

Bacteriological Examination of Milk and Milk Products Sold in Jimeta Metropolis, Yola Adamawa State Nigeria

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Abstract

The purpose of this study was to evaluate the bacteriological quality of milk sold in Jimeta metropolis. The bacteriological quality of the milk sold in Jimeta metropolis was assessed by using turbidity test to know the degree of pasteurization of the milk, total bacterial count was undertaken by using pour plate method, most probable number technique was employed for the assessment of coliform bacteria. Bacterial contamination in the untreated milk was ascertained by using methylene blue reductase test. The results of the study revealed that samples for Arewa, Nazir and Admiral'a yoghurt passed the turbidity test because they showed a turbidity value of zero. Out of the nine (9) Kindirmo sample analysed, four (4) satisfied the turbidity test while that of Nono 5 samples satisfied the turbidity test. The plate count for Kindirmo ranges from 3.0×10^6 CFU/mL to 8.3×10^7 CFU/mL, Nono range from 3.5×10^6 CFU/mL to 8.9×10^7 CFU/mL and Madara ranges from 3.3×10^6 CFU/mL to 9.0×10^6 CFU/mL. For commercial yoghurt, plate count for Arewa yoghurt fall between 3.9×10^6 CFU/mL to 4.1×10^6 CFU/mL, Nazir yoghurt plate count ranges from 4.6×10^6 CFU/mL to 4.7×10^6 CFU/mL and Admiral 'a yoghurt ranges from 3.0×10^6 CFU/mL to 3.3×10^6 CFU/mL. The results of the study also indicated that the milks have high densities of coliform bacteria in kindirmo, nono and madara compared to the new standard that said a maximum amount of coliform bacteria should not be more than 10 bacteria per milliliter (mL) of milk. However, all the commercial yoghurt analysed satisfy the standard acceptable limit of coliform bacteria. Methylene blue reductase test revealed that all the untreated samples (Madara) decolorized methylene blue in less than 30min.

Keyword: Milk, Milk Products, Kindirmo, Nono, Madara.

Keywords: Bacteriological Examination, Coliform bacteria, Milk and Milk products.

I. INTRODUCTION

Milk and its products have high biological potential, enriched nutritional values and without health risks and hazards are generally demanded for nutritional purposes (12). The compositions of raw

milk differ by species, but significant amounts of saturated fats, calcium and protein as well as vitamin C is there in milk. Cow's milk is slightly acidic with pH ranging from 6.4 to 6.8. Milk is a food of outstanding interest; it has been drunk by humans since the earliest prehistoric times and still forms the basis of national economy. It was designed by nature, or more correctly evolved naturally, to be a complete food for very young animals. It has extremely high nutritional value and as a result of that cow's milk is not only a complete food for young calves but is also an excellent food for young children and valuable for adults (4). Milk is an interesting and complex colloidal system, the properties of which are of great importance in making butter and cheese (14). Long before microorganisms were discovered, the souring of sweet milk was practiced to preserve milk and to provide a new beverage with a distinctive and desirable flavour. Microbiologists have identified many of the specific organisms responsible for the desirable changes (6). Contaminated raw milk may act as a source of many harmful bacteria leading to various diseases, such as; salmonellosis, dysentery and tuberculosis. Raw milk with a bacteria count below a specified limit is known as "certified" milk and is considered healthy (1). However, milk contains relatively few bacteria when it leaves the udder of a healthy cow, and generally these bacteria do not grow in milk under the usual conditions of handling. However, *Micrococci* species and *Streptococci* species have been recovered from aseptically drawn milk (10).

In Nigeria especially in the Northern part of the country, commonly consumed milk and milk products are Kindirmo, Nono and fresh milk called Madara. Nono is sour milk produced from fresh milk. It can be called the traditional yoghurt. Nono is produced by allowing the fresh milk to stay overnight and the fat is schemed off. The difference between nono and kindirmo is that; the fat is not schemed off during the preparation of Kindirmo (13), (16). The consumption of nono especially in the Northern part of Nigeria has been on the increase, due to the fact that nono being locally fermented milk is relatively cheaper compared to the commercial yoghurt. The fermented milk is also highly nutritious and digestible which makes it a suitable food for the elderly and children (11).

