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ON

PHYSICOCHEMICAL PROPERTIES AND MICROBIAL LOAD OF RAW COW'S MILK PRODUCED AND MARKETED IN JIMMA TOWN, SOUTH WESTERN ETHIOPIA

BY: JEMAL AHMED

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PHYSICOCHEMICAL PROPERTIES AND MICROBIAL LOAD OF RAW COW'S MILK PRODUCED AND MARKETED IN JIMMA TOWN, SOUTH WESTERN ETHIOPIA

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MSc THESIS APPROVAL SHEET

We, the undersigned, member of the board of examiners of the final open defense by **JEMAL AHMED** have read and evaluated his/her thesis entitled "**PHYSICOCHEMICAL PROPERTIES AND MICROBIAL LOAD OF RAW COW'S MILK PRODUCED AND MARKETED IN JIMMA TOWN, SOUTH WESTERN ETHIOPIA**" and examined the candidate. This is therefore to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree of Master of Science in Chemistry.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA:	Analyses of variance
AOAC:	Association of Official Analytical Chemists
°C:	Degree Celsius
CFU:	Colony Forming Unit
ES:	Ethiopian Standard
EU:	European Union
FAO:	Food and Agriculture Organization
JU:	Jimma University
Log_{10}:	Logarithm in base ten
MOA:	Ministry of Agriculture
PCA:	Plate Count Agar
PDA:	Potato Dextrose Agar
pH :	Hydrogen ion concentration
SD:	Standard Deviation
SG:	Specific Gravity
SNF:	Solid Not-Fat
SPC:	Standard Plate Count
SPSS:	Statistical Package for Social Science
TA:	Titrable Acidity
TBC:	Total Bacterial Count
TCC:	Total Coliform count
TS:	Total Solid
VRBA:	Violet Red Bile Agar
WHO:	World Health Organization
YMC:	Yeast and Mould Count

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ABSTRACT

Despite milk is a highly nutritious food, it can easily be contaminated with physicochemical and microbiological hazards. The aim of this study was to investigate the physicochemical properties and microbial load of raw cow milk produced and marketed in Jimma town. A total of 22 samples of raw cow milk were collected randomly from selected dairy farms, vendors, and cafeterias from five study sites in the town. The obtained data were analyzed by SPSS-20 statistical software. The obtained results showed that there were significant differences ($P<0.05$) observed in temperature (26.7±0.42 and 21.24±0.83 °C), total solid (12.74±0.98 and 11.05±0.59%), fat (3.63±0.07 and 3.02±0.20%), protein (3.45±0.09 and 2.57±0.44%) between dairy farms and cafeterias milk samples, respectively. However, there were no significant differences ($P>0.05$) in pH, specific gravity, lactose, solid not fat, and ash of collected milk samples among the milk value chain points. The most physicochemical quality of the examined milk samples were within the acceptable levels except milk samples from cafeterias. The overall mean values for total bacterial, total coliform, and yeast and mould count of milk samples were 6.14±0.07, 5.98±0.04, 3.94±0.17 in dairy farms, 7.63±0.20, 7.48±0.01, 4.10±0.14 in milk vendors, and 8.30±0.47, 7.52±0.02, 4.48±0.44 log $^{CFU/mL}$, in milk cafeterias respectively. All microbial load of milk samples were significantly different ($p<0.05$) and recorded greater than recommended values. Finally, it is possible to conclude that milk quality deterioration increased from dairy farms to cafeterias and may cause public health risks. Therefore, this suggests the need for improved hygienic practice and educating the public on safety issues at all milk value chain points.

Keywords: *Raw Cow's milk, physicochemical properties, Jimma town, milk value chains, total bacterial count, coliform count, yeast and mould count.*

1. INTRODUCTION

Milk is the most popular food for human consumption and is considered as a complete and nutritious food; not only for the new-born but for all age groups in both rural and urban people all over the world [1]. Milk is a complex biological fluid and by its nature, a good growth medium for many microorganisms. Because of its physico-chemical properties, it needs strict hygienic conditions to avoid contamination of milk with microorganisms. As a result, it often deteriorates and becomes inappropriate for human consumption, and processing [2].

Milk and milk products are among the most important food products of animal origin [3]. Milk is universally recognized as a complete food, which contains essential components for human nutrition. It is a colloidal composition containing water, fat, protein, lactose, minerals, and other constituents [4]. It is a vital supply of nutrients needed for the growth, maintenance, production, and correct functioning of the bodies of mammals [5].

However, milk and its products may be contaminated by various environmental pollutants from agricultural, veterinary, and hygienic practices [6]. Physiochemical analysis is an important tool to monitor the quality of milk and other dairy products. Dairy product quality starts at the farm as good dairy products can only be made from good quality of raw cow milk. So, milk should have normal composition, should be not adulterated, and should be produced under hygienic conditions [7]. Physicochemical parameters of milk and milk products can be affected by adulteration, which is done either for financial gain or lack of proper hygienic conditions of processing, storing, transportation and marketing. This ultimately leads to the stage that the consumer is either cheated or often becomes a victim of diseases [8].

Microbial load is a major factor in determining milk quality. It originates from different sources: air, milking equipment, feed, soil, faeces, and grass. The microbial load of milk reduces not only its nutritional quality but also may threaten the health of the consumers. Microorganisms may contaminate milk at various stages including production, procurement, processing, and distribution. In addition to the health of animals, the cleanness of animals, milking practices, milk handling, and equipment used may affect the quality and safety of milk [9]. Contamination of milk can leads milk to spoil which not suit for human consumption. Many milk borne diseases are spread through milk contamination. Quality milk production is necessary for fulfilling

consumers' demands. Generally, Quality milk is free from pathogenic bacteria and harmful toxic substances, free from sediment and extraneous substances, of good flavor, with normal composition, adequate in keeping quality, and low in bacterial counts [10].

Hygienic quality control of raw milk and milk products in Ethiopia is not usually conducted on a routine basis [11]. A research report at Jimma milk shed in south-western Ethiopia revealed that good hygienic milking practices are not well-practiced, and no formal quality control system to monitor the quality of milk produced and sold in the town [12]. In this study area, the limited studies have been reported on physico-chemical properties and microbial load of raw cow milk along dairy value chain from dairy farms to selling points. Besides, lack of adequate information and facts mentioned above necessitate the need for periodic investigation into the quality status of sample milk produced and marketed in the area. In the study, it was intended to find out whether the quality of the milk collected from dairy farms, and vendors or cafeterias, which were collected from the same area, was similar or not. Thus, investigation of the physicochemical properties and microbial load of raw cow milk collected from milk value chain points of Jimma town is important. Therefore, the purpose of this study is to investigate the physicochemical properties and microbial load of raw cow milk collected from milk value chain points in the study area.

1.1. Statement of the problem

Milk is a complex biological fluid and by its nature, a good growth medium for many microorganisms. Because of its physicochemical properties, it needs strict hygienic conditions to avoid contamination of milk with microorganisms [2].

The rapidly increasing human population along with growing urbanization increased the demand for milk and its products all over the world [12]. In Ethiopia, milk is produced in both urban and rural areas mostly in non-organized ways and is usually supplied to the consumers in raw form. Adulteration of milk due to lack of proper hygienic conditions such as milking, cleaning, storing, transportation, and marketing may lead to microbial contamination, and deterioration of quality of milk. These not only lead to ethical and economical problems, but also cause diseases like gastroenteritis, tuberculosis, and typhoid fever [13]. Therefore, examination of physicochemical properties and microbial load, which is a major factor in determining milk quality, should be addressed for better health of the people in the community.

Thus, the study was designed to answer the following basic research questions.

1. Do the physico-chemical properties of milk samples collected from dairy farms, vendors, and cafeterias are the same or varied?
2. What happen to the level of milk microbiological load as milk passes through dairy farms, vendors, and cafeterias in the study area?
3. Do the physico-chemical properties and microbiological loads of raw cow milk samples fit the national and international milk quality standards?

1.2. Objectives of the study

1.2.1. General objective

The main objective of this study is to assess the physicochemical properties and microbial loads of raw cow milk samples produced and marketed in Jimma town.

1.2.2. Specific objectives

- To determine the physicochemical properties such as temperature, pH, titratable acidity (TA), specific gravity (SG), fat, protein, lactose, solid not fat, and ash content of raw cow milk samples in Jimma town.
- To determine the microbiological load such as total bacteria, coliform, yeast and mould counts of raw cow milk samples in Jimma town.
- To evaluate the quality of fresh cow milk in the study area based on national and international milk quality standards.

1.3. Significance of the study

The significance of this study is to provide information on the physicochemical properties and microbial load of milk and factors that influence the quality of milk in the study area. Furthermore, this study is mainly important for producing milk of good hygienic quality, which is necessary to produce milk products with superior quality and thereby provide safe and wholesome food for the consumers. The results from the study may also be used as a baseline for further studies.

2. LITERATURE REVIEW

2.1. Milk composition and characteristics

Milk is a yellowish-white non-transparent liquid secreted by the mammary glands of all mammals. It is the primary source of nutrition and sole food for the offspring of mammals before they can eat and digest other types of food. It contains in a balanced form all the necessary and digestible elements for building and maintaining the human and animal body [14]. It is a highly nutritious substance that contains macro and micronutrients of fats, proteins, carbohydrates, vitamins, minerals and active compounds having a role in health protection. Milk protein, fat, and lactose are important sources of energy [15]. Chemical composition, particularly milk fat content is used as a quality test. The solid constituents of milk make an important food item from both nutritional as well as processing points of view. Milk Fat and protein are the most important components of different varieties of most shelf-stable milk products. Therefore it is very important to determine the major chemical compositions of milk as it is the basis of further processing into more shelf-stable products [16]. According to Ramesh [17], the major components of milk are water (87.4%), milk solids (12.60%), solids-not-fat (9.0%), fat (3.60%), protein (3.40%), milk sugar or lactose (4.90%) and ash or minerals (0.70%). The constituents may vary with genetics in terms of breed and individual cow and variability among cows within a breed and environment in terms of the interval between milking, stage of lactation, age, feeding regime, disease, and completeness of milking.

2.2. Physicochemical properties of milk

2.2.1. Milk pH

Milk pH indicates hygienic condition of milk and it should be between 6.6 and 6.8 when milk temperature is 20 °C because cooling of milk reduces the risk of growth of bacteria while high milk temperature must be considered as favorable to the growth of bacteria in milk [11]. The pH values higher than 6.8 indicate mastitis milk and pH values below 6.6 indicate increased acidity of milk due to bacterial multiplication [18]. The pH decreases with increasing temperature. At a given temperature, differences in pH and buffering capacity between individual lots of fresh milk reflect compositional variation [19].

