Original Research Article

Microbial Quality Assessment, Proximate, Vitamins, and Elemental Analysis of Fermented Milk Vended in Gombe Metropolis, Nigeria

Abstract:

Milk and milk products are highly nutritious substances and they are more prone to microbial attack and spoiled very easily. Fermented milk was analyzed to ascertain the level of contaminants and to also to quantify it is nutritional composition. Total aerobic and anaerobic bacteria contaminants as well as coliform bacteria in the fermented milk were determined. The proximate, vitamin and mineral concentration of the fermented milk were also ascertained. The pH, temperature, turbidity and acidity of the fermented milk were found to be 4.38±0.29, 10.26±1.7 °C, 6.22±4.89NTU, 0.16±0.04 respectively. The mean aerobic and anaerobic bacteria were $2.23x10^{9}$cfu/mL and $6.15x10^{3}$cfu/mL while the coliform count was 6.10±2.2MPN/100mL. However, the fermented milk analyzed was found to contain an appreciable amount of proximate constituent, vitamins and mineral element. Lack of cooling system in most of the study areas can significantly increase the pH and temperature of the milk and this will create a good environment for microorganisms which when fermented milk stay longer the bacteria will further ferment the milk and produce acid turn the milk sour.

Keywords: Proximate Analysis, Total Aerobic and Anaerobic bacteria, Coliform Count, Fermented Milk, Vitamin Analysis, Elemental Analysis

1. Introduction

Milk and milk products are important part of traditional food for Africans. Milk and its products are consumed in most of African countries including Nigeria. In Africa, the fermented milk and its products were produce using traditional and cultural methods, and fermentation of raw milk is one of the best methods of preserving the nutritional components of milk food [1]. Most traditional products are prepared without pasteurization by spontaneous fermentation, commonly initiated either by back slopping, i.e., inoculation of fresh milk with the spoiled one, or by repeated use of the same utensils in plastic or natural containers such as milk sacks, calabashes, clay pots, stone jars, and baskets [1, 2].

However, because fermented milk is highly nutritious, it is susceptible to microbial spoilage and different microorganisms such as bacteria and fungi can contaminate and spoiled fermented milk and its products. Microbial contamination of milk usually occurs either from the udder of the animal or within the udder of the animal; it can also occur from the surface of milking tools, and sometimes during handling and storage of milk [3]**.** Moreover, the health and hygienic conditions of the cow, the environments where the cows are kept and milked, and also the processes and methods used in cleaning and sanitizing the milking equipment as well as those used in milk storage, influence the number of microorganisms in milk [4]. Another important factor that leads to the deterioration of milk and provide good condition for microbial contamination of milk includes temperature and length of storage time of milk. Therefore, this study was designed and conducted to investigate the microbiological qualities of fermented milk as well as the nutritional composition of fermented milk sold within Gombe metropolis of Gombe state Nigeria.

2. Materials and Methods

2.1 Description of the Study Area

Gombe is a city in north eastern Nigeria (and a local government area) it is the capital city of Gombe state and has an estimated population of 261,536 people (2006 census) very old census. Gombe metropolitan has an area of 52sqkm and situated between latitude 9^0 30I and 12^0 30I N and longitude 8^0 45I and 11^0 45I E of the Greenwich meridian. It has two distinct climates, the dry season (November-March) and the rainy season (April–October) with an average rainfall of 850 mm. Gombe is a confluence of economic activities by its position as the meeting point for business people from the surrounding states. The states includes: Borno to the north, Yobe to the east, Taraba and Adamawa to the south and Bauchi to the west [5].

2.2 Source of Sample

For the purpose of this study, fermented milk was collected from three different markets within Gombe metropolis which includes Gombe main market, Tashan Dukku nono market, and Tashan Shongo nono market.

2.3 Sample Collection

Ninety (90) fresh milk samples were collected for the study, from each of the nono markets; thirty (30) samples were collected. After collection, the samples were transported to the laboratory in sterile corked plastic tubes packed in an iced container.