II. MATERIALS AND METHODS

A. Sample Collection

Nine (9) samples each of Madara, Nono and Kindirmo were purchased at old market, new market and shopping complex in Jimeta metropolis. The samples were collected into sterile containers after purchase and packed into a cooler contained ice blocks and were conveyed to the microbiology laboratory, Modibbo Adama University of Technology Yola. Samples were refrigerated at 4 °C until used. Also, two (2) samples each of commercial yoghurts; Nazir, Arewa and Admiral yoghurt were also purchased at Yola new market and brought to the laboratory.

B. Turbidity Test

Twenty (20) ml of the well-mixed milk was poured into a 50 ml conical flask containing 4.0 g of Ammonium Sulphate (NH₄SO₄). The mixture was then shaken thoroughly for 1 minute to dissolve all the (NH₄SO₄). The mixture was allowed to stand for 5 min and filtered into a 100ml conical flask using What's man N0.1 filter paper. 5ml of clear filtrate was transferred into a beaker of boiling water for 5 min, it was then cooled in cold water and examined for turbidity using photometer of spectrophotometer device holding it in front of an electric light suitable shaded from the eyes (9).

C. Enumeration of Total Bacteria Count

This was done using the pour plate method; the samples were serially diluted by transferring 1ml of the sample into 9 ml of distilled water. Then serial dilution was carried out. Volume of 0.5ml of the most dilutions samples was introduced on to dry nutrient agar plates after preparation. The dilution was spread evenly over the agar surface with the sterilized glass spreader and was then incubated at 37°C for 24 hr. Using colony counter (Leica Quebec Darkfield Colon Counter), the total bacterial counts were expressed in colony forming unit per mL of the samples (17).

D. Coliform Count

This was done using the “Most Probable Number (MPN) technique” also known as the Multiple Tube Fermentation Test (MTFT). Volume of 1ml of the samples was transferred into 9 ml of distilled water and a serial dilution up to 10⁻⁵ was obtained. 1ml of each the five (5) dilutions was transfer into 5 tubes containing 9 ml of lactose broth. The tubes were incubated at 37°C for 24–48 hr. Presumptive positive tubes were recorded for any growth accompanied with gas production in the Durham’s tube. Presumption positive tubes were confirmed by sub-culturing sample from any tube showing gas production into a tube of brilliant green lactose bile broth. The tubes were then incubated at 37°C for 24–48hr gas production in the Durham’s tube confirmed the positive results (7).

$$MPN = \frac{NO \text{ of Positive tubes} * 100}{\sqrt{\text{vol of samples in nagetive samples in all tubes}}}$$

The above formula was used to calculate the number of coliform per 100 ml (3).

Methylene Blue Reduction (MBR) Test

Methylene blue reduction (MBR) test was carried out as described by (5). One milliliter of methylene blue solution (1:25000) was transferred into a sterilized 20 ml screw capped test tube containing 10 ml each of the samples. The tube was capped and gently inverted three times to mix the dye with milk sample. Each of the tubes were incubated at 37°C, in a water bath and examined after every 30 minutes for a period of 4 hr. The time taken for the methylene blue in the milk to become colourless was recorded (7).

III. RESULTS

Out of the nine samples of Kindirmo, four (4) satisfied the turbidity test and five (5) of the Nono samples satisfied the turbidity test. However, all the samples of commercial yoghurt for Arewa, Nazir and Admiral’ a yoghurt passed the turbidity test with a turbidity value of zero (0), this value showed that they are sterile.

Table 1: Turbidity Test for the Different Milk Samples

Samples	Positive	Negative
Kindirmo	4	5
Nono	5	4
Arewa Yoghurt	0	2
Nazir Yoghurt	0	2
Admiral’a Yoghurt	0	2

The result plate count for Kindirmo ranges from 3.0x10⁶ CFU/mL to 8.3x10⁷ CFU/mL; that of Nono ranges from 3.5x10⁶ CFU/mL to 8.9x10⁷ cfu/ml, while that of Madara ranges from 3.3x10⁶ cfu/mL to 9.0x10⁷ CFU/mL. The lowest plate count of the 3 types of milk was found in Kindirmo sample with 3.0x10⁶ CFU/mL and the highest was Madara samples with 9.0x10⁷ CFU/mL. The results also revealed that colony counts of samples collected from new market were higher while the colonies counts were less in samples collected from Old market (Table 2).