2.2.2. Specific gravity

Specific gravity is the ratio of the density of the substance to the density of a standard substance (water). The density of a substance varies with temperature, it is necessary to specify the temperature when reporting specific gravities or densities. The specific gravity of milk is influenced by the proportion of its constituents (e.g. Composition), each of which has different specific gravity approximately as follows; Water (1.000), Fat (0.930), Protein (1.346), Lactose (1.666), Salts (4.12), and solid not fat (1.616). The specific gravity of milk is decreased by the addition of water, the addition of cream (fat), while removal of fat and reduction of temperature increases the specific gravity of milk. Generally, normally milk has a specific gravity between 1.027 and 1.035 with an average value of 1.032 at 16 °C [20].

2.2.3. Titratable acidity

Titratable acidity is a measure of freshness and bacterial activity in milk. The natural acidity of individual milk varies considerably, depending on species, breed, individuality, stage of lactation, and the physiological condition of the udder, etc. When the milk is kept for some time, the bacteria will multiply and utilize lactose and convert into lactic acid, thereby increasing the acidity and decreasing the pH value. This acidity is known as developed or real acidity. The sum of natural acidity and developed acidity is known as titratable acidity [21]. Titratable acidity of milk has long been recognized and employed as an indicator of quality. It is expressed in terms of percentage lactic acid since lactic acid is the principal acid produced by fermentation after milk is drawn from the udder. 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 [22]. The degree of bacterial contamination and the temperature at which the milk is kept are the chief factors influencing acid formation. Therefore, the amount of acid depends on the cleanliness of production and the temperature at which milk is kept [23].

2.2.4. Total solid in milk

Milk solids are non-water components of milk protein, fat, lactose, and minerals. Total solids are measured to ensure the quality of milk and milk products. The total solids in milk can be determined by indirect method from the specific gravity and fat content from lactometer reading. A direct method of gravimetric analyses can also be used. This method involves accurately

weighing a few grams of the material and subjecting it to heat until all moisture has been driven off on a water bath. The dry residue is weighed, its percentage calculated as total dry solids [24].

2.2.5. Solids non-fat

Solid non-fat is an important criterion of milk selection for further processing. Milk solids non-fat would include nitrogenous substances, milk sugar, and mineral matter. The fluid milk contains a minimum of 8.25 percent SNF. The determination of solid non-fat is done by taking a lactometer reading at 40 °C. Solids-not-fat (SNF) content was determined by the following formula [25]. SNF content (%) =TS (%) – Fat (%)……. (1)

2.2.6. Fat content

Fats are one of the most important components of all mammals' milk because they affect the cost, nutritional value, and physical and sensory characteristics of dairy products positively. Milk fat has the most complex fatty acid composition of edible fats. Over 400 individual fatty acids have been identified in milk fat. However, approximately 15 to 20 fatty acids make up 90% of the milk fat. The major fatty acids in milk fat are straight-chain fatty acids that are saturated and have 4 to 18 carbons, monounsaturated fatty acids, and polyunsaturated fatty acids. Some of the fatty acids are found in very small amounts but contribute to the unique and desirable flavor of milk fat and butter [19].

2.2.7. Protein in milk

Milk comprises casein, lactoalbumins, and lactoglobulins. About 82 percent of the protein in milk is casein and the remaining proteins are whey proteins, which are lactealbumin and lactoglobulin. Casein binds with calcium in milk and forms the calcium casein are complex, which is present in the colloidal form. Acid, rennet, alcohol, and heat can precipitate this complex. The proteins in milk are of great quality, that is to say, they contain all the essential amino acids and elements that our bodies cannot produce. It is important to remember that proteins are the building blocks of all living tissue. Milk proteins have roughly the same composition as egg protein, except for the amounts of methionine and cystine, significantly lower. Indeed, the sulfur amino acids are the limiting factors in milk. Casein and, even more, the complex milk protein contains a good proportion of all amino acids essential for growth and maintenance [26].

2.2.8. Ash content

Ash is the inorganic residue remaining after the water and organic 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. Analytical techniques for providing information about the total mineral content are based on the fact that the minerals can be distinguished from all the other components within food in some measurable way. The most widely used methods are based on the fact that minerals are not destroyed by heating, and that they have low volatility compared to other food components. The main analytical techniques used to determine the ash content of foods are based on this principle: dry ashing, wet ashing, and low-temperature plasma dry ashing [19].

2.3. Bacteriological quality of raw cow milk

Microbial quality of milk refers to the cleanness of milk. The microbial content of milk is a major feature in determining of milk quality. Milk has a complex biochemical composition and high water activity. Due to its high nutritive value, raw milk serves as a good medium for microbial growth that degrades the milk quality and shelf-life of milk. The demand of consumers for safe and high quality milk has placed a significant responsibility on dairy producers, retailers, and manufacturers to produce and market safe milk and milk products [27]. Milk from a healthy udder contains very few numbers of bacteria should be less than $3x10^4$ CFU/mL, but may become contaminated by microorganisms from the surrounding environment during milking and milk handling [28]. This is very complicated in general to attain in developing countries because of poor hygiene and cleanliness during milking and subsequent milk handling, contaminated water sources for cleaning milk utensils, lack of cooling amenities, high ambient temperature, and insufficient infrastructures for milk transportation to the market [29]. The detection of coliform bacteria, pathogens, and high microbial count in milk are major factors in determining its quality. It indicates the hygienic level exercised during milking, 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].

2.3.1. Sources of microbial contamination in milk value chain

Microbial contamination in milk comes from milk itself as it can be naturally contaminated or comes from infected or sick animals, humans, the environment, water, and equipment used for milking and storage of milk. These sources of contamination include disease-causing organisms (pathogens) shedding in milk, infected udder and/or teats, animal skin, faecal soiling of the udder, contaminated milking and storage equipment's, and water used for cleanliness. Other bacterial sources are from air, milkers, handlers, drugs or chemicals used during treatment of animal and from water used for adulteration by unscrupulous and unfaithful workers/sellers who may be contaminated and may cause additional health problems [31]. Exposure of milk to these sources or conditions may lead to increased microbial contamination and affect its quality. However sometimes recontamination may occur after processing mainly due to unhygienic conditions and poor or improper handling of milk during consumptions [32]. In general quality of milk may be lowered when it is contaminated by a number of factors such as adulteration, contamination during and after milking, presence of udder infections, mastitis (inflammation of mammary gland) disease and drugs residues used for treatments of disease which is considered to be public health concern and one of the most important causes of economic losses in the dairy industry worldwide [33].

2.3.2. Total bacterial count

Standard plate count is generally accepted as the most accurate and informative method of testing the bacteriological quality of milk [34]. The total plate count of microbes in milk provides useful general information on the microbiological quality of milk. The number of bacteria in aseptically drawn milk varies from animal to animal and even from different breasts of the same animal [35]. According to the Ethiopian Standards Agency, the bacteriological grades of quality of whole/raw cow milk according to the total number of bacterial plate count/mL is shown as follows in table 1 [36].

Table 1: Grade of raw cow milk based on Standard Plate Count (SPC)

Bacterial count CFU/mL	Grade
Not exceeding 200,000	Very good
200,000-1,000,000	Good
1,000,000-2,000,000	Bad
>2,000,000	Very bad

2.3.3. Coliform count

Coliforms are aerobic, facultative anaerobic, gram-negative, and non-spore-forming rods that ferment lactose to produce gas when incubated on agar for 48 hours at 35 °C [37]. Coliform organisms contaminate raw milk from unclean milker's hands, improperly cleaned and unsanitized or faulty sterilization of raw milk utensils especially churns, milking machines, improper preparation of the cow's flecks of dirt, manure, hair dropping into milk during milking, udder washed with unclean water, dirty towels and udder not dried before milking [38]. The presence of coliform organisms in milk indicates unsanitary conditions of production, processing, or storage. Hence their presence in large numbers of dairy products is an indication that the products are potentially hazardous to the consumers' health [34].

According to the Ethiopian Standards Agency, the bacteriological grades of quality of whole/raw cow milk according to the total number of CFU/mL are shown as follows in table 2. These standards are more or less similar to other international standards according to different works of literature [36].

Table 2: Grade of raw cow milk based on Violet Red Bile Agar (VRBA) in raw cow milk

Coliform count CFU/mL	Grade
Not exceeding 1,000	Very good
1,000-50,000	Good
50,000-500,000	Bad
>500,000	Very bad

2.3.4. Yeast and mould count

Yeasts and moulds are special class of microorganisms belonging to group fungi. Yeasts are single cell organisms larger than bacteria. They reproduce by budding and also by formation of spores. They are commonly found in soil, fruits, and dairy products [27]. Yeast and mould count is the number of colonies in the sample that grow and form countable colonies on potato Dextrose Agar (PDA) after being held at 25 °C for 3 to 5 days. Yeasts are widely distributed in the dairy environments and appear as natural contaminants in raw milk, air, and dairy utensils [9]. Total Yeast and Mold Counts (TYMC) are used to detect and quantify the amount of fungal growth on foods, and allow for identification of viable yeast and mold species present. The amount of fungi is reported as the number of colony forming units per mL (CFU/mL) [36].

3. MATERIALS AND METHODS

3.1. Description of the study area

The study was carried out in Jimma town of Oromia Regional State, southwestern Ethiopia. Jimma town is located at 355 km south-western of Addis Ababa, capital of Ethiopia, having a latitude of 7°41′N and longitude of 36°50′E and an elevation of 1704 meters above sea level. The area is characterized by a humid tropical climate of heavy annual rainfall that ranges from1200-2000 mm. About 70% of the total annual rainfall is received during the wet season, which lasts from the end of May to early September. The area has a relatively higher temperature of about 25 °C-30 °C from January to April, and a minimum temperature of 7 °C -12 °C from October to December [39].

