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PHYSICO-CHEMICAL PROPERTIES AND MICROBIAL LOAD OF RAW COW MILK OF JIMMA CITY, ETHIOPIA

BY: TESFAYE TADESSE

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PHYSICOCHEMICAL PROPERTIES AND MICROBIAL LOAD OF RAW COW MLK OF JIMMA CITY, ETHIOPIA

.

BY: TESFAYE TADESSE

ADVISOR: ABERA GURE (PhD)

CO-ADVISOR: KASSIM KEDIR (M.Sc.)

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Advisor Signature Date

Abera Gure (Ph.D) _____ _____
Associate Professor
Department of Chemistry
College of Natural Sciences,
Jimma University

Co- Advisor

Kassim Kedir (M.Sc) _____ _____
Lecturer (Analytical Chemistry)
Department of Chemistry
College of Natural Sciences,
Jimma University.

Examiners

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List of Abbreviations

AOAC:- Association of Official Analytical Chemists

ANOVA:- Analysis of variance

CFU:- Colony forming unit

CP:- Crude protein

EAC:- East African Community

ES: Ethiopian standard

FAO:- Food and Agricultural Organization.

HDL:- High density lipoprotein

LDL:- Low density lipoprotein

PCA-: Plate count Agar

PDA:- Potato Dextrose Agar

SD:- Standard deviation

SG:- Specific gravity

SH^0:- Soxhilet-Henkel Degree

SNF-: Solid not fat

SPC:- Standard plate Count

TA:- Titratable acidity

TBC:- Total Bacterial count

TCC:- Total Coliform count

TS:- Total solids.

VRBA Violet Red Bile Agar

YMC Yeast and Mould Count

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Abstract

This study was aimed to investigate physicochemical properties and microbial load of raw cow milk of Jimma city. To study the parameters 20 raw cow milk samples were purposively collected from milk venders twice in a day, in the morning and evening. Standard analytical methods were used for analysis of physicochemical and microbial load of the raw milk. The obtained data analyzed by SPSS-20 statistical software. The physicochemical parameters such as percentage of fat, protein, and ash of the studied milks were within the recommended ranges, whereas their pH was below the recommended ranges for all studied milks. The total bacteria count of Merkato, Kochi, Frustale and Jimma University were (7.00 ± 0.17, 6.57 ± 0.05, 6.94 ± 023 and 6.56 ± 0.10) $\log_{10}$cfu/mL, respectively. Total coliform counts also were recorded greater than recommended values. One way ANOVA ($p<0.05$) was used to compare the variation of the parameters among the milk venders. The obtained results showed that there were no significant differences ($P > 0.05$) in specific gravity, ash and yeast and mould count of the collected raw cow milk. But, exhibited significant differences ($P < 0.05$) in temperature, pH, titrable acidity, total solids, solids not fat, protein, fat, lactose, total bacteria count and coliform count. Therefore, it is possible to conclude that the microbial load of raw cow milk in the study area was not safe and this suggests the need for enriched hygienic practices and educating the public on safety issues.

Key words: Raw Cow Milk, physicochemical parameters, Microbial load, Total bacterial count, Coliform count

1. INTRODUCTION

Milk is used throughout the world as a human food at least in one form or more. Due to its high nutritive value, milk is considered as one of the most important diet items. Thus, milk and its products used as human diet, from birth to old age [1]. Its highly nutritious nature also makes milk to be; ideal for microbial growth. Fresh milk can be easily deteriorates to become unsuitable for processing and human consumption [2].

Milk and milk products are among the most important food products of animal origin [3]. Milk is a complex fluid containing many components. These components include water, fat, protein, lactose, mineral substances, organic acids, and miscellaneous other compounds [4]. As human milk, raw cow milk plays significant role in physical growth, cognitive development and health of children [5].

However, milk and its products may be contaminated by various environmental pollutants from agricultural, veterinary and hygienic practices [6]. Physicochemical analysis is important tools to monitor the quality milk and milk products. Physicochemical parameters of milk and milk product can be affected adulteration, which is done either for financial gain or lack proper hygienic conditions during processing and storing. This ultimately leads the consumer to become victim of diseases like bovine brucellosis and diarrhea or several days vomiting [7].

Microorganisms in raw milk can originate from different sources such as air, milking equipment, feed, soil and grass. The microorganism loads and types found in raw milk are influenced by factors such as cleanness the animal and equipment, season, temperature, storage, personnel health, and health of animal [8]. The quality milk is generally free from pathogenic bacteria, sediment and extraneous substances, and harmful toxic substances. It should have also good flavor, normal composition, and low bacterial counts [9]. In Ethiopia, milk is produced traditionally by individuals. In Jimma city, although there are milk venders, quality of milk there is not known. People also prefer some venders, suspecting that they supply quality milk than the others. Therefore, investigation of the physicochemical properties and microbial load of raw cow milk of Jimma city is important to identify where the milk venders supply similar milk quality to the community or not.

1.2. Statement of the problem

In many developing countries, people use raw milk, without making any pretreatment or quality test. But, raw milk may have less quality due to in inappropriate milking, by addition of adulterant, i.e., intentional addition of water and poor handling of milk. Adulteration of milk and its inappropriate storage may also lead to microbial contamination, and leads to deterioration of the quality of the product [11].

In Jimma city, milk venders supply raw milk for the consumers. Prior studies indicated that raw milk which is commercialized in the city. The reported study has not been exhaustively studied the physicochemical parameters and microbial load that influence the quality of milk in the city [12]. Therefore, in this the physicochemical properties and microbial load of the raw cow milk of the city was thoroughly investigated and baseline data were generated regarding the quality of raw milk in the city.

1.3. Objectives

1.3.1. General objectives

The main objective of this study was to investigate physicochemical properties and microbial load of raw cow milk of Jimma city.

1.3.2. Specific objectives

The specific objective of the study includes:

- To determine the physicochemical properties such as (temperature, pH, specific gravity, total solids, fat content, solids not fat , protein content, ash and lactose content and titratable acidity) of the cows' milk of Jimma city.
- To assess the microbiological such as total bacteria, coliform, yeast and mould counts of raw cow milk of Jimma city.
- To compare the physicochemical parameters and microbial load of the raw cow milk studied with packed milk (mama milk) commercialized in the city.
- To evaluate the quality of the raw cow milk of the city based on the national and international milk quality standard.

1.4. Significance of the study

The findings of the study could be used background information about physicochemical properties and microbial load of raw cow milk of Jimma city. It also serves as a source of information for further study who wants to conduct a research on raw caw milk and/or milk products.

2. Literature Review

2.1. Milk and its components

Milk is one of the oldest foods known to man and is defined as the normal, clean and fresh secretion, without any addition or subtraction, extracted from the udder of a healthy cow, and free from colostrum, i.e. excluding that which is got during the first seven days after calving [2].

