

Physicochemical Properties and Microbial Quality of Raw Cow Milk Produced by Smallholders in Bench Maji-Zone, Southwestern Ethiopia

Teshome Gemechu^{1*} Tesfaye Amene²

Department of Animal Science, College of Agriculture and Natural Resources, Mizan-Tepi University, P.O. Box 260, Mizan-Tepi, Ethiopia

Abstract

The study was conducted in Bench Maji-Zone, Southwestern Ethiopia, aimed to assess physicochemical properties and microbial quality of raw cow's milk produced by smallholders in the areas. A total of Forty five samples of raw cow's milk were collected from Mizan Aman, Dehub and Shei Bench Woredas. All of the samples were collected using proportional random sampling method. The mean values for pH, specific gravity, titratable acidity, fat, protein and ash contents of milk samples collected from Mizan Aman were 6.153 ± 0.114 , 1.022 ± 0.016 , 0.215 ± 0.010 , 5.867 ± 0.586 , 3.844 ± 0.475 and 0.780 ± 0.050 , respectively. The mean values for pH, specific gravity, titratable acidity, fat, protein and ash contents of milk samples collected from Dehub Bench Woreda were 6.647 ± 0.200 , 1.031 ± 0.002 , 0.174 ± 0.018 , 5.973 ± 0.730 , 3.954 ± 0.402 and 0.815 ± 0.047 , respectively. Whereas, milk samples obtained from Shei Bench Woreda had 6.627 ± 0.128 , 1.031 ± 0.001 , 0.177 ± 0.013 , 6.233 ± 0.940 , 4.140 ± 0.320 and 0.791 ± 0.066 for pH, specific gravity, titratable acidity, fat, protein and ash contents, respectively. Significant differences ($P < 0.05$) were found for the values pH, specific gravity, titratable acidity, fat, protein and ash contents between the sources of milk samples. The average (\pm SD) total bacterial count, coliform count, spore-forming bacterial count and yeast and mould count of milk samples obtained from Mizan Aman were $7.235 \pm 0.277 \log_{10}$ cfu/ml, $5.203 \pm 0.230 \log_{10}$ cfu/ml, $6.489 \pm 0.258 \log_{10}$ cfu/ml and $4.001 \pm 0.588 \log_{10}$ cfu/ml, respectively. Whereas, milk samples obtained from Dehub Bench Woreda had $7.222 \pm 0.156 \log_{10}$ cfu/ml, $5.187 \pm 0.211 \log_{10}$ cfu/ml, $6.307 \pm 0.195 \log_{10}$ cfu/ml and $3.944 \pm 0.346 \log_{10}$ cfu/ml for total bacterial count, coliform count, spore-forming bacterial count and yeast and mould count, respectively. On the other hand, the corresponding values for Shei Bench Woreda samples were $6.817 \pm 0.381 \log_{10}$ cfu/ml, $4.911 \pm 0.324 \log_{10}$ cfu/ml, $6.221 \pm 0.542 \log_{10}$ cfu/ml and $3.762 \pm 0.468 \log_{10}$ cfu/ml, respectively. Total bacterial count, coliform count, spore-forming bacterial count and yeast and mould count of milk samples obtained from Mizan Aman were significantly higher ($P < 0.05$) than milk samples obtained from Dehub and Shei Bench Woredas. Therefore, it was concluded that the physicochemical properties was adequate as compared to the standard level whereas, the microbial quality of raw cow's milk produced by smallholders in the areas was poor and this suggests the need for enriched hygienic practices and educating the public on safety issues and personal hygiene in milk handling.

Keywords: Bench Maji, Microbial quality, physicochemical properties, Raw cow milk.

INTRODUCTION

Milk has a complex biochemical composition and high water activity. Due to its high nutritive value, raw milk serves 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 (Degraaf *et al.*, 1997; Mennane *et al.*, 2007).

Adverse environmental condition is highly affecting the quality of milk and milk products. In areas where the climate is hot and humid, the raw milk gets easily fermented and spoiled during storage unless it is refrigerated or preserved. However, such storage facilities are not readily available in rural areas and cooling systems are not feasible due to lack of the required dairy infrastructure (Teshome *et al.*, 2014).