2.4 Physiochemical Analysis

Physiochemical analysis was conducted to determine the pH, temperature, turbidity and titratable acidity of the samples. This analysis was carried out according to the method described by AOAC, [6] **very old reference more updated is available up to 22nd edition.**

2.5 Determination of the Total Aerobic and Anaerobic Bacteria

Total aerobic and anaerobic bacterial count was determined by using a pour plate method as described by FAO, [7]. Serial dilution was conducted to obtain 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} dilutions and 1mL was transferred from each dilution into a duplicate petri dish labeled accordingly. 20-25mL of molten nutrient agar was added to the plates and the plates were allowed to solidify and then incubated at 37 °C for 24 hours aerobically and anaerobically. After incubation, plates containing 30-300 colonies were selected and the colonies were counted and recorded. The average was taking and the number obtained was multiplied by inverse of the dilution factor. This gives the number of colony forming units per mL of each sample (cfu/mL).

2.6 Determination of Coliform Bacteria

Coliform count was done using the "Most Probable Number (MPN) technique" also known as the Multiple Tube Fermentation Test (MTFT). In this method, the samples were serially diluted by transferring 1 mL of the sample into 9mL of distilled water. The dilutions were 10-fold (10x), 100-fold (100x) and 1000-fold (1000x). Fifteen tubes were groups into fives to make three (3) set. 1mL from each of the dilutions was transferred into first set of 5 tubes containing 9 mL of lactose broth and inverted Durham tubes; then 1ml was added to the second set of five tubes and then to the third set. The tubes were incubated at 37°C for 24 – 48hrs. 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 two loopful from any tube showing gas production into a tube of brilliant green lactose bile broth containing inverted Durham tubes. The tubes were then incubated at 37°C for

24 – 48hrs; gas production in the Durham's tube confirmed the positive results and the number of confirmed positives tubes were recorded. The Most Probable Number was determined by comparing the number of positives tubes with MPN index table [8].

2.7 Proximate Analysis

The amount of moisture, protein, fats, ash and carbohydrates present in the fermented milk was determined. The moisture, fat and ash contents of the fermented milk were determined loss in weight after drying to a constant weight as described by AOAC, [6]. The proteins content was determined by using Biuret method. The biuret method is based on the reactions that occur between cupric ions in the reagents and the peptide bonds of the protein molecules in alkaline solutions to form blue – violet or purple colored complexes. The absorbance of the color was measured using a calorimeter at 540 nm [9].

2.8 Mineral Elements Analysis

The presence of mineral elements such as Magnesium (Mg), Manganese (Mn), Calcium (Ca), Potassium (K) and Sodium (Na) in the fermented milk was ascertained by using methods described by Lawal and Adedeji, [10]. Two milliliters (2mL) of each sample was incinerated to a white ash at 550 °C in a muffle furnace for 3 hours, allowed to cool and the ash was washed into 250 mL beaker with deionized water and dissolved with a drop of HCl and filtered using Whatman no 1 filter paper. The volume was made up to 100 mL with deionized water. The minerals elements were determined using spectrometry method of Atomic Absorption Spectrophotometer (Buck 205) by Buck Scientific. Samples were aspirated and the mean signal response was recorded at each of the element respective wavelength.

2.9 Analysis of vitamins

The vitamins content of the fermented milk was determined using the official methods of the Association of Official Analytical Chemists [6] this reference is for Official methods of food analysis. The amount of vitamins such as vitamin A, vitamin C and vitamin E present in the fermented milk were analyzed by using UV/Visible spectrophotometer (GENWAY 63100).

2.10 Statistical Analysis

Analysis of variance protocol was used to confirm the significance level of the differences at ($P \leq 0.05$) in the mean values of the physiochemical parameters as well as the significance difference between fresh and fermented milk samples collected at different location in the study area.