Milk is a complex mixture of fats, proteins, carbohydrates, minerals, vitamins and other miscellaneous constituents dispersed in water [13].

Food products of animal origin play an important role in sufficient and balanced nutrition of human beings. Milk and milk products are among the most important food products of animal origin [3]. Milk is also a complex fluid containing many compounds in several States of dispersion. The components include water, fat, protein, lactose, mineral substances, organic acids, and miscellaneous other compounds [4]. Besides its general need for human health, milk proteins also provide amino acids which are needed for proper growth of adults and infants [5]

2.2. Physicochemical Properties of Milk

2.2.1. pH

The pH or the hydrogen ion concentration of milk gives a measure of the acidity of milk. In normal cow milk, the pH ranges from 6.6 to 6.8. The pH value can be lower than 6.6 due to development of acidity even though milk has normal acidity range of 0.1- 0.16% [2]. The pH value can be greater than 6.8 mastitis milk and pH values below 6.6 indicates increased acidity of milk due to bacterial multiplication [14].

The pH of milk changes over time. Milk goes sour, it becomes more acidic and the pH gets lower. This occurs as bacteria in milk convert the sugar lactose into lactic acid [7].

2.2.2. Specific Gravity

Specific gravity is the relation between the mass of a given volume of any substance and that of an equal volume of water at the same temperature. The normal cows' milk should have a specific gravity between 1.028 and 1.032 g/cm^3[8].

The specific gravity of milk is the ratio of the density of milk to density of water. The specific density should range between 1.028 g/ml – 1.036g/mL. The reading is done using a lactometer, hydrometer or pycnometer. The specific gravity in milk is lowered by addition of water and cream and is increased by addition of skim milk or removal of fat [2].

2.2.3. Titratable acidity

The titratable acidity is the percentage (%) of lactic acid in raw milk. Usually raw milk contains traces of lactic acid and has an initial natural acidity from 0.14% to 0.16%. Milk contains a large number of weak acids, weak bases and their salts, and this defines it as a buffer. When the milk is kept for some time, the bacteria will multiply and utilizes lactose and converts in to lactic acid, thereby increasing the acidity and decreasing the pH value. The sum of natural acidity and developed acidity is known as titratable acidity [5].

Fresh milk, however, does not contain any appreciable amount of lactic acid and therefore an increase in acidity is a rough measure of its age and bacterial activity [9]. In order to determine the sourness of milk, we use titration using sodium hydroxide and the degree of sourness is given by Soxhilet-Henkel Degree (SH^0). If the milk sourness is 4 to 5 SH^0, it indicates that either the milk is adulterated or there is mastitis [15]. Titratable acidity (TA) is a rapid test indicating raw milk quality and provides an indirect measure of the acid content in milk.

Generally, as milk acid content increases, TA values increase. All milk has a base acid content attributed to proteins, minerals and dissolved gasses [16]. The value of titratable acidity (TA) as an indicator of raw milk quality has been challenged recently, because milk is refrigerated within minutes after it leaves the cow until it reaches the consumer. Also, high milk protein may interfere with the test or confer falsely high TA values. Samples of milk containing less than

2.8% protein or greater than 3.8% protein were used to examine the impact of protein on titrable acidity [17].

2.2.4. Total solids contents of milk

Milk solids are the non-water components of milk – protein, lactose, and minerals. Sometimes the combination of protein, lactose and minerals is called the solids not fat content, and when the fat is included it is called total solids content [18].

There are a number of sources of errors involved in the estimation of total solids from the lactometer reading and fat test. Generally, these may consist of errors in making the lactometer reading, errors in determining the fat content, the particular formula used to calculate the results, and possibly the composition of the milk. If the calculated total solids arc to be compared with the observed total solids, then the errors involved in the determination of total solids must also be considered. The suitable methods where by such errors may be held to a minimum and to test the formula used in estimating the percentage of total solids by Lactometer [19].

2.2.5. Milk fat

Fat is one of the most important constituents of milk and is considered as economics of milk and milk products. Level of fat can vary from below 3.0 % to more than 6.0 %, a much greater range than that of any milk constituent. It is present in the milk as milk fat globules [14]. Milk fat is secreted in the form of a fat globule surrounded by a membrane. Each fat globule is composed almost entirely of triacylglycerol's and is surrounded by a membrane consisting of complex lipids such as phospholipids, along with proteins. These act as emulsifiers which keep the individual globules from coalescing and protect the contents of these globules from various enzymes in the fluid portion of the milk [10].

Milk can be classified according to its fat content as whole milk, skimmed milk, semi-skimmed milk, low-fat milk and standardized milk. The whole milk means, which water has been partly removed after heat-treating and concentrating. Skimmed milk is fat free or nonfat and standardized milk in which the fat content is adjusted to a predetermined value without altering any other constituents [20]. Low-fat, calcium-rich dairy products are generally considered to

lower blood pressure. High-fat dairy products are known to increase high density lipoprotein (HDL) and low density lipoprotein (LDL)-cholesterol concentrations. The latter normally predicts risk of cardiovascular disease [21]. Milk fat is isolated from given milk sample and is subject to butyro refract meter reading. For isolation of milk fat from milk, modified Gerber butyrometer can be used, which is open at both ends [22]

2.2.6. Milk protein

Proteins are made up of amino acids, more precisely, L-α-amino carboxylic acids. Proteins constitute an important class of compounds that are essential to all living processes. Milk proteins represent one of the greatest contributions of milk to human nutrition. About 95 % of the nitrogen in milk is in the form of proteins. When total nitrogen content is multiplied with 6.38, a Kjeldahl factor, it gives the total protein content in milk and milk products. Non protein nitrogen components comprising about 5% of the total nitrogen in fresh milk are equally important [23]. Protein is important during weight loss and subsequent weight maintenance due to the high satiating effect which helps to prevent over-consumption of energy and thereby reduces body fat stores. Whey protein (primarily in milk and yoghurt) can reduce postprandial plasma glucose concentration in type 2 diabetic subjects [24].

Cow's milk naturally contains the large amount of protein needed for her calf. Excess protein in our diets causes calcium to leach out of our bones. This can be a cause of osteoporosis [25]. The total protein determination by Kjeldahl method which served as well as an internal reference method because we used to determine protein content in milk [26].

2.6.7. Ash content

Ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter in a foodstuff. Percentage ash shows the inorganic residue remaining after these residues matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food [27].

Dry oxidation or ashing eliminates or minimizes the effect of organic materials in mineral element determination. It consists of ignition of organic compounds by air at atmospheric

pressure and at relatively elevated temperatures (450-550°C) in a muffle furnace [28]. Milk replacer has four major components: Protein, Fat, Lactose and Ash [29].

2.3. Bacteriological quality of raw milk

Microbial quality of milk refers to the cleanness of milk. The microbial content of milk is a major feature in determining its quality. It shows the hygienic level exercised during milk production and handling, that is cleanliness of the milking utensils, condition of storage, manner of transport as well as the cleanliness of the udder of the individual animal [30].