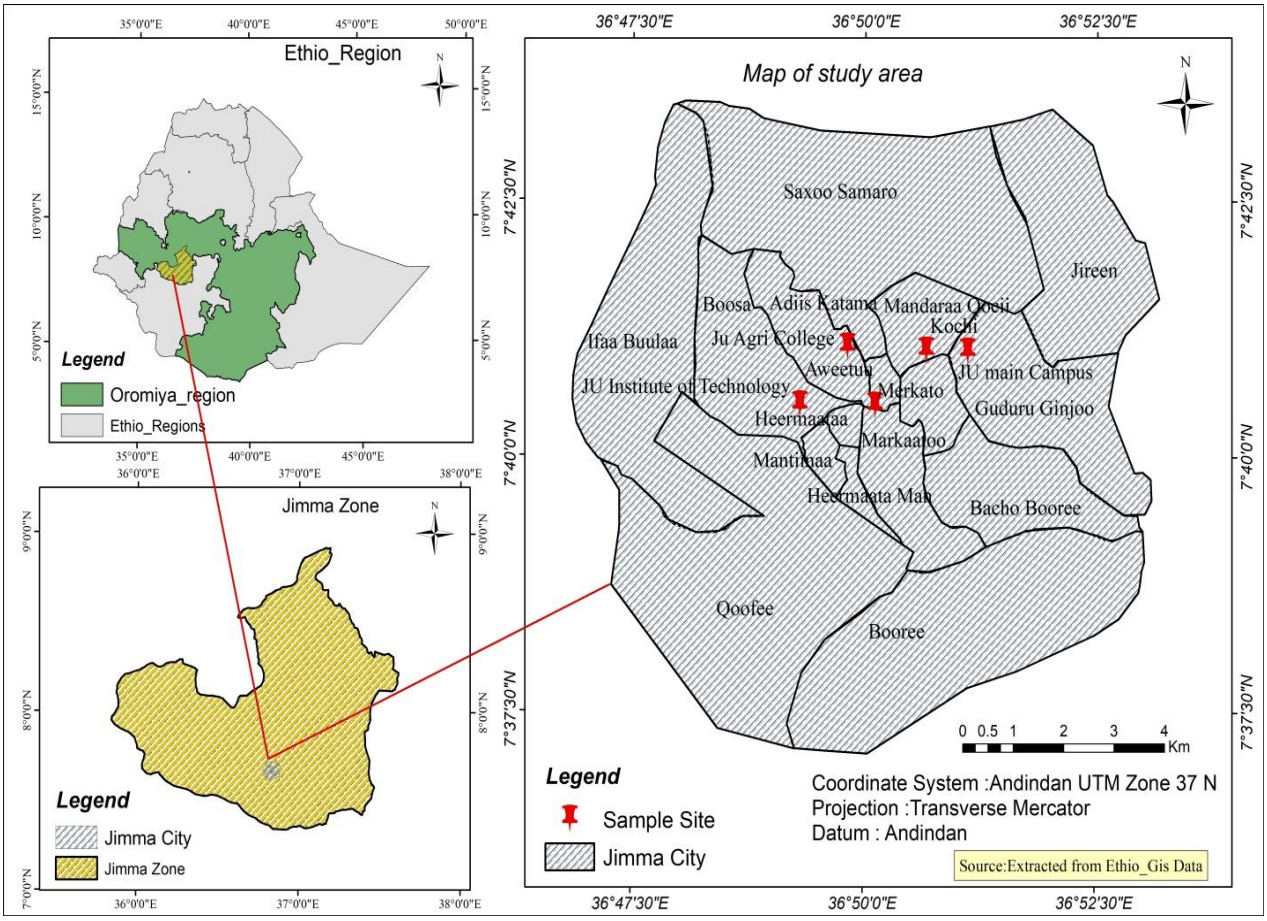

Figure 1: Map of study area

3.2. Milk sample collection and Sampling techniques

The study was carried out from November, 2021 to February, 2022 in five purposively selected study sites, namely, Kochi, Merkato, Jimma University (Main campus, College of agricultural and Veterinary medicine, and Institute of technology) in Jimma town. The raw cow milk samples were randomly collected from the three milk value chain points such as dairy farms, milk vendors, and cafeterias. A total of 22 milk samples (4 from dairy farms, 9 from milk vendors, and 9 from the cafeterias) were collected from selected study sites. Samples from the dairy farm were collected during the early morning before delivery to vendors and samples from the cafeterias were collected from bulk milk container during midday. For composite milk samples, an equal volume of milk from the same site was collected and mixed thoroughly. During collection, about 500 mL was collected aseptically from each dairy farm, vendors, and cafeterias in pre-sterilized, properly labeled, and stoppered glass sample bottles. In each day, fresh raw milk samples were collected to the laboratory in an ice-box filled with ice packs (1-5 °C) for analyses of the physicochemical properties and microbial load of raw cow's milk.

3.3. Apparatus and instruments

Apparatus and instruments used in this study includes pH meter (portable code 013 , Germen), thermometer, lactometer, butyrometer, water bath, centrifuge, analytical balance, desiccator, drying oven, muffle furnace (Serial D-6072, Model N-7 made in Germany), Kjeldahl apparatus (serial 5450200034, Model IP20 made in Germany), i.e. digestion apparatus, distillation apparatus, incubator (lab-Incubator, Model-IN-010 made in Germany), and colony-forming counter (Funke Gerber code 2013, Switzerland). Besides, common laboratory glasses wares were used in sample collection, preparation, and analysis of physicochemical quality of milk samples.

3.4. Chemicals and reagents

Different chemicals and reagents such as 1% alcoholic solution of phenolphthalein indicator obtained from UNICHEM chemical reagent (Blulux, India), potassium sulfate, copper (II) sulfate, concentrated sulphuric acid; 98% (m/m), and hydrochloric acid all from Loba Chemi Pvt.Ltd (India), sodium hydroxide (Steinheim, Germany), buffers of pH 4 and 7 (Merck), Boric acid, methyl red, bromocresol green indicator, amyl alcohol, peptone (code 64271, Germany),

standard plate count agar, violet red bile agar, and potato Dextrose agar solution were used in the study.

3.5. Analyses of physicochemical properties

3.5.1. Determination of temperature and pH

The temperature of the milk samples was determined at the collection point using a thermometer while the pH of the milk samples was determined in the laboratory using a digital pH meter [40]. The pH meter was first calibrated using buffers of pH 7.0 and 4.0 each time before the milk sample pH measurement.

3.5.2. Determination of titratable acidity

Titratable acidity of the milk samples was determined according to the method of the Association of Official Analytical Chemists (AOAC) [41]. 10 mL of milk sample was pipetted into a beaker and 5 drops of 1% phenolphthalein indicator were added to it. The milk sample was then titrated with 0.1 N NaOH solution until a faint pink color persisted. The titratable acidity expressed as %lactic acid was finally calculated using the following formula.

$$\text{Titratable acidity (\%)} = \frac{\text{mL of 0.1 N NaOH x0.009}}{\text{mL of milk sample}} \text{ x } 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

3.5.3. Determination of specific gravity

Fresh milk sample was filled sufficiently into a glass cylinder (100 mL capacity). Then lactometer was held by the tip and inserted into the milk. The lactometer was allowed to float freely until it reaches equilibrium. Then the lactometer reading at the lower meniscus was recorded immediately. At the same time, the thermometer was inserted into the milk sample and the temperature of the milk was recorded [42]. The following formula was used to calculate the specific gravity of the milk.

Specific gravity =1+ (corrected temprature reading + lactomer reading)/1000

Specific gravity = (L/1000) +1…… (3)

Where, L = corrected lactometer reading at a given temperature, i.e., for every degree above 60 °F (15.6 °C), 0.2 was added to the lactometer reading but for every degree below 60 °F, 0.2 was subtracted from the lactometer reading.

3.5.4. Determination of total solid

For the determination of total solids content, a fresh cow milk sample was thoroughly mixed and 5 g was transferred to a pre-weighed and dried flat bottom crucible. The milk samples were dried in a hot air oven at 102 °C for 3 hours. Finally, the dried samples were taken out of the oven and placed in desiccators to cool to room temperature. Then samples were weighed again and total solids were calculated by the following formula [43].

$$\text{Total solids} = \frac{\text{Crucible weight+Oven dry sample weight} - \text{Crucible weight}}{\text{Sample weight}} \text{ x100} \ldots\ldots\ldots\ldots (4)$$

3.5.5. Determination of milk fat content

The fat content was determined according to the Gerber method [8]. 10 mL of 90% sulfuric acid was pipetted into a butyrometer. Then 11 mL of milk sample was added into the butyrometer and mixed with the sulphuric acid. This was followed by the addition of 1 mL amyl alcohol into the butyrometer which was then closed with a lock stopper. Then the mixture was shaken and inverted several times until the milk was completely digested by the acid. Finally, the butyrometer was kept in a water bath for 5 minutes at 65 °C and centrifuged in a Gerber centrifuge for 5 minutes. The butyrometer was placed in a water bath again at 65 °C for 5 minutes. In the end, the butyrometer reading was recorded.

3.5.6. Determination of solids not-fat

Solids-not-fat (SNF) content was determined by difference as reported by [44] using the following formula: SNF content (%) = TS (%) – Fat (%)……. (1)

3.5.7. Determination of crude protein content

The crude protein content of milk samples were determined by the Kjeldahl method [45].

Digestion: 5 g of milk sample was warmed in the water bath at 38 °C and poured into a Kjeldahl tube. A mixture of 15 g potassium sulfate, 1 mL of copper sulfate solution, and 25 mL of concentrated sulphuric acid was added into the tube and mixed gently. The digestion was carried out for 120 minutes at 350 °C using micro-Kjeldahl digester in the presence of a catalyst (1 mL of copper sulfate and 15 g potassium sulfate) where sulphuric acid was used as an oxidizing agent. Then it was allowed to cool at room temperature for 25 minutes. The digested solution was diluted with 250 mL of distilled water.

Distillation: The Kjeldahl tube was placed in the distillation equipment. 75 mL of 40% sodium hydroxide solution was added to the tube. Then ammonia was distilled using 50 mL of 4% boric acid solution with bromocresol green/methyl red as indicators until blue color appears. Finally, the sample was titrated with 0.1 N hydrochloric acid solution until a faint pink color was formed and the burette reading was taken to the nearest 0.01 mL. A blank test was carried out using the above procedure except that water was used instead of the test sample. The percentage of nitrogen in the milk samples was calculated using the below formula [46].

$$\%N = \frac{1.4007x\ (Vs - Vb)\ X\ N\ HCl}{Weight\ of\ sample} \text{ x } 100 \quad \text{..... (5)}$$

$$\%CP = \%N \text{ x } 6.38 \quad \text{..... (6)}$$

Where, %N = percentage of nitrogen by weight, Vs = volume of HCl used for titration of a sample, Vb = volume of HCl used for titration of the blank, %CP = percent of crude protein.

3.5.8. Determination of ash content

The ash content of milk samples was determined according to the method of the Association of Official Analytical Chemists (AOAC) [43]. The dried milk samples used for the determination of total solids content were ignited in a muffle furnace at a temperature of 550 °C until they were free from carbon (residue appears grayish to white) for four hours, then the samples were transferred to desiccator to cool down. The dish containing the sample was then re-weighed after the dish was completely cooled. The ash percent of the sample was calculated as follows:

$$\%Ash = \frac{Weight\ of\ residue}{Weight\ of\ sample} \text{x}100 \quad \text{..... (7)}$$

3.5.9. Determination of lactose content

Percent lactose was determined by subtracting the fat, protein, and total ash percentages from the percentage of the total solids [47].

Percent Lactose = Percent total solids – (%fat + %protein + %ash)……. (8)

3.6. Microbial analysis

The microbial analysis of milk samples includes the determination of colony-forming units (CFUs) of total bacteria, coliform bacteria, as well as yeast and mould using appropriate media. All media used for microbial analyses were sterilized before use.

