC were in the range of 4.01-4.79 but the values for non-registered samples were in the range of 5.28-5.63. The high pH and low acidity may be due to the fact that there is no proper system of culture dosage in unbranded yogurt, which largely affects the acidity of the final yogurt (Abrar *et al*., 2009). The total bacterial counts of the registered and non-registered samples were in the range of $3.0 \times 10^3 - 9.0 \times 10^4$ and 8.2×10^4-28.4×10^5 respectively. There is significant difference in TBC among the registered and nonregistered samples (t= - 2.28 and F= 9.78 at $\alpha_{0.05}$ level). Ifeanyi *et al*. (2013) carried out a research on microbiological assessment of four brands of yogurt samples collected from vendors in Onitsha market and found the values of these four brands were in the range of 1.9×10^5-6.1×10^5. Obande and Azua (2013) worked on yogurt sold in Makurdi, Benue state, Nigeria and observed the mean value for yogurt samples was 1.4×10^7cfu/ml. The results obtained in this study for yogurt samples agree with the range obtained for yogurt by other investigators. The high count for non-registered samples especially for samples $a4_1$ and $a4_{11}$ is indicative of poor handling during processing and packaging of the product. *Bacillus* sp. was isolated from all the samples studied. The presence of *Bacillus* sp. in all the yogurt samples implies post-pasteurization contamination (Huck *et al*., 2008). *S. aureus* was isolated from samples $A4_1$, $A4_{11}$, $a2_1$ and $a2_{11}$samples. El-Malt *et al.* (2012) also detected *S. aureus* in 72% of the 100 yogurt samples they analyzed in Qena City, Egypt. Presence of *S. aureus* usually indicates contamination from food handlers (Abdel Hameed *et al*., 2009). *Aspergillus* sp. was isolated from all the samples analyzed for this research purpose. *Mucor* sp. was isolated from $A1_1$, $A1_{11}$, $a1_1$, $a1_{11}$, $a4_1$and $a4_{11}$. *Penicillium* sp. was isolated from samples $A2_1$, $A2_{11}$, $A3_1$, $A3_{11}$, $a2_1$, $a2_{11}$, $a3_1$ and $a3_{11}$. *Acremonium spp*. was isolated from $A4_1$, $A4_{11}$, $a1_1$ and $a1_{11}$. Montagna *et al.* (1998) has examined some different yogurt to verify fungal contamination and potentially toxigenic moulds in commercially available yogurt samples. 7.2% of the samples was positive for fungi such as *Penicillium* and *Cladosporium* sp. Fungi in yogurt samples generally correspond to poor cleaning practices and the use of unhygienic techniques or inadequate storage conditions. Thus the fungal contamination might to occur during transportation process and/or packaging, storage, transport and sales; besides it can survive in food by its ability to adapt at differential conditions. Though all the yogurt samples have *L. bulgaricus* and *S. thermophilus*, the organisms present in starter culture, the samples were contaminated with bacteria like *Bacillus* sp. and *S. aureus*. NAFDAC registered samples are commonly products of high standard but in this case these products are not safe for people to consume. Also the presence of fungi especially those capable of producing aflatoxins is a great concern. So there needs to be a HACCP program for transportation /packaging and storing of yogurt in Nigeria.

Corresponding Author:
Nandita De, Ph.D.
Department of Microbiology
Kaduna State University, Kaduna, Nigeria
e-mail: manadina93@gmail.com

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