According to Muriuki and Thorpe (2001), the vast majority of milk produced outside urban centers in the country is processed into milk products at household level using traditional technologies. In the study area, traditional milk production, processing and storage are a typical feature. Traditional milk products are substandard quality mainly due to inadequate dairy infrastructure such as refrigeration facility and clean water and limited knowledge of the hygienic handling of milk and milk products. Although, milk production represents an essential piece of the livelihood of the community in Bench Maji Zone, there is no published/documentated data with regards to physicochemical properties and microbial quality of raw cow's milk produced by smallholders. Therefore, the objective of this study was to assess physicochemical properties and microbial quality of raw cow's milk produced by smallholders in the Bench Maji zone.

MATERIALS AND METHODS

Description of the Study Area

Bench Maji zone is one of the 13 zones of the Ethiopian Southern Nations, Nationalities and Peoples Region State. The zone is found in Southwestern part of Ethiopia, and it is divided into 10 Woreda and one administrative town. The administrative center of Bench Maji zone (BMZ) is Mizan-Teferi which is found at distance about 561km from Addis Ababa and 830km from the regional capital Hawassa. It is bordered with Keffa Zone in North, Debub Omo in North East direction, Sheka Zone in South West, with Gambela and South Sudden Republic in South direction (BMZFED, 2015).

Agro-ecologically, BMZ, consists of 52% lowland (<1500 meter above sea level (masl), 43% mid altitude (1500-2300 masl) and 5% highland (>2300 masl). The altitude ranges from 500 to 3,000 masl. Bench Maji zone is found at 34°45'-36°10' East and 5°40'-7°40' North. The annual average temperature range from 15.1°C to 27.5°C, while the annual rainfall range from 400 to 2,000 mm (BMZFED, 2015).

Research Design

The study involved a laboratory based investigation aimed to assess physicochemical properties and microbial quality of raw cow's milk produced by smallholders in Bench Maji zone. A total of forty five samples of raw cow's milk were collected at morning from Mizan town, Debub and Shei Bench *Woredas*.

Sources of Data and Sampling Techniques

A total of 45 household milk samples (5 household from each *kebele*) were collected at morning from purposively selected nine *Kebeles* (*three kebeles from each Woreda and town*). The households were selected based on preliminary survey done from each *Kebele*. All samples were collected using proportional random sampling method. Samples of morning milk were aseptically taken twice at different times from each *kebele*. During collection, approximately 300 ml of milk sample were aseptically collected from bulk milk container of producers and placed into sterile glass bottles. Subsequently, samples were labeled and put into icebox and then transported to laboratory for analysis. The analysis was performed within three to four hours after sampling.

Physicochemical Quality of Milk

PH value

The pH of the milk samples were determined in the laboratory using a digital pH-meter based on the procedure described by O'Connor (1995).

Specific gravity

Fresh milk sample were filled sufficiently into a glass cylinder (100 ml capacity). Then, lactometer was hold by the tip and inserted into the milk. The lactometer was allowed to float freely until it reached equilibrium. Then the lactometer reading at the lower meniscus will be recorded. At the same time, thermometer were inserted into the milk sample and the temperature of the milk was recorded (O'Mahony, 1998). The following formulas were used to calculate the specific gravity of the milk.

$$\text{Specific gravity} = (L/1000) + 1$$

Where,

L = corrected lactometer reading at a given temperature, i.e., for every degree above 15.56 °C, 0.2 were added to the lactometer reading but for every degree below 15.56 °C, 0.2 were subtracted from the lactometer reading.

