Microbial Quality and Sensory Assessment of Yogurt Marketed in Akure Metropolis, Nigeria

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Abstract- Several companies have emerged in the production of yogurts due to its high consumption and health benefits, but in most of these companies, the environmental conditions involved in the production, storage and distribution of many of yogurts are not strictly monitored hence affecting its microbial and sensory qualities. In view of these, commercially available brands of yogurt drinks in Akure metropolis, Ondo State, Nigeria, were purchased and analyzed for their microbiological, physiochemical and organoleptic properties using standard methods. It was observed that (16.7%), (8.3%) and (25%) of the yogurt samples analysed lack NAFDAC number, batch number, and attractive packaging respectively. All samples had high total viable bacteria counts but the fungal load observed falls within specification. Coliforms were found in 42% of the samples. The isolated microorganisms include; *Bacillus* sp, *Streptococcus thermophillus*, *Lactobacillus bulgaricus*, *Listeria* sp, *Serratia* sp, *Klebsiella* sp, *Proteus* sp, *Escherichia coli*, *Aspergilus* sp, *Candida* sp, *Saccharomyces* sp, and *Mucor* sp. *Streptococcus thermophillus* and *Lactobacillus bulgaricus* were the most prevailing bacteria encountered (23.6%), *Aspergilus* sp. was the most encountered fungi (44.5%). pH and Total Titratable Acidity (TTA) ranged from, 3.6 to 4.6 and 1.35 to 0.72 respectively. The sensory evaluation revealed that there were significant differences ($p \leq 0.05$) in the organoleptic properties of some of the yogurts. Conclusively, The study revealed that above fifty percent (50%) of the yogurt samples bought from Akure city were still below stipulated quality standard in terms of their microbial and organoleptic qualities.

Index Terms- microbial quality, organoleptic properties, physicochemical, yogurt samples

I. INTRODUCTION

Milk is often regarded as being nature's most complete food; it earns this reputation by providing many of the nutrients which are essential for the growth of the human body (**Olasupo *et al.*, 2002)**. According to (**Oyeleke, 2009**) yogurt was described as a notoriously balanced food containing almost the nutrients present in milk but in a more assimilable form. Yoghurt is a unique fermented milk product produced as a result of controlled bacteria fermentation of milk, its uniqueness is attributable to the symbiotic fermentation (**Tamime, 2007**) carried out by a group of Heterofermentative lactic acid bacteria specifically *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. These bacteria are known as yoghurt cultures and fermentation of lactose by these bacteria produces lactic acid, which acts on milk protein to give yogurt its texture and characteristics.

Yogurts have been found to be contaminated with both spoilage and pathogenic organisms due to unhygienic pre production, production and post production processes. This is because the environmental conditions surrounding the production, storage, distribution and sales of yogurt in many parts of Nigeria is not ideal (**Oyeleke, 2009; Alli *et al.*, 2010**). As a result of this, it's microbiological and sensory qualities are not satisfactory as most of its quality parameters falls below standard and are usually not in conformance with quality specification (**Mbaeyi-Nwaoha and Egbuche, 2012; Makwin *et al.*, 2014**).

Molds and yeast are the primary contaminants in yoghurt produced commercially in Nigeria (**Oyeleke, 2009**). When these microorganisms grow in yogurt, they utilize some of the acid and produce a corresponding decrease in the acidity, which may favour the growth of putrefactive bacteria (**Agu *et al.*, 2014**).

Undesirable bacteria that can cause spoilage of dairy products include Gram-negative psychrotrophs, coliforms and lactic acid bacteria (**Willey et al., 2008; Oyeleke, 2009; Yabaya and Idris, 2012**). So to ensure the proper quality and safety of yoghurt there should be a complete check on the method by which yogurt is produced and sold in local markets and major streets (**El-Diasty *et al.*, 2009; Eissa et al., 2010; Mbaeyi-Nwaoha and Egbuche, 2012**). According to (**Younus *et al.*, 2002**), practical approach towards the quality of yoghurt is to evaluate the different samples of yoghurt sold in local markets and beverage stores for their conformity to standards as stated by food regulatory agencies. Thus, it is imperative to evaluate the microbial and sensory quality of yogurts sold in Akure metropolis, due to the high risk associated with consuming substandard or unhygienic yogurt.

