Bull. Chem. Soc. Ethiop. **2023**, 37(3), 553-563.
DOI: https://dx.doi.org/10.4314/bcse.v37i3.2

ISSN 1011-3924
Printed in Ethiopia
Online ISSN 1726-801X

PHYSICOCHEMICAL PROPERTIES AND MICROBIAL LOAD EVALUATION OF RAW COW MILKS OF JIMMA TOWN, ETHIOPIA

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(Received October 24, 2022; Revised February 2, 2023; Accepted February 14, 2023)

ABSTRACT. In this study, physicochemical properties and microbial loads of raw and sterilized cow milks of Jimma town, Ethiopia, were investigated. Raw and sterilized milk samples were collected from milk venders and local market, respectively. Standard methods were used for analysis of both physicochemical and microbial loads of the samples. The results of the study showed that physicochemical properties of the studied milk samples were within the recommended values. The total bacteria count (TBC) and coliform count (CC) of raw milk samples were above the recommended values, whereas their values in the sterilized milk sample were comparable with the recommended values. However, the yeast and mould count (YMC) of the studied milk samples were comparable with their recommended values. ANOVA ($p < 0.05$) results showed the presence of significant differences in the physicochemical properties such as temperature, pH, titratable acidity, total solids, solids not fat, protein, fat, lactose, total bacteria count and coliform count among the studied milk samples. In general, the studied raw and sterilized milk samples had acceptable physicochemical properties. However, the TBC and CC of the raw milk samples were above their respective recommended values, which might be attributed to the low hygienic practices of the venders.

KEY WORDS: Raw and sterilized cow milks, Physicochemical properties, Microbial load

INTRODUCTION

Milk and milk products are among the most important food products of animal origin [1]. That is why it 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 [2]. Its highly nutritious nature also makes milk to be; ideal for microbial growth and fresh milk can be easily deteriorates to become unsuitable for processing and human consumption [3]. The quality of milk is generally free from pathogenic bacteria, sediment and extraneous substances, and harmful toxic substances having good flavor, normal composition, and low bacterial counts [4].

Milk is a complex fluid containing many components. These components include water, fat, protein, lactose, mineral substances, organic acids, and miscellaneous other compounds [5]. As human milk, raw cow milk plays significant role in physical growth, cognitive development and health of children [6]. However, milk and its products may be contaminated by various environmental pollutants [7]. Microorganisms in raw milk can originate from different sources in which their load and types are influenced by factors such as cleanness of the animal and equipment, season, temperature, storage condition, personnel health, and health of animal [8]. Physicochemical analysis is important tools to monitor the quality of milk and milk products. Provision of milk and milk products of good hygienic quality is desirable from consumer health point of view. This ultimately leads the consumer to become safe from serious bacterial infections and diseases [9].

In Ethiopia, around 97% of the annual milk production is accounted by the traditional milk production system. Most of the milk produced in the country is accordingly processed by

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traditional technologies [10]. The consumption of raw milk and its products is common in Ethiopia [11]. The consumption of contaminated milk and its products have been reported to cause illnesses [10, 11]. This is the reason why milk testing and quality control is important. In Jimma town, although there are several milk venders, no sufficient reports were available on the quality of milk. Therefore, investigation of the physicochemical properties and microbial load of raw cow milk of Jimma town is important to identify how far quality milks the communities are consuming.

EXPERIMENTAL

Description of the study area

The study was conducted in Jimma town, which is the capital of Jimma Zone, Oromia regional state, Ethiopia. It is found at 345 km from the Addis Ababa in Southwest Ethiopia. It is located at latitude and longitude of 7°40'N36°50'E and altitude, of about 173 m above sea level. It lies in the climate zone locally known as Woyna Dega which is ideal for agriculture as well as human settlement [12]. Figure 1 shows map of the study area.

