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MICROBIAL QUALITY ASSESSMENT, PHYSIOCHEMICAL ANALYSIS AND NUTRITIONAL COMPOSITION OF FRESH MILK

***M. Bashir[1], H. K. Sambo[2], I. Ishaq[3], M. K. Adamu[4]**

[1]Department of Microbiology, Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria
[2]Department of Microbiology, Bauchi State University, Gadau, Bauchi State, Nigeria
[3]National Biotechnology Development Agency, Jalingo, Taraba State, Nigeria
[4]Department of Biology, State Collage of Education Hong, Adamawa State
***Correspondence Author: mbash1460@gmail.com, +2348063349281**

ABSTRACT

This study was designed to evaluate the microbial quality, physiochemical and nutritional composition of fresh milk samples. Ninety (90) fresh milk samples were collected and analyzed to enumerate the number of aerobic and anaerobic mesophilic bacteria, coliform bacteria, physiochemical parameters and also the nutritional composition of fresh milk samples. The mean of the pH, turbidity and titratable acidity were found to be 6.69±0.89, 10.68±5.28 and 0.15±0.03 respectively. The mean values for aerobic and anaerobic bacteria were found to be $1.99x10^{9}$cfu/mL and $7.62x10^{3}$cfu/mL respectively. The total coliform bacteria in the sample were found to have an overall mean of 7.22±4.6MPN/100mL. The proximate contents were found to be 1.07±0.2, 51.38±11.2, 9.47±1.4, 2.46±0.5 and 35.12±9.9 for moisture, protein, fat, ash and carbohydrates respectively. Vitamin A, C and E concentration of the milk samples was 169.45±24.3, 1.71±0.4 and 0.33±0.20 respectively. The elements presents in the samples includes Magnesium, Manganese, Calcium, Potassium and Sodium and they were found to be 15.41±2.0, 0.07±0.03, 270.49±22.3, 136.79±14.6 and 173.56±178.2 respectively. Proper sterilization and/or storage condition significantly increased the purity and shelf life of milk and can free milk and milk product from pathogenic microorganisms.

Keywords: Microbial, Quality, Assessment, Physiochemical, Nutritional, Fresh, Milk

INTRODUCTION

Milk has been part of the human diet for millennia and is valued as a natural and traditional food. Milk and dairy foods are considered to be one of the main food groups important in a healthy balanced diet, and as such feature in the majority of national food-based dietary guidelines from the British Eatwell and Australian plate model to the Chinese Pagoda and the Japanese Spinning top, the US pyramid, the Guatemalan pot and many others. As milk provides a substantial amount of vitamins and minerals in relation to its energy content, it is considered a nutrient dense food (Drewnowski, 2010).

Cow milk provides a wide range of essential nutrients to the diet. Milk is often recognized as a source of calcium and it is perhaps less commonly known that milk and milk products are also an important source of good quality protein, vitamin and minerals (FSA, 2002). The nutritional composition of milk makes it not only suitable for human nutrition but also ideal for microbial life. Outbreaks of milk-borne diseases have occurred despite pasteurization, as a result of either improper pasteurization or product re-contamination (Ogbonna, 2011).

Milk contamination by microorganisms generally occurs from three main sources; from within the udder, from the exteriors of the udder, and from the surfaces of milk handling and storage equipment's (Bramley and Mckinnon, 1990). Moreover, Murphy and Boor (2000) reported that the health and hygiene of the cow, the environment in which the cow is housed and milked, and the procedures used in cleaning and sanitizing the milking and storage equipment, all influence microbial numbers in milk. They also added that, temperature and length of storage time are important factors that may allow microbial contamination to reproduce. Hence, this study was design to assess the microbial quality of fresh milk, its physiological parameters as well as the nutritional composition of fresh milk.

MATERIALS AND METHODS

Source of Sample

Fresh milk samples were collected for this study and the samples were collected from three different nono markets within Gombe metropolis namely: Gombe main market, Tashan Dukku market and Tashan Shongo market.

Sample Collection

A total of ninety (90) fresh milk samples were collected in sterile corked plastic tubes in an ice container from the three different markets within Gombe metropolis using systematic random sampling as described by Patton (1990) and transported to the laboratory.

Physiochemical Analysis

The physiochemical parameters such as pH, temperature, turbidity and total titratable acidity present in the samples were determined according to the method described by AOAC, (1990) and FAO, (2010).

Enumeration of Total Aerobic and Anaerobic Mesophilic Bacteria in Fresh Milk Samples

Total aerobic and anaerobic mesophlic bacterial count was determined by using a pour plate method as described by FAO, (2010).