3.6.1. Preparation of solution

Appropriate decimal dilution was selected and samples were thoroughly mixed and serially diluted by adding 1 mL milk was mixed with 9 mL sterilized peptone water in the test tube to get a dilution of (1:10). From this, further dilutions of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} was done [46]. All diluted samples were applied to Petri plates. The Petri plates were labeled with dilution factor, media, and sample numbers.

3.6.2. Total bacterial count

For total bacterial count (TBC), appropriate decimal dilutions that would give the expected total number of colonies between 30 and 300 colonies were selected. The molten standard plate count (SPC) agar was cooled to 45 °C after sterilization before pouring into Petridish. 1 mL of milk sample was added into a sterile test tube containing 9 mL of peptone water up to a serial dilution of 10^{-7} and mixed thoroughly. Then, 1 mL of the sample from appropriate decimal dilution was placed on a petri-dish and then plate count agar medium (10-15 mL) was poured onto the petri-dish. The plated sample was allowed to solidify and finally incubated at 32 °C for 48 hours. Colony counts were made by using colony counter [48].

3.6.3. Coliform count

After mixing, the sample was serially diluted up to $1:10^{-5}$ by transferring 1 mL of the sample into 9 mL of peptone water for initial dilution and by transferring 1 mL of the previous dilution into 9 mL of peptone water and the duplicate sample (1 mL) were poured using 15-20 mL violet red bile agar solution (VRBA). The plated sample was allowed to solidify and finally incubated at 32 °C for 24 hours. Colony counts were made by using colony counter [49].

3.6.4. Yeast and mould count

Samples of milk were serially diluted up to 10^{-4} in peptone water and volumes of 0.1 mL of appropriate dilutions were plated in duplicate. A spread plate technique was employed using a

pre-sterilized dry surface of Potato Dextrose Agar supplemented with 0.1 g chloramphenicol per liter and then, dried plates were inverted and incubated at 25 °C for 3 to 5 days. The Smooth (non-hairy) colonies without an extension at the periphery were counted as yeasts whereas hairy colonies with extension at periphery were counted as moulds [49].

3.7. Statistical data analysis

The obtained data from both physicochemical properties and microbial load were reported as mean and standard deviation of replicate analyses. Data obtained was analyzed using the procedure of IBM Statistical Package for Social Sciences software version 20.0 computer programs. One way ANOVA at ($P<0.05$) was also used to evaluate the variations among the studied milk samples interms of the analyzed parameters.

4. RESULTS AND DISCUSSIONS

4.1. Physicochemical properties of raw cow milk

All the obtained results from the physicochemical properties of raw cow milk samples collected from dairy farms, vendors, and cafeterias in Jimma town compared to the standard values suggested by Ethiopian standard (ES) and European Union quality standards, and presented in Table 3-5.

Table 3: Physico-chemical properties (mean ±SD) of the studied raw cow milk samples collected from dairy farms of study sites in Jimma town

	Milk source				
Parametre	**A_1 (N=2)**	**A_3 (N=2)**	**Overall mean**	**ES [50]**	**EU [59]**
T (°C)	27±0.00	26.4±0.2	26.7±0.42	NA	NA
pH	6.78±0.03	6.64±0.005	6.71±0.98	6.6-6.8	6.6-6.8
SG	1.029±0.0	1.031±0.001	1.03±0.001	1.026-1.032	1.027-1.035
TA%	0.165±0.0	0.141±0.001	0.152±0.169	0.14-0.17	0.14-0.16
TS%	12.05±0.14	13.45±0.08	12.74±0.98	10.50-14.50	12.5-14.5
Fat%	3.58±0.032	3.69±0.01	3.63±0.07	2.50-7.0	3.5-6.0
SNF%	8.47±0.11	9.76±0.07	9.12±0.91	>8.0	8.25-10.5
Protein%	3.39±0.03	3.52±0.02	3.45±0.92	2.90-5.0	2.73-5.0
Lactose%	4.38±0.11	5.39±0.01	4.89±0.71	3.60-5.50	4.2-5.5
Ash%	0.7±0.01	0.78±0.01	0.74±0.05	0.60-0.90	0.7-0.8

A_1: Merkato, A_3: Jimma University, SD: Standard deviation, ES: Ethiopian standard, EU: European Union standard, *N: Number of samples, T °C: Temperature in degree Celsius, SG: Specific gravity, %TS: total solid percentage, %SNF: solid not fat percentage, NA; not available, %TA: Titratable acidity percentage, %TS: Total solid percentage, and SNF: Solid not fat percentage.*

Table 4: Physicochemical properties (mean ± SD) of the studied raw cow milk samples collected from vendors of study sites in Jimma town.

Parametre	Milk source			Overall mean	ES [50]	EU [59]
	A_1 (N=3)	A_2 (N=3)	A_3 (N=3)			
T (°C)	27.12±0.1	24.6±0.12	22.51±0.1	24.75±2.30	NA	NA
pH	6.383±0.0	6.46±0.01	6.65±0.01	6.49±0.13	6.6-6.8	6.6-6.8
SG	1.022±0.0	1.026±0.0	1.030±0.0	1.026±0.04	1.026-1.032	1.027-1.035
TA%	0.186±0.0	0.163±0.0	0.156±0.0	0.168±0.01	0.14-0.17	0.14-0.16
TS%	11.2±0.2	11.75±0.1	12.19±0.1	11.71±0.49	10.50-14.50	12.5-14.5
Fat%	3.16±0.02	3.29±0.04	3.51±0.03	3.32±0.17	2.50-7.0	3.5-6.0
SNF%	8.04±0.22	8.46±0.09	8.68±0.15	8.39±0.32	>8.0	8.25-10.5
Protein%	2.93±0.06	3.19±0.03	3.28±0.06	3.13±0.18	2.90-5.0	2.73-5.0
Lactose%	4.45±0.19	4.55±0.14	4.66±0.09	4.55±0.10	3.60-5.50	4.2-5.5
Ash%	0.693±0.0	0.72±0.01	0.75±0.01	0.72±0.028	0.60-0.90	0.7-0.8

A_1: Merkato, A_2: Kochi, A_3: Jimma University, SD: Standard deviation, ES: Ethiopian standard, EU: European Union standard, *N: Number of samples, T* °C*: Temperature in degree Celsius, SG: Specific gravity, %TS: total solid percentage, %SNF: solid not fat percentage, NA; not available, %TA: Titratable acidity percentage, %TS: Total solid percentage, and SNF: Solid not fat percentage.*

Table 5: Physicochemical properties (mean ± SD) of the studied raw cow milk samples collected from cafeterias of study sites in Jimma town.

Parametre	Milk source			Overall mean	ES [50]	EU [59]
	A_1(N=3)	A_2 (N=3)	A_3 (N=3)			
T (°C)	22.2±0.28	20.8±0.76	20.7±0.64	21.24±0.83	NA	NA
pH	6.31±0.01	6.45±0.05	6.61±0.05	6.456±0.15	6.6-6.8	6.6-6.8
SG	1.020±0.01	1.024±0.01	1.028±0.0	1.024±0.04	1.026-1.032	1.027-1.035
TA%	0.206±0.06	0.273±0.05	0.17±0.05	0.217±0.05	0.14-0.17	0.14-0.16
TS%	10.53±0.23	10.93±0.20	11.7±0.17	11.05±0.59	10.50-14.50	12.5-14.5
Fat%	2.82±0.02	3.01±0.08	3.23±0.02	3.02±0.20	2.50-7.0	3.5-6.0
SNF%	7.71±0.23	7.92±0.1	8.47±0.18	8.03±0.39	> 8.0	8.25-10.5
Protein%	2.13±0.025	2.59±0.04	3.01±0.03	2.57±0.44	2.90-5.0	2.73-5.0
Lactose%	4.92±0.23	4.63±0.1	4.69±0.23	4.75±0.15	3.60-5.50	4.2-5.5
Ash%	0.66±0.05	0.69±0.01	0.78±0.02	0.712±0.06	0.60-0.90	0.7-0.8

A1: Merkato, A_2: Kochi, A_3: Jimma University, SD: Standard deviation, ES: Ethiopian standard, EU: European Union standard, *N: Number of samples, T* °C*: Temperature in degree Celsius, SG: Specific gravity, %TS: total solid percentage, %SNF: solid not fat percentage, NA; not available, %TA: Titratable acidity percentage, %TS: Total solid percentage, and SNF: Solid not fat percentage.*

The overall mean temperature of raw cow milk samples was significantly different ($P < 0.05$) among three milk value chain points (Table 3-5). The overall mean temperature of milk samples collected from dairy farms (26.7 ± 0.42 °C) was significantly ($P<0.05$) higher than vendors (24.75 ±2.30 °C) and cafeterias (21.24 ±0.83 °C). This might be due to the cooling of milk from cow body temperature to the ambient temperature when transported from farm to milk markets [9]. The mean value of temperature for vendors and cafeterias milk samples of the study area were also significantly different, which might be due to temperature difference of the environment, milk handling equipment and handling techniques and time elapsed since

production [51]. Lack of cooling system and inefficient use of refrigerator in milk sellers increase the temperature of the milk samples. This could contribute to the increased number of microbial contaminants in the milk. Besides, inadequate cooling increases bacterial counts by creating a better environment for bacterial growth during storage [52].

The milk pH gives an indication of milk hygiene and freshness and it usually ranges between 6.6 and 6.8 [53]. As can be seen from table 3-5, the overall mean pH value of milk collected from the dairy farms, vendors, and cafeterias in the study areas were 6.71±0.98, 6.49±0.13, and 6.45±0.15 respectively. The mean (±SD) pH value of milk samples obtained from dairy farms was within the standards values, indicating that the milk samples were most probably obtained directly from farms shortly after milking [50]. However, the mean (±SD) pH of milk samples obtained from vendors, and cafeterias was not within recommended values set by ES [50] and EU [54]. This result indicates that milk is clearly under fermentation resulting from bacterial multiplication during the time that elapsed between production and until it reaches markets [55]. The analysis of the ANOVA showed that there was no significant difference in pH of the examined milk samples collected from three milk value chain points in the study area. However, there was a significant difference between dairy farms and cafeterias.

The mean (±SD) of the specific gravity of raw milk samples collected from dairy farms, vendors, and cafeterias were 1.030±0.001, 1.026±0.004, and 1.024±0.004 respectively. The mean (±SD) specific gravity of raw milk samples from dairy farms were within acceptable values, while the specific gravity of raw milk samples obtained from vendors, and cafeterias were below the acceptable values. These variations might be due to mixing of milk from different sources that might have been adulterated with water [55]. Statistically, it was found that there were no significant differences ($P > 0.05$) within the specific gravity of milk collected from the three milk value chain points. However, there was a significant difference between dairy farms and cafeterias. Generally, the specific gravity of milk can be affected by various factors. For instance, the specific gravity of milk decreases by the addition of water and addition of cream; while it is increased by the removal of fat and reduction of temperature [18].

