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BACTERIOLOGICAL QUALITY OF FERMENTED MILK SOLD LOCALLY IN SAMARU AND SABONGARI MARKET, ZARIA – NIGERIA

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ABSTRACT
Ten (10) fermented milk samples (nono) from Samaru and Sabongari market were collected and mesophilic bacteria count in colony forming unit per ml (cfu/ml) using plate count method was carried out. The average aerobic mesophilic bacteria count obtained ranges from 2.0 x 10^7 to 2.23 x 10^3cfu/ml. Most probable number method was employed for presumptive coliform count and out of the 10 samples analyzed, 8 samples had less than 10MPN/ml coliform which indicated that they were satisfactory and safe for consumption while the remaining two (2) samples had MPN/ml coliform greater than 10, indicating that they are unsatisfactory and may constitute health hazard. Confirmatory and completed test also confirmed the presence of *E. coli* and *Klebsiella species.* The pH of the samples determined ranges from 3.61 to 4.01, the average titratable acidity and their percentage occurrence in the samples were also calculated. *Staphylococcus* species (100%), *Escherichia* species(90%), *Streptococcus* species(80%), *Bacillus* species (40%) *and Klebsiella* species(10%) were isolated in the nono samples. Methylene blue reduction test classified the nono into good (C, D, E, F), Fair (A,B,I,J) and Poor (G,H).Though, the coliform bacterial count was less in some samples, but nevertheless the presence of these bacteria identified is an indication that the fermented milk was feacally contaminated and this can pose a health hazard to the consumers of this fermented milk. As such, the government and authorities concern should enlighten the populace that is the nono sellers on the health hazard associated with unhygienic processing of this milk so as to safeguard the health of the people.

KEYWORDS: Quality, Fermented, Milk, Contamination, Bacteria

INTRODUCTION

Milk is often described as nature's most nearly preferred single food. It is that fresh, clean and normal mammary secretion obtained by milking of one or more dairy cows that is properly feed and kept. The cow milk is a good source of animal proteins, fats, vitamins and minerals to the human body. Nutritionally less useful substances like enzymes are also present in normal milk. Some of these enzymes are used as indices in screening or quality control test

Nono is local uncontrolled fermented cow milk which forms a major part of the staple food in Northern Nigeria. They are produced mainly by the nomadic Fulani. The fresh milk is directly obtained from a cow into a properly washed semi- dried calabash and kept wide open in the sun for approximately two hours to facilitate isolation of the fat layer. Some quantity of overnight fermented milk is added thereafter to serve as a starter culture and the inoculated fresh milk is left overnight at room temperature for fermentation to get sour milk known as "kindirmo" and the addition of large volume of water to the curdle sour milk which is then stirred with a T-shaped stick to a liquid of fine consistency gives rise to "Nono". The most commonly product often mixed with nono is called "Fura" (a dumping made of millet or maize) to make a preparation called "fura da nono".

Milk from udder of healthy cow contains very few microorganisms that gained entrance to the teeth canal through the teeth opening and are flushed and purged out mechanically during milking. However, if a disease condition prevails, bacteria may be present in large number. Though, it must be emphasized that their entrance to milk is gained in most cases during and after production. Charles (1986) reported that milk produced under unhygienic conditions or from unhealthy cows may result in illness to humans. Milk from the time it leaves the udder until it's dispersed into containers for processing. Everything it comes in contact with is a potential source of microorganisms. These include the air in the environment, the milking and storage vessels and the milking personnel.

Dairy by the Fulani is a family business operated at a subsistence level without any reference to quality control. Regardless of the method of preparation, the common practice among the female Fulani maiden hawkers is the addition of stream water and other products like the milky- white supernatant of water soaked baobab tree referred to as "Kuka" to the fermented product. These all they do to increase the volume of the product and equally an attempt to improve taste and colour. Investigation into the microbiology of nono revealed that the fermented product is basically a culture of *Lactobacillus bulgaricus* growing in association with *Streptococcus thermophilus.* The two organisms are mutually beneficial in the association with both organisms converting nearly the sugar to lactic acid producing only trace amount of bi- product. *S. thermophile* produces diacetyl and *L. bulgaricus* produces cetyldehyde (Schulz and Hingst, 1954). Milk being a perishable commodity demands a strict hygienic control because it provides nutrients and near neutral pH (6.6), preferred by many micro-organisms and can as well serve as a growth medium for many pathogenic opportunistic micro-organisms (Vasada, 1988*). S. thermophilus and L. bulgaricus are* the known starter in milk fermentation that must be abundant and viable in the final fermented milk where apart from the acid production, equally adds to the fermented milk a significant flavor (Shanker, 1980). Microflora and fermented milk are specifically known to be basic ingredients for fermentation. Pathogenic bacteria in milk have been a major public health problem due to the number of diseases caused by them (Grant *et al*., 1995). In view of the health hazard associated with the consumption of contaminated food, this study is aimed at evaluating the bacteriological quality of fermented milk (nono) within the study area in order to safeguard the health of the people.