Table 2: Plate Count for Kindirmo, Nono and Madara

Source	Kindirmo CFU/mL	Nono CFU/mL	Madara CFU/mL
N. M I	8.3x10 ⁷	4.8x10 ⁷	3.7x10 ⁶
N. M II	6.3x10 ⁷	4.9x10 ⁷	3.3x10 ⁶
N.M III	5.9x10 ⁷	8.9x10 ⁷	3.5x10 ⁶

O.M I	5.9x10 ⁶	8.9x10 ⁶	3.1x10 ⁷
O. M II	8.6x10 ⁶	4.4x10 ⁶	3.3x10 ⁷
O. M III	3.3x10 ⁶	3.5x10 ⁶	9.0x10 ⁷
S. CI	3.0x10 ⁶	4.8x10 ⁷	5.4x10 ⁶
S. CII	5.4x10 ⁶	3.8x10 ⁷	5.6x10 ⁶
SC III	5.5x10 ⁶	3.5x10 ⁷	7.8x10 ⁶
Mean	2.57x10 ⁷	3.59x10 ⁷	2.04x10 ⁷

Key:
Cfu/mL: Colony forming Unit per milliliter.
N.M: New Market O.M: Old Market S.C: Shopping Complex

Plate count for Arewa yoghurt fall between 3.9x10⁶CFU/mL to 4.1x10⁶ CFU/mL with an average mean of 4.0x10⁶ CFU /mL; Nazir yoghurt plate count ranges from 4.6x10⁶ CFU/mL to 4.7x10⁶CFU/mL with average mean of 4.65x10⁶CFU/mL while that of Admiral ‘a yoghurt ranges from 3.0x10⁹CFU/mL to 3.3x10⁶CFU/mL with a mean of 3.15x10⁶ (Table 3)

Table 3: Plate Count for Commercial Yoghurts

Samples	(CFU/mL)		
	1 st	2 nd	Mean
A.Y	4.1x10 ⁶	3.9x10 ⁶	4.0x10 ⁶
N. Y	4.7x10 ⁶	4.6x10 ⁶	4.65x10 ⁶
ADY	3.0x10 ⁶	3.3x10 ⁶	3.15x10 ⁶

1.18x10⁷cfu/ml

Key:
CF/UmL: Colony-forming unit per millilitre,
AY: Arewa Youghut NY: Nazir Youghut ADY: Admineral’a Youghut.

The coliform count of the three types of milk indicated that there were high densities of coliform compared to the new standard that said a maximum amount of coliform bacteria should not be more than 10 bacteria per millilitre (mL) in milk. This showed that all the milk samples were contaminated with coliform bacteria (Table 4).

Table 4: Coliform Count for Kindirmo, Nono and Madara MPN/100mL

Sources	Kindirmo (Mpn/100mL)	Nono (Mpn/100mL)	Madara (Mpn/100mL)
N.M I	12.0	12.0	12.0
N.M II	11.0	12.0	12.0
N.M III	12.0	11.0	12.0
O.M I	11.0	4.0	11.0
O.MII	11.0	11.0	11.0
O.M III	11.0	11.0	12.0

S.CI	4.0	4.0	4.0
S.CII	4.0	10.0	10.0
S.C III	4.0	4.0	11.0

Key:
Cfu: Colony-forming Unit per milliliter
MPN: Most Probable number, N.M: New Market
O.M: Old Market, S.C: Shopping Complex
The commercial yoghurt analysed; Arewa, Nazir and Admiral’a Yoghurts have low coliform densities as shown in Table 5.

Table 5: Coliform Count for Commercial Yoghurt MPN/100mL

Samples	MPN/100mL		
	1	2	MEAN
AY	4.0	4.0	4.0
NY	4.0	3.0	3.5
AD.Y	3.0	3.0	3.0

Key: MPN: Most probable number AY: Arewa Youghut NY: Nazir Youghut ADY: Admineral’a Youghut.

Methylene blue reduction test showed that all the nine (9) sample of Madara analysed decolorized methylene blue in less than 30 min as described in Table 6.