Titrateable acidity of milk

Titrateable acidity of the milk samples was determined according to the method of the Association of Official Analytical Chemists (AOAC, 1990). Nine ml of milk sample were pipetted into a beaker and 3 to 5 drops of 1 % phenolphthalein indicator were added to it. The milk samples were then titrated with 0.1N NaOH solution until a faint pink color persisted. The titrateable acidity, expressed as % lactic acid, was finally calculated using the following formula.

$$\text{Titrateable acidity \%} = \frac{\text{N/10 NaOH (ml)} \times 0.009}{\text{Weight of milk sample}} \times 100$$

Crude protein determination

Total protein content of the milk samples were determined by Kjeldahl method (AOAC, 1990). For digestion, five gram of milk sample was warmed in a water bath at 38°C and poured into a Kjeldahl flask. 15 gram potassium sulphate, 1.0 ml of copper sulphate solution and 25 ml of concentrated sulphuric acid were added into the flask and mixed gently. The digestions were carried out in a digestion block until a clear solution appeared. Then, it was allowed to cool at room temperature.

For distillation, digestion flasks were placed in the distillation equipment and then 30 ml of distilled water and 75 ml of 50% sodium hydroxide solution were added into it. Then, ammonia was distilled and 50 ml of 40% boric acid solution using bromocresol green indicator was added until blue color appeared. Finally, the sample were titrated with 0.1 N hydrochloric acid solution from a burette until a faint pink color solution were formed and the burette reading were taken to the nearest 0.01 ml. Blank test was carried out using the above procedure except that water was used instead of test sample. The percentage of nitrogen in the milk samples was calculated as follows:

$$\%N = \frac{(V_s - V_b) \text{ HCl consumed} * \text{NHCl} * 1.4007}{\text{sample weight}} \times 100$$

$$\%CP = \%N * 6.38$$

Where,

% N: percentage nitrogen by weight

V_s: volume of HCl used for titration of sample

V_b: volume of HCl used for titration of the blank

% CP: percentage of crude protein

Determination of fat content of milk

Fat content was determined by Gerber method. Milk samples (11 ml) were mixed with commercial sulfuric acid (10 ml) having a specific gravity of 1.82 was dispensed into butyrometer and 1 ml of amyl alcohol was added into the butyrometer having the sulfuric acid and then closed with rubber cork. After closing the butyrometer using a butyrometer stopper, the content was shaken and inverted several times until all the milk samples were digested by the acid. Then the butyrometer was placed in a water bath at 65°C for five minutes. The samples were centrifuged for five minutes at 1100 rpm (rotations per minute) (Richardson, 1985). Finally, the samples were taken back to the water bath adjusted at 65°C for 5 minutes and fat percentage was recorded from the butyrometer reading (Richardson, 1985).

Determination of ash content

The ash content of the milk samples was determined gravimetrically. The dried milk samples used for determination of total solids content were ignited in a muffle furnace at a temperature of 550°C until they were free from carbon (Heating continued until black color disappeared or the ash residue appears grayish to white) for four hours, then the samples were transferred to the desiccators to cool down. Finally, the ash content was calculated according to Richardson 1985. Calculation:

$$\%Ash = \frac{\text{Residue weight}}{\text{Sample weight}} \times 100$$

Microbial Analysis

The microbial analyses of milk samples include the determination of colony-forming units (CFUs) of total bacteria, coliform bacteria, spore-forming bacteria and yeast and mould using appropriate media. All media used for microbial analyses were sterilized before use according to the manufacturer's guidelines.

Total bacterial count

For total plate count, appropriate decimal dilutions that would give the expected total number of colonies on a plate, i.e., between 30 and 300 colonies were selected (Richardson, 1985). The standard plate count (SPC) agar was cooled to 45°C before pouring. One ml of milk sample was added into sterile test tube containing nine ml peptone water up to serial dilution of 10⁻⁷ and mixed thoroughly. Total bacterial count was made by incubating surface plated duplicate decimal dilutions of milk samples on standard plate count agar (Oxoid, UK) at 32°C for 48 hours. Finally, colony count was made using colony counter (Schutt Count Plus D-37079, Germany).

Coliform count

One ml of milk sample was added into sterile test tube containing nine ml peptone water up to serial dilution of 10⁻⁷ and mixed thoroughly. Duplicate appropriate decimal dilutions were surface plated and incubated at 32°C for 24 hours on Violet Red Bile Agar (Pharma, US) and typical dark red colonies on uncrowned plates was considered as coliforms and counted. This was followed by a confirmatory test by transferring and incubating four to five typical colonies from each plate transferred into tubes containing 2% Brilliant Green Lactose Bile Broth (Oxoid, UK). Gas production within 48 hours of incubation at 35°C was considered as sufficient evidence for the presence of coliforms (Richardson, 1985).