II. MATERIAL AND METHODS

Study Area

The study was carried out in Akure city Ondo state from February 2016 to June 2016. Akure city is the capital of Ondo state which is one of the 36 states in Nigeria, it lies about $70^{o}15^{'}$ north of the equator and $50^{o}15^{'}$ east of the Meridian (**Ajibefun, 2014**). Their climatic condition follows the pattern of southwestern Nigeria where the climate is influenced mainly by the rain-bearing south west monsoon wind from the ocean and the dry northwest winds from the sahara desert. High temperature and high humidity also characterize the climate (**Ajibefun, 2014**). Various part of the city especially the indigenous core areas which is characterized by high density and low income population is experiencing environmental challenges in the area of pollution, poor housing, lack of potable water, waste disposal and sanitation as the city is growing in population (**Ajibefun, 2014**).

Sample collection

Twelve different brands of commercially prepared yogurts packaged in plastic or paper box containers were purchased in triplicates from street vendors, market places and beverage stores in different locations in Akure, capital of Ondo State, the yogurts were then refrigerated before the commencement of the analysis. Information like NAFDAC registration number, expiry date, batch number and other information on the labels of the yogurt were noted and recorded and the samples were evaluated by a random experiment with 3 repetitions for each sample and the mean values and the standard deviation of the three values were recorded.

Determination of pH

A digital pH meter (Hanna) was calibrated, 50ml of the yogurt sample was dispense into clean beaker and the calibrated pH meter was wiped with a clean tissue paper, switched on, and immersed into the yogurt mixture. The pH value of the yogurt sample was then seen displayed on the digital readout and the value was noted and recorded. This was then repeated for each of the yogurt samples (**Olugbuyiro *et al.*, 2011**).

Determination of the Total Titratable Acidity (TTA)

To obtain the TTA, a clean burrette was clamped to the retort stand and fill to the zero mark with 0.1M sodium hydroxide (NaOH), 20 ml of the yogurt sample was measured and poured into a clean conical flask, 2-3 drops of phenolphthalein was added to the solution and then NaOH was titrated against it until a pink color was observed. The titer value was noted and recorded (**Olugbuyiro *et al.*, 2011**). The titre value was then use in calculating the TTA. Using the expression;

$$\textbf{\% Lactic acid} = \frac{\mathbf{0.1M\ NaOH \times Titre\ value\ x 0.09}}{\mathbf{volume\ of\ sample}} \times \mathbf{100\%}$$

Microbial Analysis

Isolation of microorganism

Prior to the isolation, the laboratory work area and the containers of the yogurt were swabbed thoroughly with 70% ethanol before opening to avoid contamination. The yogurt samples which have assumed room temperature were shaken vigorously to suspend microbial content. To clean test tubes containing sterile distilled water, 1ml of the yogurt sample was transferred into it and diluted serially in one-tenth stepwise to 10^{-10} dilution factor. Each (1ml) of the dilution 10^{-8} and 10^{-10} were plated into the four different culture media (Nutrient Agar (NA), Potato Dextrose Agar (PDA), Eosin Methylene Blue (EMB), de Man Rogosa and Sharpe agar (MRS) in triplicate using the pour plate method. NA, EMB, and MRS plates were then incubated at 37^{o}C for 24hours while PDA plates were incubated at 25^{o}C for 3-5 days according to the methods described by (**Nester et al., 2004**).

After incubation, the colonies on NA plates were counted and used to determine the Total Viable Bacterial count of the yoghurt samples (cfu/ml), EMB (total coliform count), MRS (total lactic acid bacteria count), and PDA (total fungi count) the representative colonies or the distinct colonies on the different medium plates were then purified by sub-culturing on fresh media. The pure cultures obtained were observed for their morphological characteristics and further biochemical identification.

Biochemical characterization

Pure bacterial isolates obtained from the yogurt samples were biochemically characterize and presumptively identify using the methods describe by (**Cheesbrough, 2006**).

Identification of fungal isolate

The fungi isolates were characterized by checking their surface and reverse color or pigmentation, their hyphae, presence or absence of spores, color and structure of spores, also the characteristics of the mature fungi fruiting bodies were checked. Microscopically fungi isolate were characterized by staining (Moreira *et al.,* 2001**). For this staining procedure, a grease free slide was placed on the slab and a drop of cotton blue in lactophenol was placed on it. With the aid of a sterile inoculating needle, a part of the fungal mycelium was placed on the slide and mixed to form a smear. The slide was covered with a cover slip and viewed under ×10 and ×40 objectve lens of a binocular light microscope.**

Sensory evaluation

According to (**Obi *et al.*, 2010**), the yogurt samples were evaluated for their organoleptic characteristics (color, taste, flavor and texture) and overall acceptability by panelists who comprise of microbiology undergraduate students and students from other departments and some teaching and non-teaching staff members of Federal Unversity of Technology Akure, Ondo State, Nigeria. Using nine point hedonic scale ranging from excellent or extremely like (score = 9) to very poor or extremely dislike (score = 0) as extremes (**Obi *et al.*, 2010**), following the method stated by (**IDF, 2002**).