Normal fresh milk has an apparent acidity of 0.14 to 0.16% as lactic acid [18]. The overall mean (± SD) of titratable acidity of milk sample collected from the dairy farms, vendors, and cafeterias were 0.152±0.16%, 0.168±0.01%, and 0.217±0.05% respectively. The milk samples collected from dairy farms were within the recommended values [50, 54]. However, the milk samples collected from vendors, and cafeterias were far above the upper limit of the standard levels. This might be due to keeping of the milk samples at a normal temperature long times and highly poor handling practices till they were oversubscribed and/or consumed [56]. The result showed that there were significant differences ($P<0.05$) in milk from the dairy farms to vendors and cafeterias. Generally, this result indicates that the milk samples collected from cafeterias were no fresh milk; as it developed acidity due to bacterial growth and multiplication during transportation of milk to the selling sites and longer storage of the milk before sale [40].

The overall mean total solids content of milk collected from dairy farms, vendors, and cafeterias were 12.74±0.98%, 11.71±0.49%, and 11.05±0.59%, respectively. The result of this study also revealed that the total solid of dairy farms were within the quality standard values given by ES [50], and EU [54]. However, the sample results obtained from vendors, and cafeterias were less than standards. The variation might be due to difference management practices, in breed, and feeding which have important effects on milk composition and quality [46]. In this study, the data indicate a significant difference ($P<0.05$) in the total solids (TS) content between three milk value chain points.

Milk fat is unquestionably the most valuable constituent of milk [57]. The overall mean value of fat content in milk samples collected from the dairy farms was 3.63±0.07% followed by vendors and cafeterias (3.32±0.17% and 3.02±0.20%, respectively). The result revealed that the fat content of dairy farms were within recommended values [50, 54]. However, the sample results obtained from both vendors, and cafeterias were less than the recommended values. This might be due to removal of fat or partial skimming, and adulteration of milk [58]. The result also showed the presence of significant differences ($P<0.05$) in milk from the dairy farms to vendors and cafeterias. The mean fat content of milk from the dairy farm was significantly higher ($P < 0.05$) than the fat content of milk obtained from other milk value chain points.

The overall mean values of solid not fat (SNF) content of raw milk samples collected from dairy farms, vendors, and cafeterias were 9.12±0.91%, 8.39±0.32%, and 8.03±0.39% respectively. The result of this study also revealed that the SNF content of dairy farms and vendors were within the quality standard values [50, 54]. However, the results of the sample obtained from cafeterias were below both standards. The study exposed that there were no significant differences ($P>0.05$) within the SNF content of milk collected from dairy farms, vendors, and cafeterias.

The overall mean protein contents of milk sampled from dairy farms, vendors and cafeterias were (3.45±0.09%, 3.13±0.18%, and 2.57±0.44%) respectively. The overall values of this study for protein sampled from dairy farms, and vendors were within standard values [50, 54]. However, the Protein percent of milk sampled from the cafeteria (2.57%) was less than both standards. A significant difference ($P < 0.05$) in protein content was observed among milk value chain points. The protein content of milk obtained from the dairy farms was significantly higher ($P < 0.05$) than milk obtained from vendors, and cafeterias. The result revealed that decreasing in protein content of milk from the dairy farms to the cafeterias. This might be due to the adulteration of milk by water. In general, the composition of milk can vary depending on the breed of the animals, management practices such as feeding management, and environmental factors that influenced the milk composition [59].

The overall mean lactose content of raw cow milk collected from dairy farms, milk vendors, and cafeterias were (4.89±0.71%, 4.55±0.10%, and 4.75±0.15%) respectively. The result of this study also revealed that the lactose content of all milk value chain points were within the quality standard values [50, 54]. Statistical analysis showed that there was no significant difference ($p>0.05$) among milk value chain points. The overall mean for lactose content of milk sampled from the dairy farm was higher than that of vendors and cafeterias. This might be due to the action of lactose hydrolyzing enzymes produced by microorganisms as a result of storage temprature variation [60].

The overall mean ash contents of raw milk samples collected from dairy farms, vendors, and cafeterias were 0.74±0.56%, 0.72±0.02%, and 0.71±0.06% respectively. The mean total mineral content of the milk ranges from 0.66 to 0.78 among milk value chain points. All milk value chain points were within the acceptable values. The ash content of cow milk remains relatively

constant 0.7 to 0.8% which is influenced by breed, stage of lactation, and feed of the animal [61]. Statistically, it was found that there were no significant differences among the three critical milk value chain points.

4.2. Microbial load of raw cow milk

All the obtained results from the microbial load of raw cow milk samples collected from dairy farms, vendors, and cafeterias of the study sites in Jimma town compared to the standard values suggested by Ethiopian standard (ES) and European Union quality standards, and presented in Table 6 and 7.

Table 6: Microbial load ($\log^{CFU/mL}$) of the studied raw cow milk samples collected from dairy farms of study sites in Jimma town.

	Milk source				
Parametre	**A_1 (N=2)**	**A_3 (N=2)**	**Overall mean**	**ES [36]**	**EU [62]**
TBC	6.20±0.005	6.09±0.012	6.14±0.07	≤ 5.3	≤ 5.6
TCC	6.01±0.035	5.94±0.017	5.98±0.049	≤ 3	≤ 2.3
YMC	4.07±0.02	3.82±0.043	3.94±0.17	____	≤ 2.1

A_1: Merkato, A_3: Jimma University, SD: Standard deviation, TBC: Total bacterial count, TCC: Total coliform count, YMC: Yeast and Mould count, ES: Ethiopian standard; EU: European Union standard, *$\log^{CFU/mL}$: Logarithm in base 10 of colony-forming unit per mL.*

Table 7: Microbial loads ($\log^{CFU/mL}$) of the studied raw cow milk samples collected from vendors and cafeterias of study sites in Jimma town.

Parametre	Milk source						
	Milk value chains	**A_1(N=3)**	**A_2 (N=3)**	**A_3 (N=3)**	**Overall mean**	**ES [36]**	**EU [62]**
TBC	Milk vendors	7.86±0.02	7.53±0.036	7.49±0.019	7.63±0.20	≤ 5.3	≤ 5.6
	Cafeterias	8.64±0.01	8.49±0.15	7.76±0.044	8.3±0.47		
TCC	Milk vendors	7.5±0.00	7.47±0.00	7.48±0.00	7.48±0.015	≤ 3	≤ 2.3
	Cafeterias	7.54±0.00	7.49±0.019	7.52±0.017	7.52±0.025		
YMC	Milk vendors	4.04±0.16	4.26±0.59	4.00±0.021	4.10±0.14	____	≤ 2.1
	Cafeterias	4.97±0.71	4.10±0.020	4.38±0.012	4.48±0.44		

A_1: Merkato, A_2: Kochi, A_3: Jimma University, SD: Standard deviation, TBC: Total bacterial Count, TCC: Total coliform Count, YMC: Yeast and Mould count, ES: Ethiopian standard, EU: European Union standard, *$\log^{CFU/mL}$: Logarithm in base 10 of colony-forming unit per mL.*

The overall mean total bacterial count of milk samples collected from dairy farms, vendors, and cafeterias were 6.14±0.07, 7.63±0.20, 8.3±0.47 $\log^{CFU/mL}$ respectively. The all milk samples showed TBC higher than the acceptable levels given by ES [36], and EU [62]. This might indicate poor hygienic milk practice including unhygienic milking, unclean udder or diseased udder, unsanitary facilities, and unfavorable storage conditions [63]. The analysis of the ANOVA showed that there was a significant difference in bacterial load among milk value chain points in the study area at $P<0.05$ (Table 6 & 7). The bacterial loads of milk from the cafeterias were the poorest in bacterial quality. Generally, there was an increment in microbial load among milk value chain points of milk marketing from dairy farms to the cafeterias level.

The overall mean obtained for TCC results of milk samples collected from milk value chain points were 5.98±0.049, 7.48±0.015, and 7.52±0.025 $\log^{CFU/mL}$ from dairy farms, vendors, and cafeterias respectively. The present study showed that the coliform count of all milk samples exceeds the standards values set by ES [36], and EU [62]. This might be attributed to the hygienic condition such as dirty equipment, contact with manure of the cow during milking, and

personal hygiene of the milkers [63]. In the current study, one way ANOVA ($P<0.05$) indicated that the mean coliform count was significantly different among milk samples collected from the three milk value chain points (Table 6 & 7). The overall mean TCC of raw cow milk sampled from cafeterias, and vendors were significantly ($P<0.05$) higher than that of the milk sampled from dairy farms. Generally, the difference in the overall mean TCC observed in the study area might be associated with further contamination of the milk during transportation, not well cleaned milking utensils, and the absence or improper cooling systems at milk selling points [64].

The overall mean of YMC were 3.94±0.17, 4.10±0.14, and 4.48±0.44 $\log^{CFU/mL}$ for milk samples collected from the dairy farms, vendors, and cafeterias respectively. The presence of yeasts and moulds in milk samples collected from the dairy farms, vendors, and cafeterias were higher than the accepted levels. This might be due to poor hygiene of equipment during handling, transporting, and processing of milk, and indicates unsanitary conditions of handling and contamination from the environment [46]. Yeast and mold count showed significant differences among milk value chain points. Samples due to the sampling point of the cafeterias were significantly higher than other points ($p<0.05$). This might be attributed to contamination from dust, air, containers, water used, poor personal hygiene, and poor hygiene of milk selling environment [65]. Generally, the high YMC observed in milk obtained from cafeterias might be attributed to contamination from air, containers, or poor personal hygiene of milk sellers [66].

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

In the present study, the physicochemical parameters and microbial load of milk samples collected from dairy farms, vendors, and cafeterias in Jimma town were investigated. Most of the physico-chemical properties of milk samples collected from dairy farms and vendors were within the acceptable level. However, all the physico-chemical properties of milk samples collected from cafeterias were not within the recommended levels except lactose and ash, indicating the significance negative impact of milk adulteration and removal of fat content. The obtained results for TBC, TCC, and YMC of all examined milk samples collected from all milk value chain points were above the maximum recommended limits set by ES and EU/FAO. The overall microbial count increased from milking farm to markets, reflecting poor hygiene at milking, milk handling, and transportation. In general, the overall microbial quality of milk produced as well as marketed in the study area is poor. Therefore, adequate sanitary and control measures should be taken at all stages from production to consumer level to produce and supply wholesome milk.