3. Results

3.1 The Physiochemical Parameters

The results of physiochemical analysis showed that the pH of the fermented milk is acidic and it ranges from 4.19±0.03 to 4.59±0.27. Samples collected from Tashan Shongo nono market (TSM) has the highest temperature with 10.51±1.5 °C; while samples collected from Tashan Dukku nono market (TDM) has the lowest temperature obtained with 9.86±1.8 °C. The turbidity of fermented milk obtained ranges from 1.90±1.47 NTU to 12.07±2.75 NTU while the total titratable acidity of the fermented milk ranges from 0.15±0.03 to 0.18±0.04 as shown in Table 1.

3.2 Total Aerobic and Anaerobic Bacteria Count

The results aerobic and anaerobic bacterial count is described in Table 2. The results revealed that the aerobic bacteria in the fermented milk range from $6.53x10^8$ cfu/mL to $9.18x10^8$ cfu/mL. Also, the anaerobic bacteria count ranges from $3.60x10^3$ cfu/mL to $1.21x10^3$ cfu/mL.

3.3 Total Coliform Count

The coliform count in fermented milk samples is presented in Table 3; the results revealed a presence of coliform bacteria which ranges from 5.97±2.1 MPN/100 mL to 6.17±2.4 MPN/100mL. The overall mean coliform count is 6.10±2.2MPN/100mL.

3.4 Proximate Composition

Fermented milk has high moisture content, which ranges from 8.52±3.4mg/100mL to 51.53±12.0mg/100mL. The protein contents in fermented milk ranges from 1.58±0.9mg/100mL to 20.89±20.1mg/100mL with samples collected from TDM having the lowest protein contents Table 7. Fat content is highest in samples collected from TSM with 5.73±0.6mg/100mL and lowest in samples from GMM with 0.57±0.4mg/100mL. The ash content was found to range from 2.23±2.6mg/100mL to 2.94±2.6mg/100mL. While the carbohydrate contents of the fermented milk ranges from 40.76±14.2mg/100mL to 76.88±9.7mg/100mLas described in Table 4.

3.5 Vitamin Contents

Table 5 described the results of the analysis, the results revealed that values of vitamin A ranges from 47.48±22.1 IU/100mL to 75.23±25.2 IU/100mL. Vitamin C content of the samples ranges from 0.59±0.1mg/100mL to 0.76±0.2mg/100mL. The result also showed that fermented milk samples collected from TSM have the lowest concentration of vitamin C. However, the concentration of vitamin E ranges from 0.58±0.2mg/100mL to 0.77±0.3mg/100mL.

3.6 Mineral Constituents

Magnesium, manganese, calcium, potassium and sodium concentration of the samples are reported in Table 6 and the result is expressed mg/100mL. The magnesium concentration ranges from 15.00±20.1mg/100mL to of 23.53±21.9mg/100mL. Manganese concentration of fermented milk is highest in samples collected from Tashan Shongo Nono Market with 23.13±20.6mg/100mL and lowest in samples collected from Gombe Main Market with 16.37±20.4mg/100mL. The calcium concentrations of fermented milk range from 217.87±84.9mg/100mL to 251.65±79.1mg/100mL. The fermented milk has potassium concentration ranging from 181.47±15.5mg/100mL to 183.65±16.8mg/100mL. However, concentration of sodium ranges from 307.97±202.1 in GMM to 316.83±196.3mg/100mL in TSM samples.