Enumeration of Coliform Bacteria in Fresh Milk Samples

Coliform count was conducted using the "Most Probable Number (MPN) technique" The Most Probable Number was determined by comparing the number of positives tubes with MPN index table as described by APHA (1992).

Determination of Nutritional Composition of Fresh Milk Samples

Proximate Analysis

The level of moisture, protein, fat, ash and carbohydrate in the fresh milk samples were determined using the method of Association of Official Analytical Chemist (AOAC, 2005). The moisture contents were determined by weight difference before and after drying 5mL of the samples at 105^0C for 3hours in oven. The protein content was ascertain using biuret method which determine the reactions that occur between cupric ions in the reagents and peptide bonds of the protein molecules in an alkaline solutions to form blue-violet colored complexes which can be measured using colorimeter at 540nm. Fat was obtained by intermittent extraction with petroleum ether. The amount of ash present in the sample was determine from the residue left after incineration of 5mL of the sample at for 3hours at 550^0C in a furnance. The carbohydrate contents of the sample were determined by subtracting from 100 the sum the percentage of moisture, protein, fat and ash.

Analysis of Vitamins Content of Fresh Milk Sample

The amount of vitamins in the fresh milk samples such as vitamin A, vitamin C and vitamin E present in the samples were analyzed by using UV/Visible spectrophotometer as described by AOAC, (1990).

Analysis of Mineral Elements in the Samples

The amount of mineral elements such as Mg, Mn, Ca, K, Na present in the samples were determined by using spectrometry method of Atomic Absorption Spectrophotometer (AAS) (Buck 205) by Buck Scientific. Samples were aspirated and the mean signal response was recorded at each of the element respective wavelength as reported by Lawal and Adedeji, (2013).

Statistical Analysis of the Data

Analysis of variance protocol was used to confirm the significance level of the differences at ($P<0.05$) in the mean values of aerobic and anaerobic bacteria, coliform bacteria, physiochemical parameters and the nutritional components of the samples as well as the significance difference between the location of sample collection in the study area.

RESULTS AND DISCUSSION

Table 1 showed the results of the physiochemical analysis of the samples and the result showed that the pH of the samples range from 6.69±0.08 to 6.70±0.09. The result showed that all the samples were in the acidic range of the pH value. Similar pH values were reported by Lawal and Adedeji (2013). However, fresh cow milk has a pH value ranges from 6.6±0.11 to 6.8±0.09 (O'Connor, 1995; FAO, 1999). The results also showed that there was significant difference in the pH values between the locations of sample collection. This may be as results of differences in both milking and processing procedures between the three locations of sampling.

The results also showed that the average temperature of fresh milk samples is 21.35 ± 1.9^0. The result also showed that there was significant difference in the temperature of the samples at ($P < 0.05$) between the locations of collecting samples. The mean temperature 21.35 ± 1.9^0C of the fresh milk samples collected for this study is less than 22.83 ± 1.22^0C reported by Teshome *et al.,* (2015). Fresh milk samples obtained from Tashan Shongo market was significantly higher in temperature than those obtained from Gombe main market and Tashan Dukku market. Lack of cooling system and inefficient use of refrigerator by milk sellers increased the temperature of the milk. This could contribute to the increased number of microbial contaminants (Teshome *et al.,* 2015).

The turbidity of the fresh milk samples ranges from 5.70±2.02 NTU to 16.57±3.56 NTU. Studies on the turbidity of fresh milk samples indicated that there was significance difference ($P < 0.05$) in the turbidity of fresh milk samples between the locations of collecting samples. Samples collected from Tashan Shongo nono market have the highest turbidity with 16.57±3.56 while

Tashan Dukku market has the lowest turbidity with 5.70±2.02. However, fresh milk samples fail to satisfy the turbidity standard value of zero (0) which said that; milk is considered sterile when it shows no turbidity (FAO, 2010).

However, fresh milk samples collected from Tashan Dukku market has the peak of acidity 0.17±0.03 and those collected from Gombe main market has the lowest acidity value with 0.12±0.02. The result of the analysis showed that there was significant difference in the total titratable acidity between the locations of sample collection. The fresh milk samples have an overall titratable acidity of 0.15±0.03 with TDM having the highest with 0.17±0.03 while the GMM has the lowest with 0.17±0.03. However, when the total titratable acidity is greater than 0.16±0.04 indicated that the milk samples were kept at room temperature for a longer period of time and it indicated a poor handling (Teshome *et. al.*, 2015). Higher acidity value (0.194 ±0.006) was reported by Teshome *et al.*, (2015). Asaminew and Eyassu (2011) reported a higher acidity for milk samples collected individual farmers (0.023 ± 0.01). Similarly Zelalem and Faye (2006) also reported higher acidity (0.27).