Statistical analysis

The data obtained were statistically analysed using SPSS version 20, the results obtained were statistically analysed using analysis of Variance (ANOVA), and the tests of significance were carried out by Duncan's multiple range test at $p \leq 0.05$. The results obtained were computed as mean of triplicate ± standard deviation.

III. RESULTS AND DISCUSSION

Yogurt Pack Information

The information on the yogurt samples were noted and documented in **Table 1**. Out of the 12 yogurt brands evaluated, two lacks NAFDAC number, one lacks production and expiry date, and three of them have unattractive packaging.

Table 1: Yogurt Pack Information

Information	Number of yogurt	Yes (%)	No (%)
NAFDAC Number	12	10 (83.3)	2 (16.7)
Production date	12	11(91.67)	1 (8.33)
Expiry date	12	11 (91.67)	1 (8.33)
Batch number	12	11 (91.67)	1 (8.33)
Manufacturing address	12	12 (100)	0 (0.00)
Package Attractiveness	12	9 (75)	3 (25)

Total Microbial Count

The total viable count of the various yogurt samples was obtained by inoculating the sample on nutrient agar (NA). The number of isolates obtained vary from one yoghurt to the other, sample C has the highest bacteria count in cfu/ml ($60.00 \pm 1.00 \times 10^8$), while sample H has the least viable bacterial count ($6.00 \pm 0.50 \times 10^8$) (**Table 2**). The lactic acid bacteria counts also vary from one yogurt to the other. Sample E, H and I has the least lactic acid bacteria count in cfu/ml ($1.00 \pm 0.00 \times 10^8$), on the other hand sample F has the maximum count ($9.00 \pm 0.50 \times 10^8$) (Table 2). Sample A, F, G, K, L do not have coliform bacteria, samples J, H, B, C has the least count ($1.00 \pm 0.50 \times 10^8$) while sample E has the highest ($5.00 \pm 0.50 \times 10^8$) cfu/ml, (**Table 2**). Samples A, F, K have no fungal growth at 10^8 dilution, the least fungi count was seen in samples E, H ,I ,L ($1.00 \pm 0.00 \times 10^8$) (Table 2) and sample C has the highest ($5.00 \pm 0.50 \times 10^8$).

Table 2: Total viable bacteria count, total lactic acid bacteria count, total coliform count and total fungi count.

Sample	TVB (cfu/ml × 10^8)	TLC (cfu/ml × 10^8)	TCC (cfu/ml × 10^8)	TFC (cfu/ml × 10^8)
A	$20.00^{d} \pm 0.50$	$2.00^{b} \pm 0.50$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
B	$12.00^{c} \pm 0.00$	$4.00^{d} \pm 0.50$	$1.00^{b} \pm 0.00$	$2.00^{c} \pm 0.50$
C	$60.00^{k} \pm 1.00$	$3.00^{cd} \pm 0.76$	$1.00^{b} \pm 0.50$	$5.00^{e} \pm 0.50$
D	$40.00^{i} \pm 0.50$	$6.00^{f} \pm 0.50$	$2.00^{c} \pm 0.00$	$3.00^{d} \pm 0.50$
E	$22.00^{e} \pm 0.50$	$1.00^{a} \pm 0.50$	$5.00^{d} \pm 0.50$	$1.00^{b} \pm 0.50$
F	$50.00^{j} \pm 1.00$	$9.00^{h} \pm 0.50$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
G	$35.00^{h} \pm 0.50$	$5.00^{e} \pm 0.50$	$0.00^{a} \pm 0.00$	$2.00^{c} \pm 0.50$
H	$6.00^{a} \pm 0.50$	$1.00^{a} \pm 0.00$	$1.00^{b} \pm 0.50$	$1.00^{b} \pm 0.00$
I	$10.00^{b} \pm 0.50$	$1.00^{a} \pm 0.20$	$2.00^{c} \pm 0.20$	$1.00^{b} \pm 0.20$
J	$30.00^{h} \pm 0.40$	$7.00^{g} \pm 0.20$	$1.00^{b} \pm 0.00$	$3.00^{d} \pm 0.50$
K	$25.00^{g} \pm 0.00$	$4.00^{d} \pm 0.10$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
L	$20.00^{d} \pm 0.20$	$3.00^{c} \pm 0.10$	$0.00^{a} \pm 0.00$	$1.00^{b} \pm 0.20$