Table 1: Results of the Physiochemical Parameters

S/N	Location	Samples Collected	pH	Temperature (^{o}C)	Turbidity (NTU)	Total Titratable Acidity
1	GMM	30	4.59±0.27	10.43±1.8	4.70±2.54	0.18±0.04
2	TDM	30	4.47±0.26	9.86±1.8	1.90±1.47	0.16±0.04
3	TSM	30	4.19±0.34	10.51±1.5	12.07±2.75	0.15±0.03
4	Total	90	4.38±0.29	10.26±1.7	6.22±4.89	0.16±0.04

Key: GMM = Gombe Main Market, TDM = Tashan Dukku Market, TSM = Tashan Shongo Market

Table 2: Total Aerobic and Anaerobic Bacterial Count in cfu/mL

S/N	Location	Samples Collected	Aerobic Bacteria count (cfu/mL)	Anaerobic Bacteria Count (cfu/mL)
1	GMM	30	$9.18x10^{8}$	$1.21x10^{3}$

2	TDM	30	$6.57x10^{8}$	$1.34x10^{3}$
3	TSM	30	$6.53x10^{8}$	$3.60x10^{3}$
4	Total	90	$2.23x10^{9}$	$6.15x10^{3}$

Key: GMM = Gombe Main Market, TDM = Tashan Dukku Market, TSM = Tashan Shongo Market

Table 3: Total Coliform Count of Fermented Milk MPN/100mL

S/N	Location	Samples Collected	Total Coliform Count
1	GMM	30	6.17±2.4
2	TDM	30	6.17±2.1
3	TSM	30	5.97±2.1
4	Total	90	6.10±2.2

Key: GMM = Gombe Main Market, TDM = Tashan Dukku Market, TSM = Tashan Shongo Market.

Table 4: Proximate Composition of Fermented Milk; it is expressed in mg/100mL

S/N	Location	Samples Collected	Moisture	Protein	Fat	Ash	Carbohydrate
1	GMM	30	51.53±12.0	4.28±3.8	0.57±0.4	2.94±2.6	40.76±14.2
2	TDM	30	15.50±8.9	1.58±0.9	3.73±1.3	2.31±2.8	76.88±9.7
3	TSM	30	8.52±3.4	20.89±20.1	5.73±0.6	2.23±2.6	62.54±21.3
4	Total	90	25.18±20.9	8.92±14.5	3.34±2.3	2.49±2.6	60.06±21.6

Key: GMM = Gombe Main Market, TDM = Tashan Dukku Market, TSM = Tashan Shongo Market.

Table 5: Vitamin Contents of Fermented Milk

S/N	Location	Samples Collected	Vitamin A (IU/100mL)	Vitamin C (mg/100mL)	Vitamin E (mg/100mL)
1	GMM	30	58.58±21.2	0.76±0.2	0.58±0.21
2	TDM	30	75.23±25.2	0.72±0.2	0.77±0.33
3	TSM	30	47.48±22.1	0.59±0.1	0.62±0.24
4	Total	90	60.43±25.4	0.69±0.2	0.66±0.27

Key: GMM = Gombe Main Market, TDM = Tashan Dukku Market, TSM = Tashan Shongo Market

Table 6: Mineral Constituents of Fermented Milk; it is expressed in mg/100mL.

S/N	Location	Samples	Magnesium	Manganese	Calcium	Potassium	Sodium

1	GMM	30	15.00±20.1	16.37±20.4	217.87±84.9	181.47±15.5	307.97±202.1
2	TDM	30	17.63±20.5	17.67±20.6	225.25±86.8	183.65±16.8	316.67±196.4
3	TSM	30	23.53±20.9	23.13±20.6	251.65±79.1	183.65±16.8	316.83±196.3
4	Total	90	18.72±20.6	19.06±20.5	231.59±83.9	182.92±16.2	313.82±196.1

Key: GMM = Gombe Main Market, TDM = Tashan Dukku Market, TSM = Tashan Shongo Market