Contamination of milk with high levels of spoilage bacteria is usually unsuitable for further processing since it does not meet the consumer's expectations in terms of health (nutritional value), safety (hygienic quality) and satisfaction (sensory attributes)[31]. As a result, total bacterial counting has become one of the accepted criteria for grading milk intended for consumption and processing for dairy products. The importance of various etiological agents in milk-borne diseases has changed dramatically over time. Lack of knowledge about clean milk production, use of unclean milking equipment and lack of potable water for cleaning purposes were some of the factors which contributed to the poor hygienic quality of raw milk [32].

Milk is an important vehicle for transmission of pathogenic microorganisms to human beings unless it is produced and handled under good hygienic conditions. Thus, hygienic production of milk has to get attention in order to provide more and better quality milk for the general public. The detection of coliform bacteria, pathogens and high microbial count in milk are major factors in determining its quality [33].

2.3.1. The source of milk contamination

Bacterial contamination could arise from the cow’s udder, barn, milk collection materials, various ingredients added to dairy products and dairy farm workers [34].

The milk market requires and offers safe and high-quality products, preventing a contamination source by good hygiene practices to reduce a possible exposure of food-borne pathogens and chemical milk residues [34]. The others sources of milk contamination are poor hygiene milk

practices, mastitis, infectious pathogens in infected cows and the presence of environmental pathogens by poor animal hygiene [36]. The microbiological quality of dairy products reflects good hygienic practices during the dairy milking process; raw milk contamination may occur in diseased or infected cows with environmental bacteria [37].

Milk is a perishable product and an ideal medium for the growth of a wide variety of bacteria. When it is secreted from a healthy udder, raw milk contains only a very few bacteria of about 500 to 1,000 bacteria per milliliter. After milking environmental contamination occurs, which in turns increases the total bacteria count up to 50,000 per ml or may even reach several millions bacteria per milliliter [38].

Many countries have implemented laws and regulations concerning the composition and hygienic quality of milk and milk products to protect both the consumers and the public health. Unfortunately, these laws and regulations are not often adhered in developing countries making milk-borne diseases a higher health risk to public [39].

2.3.2. Total bacteria count

The total bacteria count is the number of bacteria in a sample that can grow and form countable colonies on Standard Methods Agar after being held 48 hours. The total number of bacteria in raw milk should not exceed more than ranging between 1000,000 bacteria cells per milliliter in milk [40]

In the last five years a greater number of literatures reported bacterial count, for example, between colony forming units per milliliter (cfu/mL). According to these values exceed the levels acceptable in the East African community (EAC) countries. This condition causes increased number of bacteria in the milk and contributes to loss of milk quality [41].

Table 1: Grade of raw cow milk based on standard plate count (SPC) [41].

Bacterial count CFU/mL	Grade
Not exceeding 200,000	Very good
200,000 -1,000,000	Good
1,000,000-5,000,000	Fair
>5,000,000	Poor

2.3.3. Coliform Count

Coliforms are among the many groups of microorganisms that are normally present in raw milk. Common coliform genera in raw milk include Citrobacter, Enterobacter, Escherichia, and Klebsiella [42]. The term "total coliforms" also refers to a large group of Gram-negative, rod-shaped bacteria that share several characteristics. The presence of total coliforms may or may not indicate faecal contamination. In extreme cases, a high count for the total coliform group may be associated with a low, or even zero, count for thermo tolerant coliforms. Such a result would not necessarily indicate the presence of contamination.

According to the European Union standards for coliform counts of raw milk should be less than10^2 cfu/mL [43]. The coliform count is related to the unsanitary milking process and dirty cow's environment. Raw milk contains TCC of < 100 cfu/mL consumed [44]. The use of coliforms as indicator organisms for the presence of unsanitary conditions in milk handling is increasingly under scrutiny, it is clear that coliforms are not appropriate index organisms for the presence of public health hazards in dairy products [42]. Coliform contamination of milk has been associated with milking equipment in case of hand milking, water, animal mastitis and milk containers. According to the Ethiopian standard Agency (ESA) the total coliform count per milliliter set to be less than 1000 cfu/mL in raw cow milk is very good quality [45].

Table 2: Grade of raw cow milk based on violet red bile agar (VRBA) in raw cow milk [45]

Coliform count CFU/mL	**Grade**
Not exceeding 1000	Very good
1,000 – 50, 000	Good
50,000-500,000	Fair
>500,000	Poor

2.3.4. Yeast and mould count

Yeast and mould count is the number colonies in a sample that grow and form countable colonies on but Potato Dextrose Agar (PDA) after being held at 25 ^{0}c for 3 to 5 days [41]. Yeasts are widely distributed in the dairy environments and appear as natural contaminants in raw milk, air, and dairy utensils [46]

3. Materials and Methods

3.1 Description of the Study area

Study was conducted in Jimma city, which is the capital of Jimma Zone, Oromia regional state, Ethiopia. It is located at 345 km the Addis Ababa in Southwest Ethiopia. It is available at latitude and longitude of $7^{o}40'N36^{o}50'E$ and altitude, of about 173 m above sea level. It lies in the climate zone locally known as Woyna Dage which is ideal for agriculture as well as human settlement [12]. Figure 1 shows map of the study Jimma zone and Jimma city.