MATERIALS AND METHODS

Sample collection

Nono samples were purchased randomly from Samaru and Sabongari market Zaria- Nigeria. Five samples from each market. About 250mls of the sample was collected in a sterile conical flask, stoppered and transported in ice- packed cooler to the laboratory with minimal delay and held at refrigeration temperature until needed for analysis.

Enumeration and isolation of bacteria

Nutrient agar was used in enumeration and isolation of bacteria as described by American Public Health Association (APHA, 1976).

Characterization of bacteria

The bacterial isolates were characterized based on the following biochemical tests: Grams stain, spore stain, coagulase, motility, indole, catalase, MR-VP and citrate utilization.

The pH of the samples was also analysed using model 97 pH meter. The presence or absence of gas bubbles in the samples was also determined physically by shaking the samples placed in universal bottles and observed.
The most probable number (MPN) method was used in order to estimate the number of coliform bacteria in the samples. The presumptive, confirmatory, and completed tests were also carried out to identify the type of coliform bacteria present in the samples. The results were compared with the World Health Organization Standard to determine whether the milk is safe for consumption or not.

RESULTS AND DISCUSSION

Table 1 shows the aerobic mesophilic bacterial count of the different nono sample sourced from Samaru and Sabongari market respectively. It also shows their various ranges and averages. However, the overall range of the aerobic mesophillic bacteria count is $2.0x10^7$ to $2.23x10^8$cfu/ml. Table 2 shows the presumptive, confirmatory and complete tests of the coliform bacteria analysis. Coliform bacteria are indicators of some degree of potentially hazardous contamination. According to subjected standard by World Health Organization, (2001), MPN/ml coli form less than 10, greater than 100 and the range of 500 to 2500 are regarded as satisfactory, unsatisfactory and grossly contaminated respectively. Therefore, some of the tested samples were bacteriologically safe and satisfactory for consumption in spite of the presumptive coliform count per ml value of the range from 2.5 to 4.5 while two samples from Sabongari were unsatisfactorily and may constitute a health hazard as the coliform may be the pathogenic members of the family *enterobacteriaceae.* The presence of gas bubbles in some of the samples may be an indication of gross contamination. It may be attributed to poor storage conditions, and contamination with coliform bacteria.

Table 1: Aerobic mesophilic bacterial count

Source of Sample	Sample	Bacterial count (cfu/ml)	
		Range	Average
Samaru	A	$2.5 \times 10^5 - 4.45 \times 10^8$	$2.23X\ 10^8$
"	B	$1.28 \times 10^6 - 9 \times 10^7$	$4.56X\ 10^7$
"	C	$6.0 \times 10^4 - 6.0 \times 10^7$	$3.00X\ 10^7$
"	D	$2.25 \times 10^5 - 4.80 \times 10^7$	$2.41X\ 10^7$
"	E	$1.15 \times 10^5 - 4.0 \times 10^7$	$2.0\ X\ 10^7$
Sabongari	F	$2.4\ X10^5 - 1.9\ X10^8$	$9.5\ X\ 10^7$
"	G	$1.1\ X10^6 - 3.5\ X10^8$	$1.76X\ 10^8$
"	H	$9.75X\ 10^5 - 2.75\ X\ 10^8$	$1.38X\ 10^8$
"	I	$2.95\ X\ 10^5 - 9.0\ X\ 10^7$	$4.5\ X\ 10^7$
"	J	$1.43\ X10^6 - 1.75\ X\ 10^8$	$8.8\ X\ 10^7$

KEY
A,B,C…….. J represent nono samples from Samaru and Sabongari market.

Table 2: Presumptive, confirmatory and completed coliform bacteria count

Sample Source	Sample	MPN/ml	Growth on EMBA	Growth on NA	Gram Reaction
Samaru	A	2.5	_	X	-ve rods
"	B	2.5	+	X	– ve rods
"	C	4.5	+	X	-ve rods
"	D	2.5	+	X	-ve rods
"	E	4.5	+	X	-ve rods
Sabongari	F	2.5	+	X	-ve rods
"	G	11.5	+	X	-ve rods
"	H	11.5	+	X	-ve rods
"	I	4.0	+	X	-ve rods
"	J	4.0	+	X	-ve rods

KEYS
- = dark pink mucoid colonies
+ = presence of green metallic sheen
X = presence of growth on nutrient agar slant
-ve = negative