5.2. Recommendations

The following recommendations are forwarded based on the finding that showed poor quality of the milk in the study area.

- Good hygienic and sanitation practices should be applied across the milk value chains from the stages of production to consumption.
- Producers, vendors, consumers, and other stakeholders should be trained on factors that deteriorate the quality of milk.
- Proper sterilization and storing of the milk should be followed seriously across all the milk value chains especially at cafeteria level.
- Further research is required to assess contamination of milk by giving attention to those pathogens that have a human health hazards.

6. REFERENCES

[1]. Fufa, A.; Nigus, T.; Fikru, R.; Dinka, A., and Kebede, A. Handling Practices, Quality and Safety of Milk along the Dairy Value Chains in Selected Sub Cites of Addis Ababa, Ethiopia. *Bio. J. sci. Tech. res.*, **2019**, 13(1), 2574-1241.

[2]. Kibebew, B.; Mitiku, E., and Firew, K. Hygienic Production Practices and Microbial Quality of Cow Milk in Cheha District of Gurage Zone, Southern Ethiopia. *Open J. Ani. Sci.*, **2020,** 10(3). DOI: 10.4236/ojas.2020.103038.

[3]. Tasci, F. Microbiological and Chemical Properties of Raw Milk Consumed in Burdur. *J. Anim. Vet. Adv*., **2011**, 10(5), 635-4.

[4]. Walstra, P.; Wouters, J. T. M.; and Geurts, T. J. Milk: Main Characteristics, In Dairy Science and Technology, 2nd ed. CRC Press; Boca Raton: 2006, pp12.

[5]. Neumann, C. G.; Harris, D. M.; and Rogers, L. M: Contribution of Animal Source Foods in Improving Diet Quality and Function in Children in the Developing. *World. Nutri. Rese.* , **2002**, 22,193-220.

[6]. William, F. "contaminants of milk and dairy products: Contamination results from Farm and dairy practices." Module in food science, 2015, 1-13.

[7]. Marimuthu, M.; Sankar, N.; Sathish, A.; Vivek, S.; and Mohan Raj, N. Comparative Study on Physiochemical Quality of Raw Milk Samples Collected from Different Villages of Karur District, Tamilnadu, India. *Int. J. Pharmaceut. Chem. and Biol. Sci*., **2013**, 3(3), 635-8.

[8]. Teklemichael, T.; Ameha, K.; Eyassu, S. Physico Chemical Properties of Cow Milk Produced and Marketed in Dire Dawa Town, Eastern Ethiopia. *F. Sci. Qua. M.*, **2015**, 42, 2225-0557.

[9]. Mitiku, E.; Alemnesh, Y. and Firew, K. Microbial Quality and Safety of Raw Cow Milk in Girar Jarso District of Oromia Regional State, Ethiopia. *Eth. J. Anim. Prod*. **2019**, 19(1):37-55.

[10]. Khan, M.T.G.; Zinnah, M.A.; Siddique, M.P.; Rashid, M.H.A.; Islam, M. A., and Choudhury, K.A. Physical and microbial qualities of raw milk collected from Bangladesh Agricultural University Dairy Farm and the Surrounding Villages, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh, Bangl. *J. Veteri. Medi.* **2008**, 6, 217–221.

[11]. Bruktawit, S. physicochemical properties and microbial quality of cow milk collected from selected sub city of Addis Ababa, Ethiopia MSc Thesis College of veterinary Medicine and Agriculture, Addis Ababa University, 2016.

[12]. Gashaw, A.; Gebrehiwot, E. Study on Milk Hygiene, Quality Control in the Market Chain in Jimma. *J Adv. Dairy Res*. **2018**, 6: 213. doi:10.4172/2329-888X.1000213.

[13]. Mboya, N.J. microbiological and compositional quality of raw milk delivered by small scale dairy farmers to the milk collection centers in hai district, tanzania MSc thesis, dairy science and technology morogoro, tanzania, 2016 .

[14]. Pandey, G.S. and Voskuil, G.C.S. Manual on Milk safety, quality and hygiene. Golden Valley Agricultural Research Trust, Zambia, 2011, pp52.
[15]. Merwan, A.; Nezif, A.; and Metekia, T. Review on milk and milk product safety, quality assurance and control. *Int.J. Livestock Prod*., **2018**, 9(4), 67-78.
[16]. Goff, H.D. Introduction to dairy science and technology: Milk history, consumption, production, and composition. Dairy science and Technology University of Guelph. 2010. (www.foods.Guelph.ca\dairyedu\itro.Html, Reviewed on 16/2/2015).
[17]. Ramesh, C.C. Manufacturing Yogurt and Fermented Milks, 6th ed., Blackwell Publishing Ltd, Oxford, Uk, 2006, pp7-40.
[18]. O'Connor, C.B. Rural Dairy Technology ILRI Training Manual I, International Livestock Research Institute, Addis Ababa, Ethiopia; 1995.
[19]. McCarthy, O. J. Physical and Physico-Chemical Properties of Milk, Massey University, Palmerston North, New Zealand; 2002.
[20]. Morris, B. Jacobs. The chemical analysis of foods and food products. Third edition. CBS, Publishers and Distributors. New Delhi, India, 1999.
[21]. Krishnaiah, N. Quality control of milk and processing. Dept. of Veterinary Public Health. ANGRAU, Rajendra Nagar, Hyderabad, 2005.
[22]. Lampart, L.M. Milk and dairy products, their composition, food value, chemistry, bacteriology and processing. Chemical Publishing Co., Inc., Brooklyn, N.Y; 2005, pp242.
[23]. Heineman, P. G. Milk., W. B. Saunders Company, Philadelphia and London; 2001, pp266-337, 195-197.
[24]. Berhe, T. A.; Teklit, B.K. Physicochemical Properties and Comparisons of Goat and Cow Milk. Review *IJEDR*, **2018,** 6, (3): 2321-9939.
[25]. Harris, B. and Bachman, K.C. Nutritional and Management Factors Affecting Solids-Not-Fat, Acidity and Freezing Point of Milk Original publication date December 1988. Reviewed June, 2003.
[26]. Guetouache, M.G.; Bettache and Medjekal, S. Composition and nutritional value of raw milk Vol.2 (10), December, 2014, pp115-122.
[27]. Mennane, Z.; Ouhssine, M.; Khedid, K.; Elyachioui, M. Hygienic Quality of Raw Cow's Milk Feeding from Domestic. *Int. J. Agri. Biol*., **2007**, 9(1):1560–8530.
[28]. Robinson R. K. Dairy Microbiology Hand Book, Third Edition. John Wiley and Sons Inc., New York, 2002.
[29]. Bille, P. G.; Haradoeb, B.R.; Shigweda, N. Evaluation of chemical and bacteriological quality of raw milk from Neudamn Dairy Farm in Namibia. *AJANDev.*, **2009**, 9 (7):1511:1523.
[30]. Estifanos, H.; Tarekegn, G.; Yonas ,H.; Eyassu, S.; Mengistu, K.; Mohammed, A. Physicochemical Properties and Microbial Quality of Raw Cow Milk Collected from Harar Milkshed, *Eastern Ethiopia J. Biol. Chem. Research.* , **2015**, 32(2):606-616.

[31]. Swai, E.S. and Schoonman, L. Microbial quality and associated health risks of raw milk marketed in the Tanga region of Tanzania. *APJTB*. , **2011**, 1(3): 217 - 222.
[32]. Parekh, T.S. and Subhash, R. Molecular and bacteriological examination of milk from different milch animals with special reference to Coliforms. *Cur. Rese. Bacterio*. , **2008**, 1(2): 56 - 63.
[33]. Mdegela, R.H.; Ryoba, R.; Karimuribo, E.D.; Pire, E.J.; Løken, T.; Reksen, O.; Mtengeti E. and Urio, N.A. Prevalence of clinical and subclinical mastitis and quality of milk in smallholder dairy farms in Tanzania. *JSAVA*. , **2009**, 80(3): 163 - 168.
[34]. Godefay, B. and Molla, B. Bacteriological quality of raw milk from four dairy farms and milk collection center in and around Addis Ababa. Berl. Munch. Tierärztl.Wschr. 2000, pp.113.
[35]. O'Connor, C.B. Rural Dairy Technology, ILRI Training Manual No.1. International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia, 1994.
[36]. Tamiret, T. Microbiological quality analyses of Raw and pasteurized milk samples collected from Addis Ababa and its surrounding in Ethiopia. *Appro. Poultry, Dai. Veter. sci.*, **2018**, 4(5), 2576-9162.
[37]. FAO (Food and Agricultural Organization). Manuals of food quality control, food analysis, quality adulteration and tests of identity, Food and Nutrition paper and Agricultural Organization of the United Nations, Rome, 1986.
[38]. Saba, H. Quality Assessment of Cattle Milk in Adea Berga and Ejerie Districts of West Shawa Zone, Ethiopia MSc thesis School of Animal and Range Science, Haramaya University, Haramaya, 2015.
[39]. Office of Planning and Economic Development for Jimma Zone (OPEDJZ).Statistical Abstract. Jimma, Oromia, Ethiopia.2002, pp161.
[40]. Gurmessa, T.; Debela, M. E., and Alemayehu, R. Physico-chemical qualities of raw cow milk in Ethiopia. The case of Borana zone, Yabello District. *G. J. Dai. Farm. Prod*., **2015**, 3 (2), 2449-1861.
[41]. Zerihun, A.; Getenesh, T. Physical Properties and Chemical Compositions of Raw Cow Milk in Milk Shades Around Addis Ababa, Ethiopia. *J.N. Sci. Res*., **2019**, 9 (19), 2224-3186.
[42]. O' Mahoney, F. Rural Dairy Technology- Experiences in Ethiopia. ILCA manual No 4.1998.
[43]. Negash, F; Tadesse, E. and Woldu, T. Microbial quality and chemical composition of raw cow milk in the Mid –Rift valley of Ethiopia. *Afri. J. Agri. Res.*, **2012**, 7 (29), 4167-4170.
[44]. Getachew, F. "Assessments report on the dairy subsector in Ethiopia". FAO (Food and Agriculture Organization of the United Nations), Rome, Italy, 2003.
[45]. AOAC. "Official Methods of Analysis", AOAC, Arlington, VA, 2005.
[46]. Teshome, G.; Tesfaye, A. Physicochemical Properties and Microbial Quality of Raw Cow Milk Produced by Smallholders in Bench Maji-Zone, Southwestern Ethiopia. *F. Sci. Qual. Man.*, **2016**, 54, 2224-6088.

[47]. Teshome, G.; Fikadu, B. and Mitiku, E. physical and chemical quality of raw cow's milk produced and marketed in Shashemene town, southern Ethiopia. *J. f. agri. Sci.,* **2015**, 5(2), 7-13.