4. Discussion

The study revealed that there is no significant difference in the pH values between the locations of sample collection. As pH measure the degree of acidity and alkalinity of sample of products, the study showed that all the samples are within the acidic range. The average pH of the fermented milk was found to be 4.4±0.21. This pH value obtained in this study is closely similar with the pH values by Lawal and Adedeji, [10] on their work Nutritional and elemental analysis of warankasi (fermented milk product) sold in Lagos metropolis. Analysis of temperature of the milk samples showed that there was significant difference in the temperature of the samples at 5% level of significant between the locations of collecting samples used in this study. The mean of temperature 10.26±1.7^{o}C obtained in this study is slightly higher than the 7-10^{o}C recommended by FAO, [7]**.** Higher temperature of 22.83±1.22 was reported by Gemechu *et al.*, [11] in their work on physical and chemical quality of raw cow's milk produced and marketed in Shashemene town, Southern Ethiopia. Lack of cooling system and inefficient use of refrigerator by milk sellers in the study area can increase the temperature of the milk and also increase the number of microbial contaminants [11]

Studies on the turbidity of fermented milk samples indicated that there was significance difference between locations of collecting samples. The lowest turbidity was found to be 1.90±1.47NTU. However, the samples fail to satisfy the turbidity standard value of zero (0) which said that milk is considered sterile when it shows no turbidity [7]. The mean total titratable acidity of 0.16±0.04 was found in the study. Lawal and Adedeji, [10] reported higher titratable acidity than those found in this study. Moreover, higher acidity in milk indicated that the milk was kept at room temperature for a longer period of time [11]. Higher acidity value (0.194 ±0.006) was also reported by Gemechu *et al.*, [11]**.** When there is high acidity in milk, the milk can become sour as a result of high accumulation of

lactic acid bacteria.

The average mean of aerobic bacteria present in the fermented milk sample was found to be $2.23x10^9$cfu/mL; this value significantly lower than $3.2x10^4$cfu/mL reported by Godic and Slavica, [12]**.** Nzabuheraheza and Nyiramugwera, [13] also reported $7.21x10^7$cfu/mL in traditionally fermented milk; this result is also higher than the result obtained in this study. The result is also in-line with result reported by Ogbonna, [14] on microbiological analysis and safety evaluation of nono: A fermented milk product consumed in most parts of Northern Nigeria. This low number in aerobic bacteria count may be due to either high microbiological quality of the milk under process and/or heating process in fermented milk. However, analysis on the anaerobic bacteria count revealed that there was no significance difference in the anaerobic count between the locations of collecting samples. The findings also revealed that the fermented milk samples have an average mean of $6.15x10^3$cfu/mL of anaerobic count. Analysis of the fermented milk samples for assessing the total number of coliform bacteria in the samples by means of coliform count showed that there was a significant difference between the locations of collecting samples. The average mean of coliform bacteria was found to be 6.10±2.2MPN/100mL. The low coliform count obtained in this study could be as a result of good hygienic method of milking practiced observed by the milk producers.

Moisture content is a measure of the water content in a product sample [10]. There was significance difference between the moisture content from the sample collected from the three locations of collecting samples. The fermented milk has a moisture content of 25.18±20.9mg/100mL. The result obtained is lower than 68.4% obtained by Lawal and Adedeji, [10]. According to Aworh and Akinniyi, [15] the moisture content accounts for the textural property of the product sample. The significant of moisture content in milk is that, high moisture content supports microbial activities and reduces the shelf life of the milk. Low moisture contents on the other hand, implies low water activities, low water activities causes reduction in microbial growth and the predominant microbial culture consequently increasing the shelf life of the milk samples as a result of low availability of water for microbial growth [16].

The protein content of milk is an essential feature of its market value since higher protein content

enhances performance of technological transformation [17]. There was significant difference in protein content between the locations of collecting sample. The results indicated that fermented milk have a mean protein of 8.92±14.5mg/100mL. This low protein may be because most of the proteins are destroyed by heat during pasteurization of the milk. The result is lower than the result obtained by Lawal and Adedeji, [10]**.** These values were higher than that found by Ahmad *et al.,* [18] in raw milk and Gemechu *et al.,* [11].