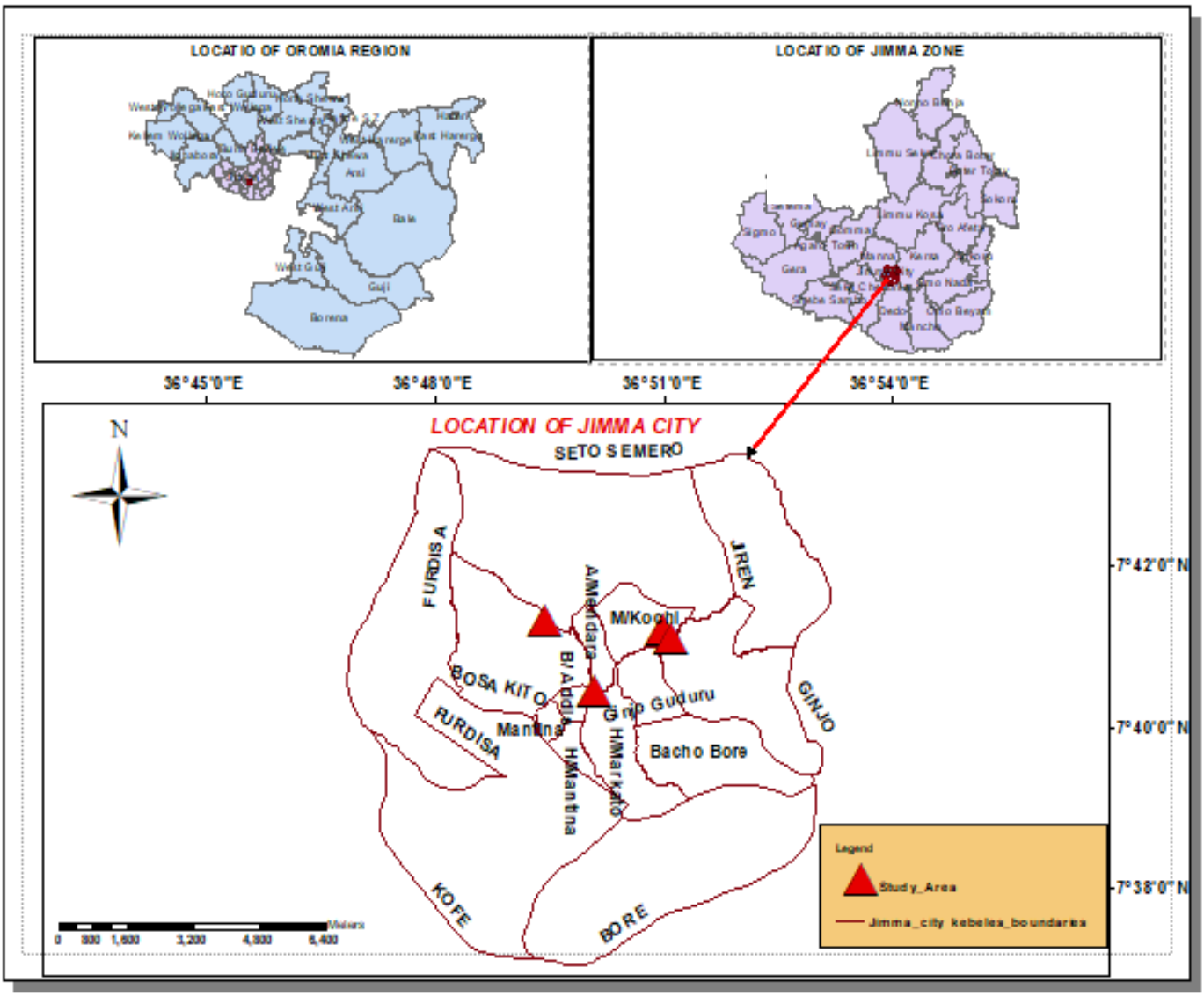

Figure 1: Map of the study area

3.2. Selection of sampling

The raw cow milk samples were collected from 4 sites (milk venders), which were purposively selected from different places of the city. The selected sites were (Markato, Kochi, Frustale and Jimma University).

3.3. Sample collection

Totally 20 raw cow milk samples, each sample 500 mL were collected from milk venders. From each sampling sites 3 samples, from different containers, were collected from the venders twice a day, in the morning and in the evening. Then, milk samples collected from the same site were mixed up to make a composite sample. Similarly, 3 sterilized and packed milk (mama) samples were also collected from the local market. Similarly, by mixing equal volume of mama milk, a composite sample was prepared. Each day, fresh raw milk samples were collected for analysis of the physicochemical parameters and microbial load.

3.4. Apparatus and Instruments

Different types of instruments and apparatus such as Thermometer, Pycnometer, pH-meter (portable code 013,German), Butrometer, Kjeldahl apparatus, , muffle furnace, incubator, colony forming counter (Funke Gerber code 2013, Switzerland) and different common laboratory glasses were used during sample collection, preparation and analysis of physicochemical properties and microbial load of milk samples.

3.5. Chemicals and reagents

Chemicals such as 1% Phenolphthalein indicator (98%) obtained from UNICHEM Chemical reagent (Blulux, India), sulfuric acid(98%), hydrochloric acid(37% w/v) and potassium sulfate all from Loba Chemie Pvt.Ltd (INDIA), copper sulphate, sodium hydroxide, boric acid, methyl red indicator, bromocresol green indicator, amyl alcohol, peptone, peptone and saline (code 64271, German) and Potato Dexrose agar solution were used during the experiments.

3.6. Analysis of physicochemical properties

3.6.1. Determination of temperature and pH

The temperature of the milk samples was determined at sampling sites using thermometer. pH of the milk samples was determined in the laboratory using a digital pH-meter. The pH meter was calibrated by using known standard buffer solution of pH 7.0 and 4.0. After calibrating the pH meter with the standard buffer, the pH of milk was measured by immersing the electrode into the beaker containing milk sample and reading was then recorded. The pH-meter was calibrated before and after the pH of the sample was measured [47].

3.6.2. Determination of titratable acidity

Titratable acidity was determined to the method of the AOAC [9]. Accordingly, 10 mL milk sample was pipetted into a beaker and then, 3-5 drops of 1% phenolphthalein indicator was added. The milk sample was then titrated with 0.1N NaOH solution until a faint pink color was appeared. Finally, Titratable acid of milk samples, which was expressed as percentage of lactic acid was calculated by the following formula [48].

$$TA = \frac{0.1N\ NaOH(mL) \times 0.09 \times 100}{\text{Weight of milk sample}}$$

3.6.3. Determination of Specific gravity

The specific gravity (SG) of the samples was determined using pycnometer method [49]. To measure SG, masses of equal volumes of milk sample and distilled water were separately measured. Then, the specific gravity of a substance which is expressed as ratio of density of milk to the density of water is determined by the following formula [49].

$$SG = \frac{\text{mass of milk}}{\text{mass of distilled water}}$$

3.6.4. Determination of total solids

Fresh raw cow milk sample was thoroughly mixed and then, 5 g of the sample was transferred to the pre-weighed and dried crucible. Afterwards, the sample was dried in an oven at 102 ℃ for 3 h. The dried sample was taken out of the oven and placed in a desiccator to cool to room temperature. Finally, the dried sample was weighed to determine the total solids by using the following formula [50].

$$TS = \frac{\text{Weight of crucible + weight of Ovendrysample - weight of crucible}}{\text{Weight of sample}} x_{100}$$

3.6.5. Determination of fat content

The fat content was determined by the Gerber method [7]. Accordingly, 10 mL conc. H_2SO_4 was pipetted into a butyrometer. Then 10 mL of milk sample was added. Then, after addition of 1 mL amyl alcohol and butyrometer was closed with a lock stopper and the mixture was shaken and inverted several times until the milk was completely digested by the acid. The content was placed in water bath at 65 ℃ for 5 min. and then centrifuged in a Gerber centrifuge for 5 min. The butyrometer was again placed in water bath at 65℃ for 5 min. At the end, the butyrometer reading was recorded [51].