Spore-forming bacteria count

The enumeration of spore-forming bacteria was done using plate count agar following the methods recommended by McLandsborough (2005). Milk samples were heated at 80°C for 10 minutes in water bath and volumes of 0.1 ml of appropriate dilutions were surface plated as for the standard plate count using plate count agar. All plates were incubated in an inverted position for 3 days at 30°C and colonies were counted.

Yeast and mould count

Samples of milk were serially diluted following similar methods as for total bacterial count but dilutions were surface plated on Potato Dextrose Agar (PDA) (Oxoid, Pvt. Ltd. MU 096: UK). 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 moulds (Yousef and Carlstrom, 2003).

Statistical Analysis

Data from microbial counts were first transformed to logarithmic values (\log_{10}) before statistical analysis. Then, data on the physicochemical properties and the transformed microbial count values were analyzed using General Linear Model (GLM) procedure of SAS (SAS, 2009). Mean separation was carried out using the Least Significant Difference (LSD) technique when analysis of variance shows significant differences between means and differences were considered significant at $p < 0.05$.

The following model was used for the analysis of the physicochemical properties and microbial quality of milk:

$$Y_{ij} = \mu + \alpha_i + e_{ij}$$

Where,

Y_{ij} = The value of the respective variable mentioned above pertaining to the i *Woreda* ($i=3$, Mizan Aman, Bench and Shei Bench)

μ = Overall mean of the respective variable

α_i = The effect of i^{th} *Woreda* (Mizan Aman, Debub and Shei Bench) on the respective variable

e_{ij} = The error term

RESULTS AND DISCUSSION

Physicochemical Properties of Raw Cow Milk

The physicochemical properties of raw cow milk samples collected from milk producers in study areas were shown in Table 1. The mean pH value of raw milk samples were significantly different ($P < 0.05$) among *Woreda*. On the other hand, there was no marked difference between milk samples collected from Debub and Shei Bench. The pH value of milk samples collected from Mizan Aman town was more acid than those of the Debub and Shei Bench *Woredas*. This might be due to variations in the milk hold equipment, age of milk and handling techniques.

The pH of milk samples collected from Mizan Aman town was significantly ($P < 0.05$) lower than the pH of milk obtained from Debub and Shei Bench *Woredas* (Table 1). The average (\pm SD) pH of milk samples obtained from Mizan Aman town (6.153 ± 0.114) were not within the normal pH range indicating that there were bacterial growths in the milk samples. However, the average pH value of milk samples obtained from Debub Bench (6.647 ± 0.200) and Shei Bench (6.627 ± 0.128) (6.67 ± 0.03) were within the normal pH range of fresh cow milk indicating that the milk were most probably obtained directly from households shortly after milking. Fresh cow milk has a pH value that ranges from 6.6 to 6.8 (O'Connor, 1995 and FAO, 1999). The pH values higher than 6.8 indicates mastitic milk and pH values below 6.6 indicates increased acidity of milk due to bacterial multiplication (O'Connor, 1995). Consequently, the low pH of milk collected from Mizan Aman town might probably be due to the production of acid resulting from bacterial growth and multiplication in the milk samples.

There was a significant difference in milk specific gravity among Mizan Aman, Debub and Shei Bench *Woredas* (Table 1). However, there was no marked difference between milk samples collected from Debub and Shei Bench *woredas*. The specific gravity of normal milk ranges from 1.027 – 1.035 g per ml with a mean value of 1.032 g per ml (Tamime, 2009). FAO (1988) also reported that the specific gravity of normal milk ranges from 1.028-1.033 gram per milliliter. In the current study, the result of milk samples collected from Debub and Shei Bench *woredas* within the ranges of Tamime (2009) and FAO (1988) findings. Conversely, the result of milk samples collected from Mizan Aman town did not exist within a ranges of Tamime (2009) and FAO (1988) findings. This might be indicating the adulteration of milk with water. According to O'Connor (1993) the higher value of specific gravity (1.035) indicates skimming off fat whereas, the lower value than normal value of specific gravity of milk (1.020) is indicative of addition of water. Similar on-farm result of specific gravity of 1.030 was reported by Zelalem and Ledin, (2001). Furthermore, adulteration of milk with water that was usually done in order to increase the quantity of milk lowers milk's specific gravity while addition of solids such as flour