The fat contents from various locations were found have a significant difference. The average mean obtained is 3.34±2.3mg/100mL and it is lower than those reported by Lawal and Adedeji, [10] and Gemechu *et al.,* [11]. Low fat concentration obtained in this study may result from the fact that during fermentation of milk most fats are removed. Hence the amount of fat present the fermented milk is usually less than those present in the fresh milk.

The study shows that there was no significant difference in ash contents between the locations of collecting sample. The mean ash content for fermented milk samples was found to be 2.48±1.9mg/100mL. This value is lower than the result reported by Lawal and Adedeji, [10]. The value is higher than the value reported by Ponka *et al.,* [17]; Gemechu *et al.,* [11]. The amount ash present in a particular sample can determine the amount of mineral element available in that sample.

The carbohydrate content in the sample was obtained by subtracting from 100 the percentage of moisture, protein, fat and ash. The average carbohydrate content is 47.59±20.9mg/100mL which is higher than those reported by Lawal and Adedeji, [10] and higher than those reported by Ajai *et al.,* [16]. Significantly, carbohydrate provides energy to the body [10].

Vitamin A and E concentration of the samples between the locations of collecting samples were remarkably different. The results of the analysis also showed that the fermented milk samples have an average mean of 60.43±25.4IU/100mL. This value is significantly lower than 264.5IU/100mL reported by Yasmin *et al.,* [19]. Vitamin A is important in normal vision, gene expression, growth and immune function by its maintenance of epithelial cell functions [20]. The result also showed that there was significant difference in the vitamin C content between the locations of collecting samples. The results of the analysis indicated that an average mean of 0.69±0.2mg/100mL for fermented milk samples. The

concentration of vitamin C obtained in this study is lower than those reported by Yasmin *et al.,* [19]. Vitamin C is a potent antioxidant that facilitates the transport and uptake of non-heme iron at the mucosa, the reduction of folic acid intermediates and the synthesis of cortisol. It is deficiency of vitamin C fragility to blood capillaries, gum decay and scurvy [20]. The result showed 0.66±0.27mg/100mL concentration of vitamin E in fermented milk. The result obtained in this study is higher the result reported by Yasmin *et al*., [19]. Vitamin E is a powerful antioxidant which helps to protect cells from damage by free radicals and it is vital to the formation and normal function of red blood cell and muscles [21].

There was no significant difference in the mineral contents of the fermented milk analyzed between the locations of collecting sample. Magnesium concentration of fermented milk has a mean of 18.72±20.6mg/100mL. The value obtained was lower than the value reported by Ponka *et al.,* [17] and lower than 12.7-13.7mg/100mL reported by Ajai *et al.,* [16]**;** and higher than 5.12mg/100mL reported by Lawal and Adedeji, [10]. There is an overall mean of 251.40mg/100mL of calcium content. This value is lower than the value reported by Ajai *et al.,* [16] which ranged between 2000.10mg/100mL to 2830.50mg/100mL. The overall average mean of the potassium (K) content is 159.86mg/100mL. This value was lower than the value reported by Ajai *et al.,* [16] in Minna Nigeria which ranged between 1065.50mg/100mL to 1611.44 mg/100mL. The overall average mean of sodium content is 243.69mg/100mL. This value is lower than the value 293±49.39mg/100mL reported by Ponka *et al.,* [17] and higher than 2.23mg/100mL reported by Lawal and Adedeji, [10].

5. Conclusion

The fermented milk analyzed was found to contain high numbers of aerobic and anaerobic bacteria and this high in number may result from lack of cooling system and lack of good storage condition. However, in spite of that, the fermented milk examined contained an appreciable amount of vitamin A, vitamin E and vitamin C. They also contained elements in good quantity of calcium, potassium, sodium, and magnesium but least in manganese although within the recommended values. In terms of proximate composition it was found to contain abundant moisture, protein, carbohydrate, ash and fat.

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