3.6.6. Determination of solids not fat

Solids-not-fat (SNF) content was determined as the difference between the percentage of total solids and fat percentage using the following formula [52].

$$\text{SNF content (\%)} = \text{TS (\%)} - \text{Fat (\%)}$$

3.6.7. Determination of crude protein content

The crude protein content of milk samples were determined by the Kjeldahl method AOAC [24]. Thus, 5 g of milk sample was warmed in water bath at 38 ^{0}C for 3 min. and then transferred to Kjeldahl tube. Then, a mixture of 15 g K_2SO_4, 1 mL $CuSO_4$ (98%) solution and 25 mL of concentrated H_2SO_4 were added into the tube and mixed gently. The digestion was carried out

for 2 h at 350 ^{0}C. Then it was allowed to cool at room temperature for about 25 min. The digested solution was diluted with 250 mL of distilled water [53].

After the Kjeldahl tube was placed in the distillation equipment, 75 mL of 50% NaOH solution was added. Then, ammonia was distilled using 50 mL of 4% boric acid solution with methyl red/bomocresol green as indicators until blue color was appeared. Finally, the sample was titrated with 0.1N HCl solution until a faint pink color was formed.

Blank test was also prepared the same procedure except that water was used instead of the milk sample. The percentage of nitrogen, indicating percentage of protein, in the milk samples was then calculated by using the following formula [54].

$$\%N = \frac{(Vs - V\,b)_{1.4007} \times N\ HCl}{\text{Weight of sample}} \times_{100}$$

Where Vs and Vb = Volume of HCl consumed by sample and blank, respectively.

$$\%CP = \%N \times 6.38$$

3.6.8. Determination of ash content

Dried milk sample which was used for determination of total solids content was ignited in a muffle furnace at a temperature of 550 °C. It was ignited for 4 h until black color was disappeared or the ash was changed to grayish to white. Then, the sample was transferred to the desiccators to cool. Finally, the percentage of ash content was calculated using to the following formula [55].

$$\%\ Ash = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

3.6.9. Determination of lactose content

The percent of lactose was determined by subtracting the sum of fat, protein and ash contents from the total solids [56].

% Lactose = % Total solids - (%Fat + % Protein+ % ash).

3.7. Microbial Analysis

3.7.1. Preparation of solution

Each milk sample was diluted using sterilized distilled water before applying to the plate. So, 1 mL milk was mixed with 9 mL sterilized distilled water in a test tube to get a dilution of (1:10). From this, further dilutions of 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} [57] were prepared. All diluted samples were applied to petri plates. The petri plates were labeled with dilution factor and sample numbers [57].

3.7.2. Total bacterial count

Total bacterial count (TBC) is a rough gauge to measure the quality of milk, herd health, efficacy of farm sanitation, milk handling and storage as well as transportation temperature [58].
To measure the TBC, standard plate count (SPC) agar was cooled to 45°C before pouring. 1 mL milk sample was added into sterile test tube containing 9 mL peptone water. TBC was made by incubating surface plated duplicate decimal dilutions of milk samples on standard plate count agar at 32^0C for 48 h. For total plate count, appropriate decimal dilutions that would give the expected total number of colonies on a plate between 30 and 300 colonies were selected [42].

3.7.3. Coliform count

To determine coliform count (CC), 1 mL milk sample was added into sterile test tube containing 9 mL peptone water. Duplicate appropriate decimal dilutions were surface plated and incubated at 32^0C for 24 h on Violet Red Bile Agar and typical dark red colonies on uncrowned plates were considered as coliforms and counted. Gas production within 48 h of incubation at 35^0C was considered as sufficient evidence for the presence of coliforms [42].

3.7.4. Yeast and mould count

Yeast and mould count (YMC) of milk samples were determined following similar methods as for TBC, but dilutions were surface plated on Potato Dextrose Agar (PDA). The dried plates

were then incubated at 25 ^{0}C for 3 to 5 days. Colonies with a blue green color was counted as yeasts and mould [42].

3.8. Statistical data analysis

The obtained data from both physicochemical properties and microbial load were reported as mean and standard deviation of replicate analysis. IBM SPSS Statistics 20 version software was used to process data. One-way ANOVA at ($p< 0.05$) was also used to evaluate the variations among the studied milk samples in terms of the analyzed parameters.

4. Result and Discussion

4.1 Physicochemical Properties of Raw cow milk

The obtained results for physicochemical properties of the studied raw cow milk samples of Jimma city are presented in Table 3.

Table 3: Physicochemical properties (mean ± SD) of raw cow's milk of Jimma city.

Parameter	Milk source					
	Merkato	**Kochi**	**Frustale**	**JU**	**Mama**	**FAO** [59]
Temp. (℃)	28.43 ± 0.71	26.10 ± 0.62	26.73±0.51	23.73 ± 0.32	21.33 ± 0.31	NA
pH	6.32 ± 0.03	6.56 ± 0.03	6.50 ± 0.09	6.58± 0.03	6.61 ± 0.02	6.60 - 6.80
SG	1.02 ± 0.01	1.03 ± 0.00	1.02 ± 0.05	1.03 ± 0.01	1.03 ± 0.01	1.03 - 1.03
%TA	0.25 ± 0.02	0.13 ± 0.01	0.23 ± 0.02	0.14 ± 0.01	0.14 ± 0.10	0.14 - 0.16
%TS	11.20 ± 0.40	12.23 ± 0.15	11.90 ± 0.2	12.43± 0.15	12.45 ± 0.13	12.52 - 14.56
%Fat	3.13 ± 0.15	3.53 ± 0.15	3.67 ± 0.21	3.00 ± 0.01	2.96 ± 0.18	2.50 - 6.00
%SNF	7.90 ± 0.25	8.70 ± 0.30	8.53 ± 0.06	9.43 ± 0.20	9.64 ± 0.16	8.42 - 10.5
%Protein	4.02 ± 0.07	3.90 ± 0.07	3.54 ± 0.04	3.61 ± 0.29	3.50 ± 0.04	2.90 - 5.00
%Ash	0.73 ± 0.05	0.70 ± 0.03	0.74 ± 0.04	0.75 ± 0.04	0.77 ± 0.01	0.70 - 0.80
%Lactose	3.32 ± 0.37	4.11 ± 0.38	4.25 ± 0.06	5.07 ± 0.53	5.48 ± 0.20	3.60 - 5.50

SD: standard deviation, FAO: Food and Agricultural Organization, NA:not available, JU:Jimma University, SG: specific gravity and %TA: Titrable acidity percentage, %TS: Total Solid percentage, and %SNF: Solid not fat percentage.

It was observed that the mean temperature of cow's milk samples were significantly different ($P < 0.05$) among milk sample sites. Milk sample, from Merkato had the highest temperature. This might be due to variations in the milk handling equipment and handling techniques [59].

Lack of refrigerator for milk storage, by milk vendors may increase the temperature of the milk. This could contribute to the increased number of microbial contaminant in the milk. Inadequate cooling will increase bacterial counts by allowing a better environment for bacterial growth during storage above 16 ℃ temperature [60].