[48]. Birhanu, Y.; Asaminew, T.; Hailu, Microbiological Quality of Raw Cow's Milk In and Around BahirDar City, Ethiopia. *F. Sci. Qual. Man.,* **2019**, 88, 2225-0557.

[49]. Bekele, A.; Fekadu, B.; and Mitiku, E. Chemical Composition and Microbial Quality of Cow Milk in Urban and Peri Urban Area of Dangila Town, Western Amhara Region, Ethiopia. *Int.J.Curr.Res.Aca.Rev. ,* **2020**, 8(11): 78-84.

[50]. Zerihun, A.; Getenesh, T. Physical Properties and Chemical Compositions of Raw Cow Milk in Milk Shades Around Addis Ababa, Ethiopia. *J.N. Sci. Res*., **2019**, 9 (19), 2225-0921.

[51]. Amanual, B; Ulfina, G. Review on hygienic milk products practice and occurrence of Mastitis in cow's milk. *Agri. Res & Tech.* **2018**, 18(2), 556053.

[52]. Reinemann, J.D.; Mein, G.A.; Bray, D.R.; Britt, J.S. "Trouble shooting High Bacteria Counts in Farm Milk". Sources of Bacterial Contamination in Raw Milk, 2005.

[53]. Food and Agriculture Organization of the United Nations (FAO) Extension Services for Quality Milk Production. Proceedings of an International Workshop in Conjunction with the East-West-Forum of the Federal Ministry for Food, Agriculture and Forestry, Germany pp35, 1999.

[54]. Raff, H. Market implications of changing fat content of milk and dairy products, fat content and composition of animal products. *J. Food Sci. Technol*., **2011**, 5 (2): 6 - 17.

[55]. Mitiku, E.; Mekdes, S.; Yesihak, Y. M. Milk production, marketing practices and qualities along milk supply chains of Haramaya District, Ethiopia. *Afri. J. Agri. Res*., **2019**, 14(35), 1991-637, DOI: 10.5897/AJAR.

[56]. Kearson, D. The Chemical Analysis of Food. 7th Ed., Chemical Publishers Co., Berlin, pp9-11, 2005

[57]. Tamime, A.Y. Milk Processing and Quality Management. Society of Dairy Technology, United Kingdom, 2009.

[58]. Haftu, K. S., and Degnet, H. M. Determination of Adulteration and Chemical Composition of Raw Milk Sold in Hossana Town, South Ethiopia. *Dairy and Vet Sci J*., **2018**, 6(5): DOI: 10.19080/JDVS.2018.06.555699.

[59]. Jalel, F.; Berhan, T.; Ulfina, G., and Kefena, E. Physico-Chemical Analysis of Milk Collected From Urban Areas of Oromia Special Zone Surrounding Finfinne, Ethiopia. *IJDST*, **2021**, 5(2), 1716-3498.

[60]. Shibru, D.; Tamir, B.; Kassa, F.; Goshu, G. Physicochemical Quality of Raw and Pasteurized Milk from Sebeta and Bishoftu Urban Areas, Oromia, Ethiopia. *Euro. Exp. Biol*., **2019**, 9 (6), 2248-9215.

[61]. Amistu, K.; Degefa, T.; Melese, A. Assessment of Raw Milk Microbial Quality at Different Critical Points of Oromia to Milk Retail Centers in Addis Ababa. . *F. Sci. Qual. Man.,* **2015**, 38, 2225-0557.

[62]. Fernandes, R. Microbiology Handbook Dairy Products. pp.100, 2009.
[63]. Alemnesh, Y., Mitiku, E., and Firew, K. Producing, Processing, Marketing and Hygiene of Cow Milk in the Supply Chain of Girar Jarso District of Oromia Regional State, Ethiopia. *East Afr. J. Sci*., **2020**, 14 (2), 151-162.
[64]. Adugna, C.; Eshetu, M. Hygienic practice, microbial quality and physco-chemical properties of milk collected from farmers and market chains in Eastern Wollega zone of Sibu Sire districts, Ethiopia. *J. Agric S.c Food Technol.*, **2021**, 7(1): 125-132. DOI: 10.17352/2455-815X.000099.
[65]. Mitiku, E. G.; Mulu, M. A. & Yesihak, Y. M. Milk Production, Marketing and Quality in Meta District of Eastern Hararghe Zone, Ethiopia. *J. Agri. Sci.*, **2019**, 11(5), 1916-9760.
[66]. Gurmessa, T. D. Microbiological quality and impact of hygienic practices on raw cow's milk obtained from pastoralists and market. The case of Yabello District, Borana zone, Ethiopia. *G. J. F. Sci. and Techno.* , **2015**, 3(2), 2408-5472.

APPENDICES

Appendix 1: Microbial load plates

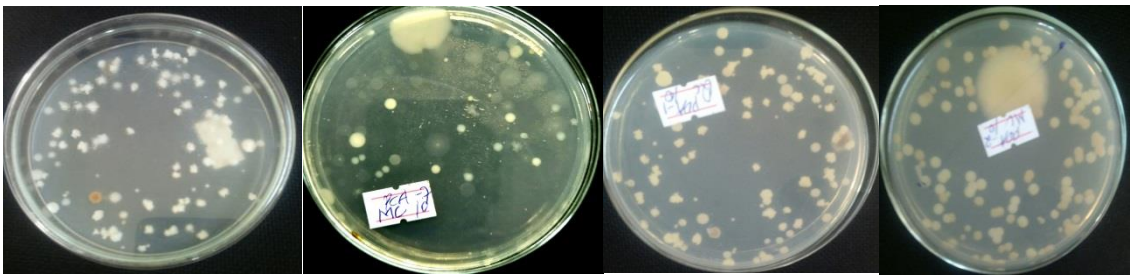

Figure 2: Total bacteria colony in milk samples

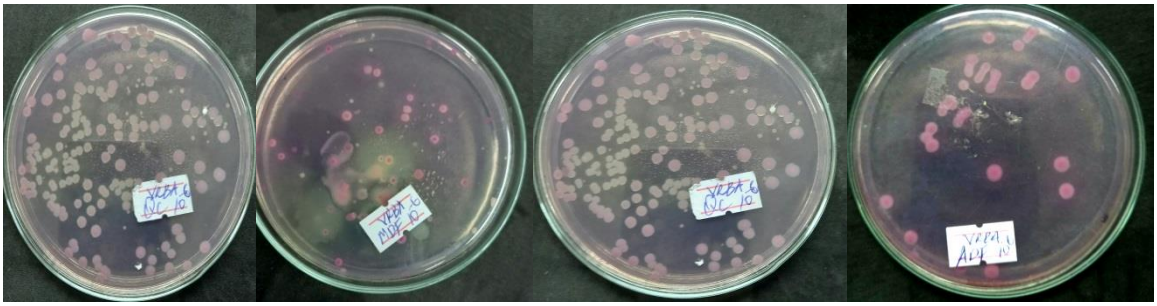

Figure 3: Total coliform colony in milk samples

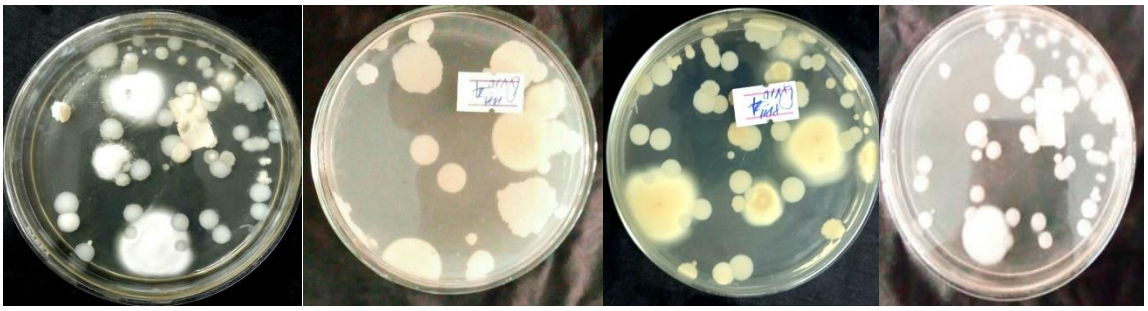

Figure 4: Yeast and Mould colony in milk samples

Appendix 2: List of tables for statistical analyses of milk samples

ANOVA of physicochemical properties of milk obtained from the study area

Table 1: ANOVA Table for Physicochemical Properties of Raw Cow Milk

		Sum of Squares	Df	Mean Square	F	Sig.
T	Between Groups	39.178	2	19.589	8.003	.028
	Within Groups	12.238	5	2.448		
	Total	51.416	7			
PH	Between Groups	.084	2	.042	2.252	.201
	Within Groups	.093	5	.019		
	Total	.177	7			
SG	Between Groups	.000	2	.000	1.648	.282
	Within Groups	.000	5	.000		
	Total	.000	7			
TA	Between Groups	.006	2	.003	2.503	.176
	Within Groups	.006	5	.001		
	Total	.012	7			
TS	Between Groups	3.455	2	1.728	3.963	.093
	Within Groups	2.179	5	.436		
	Total	5.634	7			
FAT	Between Groups	.459	2	.230	7.513	.031
	Within Groups	.153	5	.031		
	Total	.612	7			
SNF	Between Groups	1.414	2	.707	2.615	.167
	Within Groups	1.352	5	.270		
	Total	2.765	7			
Protein	Between Groups	1.005	2	.502	5.438	.056
	Within Groups	.462	5	.092		
	Total	1.467	7			
Lactose	Between Groups	.139	2	.069	.600	.584
	Within Groups	.579	5	.116		
	Total	.718	7			
ASH	Between Groups	.001	2	.000	.191	.832
	Within Groups	.012	5	.002		
	Total	.013	7			

DF: Degrees of freedom, TA: titratable acidity, T: Temprature, SG: specific gravity, TS: total solid percentage, %SNF: solid not fat percentage, %TA: Titratable acidity percentage, , %TS: Total solid percentage, and SNF: Solid not fat percentage, sig.: significant value at 0.05.

Table 2: ANOVA Table microbial load of the studied raw cow milk

		Sum of Squares	Df	Mean Square	F	Sig.
TBC	Between Groups	5.624	2	2.812	26.438	.002
	Within Groups	.532	5	.106		
	Total	6.156	7			
TCC	Between Groups	9.079	2	4.540	12.051	.012
	Within Groups	1.884	5	.377		
	Total	10.963	7			
YMC	Between Groups	12.919	2	6.460	7.416	.032
	Within Groups	4.355	5	.871		
	Total	17.274	7			

DF: Degrees of freedom, TBC: Total bacterial count, TCC: Total coliform count, YMC: Yeast and Mould count, sig.: significant value at 0.05.