Values are mean of triplicates ± standard deviation. Values in the same column carrying the same superscript are not significantly different according to Duncan's multiple range tests at ($P < 0.05$)

KEYS: TVB = Total Viable Count, TLC = Total Lactic Acid Bacteria Count, TCC =Total Coliform Bacteria Count, TFC = Total Fungal Count

Percentage occurrence of microorganisms isolated from the yogurt samples

Table 3 and **4** gives percentage occurrence of bacterial and fungal isolates respectively. The isolated bacteria were identified as; *Bacillus spp, Streptococcus thermophillus, Lactobacillus bulgaricus, Proteus spp, Serratia spp.* and *Klebsiella spp., Listeria spp., Escherichia coli.* Total number of bacteria and fungi isolated is 43 and 18 respectively. The frequency most occurred bacteria are *Streptococcus thermophillus* 10(23.26%) and *Lactobacillus bulgaricus* 10(23.26%) while the most occurred fungi is *Aspergillus spp* 8 (44.5).

Table 3: Percentage occurrence of bacteria isolates

Isolated microorga	A	B	C	D	E	F	G	H	I	J	K	L	Frequency And

nism													Percentage occurrence%
Bacillus spp	+	+	+	+	+	-	-	-	-	+	-	-	6 (13.95)
Streptococcus thermophillus	+	+	+	+	-	+	+	-	+	+	+	+	10 (23.26)
Lactobacillus bulgaricus	+	+	+	+	-	+	+	+	-	+	+	+	10 (23.26)
Proteus spp	-	-	+	-	+	-	-	+	-	-	-	-	3 (6.98)
Serratiasp	-	+	-	+	-	-	-	-	-	-	-	-	2 (4.65)
Klebsiella spp	-	+	+	-	+	-	-	-	-	-	-	-	3 (6.98)
Listeria spp	-	+	-	-	-	-	+	+	-	+	-	-	4 (9.30)
Escherichia coli	-	+	+	+	-	-	-	-	+	+	-	-	5(11.60)
Total	3(6.97)	7(16.28)	6(13.95)	5(11.63)	3(6.97)	2(4.65)	3(6.97)	3(6.97)	2(4.65)	5(11.63)	2(4.65)	2(4.65)	43 (100)

Keys: + = present, - = absent, A- L = yogurt samples A to L

Table 4: Percentage occurrence of fungi

Isolated Fungi	A	B	C	D	E	F	G	H	I	J	K	L	Frequency and Percentage of occurrence (%)
Aspergillus spp	-	+	-	+	+	-	+	+	+	+	-	+	8 (44.5)
Candida spp	-	+	+	+	-	-	-	+	+	+	-	-	6(33.3)
Mucorspp	-	-	-	+	-	-	-	-	+	-	-	-	2(11.1)
Saccharomyces spp	-	-	-	+	+	-	-	-	-	-	-	-	2(11.1)
Total	0(0.00)	2(11.1)	1(5.6)	4(22.2)	2(11.1)	0(0.00)	1(5.6)	2(11.1)	3(16.7)	2(11.1)	0(0.0)	1(5.6)	18100)

Keys: - = absent**,** + = present**,** A-L= yogurt samples A- L

pH and Total Titratable Acidity TTA of the yogurt samples

The pH and TTA values of the yogurt samples are represented in **Figure 1**, Sample E has the least mean pH value (3.6 ± 0.2) while sample I has the highest (4.6 ± 0.2). Considering the Total Titratable acidity (TTA), sample B has the least value (0.720 ±0.2), while sample F has the highest value (1.350 ± 0.2).