As can be seen from Table 3, the pH of milk samples obtained from Merkato, Kochi, Frustale and JU: 6.32± 0.03, 6.56 ± 0.03, 6.50 ± 0.09 and 6.58 ± 0.03, respectively, and all the studied samples exhibited pH values slightly below the standard pH range [59]. This may indicate that there might be bacterial growths in the milk samples. However, the pH of mama milk sample was within normal pH range (6.6 - 6.8), indicating that the mama milk is free from bacteria [60]. One way ANOVA ($p < 0.05$) indicated the presence of significance differences in pH of the studied milk samples.

The SG of normal milk ranges from 1.03 – 1.04 g/mL with a mean value of 1.03 g/mL [61]. The obtained SG values of Kochi, JU and Mama milk samples were within the recommended normal range. Merkato and Frustale milk samples exhibited SG values slightly below the lower limit of the standard value [61]. But, one way ANOVA ($p > 0.05$) indicated that there was no significant difference in the SG of the studied milk samples. . The SG of milk is decreased by addition of water or cream (fat), while removal of fat and reduction of temperature increase SG of milk [9]. Based on the SG results, the studied milk samples satisfy the quality of raw milk quality standard.

Normal fresh milk has an apparent acidity of 0.14 to 0.16% as lactic acid [62]. In this study, the %TA of Kochi, JU and Mama Milk samples were 0.13± 0.01%, 0.14 ± 0.01% and 0.14 ± 0.01%, respectively, which are within acceptable range. However, Merkato and Frustale milk samples demonstrated 0.25 ± 0.02% and 0.23 ± 0.02%, respectively, which were far above the upper limit of the normal %TA range in raw milk samples. This may be due to bacterial growth and multiplication during transportation to the selling sites. The high %TA is generally indicating the presence of high bacterial activity [63]

The %TS of all the studied raw cow milk and mama were below the normal recommended %TS values. This might be occurred due to the animals' food, breeding, climate and management practices which have important effects on milk composition and quality [64].

The fat content of milk obtained from Merkato, Kochi, Frustale, JU and Mama were 3.13 ± 0.15%, 3.53 ± 0.15%, 3.67 ± 0.21%, 3.00 ± 0.01% and 2.96 ± 0.18%, respectively. All the studied milk samples were exhibited similar fat content which were within the recommended

values [60]. Although, the observed variations was not significant in this study, generally, fat content of milk is highly affected by animals' food, parity, breeding and stage lactation [9]

The obtained SNF contents the studied milk samples were for Merkato (7.90 ± 0.25%), Kochi (8.70 ± 0.30%), Frustale (8.53 ± 0.06%), JU (9.43±0.20%) and Mama (9.64 ± 0.16), with the exception of Markato milk sample, the SNF contents obtained from other sampling sites and that of mama milk were within the recommended levels. The variation of Markato milk from the others could be due to differences in the feeding practices, milking method and lactation period [32].

The protein contents obtained from milk samples were Merkato (4.02 ± 0.07%), Kochi (3.9 ± 0.07%), Frustale (3.54 ± 0.04%), JU(61 ± 0.29%) and Mama (3.50 ± 0.16%) and all were agreed with the standard protein contents of raw milk . The highest protein (4.02 ± 0.07) was obtained from Merkato site. The blending of the samples from different sources, genotypic variation and nutritional level of cows may contribute for the rise of protein content of the milk of this site [11].

Ash content of milk samples obtained from Merkato, Kochi, Frustale, JU and Mama were 0.73 ± 0.05%, 0.70 ± 0.03%, 0.74 ± 0.04%, 0.75 ± 0.04% and 0.77± 0.01%, respectively and all are within the recommended standard ranges [60]. The ash contents of all raw milk samples were lower than that of mama sample. Ash content of milk can be affected by breed, stage of lactation and animals' food [41]. .

The obtained lactose contents of milk samples were Merkato (3.32 ±0.37%), Kochi (4.11 ± 0.38%), Frustale (4.25 ± 0.06%), JU (5.07 ± 0.53%) and Mama (5.48 ± 0.20%), except for Merkato sample, the remaining milk samples have %lactose contents within the recommended range (3.60 - 5.50%) [60]. The Merkato sample lower than Kochi, Frustale, JU and mama samples. This might be due to bacterial activities. One way ANOVA ($p < 0.005$) demonstrated the presence of significance differences in lactose contents the studied milk samples. Merkato milk sample contained the lowest lactose content and the highest was determined in Mama milk. This variation might be occurred due to bacterial activities [9]

4.2. Microbial load in milk samples

The obtained results for microbial load of milk samples such as total bacteria count, coliform count and yeast and mould count cow milk collected from Merkato, Kochi, Frustale, JU and mama milk in the Jimma city are presented with mean and standard deviation are presented in table 4.

Table 4: Microbial counts (log(cfu/mL)) of raw cow's milk samples.

Parameter	Milk Source					
	Merkato	**Kochi**	**Frustale**	**JU**	**Mama**	**ES** [45]
TBC	7.00± 0.17	6.57 ± 0.05	6.94 ± 0.23	6.56 ± 0.10	4.90 ± 0.52	≤ 6.00
CC	7.14 ±0.03	6.35 ±0.08	7.46 ±0.63	5.57 ±0.07	4.91 ±0.06	≤ 4.70
YMC	5.10± 0.41	5.18 ± 0.03	5.26 ± 0.07	5.36 ± 0.05	5.12 ± 0.10	≤ 5.00

ES: Ethiopian Standard, TBC: Total Bacteria count, CC: Coliform count and YMC: Yeast and Mould count.

The obtained TBC of raw cow milk samples in $\log^{(CFU/mL)}$ were from Merkato (7.00 ± 0.17), Kochi (6.57 ± 0.05), Frustale (6.94 ± 0.23) and JU (6.56 ± 0.10). The all milk samples showed TBC higher than the acceptable level of 6.00 $\log^{CFU/mL}$. This might indicate poor hygienic milk handling practices including unhygienic milking, unclean or diseased udder, unsanitary facilities and/or unfavorable storage condition [64]. One way ANOVA ($p < 0.05$) indicated the presence significant differences of TBC among the studied cow milk samples

Generally, the TBC of the studied milk samples are higher than the recommended maximum TBC set by Ethiopian standard Agency, 4.70 $\log^{CFU/mL}$ and East Africa Community Standard, 6.30 $\log^{CFU/mL}$ [45]. The higher TBC indicates that total bacterial count is a good indicator for monitoring the sanitary conditions practiced during collection and handling of raw milk [65].

In the current study, compared to other sample sites, higher CC was obtained in Merkato (7.14 ± 0.03) and Frustale (7.46 ± 0.63). This may indicate the contamination of the raw cow milk samples either from poor milk handling such as improper handling practices, poor hygiene of

milkers and container or the unhygienic environment [65]. One way ANOVA ($p < 0.05$) revealed as there were significant differences in CC among the raw cow milk samples studied.