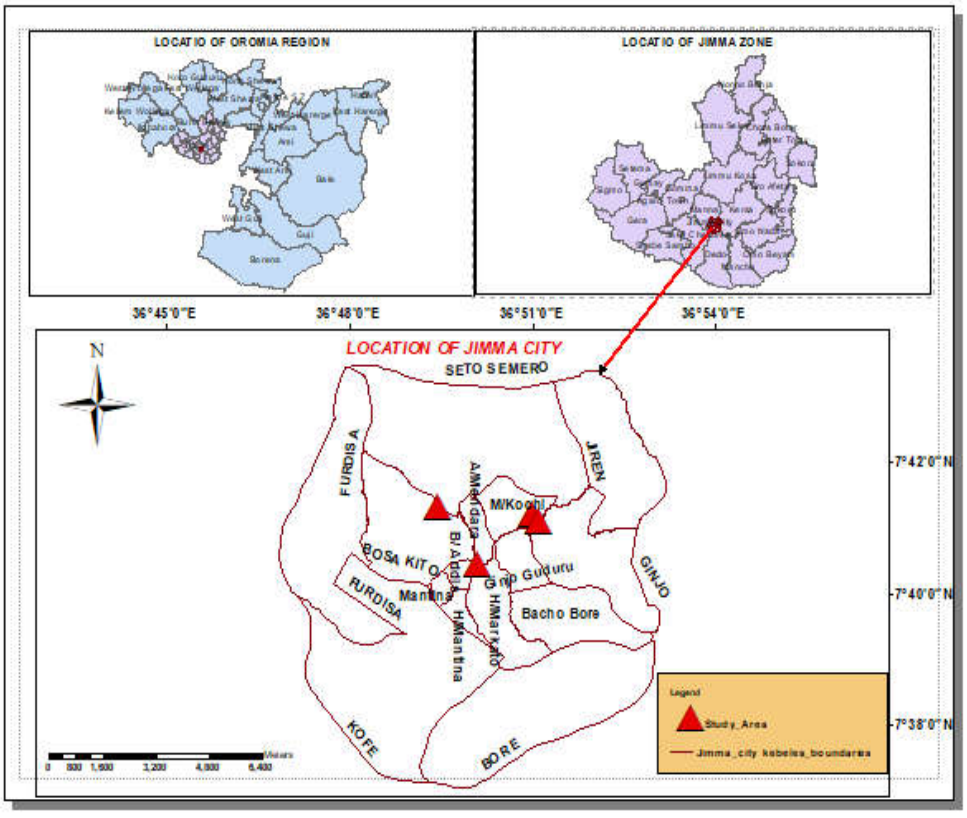

Figure 1. Map of the study area.

Sampling and sample collection

The raw cow milk samples were purposely collected from 4 sites (milk venders). The selected sites were (Markato, Kochi, Frustale and Jimma University). Sterilized and packed milk samples were also collected from local super markets in the town. Totally 12 raw cow milk samples, each sample 500 mL were collected from milk venders. From each sampling sites, 3 samples were collected from different containers twice in a day, in the morning and evening. Then, milk samples collected from the same site were mixed up to make a composite sample. Similarly, sterilized milk sample was taken from the local market. In each day, fresh raw milk samples were collected for analysis of the physicochemical parameters and microbial load.

Chemicals, reagents and media

Analytical grade chemicals and reagents 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 sulfate, sodium hydroxide, boric acid, methyl red indicator, bromocresol green indicator, amyl alcohol, peptone, peptone and saline (code 64271, German) and Potato Dexrose agar, Standard plate count, and Violet Red Bile media were used during the experiments.

Apparatus and instruments

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

Analysis of physicochemical properties

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 4.0 and 7.0. After calibrating the pH meter, 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 [13].

Determination of titratable acidity

Titratable acidity was determined to the method of the AOAC [4]. 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 0.1 N 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 [14].

$$\text{TA\%} = \frac{\text{0.1N NaOH x 0.09}}{\text{Weight of milk sample}} \times 100$$

Determination of specific gravity

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 the ratio of the density of milk to the density of water is determined by the following formula [15].

$$\text{SG} = \frac{\text{Density of milk}}{\text{Density of water}}$$

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 ^{o}C for 3 h. The dried sample was taken out of the oven and placed in a desiccator to cool at room temperature. Finally, the dried sample was weighed to determine the total solids by using the following formula [16].

$$\text{TS\%} = \frac{(\text{Weight of Crucible} + \text{Weight of oven dry sample}) - \text{Weight of Crucible}}{\text{Weight of sample}} \times 100$$

Determination of fat content

The fat content was determined by the Gerber method [17]. Accordingly, 10 mL conc. H_2SO_4 was pipetted into a butyrometer. Next, 10 mL of milk sample and 1 mL of amyl alcohol were added into a butyrometer, respectively. Then, butyrometer was closed with a lock stopper and then the mixture was shaken properly several times until the milk was completely digested by the acid. The content was placed in water bath at 65 ^{o}C for 5 min. and then centrifuged in a Gerber centrifuge for 5 min. The butyrometer was again placed in water bath at 65 ^{o}C for 5 min. Finally, the butyrometer reading was recorded.

Determination of solids not fat

Solids-not-fat (SNF) content was determined as the difference between the percentage of total solids (TS) and fat percentage using the following formula [18].