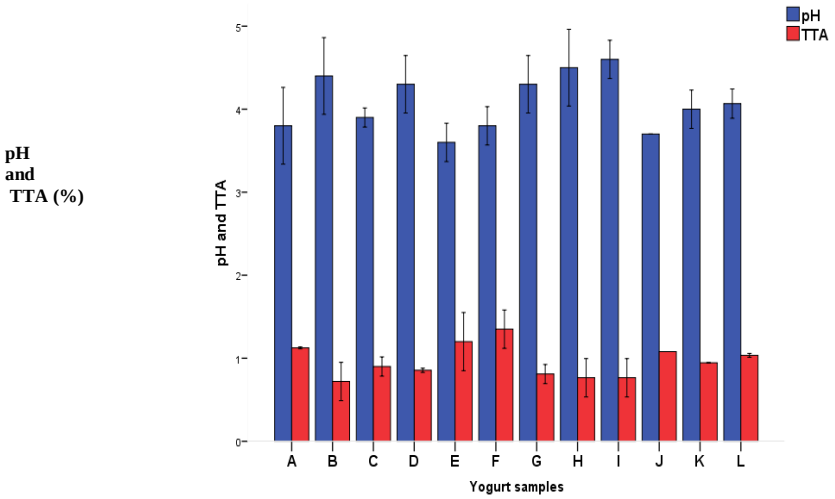

Figure 1: pH and Total Titratable Acidity (TTA) of the yogurt samples.

Sensory evaluation of the yogurt samples

Table 5 gives the observed sensory attributes or characteristics of the yogurt samples with reference to (**USDA, 2001**). The mean scores of the yogurts sensory evaluation conducted by 15 panelists using 9 point hedonic scale were shown in **Table 6**. Sample E has the least mean score for color and taste, Sample H has the least mean score for flavor, Sample L has the least mean score for texture, Sample D and E have the least mean score for overall acceptability.

Table 5: Sensory characteristics of the yogurts sample

Sample	Color	Texture	Taste	Flavor	Body
A	Off white	Smooth	Sweet	Pleasant	Watery
B	Off white	Rough	Sour	Fair	Fairly thick
C	Cream	Rough	Sweet and fairly sour	Pleasant	Thick
D	Off white	Rough	Stale	unpleasant	Watery
E	Cream	Rough	Bitter	Fair	Too thick
F	Bright White	Smooth	Sweet and fairly sour	Pleasant	Fairly thick
G	Bright White	Smooth	Sweet and fairly sour	Pleasant	Fairly thick
H	Off white	Fairly smooth	Sour	Fair	Thick
I	Cream	Rough	Fairly sweet	Not pleasant	Thick
J	Off white	Fairly smooth	Sweet and fairy sour	Pleasant	Thick
K	Cream	Smooth	Sweet and fairly sour	Pleasant	Thick
L	Cream	Smooth	Sweet	Pleasant	Watery

Keys: A-L = yogurt samples A-L

Table 6: The mean score of the sensory evaluation with the standard deviation

Sample	Color	Taste	Flavor	Texture	Overall acceptability
A	7.2 ± 0.94^{ab}	6.93 ± 0.88^{abcd}	7.20 ± 1.26^{ab}	7.20 ± 0.94^{ab}	7.13 ± 0.83^{ab}
B	6.8 ± 1.57^{bc}	6.67 ± 0.98^{bcd}	6.00 ± 1.70^{de}	6.20 ± 1.08^{b}	6.60 ± 0.99^{ab}
C	7.93 ± 0.88^{a}	7.73 ± 1.10^{a}	7.60 ± 1.35^{a}	7.40 ± 1.35^{a}	7.20 ± 1.52^{a}
D	5.87 ± 1.51^{cd}	5.73 ± 0.96^{ef}	5.80 ± 1.26^{e}	6.40 ± 1.35^{ab}	6.13 ± 1.30^{b}
E	5.60 ± 1.59^{d}	5.20 ± 1.47^{f}	6.33 ± 1.23^{bcde}	6.27 ± 1.10^{b}	6.13 ± 1.41^{b}
F	7.00 ± 1.20	7.07 ± 1.03^{abc}	6.93 ± 1.44^{abcd}	6.73 ± 1.28^{ab}	7.00 ± 1.07^{ab}

	abc				
G	6. 87± 1.81 abc	6.67 ± 1.40 bcd	7.27 ± 1.33 ab	6.53 ± 1.55 ab	6.80 ± 1.42 ab
H	6.80 ± 1.15 bc	6.07 ± 1.39 de	5.73 ± 1.28 e	6.27 ±1.44 b	6.40 ± 0.91 ab
I	6.47 ± 1.25 bcd	6.27 ± 1.03 cde	6.13 ± 1.25 cde	6.60 ± 1.18 ab	6.93 ± 1.33 ab
J	7.00 ± 1.46 abc	7.40 ± 1.12 ab	7.27 ± 1.46 ab	6.87 ± 1.30 ab	7.00 ± 1.00 ab
K	7.00 ± 1.00 abc	7.33± 0.72 ab	6.67±0.98 abcde	6.67 ± 1.05 ab	6.60 ± 1.24 ab
L	6.40 ± 1.40 bcd	6.53 ± 1.30 bcd	7.07 ± 1.16 abc	6.20 ± 1.26 b	6.40 ± 1.06 ab