The YMC were 5.10 ± 0.41, 5.18 ± 0.03, 5.26 ± 0.07, 5.36 ± 0.05 and 5.12 ± 0.10 $\log^{CFU/mL}$ for milk samples collected from Merkato, Kochi, Frustale, JU and Mama, respectively. The obtained YMC in all samples were higher than the recommended maximum limit set by ES [46]. This might be due to the contamination of cow milk from environment such as from air, unclean containers and poor personal hygiene of milk handler [43].

5. CONCLUSIONS AND RECOMMENDATION

5.1. CONCLUSIONS

In the present study, the physicochemical parameters and microbial load of milk samples collected from Jimma city vendors were investigated. Most of the physicochemical parameters study results were within the recommended milk quality safe range, indicating that the studied milk samples are free from adulteration. However, among the studied milk samples, milk sample which was collected from Merkato exhibited variations in some parameters. The obtained results for TBC, CC and YMC of all the studied milk samples were above the maximum recommended limits set by ES, indicating that studied milk were not safe for consumption in terms of microbial load. Therefore, sanitary measures should be taken at all milk sellers.

5.2. RECOMMENDATION

Based on the findings of the present study, the researcher would like to forward the following recommendation.

- To obtain quality milk products, milk suppliers and consumers should be trained on factors that deteriorate quality of raw milk.
- To minimize the impact of microbial on health of the user, raw milk should be boiled before using.
- Further investigations are recommended to identify contaminants at species level by giving attention to those pathogens that have human health hazard.

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APPENDICES

Appendix A. Microbial load plates

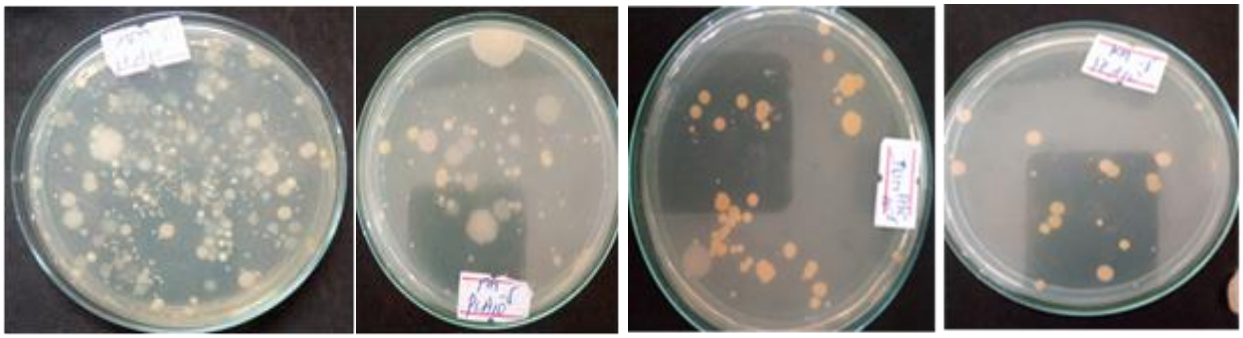

Figure 2 Total bacteria colony in milk samples

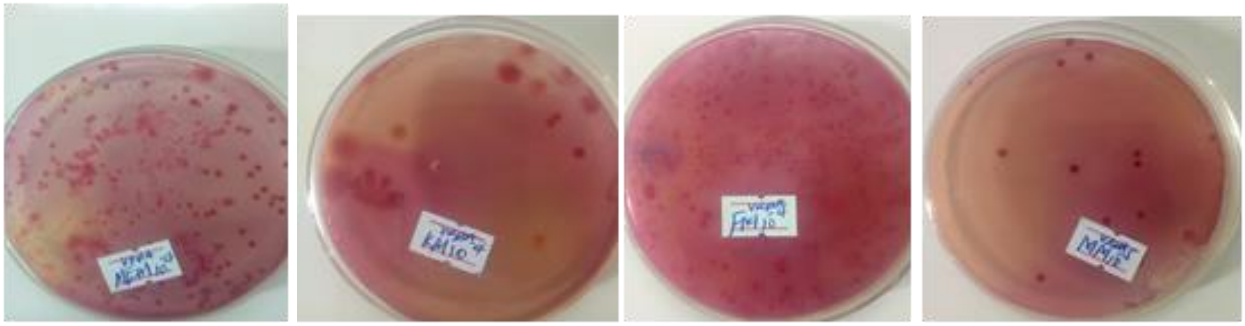

Figure 3 Total Coliform counts colony in the milk samples

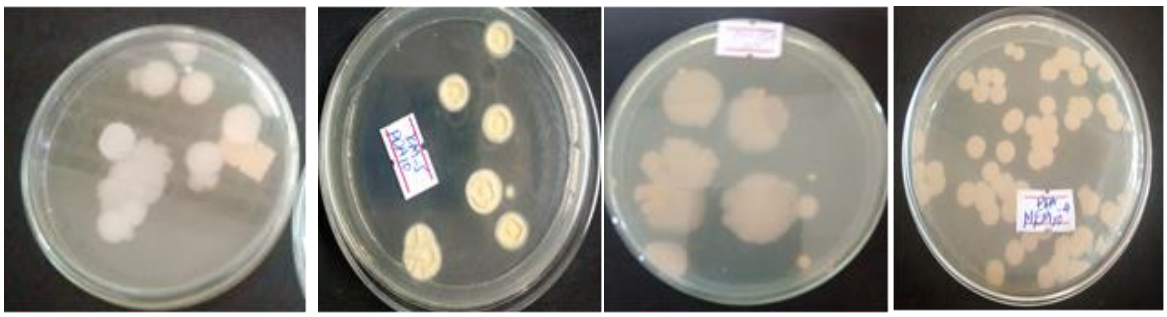

Figure4. Mould counts colony in milk samples

Appendix B. List of Tables

Appendix ANOVA of physicochemical properties of milk obtained from the study area.

Appendix Table 1 Average composition of mama milk

Parameter	Average (%)
Fat	2.7
Protein	3.5
Lactose	4.7
Ash	0.8

Source: Labeled by Sebeta Agrio Industry

Appendix Table 2 ANOVA of physicochemical properties of milk obtained from the study area.