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

Determination of crude protein content

The crude protein content of milk samples were determined by the Kjeldahl method AOAC [19]. Thus, 5 g of milk sample was warmed in water bath at 38 ^{o}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 thoroughly. The digestion was carried out for 2 h at 350 ^{o}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 [20].

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/bromocresol green as indicators until blue color was appeared. Finally, the sample was titrated with 0.1 N HCl solution until a faint pink color was formed.

A blank test was also prepared in the same procedure except that water was used instead of the milk sample. The percentage of nitrogen, indicating the percentage of protein, in the milk samples was then calculated by using the following formula [21].

$$N\% = \frac{(V_s - V_b)_{1.4007} \times N_{HCl}}{\text{Weight of sample}} \times 100$$

where Vs and V_b = Volume of HCl consumed by sample and blank, respectively.

$$\text{Crude Protein}\% = N\% \text{ x } 6.38$$

Determination of ash content

A dried milk sample which was used for the determination of total solids content was ignited in a muffle furnace at a temperature of 550 °C. It was ignited for 4 h until the black color disappeared or the ash changed from grayish to white [22]. Then, the sample was transferred to the desiccators to cool. Finally, the percentage of ash content was calculated using the following formula.

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

Determination of lactose content

The percent of lactose was determined by subtracting the sum of fat, protein and ash contents from the total solids [23].

$$\text{Lactose } \% = \text{Total solids } \% - (\text{Fat } \% + \text{Protein } \% + \text{ash } \%)$$

Microbial analysis

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} [24] were prepared. All diluted samples were applied to petri plates. The petri plates were labeled with dilution factor and sample numbers.

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 [25]. 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 °C 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 [26].

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 °C 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 °C was considered as sufficient evidence for the presence of coliforms [26].

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 °C for 3 to 5 days. Colonies with a blue green color was counted as yeasts and mold [26].

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.

RESULTS AND DISCUSSION

Physicochemical properties of raw sterilized cow milk

The results of physicochemical properties of raw milk samples of Jimma town are presented in Table 1.

Table 1. Physicochemical properties (mean ± SD) of raw and sterilized cow milk of Jimma town.

Parameters	Milk source					
	Merkato	Kochi	Frustale	JU	Sterilized	FAO [27]
Temp. (°C)	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	1.028 - 1.031
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: Titratable 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 storage techniques [27]. Lack of refrigerator for milk storage, 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 °C temperature [28].

As can be seen from Table 1 the mean pH values 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 of them exhibited pH values slightly below the standard pH range [27]. This may indicate that there might be bacterial growths in the milk samples. However, the mean pH value of sterilized milk sample was within normal pH range (6.6 - 6.8), this could be due to the fact that the sterilized milk is pasteurized and packed prior to market distribution [28]. 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 1.028 - 1.031 with a mean value of 1.03 [29]. The obtained SG values of Kochi, JU and sterilized 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 [29]. There was no significant variation ($p > 0.05$) in the SG among 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 [30]. Based on the SG results, the studied milk samples satisfy raw milk quality standard.

Normal fresh milk has an apparent acidity of 0.14 to 0.16% as lactic acid [30]. In this study, the TA% of Kochi, JU and sterilized 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 might 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 [31].

The TS% of all the studied raw cow milk and sterilized 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 [32].

The fat content of milk obtained from Merkato, Kochi, Frustale, JU and sterilized 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 nearly similar mean fat contents which were within the recommended values [28]. Although, the observed variations was not significant in this study, generally, fat content of milk is highly affected by animals' food, breeding and stage of lactation [32].

The obtained mean of SNF% of the studied milk samples were: Merkato (7.90 ± 0.25%), Kochi (8.70 ± 0.30%), Frustale (8.53 ± 0.06%), JU (9.43 ± 0.20%) and sterilized (9.64 ± 0.16), with the exception of Markato milk sample, the SNF contents obtained from other sampling sites and that of sterilized milk were within the recommended levels. The variation of Merkato milk from the others could be due to differences in the feeding practices, milking method and lactation period as also indicated in literatures [32, 33].

The protein contents obtained from milk samples were: Merkato (4.02 ± 0.07%), Kochi (3.9 ± 0.07%), Frustale (3.54 ± 0.04%), JU (3.61 ± 0.29%) and sterilized (3.50 ± 0.04%) 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 [32, 33].

Ash content of milk samples obtained from Merkato, Kochi, Frustale, JU and sterilized 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 [34]. The ash contents of all raw milk samples were lower than that of sterilized sample. Ash content of milk can be affected by breed, stage of lactation and animals' food [35].