Table 6: Methylene Blue Reductase Test for Madara in Minutes

Source	Madara (min)
N.M I	28
N.M II	27
N.M III	28
O.M I	25
O.M II	22
O.M III	24
S.C I	27
S. C II	26
S.C III	26

Key:
N.M: New Market
O.M: Old Market
S.C: Shopping Complex

IV. DISCUSSION

Fresh cow milk (Madara) as well as locally produced milk products; Kindirmo and Nono were highly contaminated with microorganisms. The highest bacterial plate count of Kindirmo was 8.3x10⁷ CFU /mL while that of Nono was 8.4x10⁷ cfu /mL.

The results obtained were higher than 6.7×10^6 CFU/mL reported (9) from locally processed nono milk samples and it is lower than 2.92×10^{10} CFU/100 mL to 3.2×10^9 CFU/100mL reported by (5). The high bacterial count of fresh milk (madara) (9.0×10^7 cfu/ml) may be due to the original microflora in the collecting bowl which may not be sterilized prior to milking as well as the milking personnel. The natural flora from the udder of the cows has been observed to contain 6.0×10^5 CFU/mL (15). The high bacterial count from “Kindirmo” and Nono may be due to the use of contaminated containers (calabash and plastic dishes) used for selling the products by hawkers, especially those from new market, where the containers used for selling the products have wide mouth opening making it easily accessible to flies and air microflora. In new market, the fresh cow milk and the two locally processed milk products were found to have high coliform densities (>11.0 MPN/100mL). It is likely that the handling manner and preparation process and utensils used could have influenced the increasing plate count results that were obtained. This is because (18) reported plate counts as high as those obtained in this study from food contacts surfaces and palms of food handlers. Madara is considered satisfactory because all the samples decolorized methylene blue in less than 30minutes (7). High coliform density in Nono and Kindirmo had earlier been reported by (2) who observed increase in coliform densities in the fermented products with high pH level. The pH may not be acidic enough so as to inhibit the proliferation of coliform bacteria. In the 9 samples, each of Kindirmo and Nono, 5 samples of Kindirmo failed to satisfy the turbidity test (that is, contained bacteria). Also for Nono, 4 out of the 9 samples did not satisfy the turbidity test. Arewa, Nazir and Admiral’a yoghurt conform to the turbidity test. This indicates that these products have been properly pasteurized (72°C for 15 sec). Most coliforms originate from the intestine of warm blooded animals, including people. Since most coliform bacteria are not harmful, the finding of coliforms in milk does not necessarily mean that a disease causing or pathogenic form of the bacteria is present. However, an elevated coliform count in milk and dairy products suggests that unsanitary conditions exist during production, processing or packaging (11). In the dairy farm setting, a coliform count is a useful indicator of sanitation and has been a common tool in public health protection for many years, for example, the presence of coliform is used as an indication of environmental contamination of milk product (5). The process of pasteurization easily kills coliforms in pasteurized products, this shows some level of sterility during product manufacturing or packaging. For milk sold raw, where no intervening pasteurization step is utilized, coliform counts reflects poor hygiene and sanitation and practices throughout milk handling, from the cow to final packaging, in addition to food safety and public health concerns,

coliforms, along with other bacteria, may produce off flavours in milk and reduce shelf life of dairy products.

V. CONCLUSION

This study revealed that commercial yoghurts analysed are fully pasteurized since they have a turbidity value of zero while kindirmo and nono fail to satisfy the turbidity test and hence they are not fully pasteurized. Also, there is bacterial contamination of the milk by coliform bacteria since all the samples of kindirmo, nono and madara have coliform counts greater than the standard limit of less than 10 coliform bacteria. An important source of microbial contamination of the milk is faecal pollution probably from cow’s dung. Furthermore, some of the microorganisms encountered may have direct consequence on dairy products and utilization. Most of the local Fulani’s who serve as principal suppliers of these products are ignorant of the level of microorganisms and contamination of these products. It is therefore recommended, that these milk handlers should adhere to good hygiene and environmental sanitation practices such; washing of cows teats, hands and other milking equipment adequately with a suitable antiseptic to prevent contamination of raw milk. Subsequently, milk should be stored in the aluminium cans recommended by the FAO (which are easier to clean), and in the absence of refrigeration, milk should be transported in ice packs and properly covered.

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