Values are means of result from 15 panelists' ± standard deviation. Values in the same column carrying the same superscript are not significantly different according to Duncan's multiple range tests at ($p<0.05$).
Keys :
A-L = A-L are the yogurt samples

IV. Discussion

In the course of sample collection, it was also observed that many of the yogurts purchased from street vendors were not properly handled unlike the ones purchased from supermarkets which were provided with suitable environmental condition that supports the sustenance of their quality. The quality and integrity of yogurt can be checked using microbiological evaluation, and this has proven effective in ensuring the safety of the products (**Mbaeyi-Nwaoha and Egbuche, 2012**). The absence of NAFDAC number on some of this yogurt is an indication that the yogurt is not produced under standard environmental condition and the integrity of the yogurt is questionable. Yoghurt samples marketed in Akure metropolis have good physiochemical quality in terms of pH and total titratable acidity when compared to international and national standards, but the presence of high viable bacteria counts in the yogurt samples makes their quality and safety to be questionable.

Apart from the high microbial load of the yogurt samples, the isolation of pathogenic organisms like *Bacillus* spp, *Klebsiella* sp, *Serratia* sp, *Proteus* sp, *Listeria* sp, and *Escherichia coli* in some of the yogurt brands is an indication of either processing or post processing feacal contamination of the yogurt (**Agu *et al.*, 2014**), which could be from the raw materials (the milking animal), production lines production personnel, failure in any of the equipments used and contamination from sales personnel. The National Agency of Foods and Drugs Administration Control (NAFDAC) stipulated that *E.coli* and other coliforms generally must not be detectable in 100 ml of yogurt samples products (**Mbaeyi-Nwaoha and Egbuche, 2012).** The isolation of *Escherichia coli, Serratia* sp, *Klebsiella* sp, and *Proteus* sp from the yogurt samples, was not surprising considering the low level of hygiene and development involved in the production, distribution and handling of many of these yogurts. The presence of these organisms is of public health concern as these organisms have been implicated as the causal agents of one or more human diseases such as gastrointestinal discomfort or disorders, pneumonia, listerosis and others. Similarly, the isolation of fungi like *Mucor* sp, *Aspergillus* sp, and *Candida* sp is also a threat to the health of consumers as they have also been implicated as the causal agent of diseases such as aspergillosis, candidiasis and others.

It has been observed that the quality or integrity of a particular food sample can be determined by evaluating its sensory characteristic (**USDA, 2001**). Considering this, it was observed that some of the yogurts sample conformed to the specification of (**USDA, 2001**), and that of (**Childs and Drake, 2008**). As graded by fifteen (15) panelists, the mean score of the organoleptic properties of the yogurts samples were found to vary and differ from each other. These differences could be as a result of variation in the standardization of raw materials and manufacturing techniques employed (**Chandan and O'Rell, 2006**). However, it was also observed that some yogurt brands were not significantly different from each other. Color, texture and thickness of yogurt are important quality characteristics, but the flavor and taste of the product is generally considered the most critical and important indicator of consumer acceptance (**Olugbuyiro and Oseh, 2011**). As it had been earlier stated by (**Olugbuyiro and Oseh, 2011**) that low score in average overall acceptability is a function of flavor, taste and smell, the results obtained from this research conform to this statement.

V. Conclusion

Based on the results of this research, it can be concluded that the fifty percent (50%) of the yogurt samples bought from Akure city were still bellow stipulated quality standard in terms of their microbial and organoleptic qualities. This is because some of the yogurts do not possess adequate bacteriological and sensory quality which may be due to variations in conditions surrounding the formulation, production, packaging, distribution, storage and sales of the products. Therefore, the food regulatory agencies in charge of yogurt should intensify their effort in the evaluation of yogurts, to accurately ascertain its microbial and organoleptic quality.

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