Table 1: The Mean Physiochemical Parameters of the Fresh Milk Samples

Location	No. of Samples	pH	Temperature (^{0}C)	Turbidity (NTU)	Total Titratable Acidity
GMM	30	6.69±0.11	20.45±1.3	9.77±2.53	0.17±0.03
TDM	30	6.69±0.08	20.97±2.0	5.70±2.02	0.17±0.03
TSM	30	6.70±0.09	22.62±1.7	16.57±3.56	0.16±0.02
Mean	90	6.69±0.89	21.35±1.9^{0}C	10.68±5.28	0.15±0.03

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

The result in table 2 showed a wide range of aerobic and anaerobic bacteria in fresh milk. There was a significant difference in the total aerobic bacteria between the locations of sampling. The aerobic plate counts of fresh milk range from 5.18x10^{8}CFU/mL to 8.73x10^{8}CFU/mL with Gombe main market having the highest number of aerobic plate count while Tashan Dukku market has the lowest aerobic mesophilic plate count. The average mean is 1.99x10^{9}CFU/mL; this value significantly higher than 3.2x10^{4}CFU/mL reported by (Godic Torkar and Golc Teger, 2008) and 5.5x10^6 CFU/mL reported by Aaku *et al*., (2004). This high number in Aerobic mesophilic plate count may be due to either low microbiological quality of the milk under process and/or contamination after heating process in fermented milk (Godic and Slavica 2008). However, if these types of milk are consumed without taking proper attention to pasteurization then there will be a serious health problem.

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 revealed that fresh milk samples have the highest number of anaerobic bacteria in samples collected from Gombe Main Market with $2.65x10^3$cfu/mL and lowest in those collected in TDM with $2.33x10^3$cfu/mL. However, all the samples analyzed falls within the recommended/ legal limit of pasteurized and non-pasteurized milk which is $2.0x10^4$cfu/mL and $1.0x10^5$cfu/mL respectively (FDA, 2007).

Table 2: Total Aerobic and Anaerobic Mesophilic Bacterial Count of the Fresh Milk Samples expressed in (CFU/mL)

Location	No. of Samples	Aerobic Bacteria Count	Anaerobic Bacteria Count
GMM	30	$8.73x10^8$	$2.65x10^3$
TDM	30	$5.18x10^8$	$2.33x10^3$
TSM	30	$6.06x10^8$	$2.64x10^3$
Total	90	$1.99x10^9$	$7.62x10^3$

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

The total coliform count in the fresh milk samples collected from Tashan Shongo Market is significantly higher than those collected from Gombe Maim Market and Tashan Dukku Market. The coliform count ranges from 4.93±2.3MPN/100mL to 10.07±4.9MPN/100mL with an overall mean of 7.22±4.6MPN/100mL (Table 3). The average mean obtained is greater than 6.57±6.4MPN/100mL reported by Okeke *et al.*, (2014). The high coliform count obtained in this study could be a result of poor hygienic practice during milking by local milk producers.

Table 3: Result of the Total Coliform Count of the Fresh Milk Samples expressed in MPN/100mL

Location	No. of Samples	Total Coliform Count
GMM	30	6.67±4.5
TDM	30	4.93±2.3
TSM	30	10.07±4.9
Total	90	7.22±4.6

Standard acceptable value: Not exceed 10 MPN/100mL APHA (1992)
Key: Gombe Main Market (GMM), Tashan Dukku Market (TDM), Tashan Shongo Market (TSM)

The result of proximate analysis is described in table 4; the moisture content in the samples ranges from 0.86±0.2mg/100mL to 1.24±0.2mg/100mL. The result also revealed that the moisture has an overall mean of 1.07±02. Moisture content is a measure of the water content in a product sample; there was significance difference in the moisture content between the locations

of collecting samples. The significant of moisture content in milk is that, high moisture content implies high water activity which supports microbial growth consequently reducing the shelf life of the milk sample. 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 (Ajai, *et al.,* 2012).

There was significant difference in protein content between the locations of collecting sample. The sample has the highest protein contents of 52.50±9.9mg/100mL and lowest value of 49.58±13.9mg/100mL; samples collected from Tashan Dukku market have the lowest protein contents. The milk protein is an essential feature of its market value since higher protein content enhances performance of technological transformation Ponka *et al.,* **(**2013). The results indicated that the fresh milk samples have a mean average of protein with 51.38±11.2mg/100mL. The results obtained in this study agree with the work carried out by of Lawal and Adedeji (2013). The values obtained in this study were higher than those found by Ahmad *et al*., (2008) in raw cow milk; this may be as a result of differences in the types of food used to feed the animals.