or sugar into milk and removing the butterfat increases the specific gravity of milk beyond 1.035 gram per milliliter (O'Connor, 1995; Omore *et al.*, 2005).

The mean titratable acidity was significantly different ($P < 0.05$) among milk samples collected from Mizan Aman, Debub and Shei Bench *Woredas* (Table 1). On the other hand, there was no marked difference among milk samples collected from Debub and Shei Bench *Woredas*. In the current study, the milk samples collected from three areas had a titratable acidity value of greater than 0.16% which indicates that the milk samples were kept at room temperature for longer period of time and under poor handling practices until they were sold and/or consumed. Normal fresh milk has an apparent acidity of 0.14 to 0.16% as lactic acid (O'Connor, 1995).

The titratable acidity milk obtained from Mizan Aman town was significantly ($P < 0.05$) higher than that of Debub and Shei Bench *Woredas* (Table 1). The higher titratable acidity of raw milk samples collected from Mizan Aman town may be due to bacterial growth and longer storage of the milk before sale. Asaminew and Eyassu (2011) reported higher acidity for milk samples collected from individual farmers ($0.23 \pm 0.01\%$ lactic acid) in Bahir Dar Zuria *Woreda*

Table 1. Mean (\pm SD) physicochemical properties of raw cow's milk samples collected from three *Woredas* (n=45)

| Variables | <i>Woredas</i> | | | Overall mean |
|------------------|--------------------------------|--------------------------------|--------------------------------|-------------------|
| | Mizan Aman (n=15) | Debub Bench (n=15) | Shei Bench (n=15) | |
| pH value | 6.153 \pm 0.114 ^b | 6.647 \pm 0.200 ^a | 6.627 \pm 0.128 ^a | 6.477 \pm 0.273 |
| Specific gravity | 1.022 \pm 0.016 ^b | 1.031 \pm 0.002 ^a | 1.031 \pm 0.001 ^a | 1.028 \pm 0.010 |
| TA(%LA) | 0.215 \pm 0.010 ^a | 0.174 \pm 0.018 ^b | 0.177 \pm 0.013 ^b | 0.190 \pm 0.023 |
| Fat | 5.867 \pm 0.586 | 5.973 \pm 0.730 | 6.233 \pm 0.940 | 6.024 \pm 0.763 |
| Protein | 3.844 \pm 0.475 | 3.954 \pm 0.402 | 4.140 \pm 0.320 | 3.980 \pm 0.414 |
| Ash | 0.780 \pm 0.050 | 0.815 \pm 0.047 | 0.791 \pm 0.066 | 0.795 \pm 0.056 |

Means followed by different superscript letters within a row are significantly different ($P < 0.05$), TA = Titratable acidity, LA= Lactic acid, n= number of respondents, n= number of samples

There was no significant difference ($P > 0.05$) in fat content observed among three study areas. The average fat content of milk obtained from three areas (5.867 ± 0.589 , 5.973 ± 0.730 and $6.233 \pm 0.940\%$ of Mizan Aman, Debub and Shei Bench *Woredas*, respectively) were greater than the earlier findings of Mansson *et al.* (2003), Janštová *et al.* (2010) and Teklemichael (2012) who reported a fat content of 4.3%, $3.79 \pm 0.18\%$ and $3.862 \pm 0.412\%$, respectively for milk produced in dairy farms. According to European Union quality standards for unprocessed whole milk, fat content should not be less than 3.5% (Tamime, 2009). Consequently, the average fats content ($6.024 \pm 0.763\%$) observed from three areas were within the recommended standards.