Parameter	DF	SS	MS	F	P-value
Temperature	4	92.08	23.02	85.06	0.00*
pH	4	0.16	0.04	18.87	0.00*
SG	4	0.00	0.00	0.42	0.79
TS	4	3.26	0.81	15.43	0.00*
SNF	4	5.67	1.42	26.88	0.00*
Fat	4	1.28	0.32	12.01	0.001*
Protein	4	0.14	0.03	6011.81	0.00*
Ash	4	0.01	0.00	1.97	0.18
Lactose	4	6.58	1.64	28.30	0.00*

DF=Degrees of freedom, SS=Sume of Square, MS=Mean Square, TA= titrable acidity, SG= specific gravity, TS= Total solids, SNF= Solids not fat, = Significant at P<0.05.*

Appendix Table 3: ANOVA for microbial count (log10cfu/ml) of milk obtained from sellers

Parameter (%)	DF	SS	MS	F	P-value
TBC	4	8.86	2.21	30.72	0.00*
CC	4	12.73	3.18	7.79	0.04*
YMC	4	0.23	0.06	1.58	0.25

DF=Degrees of freedom, SS=Sum of Square, MS=Mean Square, TBC= Total bacterial count, CC= Coliform count, YMC = Yeast and mould count, = Significant at P<0.05.*

Appendix Table 5 Descriptive Analysis of physicochemical properties

Appendex 4 Descriptive analysis of Physicochemical properties

Parameter	Site	N	Mean	Std. Deviation	95% Confidence Interval		Min.	Max.
					Lower Bound	Upper Bound		
Temp.	Merkato	3	28.4333	.70946	26.6709	30.1957	27.80	29.20
	Kochi	3	26.1000	.62450	24.5487	27.6513	25.40	26.60
	Frustale	3	26.7333	.51316	25.4586	28.0081	26.30	27.30
	JU	3	23.7333	.32146	22.9348	24.5319	23.50	24.10
	Mama	3	21.3333	.30551	20.5744	22.0922	21.00	21.60
pH	Merkato	3	6.3167	.02887	6.2450	6.3884	6.30	6.35
	Kochi	3	6.5600	.02646	6.4943	6.6257	6.53	6.58
	Frustale	3	6.5000	.08888	6.2792	6.7208	6.40	6.57
	JU	3	6.5800	.03000	6.5055	6.6545	6.55	6.61
	Mama	3	6.6067	.02082	6.5550	6.6584	6.59	6.63
SG	Merkato	3	1.02300	.011790	.99371	1.05229	1.010	1.033
	Kochi	3	1.02933	.001528	1.02554	1.03313	1.028	1.031
	Frustale	3	1.02500	.014526	.98892	1.06108	1.010	1.039
	JU	3	1.02767	.004726	1.01593	1.03941	1.024	1.033
	Mama	3	1.03100	.001000	1.02852	1.03348	1.030	1.032
TA								
	Merkato	3	.25067	.020033	.20090	.30043	.230	.270
	Kochi	3	.13200	.010392	.10618	.15782	.126	.144
	Frustale	3	.23400	.018000	.18929	.27871	.216	.252
	JU	3	.13800	.010392	.11218	.16382	.126	.144
	Mama	3	.14400	.018000	.09929	.18871	.126	.162
TS	Merkato	3	11.20000	.400000	10.20634	12.19366	10.800	11.600
	Kochi	3	12.23333	.152753	11.85388	12.61279	12.100	12.400
	Frustale	3	11.90000	.200000	11.40317	12.39683	11.700	12.100
	JU	3	12.43333	.152753	12.05388	12.81279	12.300	12.600
	Mama	3	12.45000	.130767	12.12516	12.77484	12.300	12.540
Fat	Merkato	3	3.1333	.15275	2.7539	3.5128	3.00	3.30
	Kochi	3	3.5333	.15275	3.1539	3.9128	3.40	3.70
	Frustale	3	3.3667	.20817	2.8496	3.8838	3.20	3.60
	JU	3	3.0033	.10017	2.7545	3.2522	2.90	3.10
	Mama	3	2.6900	.18248	2.2367	3.1433	2.57	2.90
SNF	Merkato	3	8.0667	.32146	7.2681	8.8652	7.70	8.30
	Kochi	3	8.7000	.30000	7.9548	9.4452	8.40	9.00
	Frustale	3	8.5333	.05774	8.3899	8.6768	8.50	8.60
	JU	3	9.4300	.20421	8.9227	9.9373	9.20	9.59
	Mama	3	9.7600	.15875	9.3657	10.1543	9.64	9.94

Protein	Merkato	3	2.32233	.002517	2.31608	2.32858	2.320	2.325
	Kochi	3	2.27367	.002517	2.26742	2.27992	2.271	2.276
	Frustale	3	2.10633	.003215	2.09835	2.11432	2.104	2.110
	JU	3	2.10500	.001000	2.10252	2.10748	2.104	2.106
	Mama	3	2.10667	.002082	2.10150	2.11184	2.105	2.109
Ash	Merkato	3	.7300	.05000	.6058	.8542	.68	.78
	Kochi	3	.6967	.02517	.6342	.7592	.67	.72
	Frustale	3	.7433	.03512	.6561	.8306	.71	.78
	JU	3	.7533	.04163	.6499	.8568	.72	.80
	Mama	3	.7733	.01155	.7446	.8020	.76	.78
Lactose	Merkato	3	5.0133	.32332	4.2102	5.8165	4.64	5.20
	Kochi	3	5.7400	.33045	4.9191	6.5609	5.40	6.06
	Frustale	3	5.7100	.06245	5.5549	5.8651	5.66	5.78
	JU	3	6.5533	.21939	6.0083	7.0983	6.30	6.68
	Mama	3	6.8733	.15695	6.4834	7.2632	6.75	7.05

Appendix Table 5 Descriptive Analysis of Microbial load

Parameter	Site	N	Mean	Std. Deviation	95% Confidence Interval		Min.	Max.
					Lower Bound	Upper Bound		
TBC	Merkato	3	7.0000	.16523	6.5896	7.4104	6.81	7.11
	Kochi	3	6.5700	.04583	6.4562	6.6838	6.52	6.61
	Frustale	3	6.9367	.23116	6.3624	7.5109	6.67	7.08
	JU	3	6.5600	.10440	6.3006	6.8194	6.49	6.68
	Mama	3	4.9000	.51643	3.6171	6.1829	4.53	5.49
CC	Merkato	3	7.1400	.53731	5.8053	8.4747	6.81	7.76
	Kochi	3	6.3533	.26274	5.7006	7.0060	6.15	6.65
	Frustale	3	7.4600	.56930	6.0458	8.8742	6.81	7.87
	JU	3	5.7900	.38432	4.8353	6.7447	5.52	6.23
	Mama	3	4.9100	1.10077	2.1755	7.6445	4.23	6.18
YMC	Merkato	3	5.0100	.38223	4.0605	5.9595	4.57	5.26
	Kochi	3	5.1767	.02517	5.1142	5.2392	5.15	5.20
	Frustale	3	5.2567	.07371	5.0736	5.4398	5.20	5.34
	JU	3	5.3633	.04509	5.2513	5.4753	5.32	5.41
	Mama	3	5.1167	.10408	4.8581	5.3752	5.00	5.20

TBC: Total bacterial count, CC: Coliform count, YMC: yeast and mould count