Table 3: Descriptive analysis for Physicochemical Properties of Raw Cow Milk

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
T	Dairy farms	2	26.7000	.42426	.30000	22.8881	30.5119
	Vendors	3	24.7500	2.30775	1.33238	19.0172	30.4828
	Cafeterias	3	21.2333	.83865	.48419	19.1500	23.3167
	Total	8	23.9188	2.71019	.95820	21.6530	26.1845
PH	Dairy farms	2	6.71000	.098995	.070000	5.82057	7.59943
	Vendors	3	6.49667	.138684	.080069	6.15216	6.84118
	Cafeterias	3	6.45667	.150111	.086667	6.08377	6.82956
	Total	8	6.53500	.159194	.056284	6.40191	6.66809
SG	Dairy farms	2	1.03000	.001414	.001000	1.01729	1.04271
	Vendors	3	1.02600	.004000	.002309	1.01606	1.03594
	Cafeterias	3	1.02400	.004000	.002309	1.01406	1.03394
	Total	8	1.02625	.003955	.001398	1.02294	1.02956
TA	Dairy farms	2	.15300	.016971	.012000	.00053	.30547
	Vendors	3	.16833	.015695	.009062	.12934	.20732
	Cafeterias	3	.21733	.050954	.029418	.09076	.34391
	Total	8	.18288	.041326	.014611	.14833	.21742
TS	Dairy farms	2	12.75000	.989949	.700000	3.85566	21.64434
	Vendors	3	11.71333	.496017	.286376	10.48116	12.94551
	Cafeterias	3	11.05333	.594671	.343333	9.57609	12.53058
	Total	8	11.72500	.897170	.317198	10.97495	12.47505
FAT	Dairy farms	2	3.63500	.077782	.055000	2.93616	4.33384

	Vendors	3	3.32000	.176918	.102144	2.88051	3.75949
	Cafeterias	3	3.02000	.205183	.118462	2.51030	3.52970
	Total	8	3.28625	.295729	.104556	3.03901	3.53349
SNF	Dairy farms	2	9.11500	.912168	.645000	.91950	17.31050
	Vendors	3	8.39333	.325167	.187735	7.58557	9.20109
	Cafeterias	3	8.03333	.392471	.226593	7.05838	9.00828
	Total	8	8.43875	.628546	.222224	7.91327	8.96423
Protein	Dairy farms	2	3.45500	.091924	.065000	2.62910	4.28090
	Vendors	3	3.13333	.181751	.104934	2.68184	3.58483
	Cafeterias	3	2.57667	.440151	.254122	1.48327	3.67006
	Total	8	3.00500	.457759	.161842	2.62230	3.38770
Lactose	Dairy farms	2	4.88500	.714178	.505000	-1.53163	11.30163
	Vendors	3	4.55333	.105040	.060645	4.29240	4.81427
	Cafeterias	3	4.74667	.153080	.088380	4.36640	5.12694
	Total	8	4.70875	.320243	.113223	4.44102	4.97648
ASH	Dairy farms	2	.74000	.056569	.040000	.23175	1.24825
	Vendors	3	.72100	.028513	.016462	.65017	.79183
	Cafeterias	3	.71200	.061579	.035553	.55903	.86497
	Total	8	.72238	.043687	.015446	.68585	.75890

Table 4: Descriptive analysis for microbial load of the studied raw cow milk

						95% Confidence Interval for Mean	
		N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound
TBC	Dairy farms	2	6.14500	.077782	.055000	5.44616	6.84384
	Vendors	3	7.62667	.203060	.117237	7.12224	8.13110
	Cafeterias	3	8.29667	.470779	.271805	7.12719	9.46615
	Total	8	7.50750	.937744	.331543	6.72353	8.29147
TCC	Dairy farms	2	5.04000	1.371787	.970000	-7.28502	17.36502
	Vendors	3	7.48333	.015275	.008819	7.44539	7.52128
	Cafeterias	3	7.51667	.025166	.014530	7.45415	7.57918
	Total	8	6.88500	1.251433	.442449	5.83878	7.93122
YMC	Dairy farms	2	3.94500	.176777	.125000	2.35672	5.53328
	Vendors	3	4.10000	.140000	.080829	3.75222	4.44778
	Cafeterias	3	6.66000	1.463660	.845044	3.02407	10.29593
	Total	8	5.02125	1.570909	.555400	3.70794	6.33456

Table 5: Multiple Comparisons test for Physicochemical Properties of Raw Cow Milk

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
T	Dairy farms	Vendors	1.95000	1.42817	.230	-1.7212	5.6212
		Cafeterias	5.46667*	1.42817	.012	1.7954	9.1379
	vendors	Dairy farms	-1.95000	1.42817	.230	-5.6212	1.7212
		Cafeterias	3.51667*	1.27740	.040	.2330	6.8003
	Cafeterias	Dairy farms	-5.4667*	1.42817	.012	-9.1379	-1.7954
		Vendors	-3.5167*	1.27740	.040	-6.8003	-.2330
PH	Dairy farms	Vendors	.213333	.124722	.148	-.10727	.53394
		Cafeterias	.253333	.124722	.098	-.06727	.57394
	vendors	Dairy farms	-.213333	.124722	.148	-.53394	.10727
		Cafeterias	.040000	.111555	.735	-.24676	.32676
	Cafeterias	Dairy farms	-.253333	.124722	.098	-.57394	.06727
		Vendors	-.040000	.111555	.735	-.32676	.24676
SG	Dairy farms	Vendors	.004000	.003317	.282	-.00453	.01253
		Cafeterias	.006000	.003317	.130	-.00253	.01453
	vendors	Dairy farms	-.004000	.003317	.282	-.01253	.00453
		Cafeterias	.002000	.002966	.530	-.00563	.00963
	Cafeterias	Dairy farms	-.006000	.003317	.130	-.01453	.00253
		Vendors	-.002000	.002966	.530	-.00963	.00563
TA	Dairy farms	Vendors	-.015333	.031552	.648	-.09644	.06577
		Cafeterias	-.064333	.031552	.097	-.14544	.01677
	vendors	Dairy farms	.015333	.031552	.648	-.06577	.09644
		Cafeterias	-.049000	.028221	.143	-.12155	.02355
	Cafeterias	Dairy farms	.064333	.031552	.097	-.01677	.14544
		vendors	.049000	.028221	.143	-.02355	.12155
TS	Dairy farms	vendors	1.03666	.602679	.146	-.51257	2.58590
		Cafeterias	1.69667*	.602679	.037	.14743	3.24590
	vendors	Dairy farms	-1.03666	.602679	.146	-2.58590	.51257
		Cafeterias	.660000	.539053	.275	-.72568	2.04568
	Cafeterias	Dairy farms	-1.6967*	.602679	.037	-3.24590	-.14743
		vendors	-.660000	.539053	.275	-2.04568	.72568
FAT	Dairy farms	vendors	.315000	.159609	.105	-.09529	.72529
		Cafeterias	.615000*	.159609	.012	.20471	1.02529
	vendors	Dairy farms	-.315000	.159609	.105	-.72529	.09529
		Cafeterias	.300000	.142759	.090	-.06697	.66697
	Cafeterias	Dairy farms	-.61500*	.159609	.012	-1.02529	-.20471
		Vendors	-.300000	.142759	.090	-.66697	.06697
SNF	Dairy farms	Vendors	.721667	.474620	.189	-.49838	1.94172
		Cafeterias	1.08166	.474620	.072	-.13838	2.30172
	vendors	Dairy farms	-.721667	.474620	.189	-1.94172	.49838

		Cafeterias	.360000	.424513	.435	-.73124	1.45124
	Cafeterias	Dairy farms	-1.08166	.474620	.072	-2.30172	.13838
		Vendors	-.360000	.424513	.435	-1.45124	.73124
Protein	Dairy farms	Vendors	.321667	.277484	.299	-.39163	1.03496
		Cafeterias	.878333*	.277484	.025	.16504	1.59163
	vendors	Dairy farms	-.321667	.277484	.299	-1.03496	.39163
		Cafeterias	.556667	.248189	.075	-.08132	1.19466
	Cafeterias	Dairy farms	-.87833*	.277484	.025	-1.59163	-.16504
		Vendors	-.556667	.248189	.075	-1.19466	.08132
Lactose	Dairy farms	Vendors	.331667	.310640	.334	-.46686	1.13019
		Cafeterias	.138333	.310640	.675	-.66019	.93686
	vendors	Dairy farms	-.331667	.310640	.334	-1.13019	.46686
		Cafeterias	-.193333	.277845	.518	-.90756	.52089
	Cafeterias	Dairy farms	-.138333	.310640	.675	-.93686	.66019
		Vendors	.193333	.277845	.518	-.52089	.90756
ASH	Dairy farms	Vendors	.019000	.045479	.693	-.09791	.13591
		Cafeterias	.028000	.045479	.565	-.08891	.14491
	vendors	Dairy farms	-.019000	.045479	.693	-.13591	.09791
		Cafeterias	.009000	.040678	.834	-.09557	.11357
	Cafeterias	Dairy farms	-.028000	.045479	.565	-.14491	.08891
		Vendors	-.009000	.040678	.834	-.11357	.09557
*. The mean difference is significant at the 0.05 level.							

Table 6: Hoc-Post Multiple Comparisons test for microbial load of the studied raw cow milk

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
TBC	Dairy farms	vendors	-1.481667*	.297709	.004	-2.24695	-.71638
		Cafeterias	-2.151667*	.297709	.001	-2.91695	-1.38638
	vendors	Dairy farms	1.481667*	.297709	.004	.71638	2.24695
		Cafeterias	-.670000	.266279	.053	-1.35449	.01449
	Cafeterias	Dairy farms	2.151667*	.297709	.001	1.38638	2.91695
		vendors	.670000	.266279	.053	-.01449	1.35449
TCC	Dairy farms	vendors	-2.443333*	.560288	.007	-3.88360	-1.00307
		Cafeterias	-2.476667*	.560288	.007	-3.91693	-1.03640
	vendors	Dairy farms	2.443333*	.560288	.007	1.00307	3.88360
		Cafeterias	-.033333	.501136	.950	-1.32155	1.25488
	Cafeterias	Dairy farms	2.476667*	.560288	.007	1.03640	3.91693
		vendors	.033333	.501136	.950	-1.25488	1.32155
YMC	Dairy farms	vendors	-.155000	.851963	.863	-2.34504	2.03504
		Cafeterias	-2.715000*	.851963	.024	-4.90504	-.52496
	vendors	Dairy farms	.155000	.851963	.863	-2.03504	2.34504

		Cafeterias	-2.560000*	.762019	.020	-4.51883	-.60117
	Cafeterias	Dairy farms	2.715000*	.851963	.024	.52496	4.90504
		vendors	2.560000*	.762019	.020	.60117	4.51883
*. The mean difference is significant at the 0.05 level.							