The fat content was found to be significantly different between the locations of collecting samples. The fat content is highest in samples collected from Tashan Shongo market with 9.67±0.9mg/100mL and lowest in Gombe main market with 9.27±1.9mg/100mL. The fresh milk samples have average fat contents of 9.47±1.4mg/100mL. According to European Union quality standards for unprocessed whole milk, fat contents should not be less than 3.5mg/100ml (Tamime, 2009). Consequently, percentage of fat obtained in this study fall within the recommended standard. The percentage fat content obtained is higher than those reported by Teshome *et al.,* (2015).

The ash contents of the samples ranges from 2.36±0.5mg/100mL to 2.52±0.5mg/100mL. The study shows that there was no significant difference in ash contents between the locations of collecting sample. Samples collected from Gombe main market have the highest ash content with 2.52±0.5mg/100mL and those collected from Tashan Shongo market has the lowest ash contents with 2.36±0.5mg/100mL. The mean ash content for the fresh milk samples was 2.46±0.5mg/100mL. This value is lower than 7.2mg/100mL reported by Lawal and Adedeji (2013). However, the value is higher than 0.63±0.07 reported by Ponka *et al.,* **(**2013); and 0.65 reported by Sanz Ceballos *et al.,* (2009). The amount of ash present in the milk sample determine

the mineral elements that could be found in the milk samples, low concentration of ash indicated that there is low concentration of minerals elements in the milk samples.

The result of carbohydrate content showed that samples collected from Tashan Dukku market have the highest carbohydrate contents with 36.10±10.4mg/100mL and it is lowest in samples collected from Gombe Main Market with 34.60±10.4mg/100mL. The Average carbohydrate content of the samples is 35.12±9.9mg/100mL which is higher than 4.27mg/100mL reported by Lawal and Adedeji (2013) and also higher than the value reported by Ajai, *et al.,* (2012) which ranged from 9.10mg/mL to 22.27mg/100mL. Significantly, carbohydrate is essential to the body by providing energy needed by the body for its metabolic activities.

Table 4: The Mean Proximate Contents of the Fresh Milk Samples; Expressed in mg/100mL

Location	No. of Samples	Moisture	Protein	Fat	Ash	Carbohydrate
GMM	30	1.11±0.2	52.50±9.9	9.27±1.9	2.52±0.5	34.60±10.4
TDM	30	0.86±0.2	49.58±13.9	9.48±1.4	2.51±0.5	36.10±10.4
TSM	30	1.24±0.2	52.06±9.5	9.67±0.9	2.36±0.5	34.67±9.4
Total	90	1.07±0.2	51.38±11.2	9.47±1.4	2.46±0.5	35.12±9.9

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

The results of the analysis for vitamin showed that the fresh milk samples have significant quantity of vitamin A and C with little amount of vitamin E. the result of vitamins analysis is described in table 5. Vitamin A concentration of the samples between the locations of collecting samples was remarkably different. The result revealed that vitamin A concentration ranges from 156.23±19.7 IU/100mL to 179.78±26.8 IU/100mL. The mean concentration of vitamin A obtained is 169.45±24.3 IU/100mL. This value is significantly lower than 264.5IU/100mL reported by Yasmin *et al.,* (2012). Vitamin A is important in normal vision, gene expression, growth and immune function by its maintenance of epithelial cell functions (Achikanu *et al*., 2013).

The result showed that there was significant difference in the vitamin C content between the locations of collecting samples. The result of the analysis also showed that milk samples collected from Tashan Shongo market have the lowest concentration of vitamin C. The results of the analysis indicated that fresh milk samples have high vitamin C concentration with 1.85±0.4mg/100mL in milk samples collected from Tashan Dukku market and lowest in those

collected from Tashan Shongo market with 1.59±0.3mg/100mL. The mean 1.71±0.4mg100mL concentration of vitamin C obtained in this study is lower than those reported by Yasmin *et al.*, (2012) which ranged from 4.43mg/100mL to 4.88mg/100mL. 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. Deficiency of vitamin C causes fragility to blood capillaries, gum decay and scurvy (Achikanu *et al*., 2013).

The vitamin E content of the fresh milk samples was found to have a mean average of 0.33±0.20mg/100mL. The result obtained in this study is higher than the result reported by Yasmin *et al*., (2012) which ranged from 0.041mg/100 g to 0.226mg/100mL. The result also showed that there was no significant difference in the vitamin E content obtained between the locations of collecting samples. The results also revealed that the concentration of vitamin E ranges from.32±0.2mg/100mL to 0.34±0.20mg/100mL.very importantly, 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 (Lukaski, 2004).