The obtained lactose contents of milk samples were for Merkato (3.32 ± 0.37%), Kochi (4.11 ± 0.38%), Frustale (4.25 ± 0.06%), JU (5.07 ± 0.53%) and sterilized (5.48 ± 0.20%), except for Merkato sample, the remaining milk samples have %lactose contents within the recommended range (3.60 - 5.50%) [28]. Merkato milk sample contained the lowest lactose content and the

highest was determined in sterilized Milk. This variation might be occurred due to bacterial activities and lactation period [4]. The presence of significant variation in lactose contents were observed among the studied milk samples.

Microbial load in milk samples

The obtained results for microbial load of milk samples such as total bacteria count (Figure 2), coliform count (Figure 3) and yeast and mould count (Figure 4) of cow milk samples collected from Merkato, Kochi, Frustale, JU and sterilized milk in the Jimma town are presented Table 2.

Table 2. Microbial counts log (cfu/mL) of raw and sterilized cow milk samples.

Parameter	Milk samples					
	Merkato	Kochi	Frustale	JU	Sterilized	ES [37]
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.

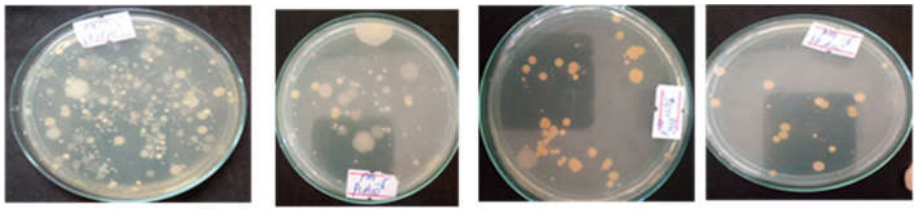

Figure 2. Total bacteria colonies in milk samples.

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 [32]. 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). [33]. The total bacterial count is a good indicator for monitoring the sanitary conditions practiced during collection and handling of raw milk [35].

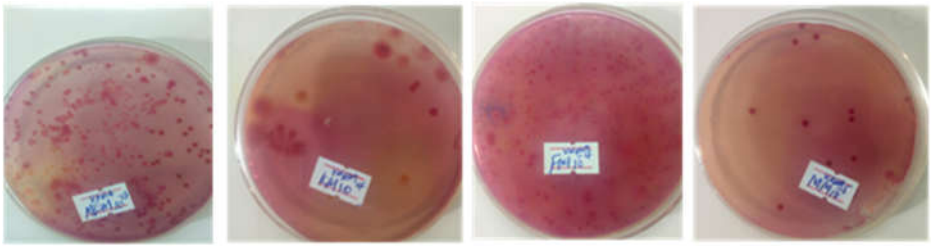

Figure 3. Total Coliform counts colony in the milk samples.

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 [35]. One way ANOVA ($p < 0.05$) revealed as there were significant differences in CC among the raw cow milk samples studied.

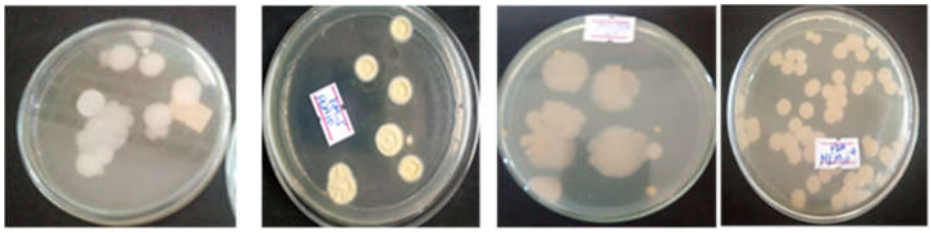

Figure 4. Yeast and Mould colonies in milk samples.

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 [36]. 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 [37].

CONCLUSION

In the present study, the physicochemical parameters and microbial load of milk samples collected from Jimma town vendors were investigated. Most of the physicochemical parameters study results were within the recommended milk quality standard range, indicating that the studied milk samples are free from adulteration. However, milk sample which was collected from Merkato exhibited variations in some parameters. The obtained results for total bacteria count, Coliform count and Yeast and mould count of all the studied milk samples were above the maximum recommended limits set by Ethiopian Standard, indicating that studied milk were not safe for consumption in terms of microbial load. Therefore, sanitary measures should be taken at all milk sellers and chain suppliers through awareness creation and boiling raw milk is recommended for the consumer to minimize health impact of the microbes. 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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