Table 3: Shows the physicochemical parameters of "nono" samples which comprises of the average percentage titratable acidity, pH, gas bubbles and the methylene blue reduction test result. Many factors affect the methylene blue reduction test and therefore, the steps of operation should be uniform since the oxygen content must be used up before the colour disappears, any manipulation that increases the oxygen affects the test. Cold milk holds more oxygen than warm milk, pouring milk back and forth from one container to another increase the amount and at milking time much oxygen may be absorbed. The kind of organisms affects the rate of reduction. The coliform appear to be the most rapidly reducing organisms, closely followed by *Streptococcus lactis.* Some the Fecal streptococci and certain *Micrococci*, Thermoduric and psychrotrophic bacteria reduce methylene blue very slowly if at all; a large number of leucocytes affect the reduction time materially. Light hastens reduction and therefore the tests should be kept covered. The concentration of the dye should be uniform as an increased concentration lengthens the time of reduction, increasing the incubation temperature, augments the activity of the bacteria and therefore shortens the reduction time. The pH ranges from 3.81 to 4.01 and the average titratable acidity ranges from 0.72 to 1.10. This pH may limit the multiplication or survival of pathogens at ambient temperatures (Kornacki and Marth, 1982). This pH prevents the growth of pathogens. However, the organisms isolated were able to survive the pH because they are acidophiles and were able to withstand the pH

at that period of analysis. Any contamination at this pH could be attributed to poor use of contaminated sour milk as starter.

Table 4: shows the Gram and biochemical reactions of the isolates and their percentage occurrences. The organisms isolated are *Staphylococcus* spp (100%), *Bacillus* spp (40%), *Klebsiella* spp (10%), *Streptococcus spp*(80%) *and Escherichia spp*(90%). Among the genera of bacteria isolated, *Staphylococcus* spp was predominant in the sample tested. The contamination of the samples with this organism could have originated as a result of discharge into the atmosphere as aerosols by animals through sneezing or coughing or due to the manner in which the food is hawk and sold that continually predispose it to contamination. The presence of *Staphylococcus* spp in milk and manufactured dairy products have made them involved in an outbreak of *Staphylococcal* food poisoning which is an intoxication caused by *Staphylococcus aureus* in food (Park *et al.*, 1990). The predominant occurrence of *Staphylococcus* spp in nono samples (100%) agrees with what was reported in the studies carried out by Umoh, 1989 where 99% of "fura da nono" samples examined were contaminated with *Staphylococci.* Sample G and H had an unsatisfactory MPN/ ml of coliform bacteria while other samples were satisfactory. The range of mesophilic bacteria is 2.0 x 10^7 to 2.23 x10^8cfu/ml. It also shows the confirmatory and complete test for coliform bacteria which had *E. coli* and *Klebsiella* spp . The occurrence of *Klebsiella* spp (10%) which must have gained entrance into milk from the soil, grains and other places indicates nono contamination from the surrounding after milking.

Table 3: Physicochemical characteristics of the samples

Sample source	Sample	% Acid	pH	Gas bubble	MBRT	Time (hours)	Remark
Samaru	A	0.79	3.81	+	0	5	Fair milk
"	B	1.04	3.90	-	0	6	Fair milk
"	C	1.08	3.92	-	X	7	Good milk
"	D	0.72	3.79	-	X	7.30	Good milk
"	E	0.90	3.90	-	X	7.20	Good milk
Sabongari	F	1.10	4.01	-	X	7.40	Good milk
"	G	1.05	4.00	+	√	2	Poor milk
"	H	1.02	3.90	-	√	2	Poor milk
"	I	0.90	3.81	-	0	5	Fair milk
"	J	0.90	3.81	+	0	6	Fair milk

KEY
MBRT= Methylene Blue Reduction Test
+ = Present
- = Absent
X =Represent decolorized in less than 8hrs but not less than 6hrs
0 = Represent decolorized 6 and 5hrs
√ = Represent decolorized in 2hrs

Table 4: Bacteria isolated and their percentage occurrence

S/NO	Bacteria	% Occurrence
1	*Staphylococcus aureus*	100
2	*Bacillus* spp	40
3	*Klebsiella* spp	10
4	*Streptococcus* spp	80
5	*Escherichia* spp	90

CONLUSION
Considering the number of samples analysed, the fermented milk was grossly contaminated with aerobic mesophylls. Though, the coliform bacterial count was less, but nevertheless the presence of these bacteria identified is an indication that the fermented milk was feacally contaminated and this can pose a health hazard to

the consumers of this fermented milk. As such, the populace that is the nono sellers should be enlightened by the Government and authorities concern on the health hazard associated with unhygienic processing of this milk so as to safeguard the health of the people.

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