Table 5: The Mean Values of Vitamin A, C and E Concentration of the Fresh Milk Samples

Location	No. of Samples	Vitamin A IU/100mL	Vitamin C mg/100mL	Vitamin E mg/100mL
GMM	30	156.23±19.7	1.69±0.3	0.32±0.20
TDM	30	179.78±26.8	1.85±0.4	0.34±0.20
TSM	30	172.33±20.3	1.59±0.3	0.33±0.21
Total	90	169.45±24.3	1.71±0.4	0.33±0.20

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

The mineral element presents in the fresh milk samples were analyzed and reported in table 6. The concentration of magnesium in the fresh milk samples ranges from 15.07±2.0mg/100mL to 15.63±2.1mg/100mL. Magnesium concentration between the locations of sampling did not differ significantly. The result of the analysis showed that fresh milk samples has average mean of 15.41±2.0mg/100mL. The value obtained was lower than 103±14.63mg/100mL the value reported by Ponka *et al.*, **(**2013). This result is higher than 5.12mg/100mL reported by Lawal and Adedeji (2013).

Samples obtained from Tashan Dukku market have the highest manganese content with 0.08±0.03mg/100mL while samples collected from Gombe main market and those collected from Tashan Shongo market have manganese concentration of 0.07±0.03mg/100mL. The

average of the manganese concentration of all the samples was 0.07±0.03mg/100mL, the value lower than 12.7-13.7mg/100mL reported by Ajai, *et al.,* (2012).

The amount of Calcium in the milk samples ranges from 267.47±21.9mg/100mL to 274.28±23.8mg/100mL. The result of the analysis showed that there is no significant difference in calcium content between the sampling locations at $P < 0.05$. However, the calcium content has an overall mean value of 270.49±22.3mg/100mL of calcium content. This value is lower than the value reported by Ajai, *et al.,* (2012) which ranged between 2000.10mg/100mL to 2830.50mg/100mL. The values are also lower than the values reported by Dirienzo, (2001) in Whey and milk products which ranged between 500mg/100mL to 2000mg/100mL.

The result also revealed Potassium concentration of the samples ranging from 136.35±15.4mg/100mL to 137.67±13.4mg/100mL. Both the samples obtained from Tashan Dukku market and those obtained from Tashan Shongo market have 136.35±15.4mg/100mL which is lowest value of potassium (K) concentration of the fresh milk samples. The overall average mean of the potassium (K) content is 136.79±14.6mg/100mL. This value was lower than the value reported by Ajai, *et al.,* (2012) which ranged between 1065.50mg/100mL to 1611.44 mg/100mL. The low value obtained may results from the type of feed used by the animals.

Sodium concentration ranges from 153.50±165.7 mg/100mL to 184.00±184.3mg/100mL with Tashan Dukku Market having the highest concentration of sodium in the fresh milk samples. The overall average mean of sodium content is 173.56±178.2mg/100mL. This value is lower than the value 293±49.39mg/100mL reported by Ponka *et al.,* **(**2013). This value is higher than 2.23mg/100mL reported by Lawal and Adedeji (2013).

Table 6: The Mean Value of the Mineral Elements Presents in the Fresh Milk Samples Expressed in mg/100mL

Location	No. of Samples	Magnesium	Manganese	Calcium	Potassium	Sodium
GMM	30	15.53±2.0	0.07±0.03	274.28±23.8	137.67±13.4	153.50±165.7
TDM	30	15.63±2.1	0.08±0.03	267.47±21.9	136.35±15.4	183.17±188.1
TSM	30	15.07±2.0	0.07±0.02	269.72±21.4	136.35±15.4	184.00±184.3
Total	90	15.41±2.0	0.07±0.03	270.49±22.3	136.79±14.6	173.56±178.2

Key:
Gombe Main Market (GMM)
Tashan Dukku Market (TDM)
Tashan Shongo Market TSM)

CONCLUSION

The study revealed that fresh milk samples analyzed contained physiochemical parameters that are in-line with the internationally acceptable limit. However, the number of aerobic and anaerobic mesophilic bacteria found in this study is greater than $1.0x10^5$cfu/mL and $2.0x10^4$cfu/mL which is recommended by FDA (2007). The total coliform bacteria was found be within the acceptable limit of not exceeding 10 MPN/100mL as recommended by APHA (1992). The result of the study indicated that the fresh milk is safe for drinking in terms of coliform count although measures need to be taking in order to ensure the supply of good and hygienic milk for consumption by public by adopting aseptic techniques during milking and/or processing of the milk.

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