Protein content of milk obtained from Mizan Aman, Debub and Shei Bench *Woredas* were 3.844 ± 0.475 , 3.954 ± 0.402 and 4.140 ± 0.320 , respectively (Table 1). There was no marked difference ($P > 0.05$) among milk samples three areas. The average protein content of raw milk obtained from Mizan Aman, Debub and Shei Bench *Woredas* were higher than the earlier findings of Abd Elrahman *et al.* (2009) who reported a protein content of 3.48% for milk produced in dairy farms. Correspondingly, Fikrineh *et al.* (2012) reported lower protein content ($3.46 \pm 0.04\%$) for milk samples collected from households producing local and crossbred cows. According to European Union quality standards for unprocessed whole milk, total protein content should not be less than 2.9%, (Tamime, 2009). Therefore, the average proteins content ($3.980 \pm 0.414\%$) observed from three areas were within the recommended standards.

Ash content of raw milk obtained from Mizan Aman, Debub and Shei Bench *Woredas* averaged 0.780 ± 0.050 , 0.815 ± 0.047 and 0.791 ± 0.066 , respectively (Table 1). The ash contents of milk samples collected from three sampling areas was not significantly ($P < 0.05$) different. The ash content of cow milk remains relatively constant 0.7 to 0.8% and it is influenced by breed, stage of lactation and feed of the animal (O'Connor, 1995). The composition of milk can vary depending on breed of the animals, interval between milkings, completeness of milking, stage of lactation, feed of the animal, age and health status of the milking cows. Microbial activities such as degradation of proteins and lipids of milk can also change the composition of milk (O'Connor, 1995).

Microbial Quality of Raw Cow Milk

Total bacterial count

Mean total bacterial count was significantly different ($P < 0.05$) among milk samples collected from Mizan Aman, Debub and Shei Bench *Woredas* (Table 2). On the other hand, there was no marked difference among milk samples collected from Mizan Aman and Debub Bench *Woredas*. The total bacterial count obtained in this study is generally high compared to the acceptable level of 1×10^5 bacteria per ml of raw milk (O'Connor 1994).

The total bacterial count obtained from Mizan Aman and Debub Bench were significantly higher ($P <$

0.05) than milk samples collected from Shei Benchi *Woreda* (Table 2). This might be due to further contamination of the milk during transportation, use of poorly cleaned milk containers and absence of cooling system. In general, higher total bacterial count of milk samples obtained from study areas could be attributed to improper cleaning of the udder and milking containers before and after milking, failure to use separate towel for each cow, improper cooling system and milk contamination from the hands of producers.

Table 2. Mean (\pm SD) microbial counts (\log_{10} cfu/ml) of raw cow's milk samples collected from three *Woredas* (n=45)

| Variables | <i>Woredas</i> | | | Overall mean |
|-----------|--------------------------------|---------------------------------|--------------------------------|-------------------|
| | Mizan Aman (n=15) | Dehub Bench (n=15) | Shei Bench (n=15) | |
| TBC | 7.235 \pm 0.277 ^a | 7.222 \pm 0.156 ^a | 6.817 \pm 0.381 ^b | 7.091 \pm 0.342 |
| CC | 5.203 \pm 0.230 ^a | 5.187 \pm 0.211 ^a | 4.911 \pm 0.324 ^b | 5.100 \pm 0.288 |
| SFBC | 6.489 \pm 0.258 ^a | 6.307 \pm 0.195 ^{ab} | 6.221 \pm 0.542 ^b | 6.341 \pm 0.371 |
| YMC | 4.001 \pm 0.588 | 3.944 \pm 0.346 | 3.762 \pm 0.468 | 3.902 \pm 0.477 |

Means followed by different superscript letters within a row are significantly different ($P < 0.05$), TBC=Total bacterial count, CC= Coliform count, SFBC= Spore forming bacterial Count, YMC= Yeast and mould count, n= number of samples

In the present study, total bacterial count of raw cow milk collected from three areas were lower than that reported by Ahmed *et al.* (2008) who found high total bacterial count of ($9.089 \pm 0.281 \log_{10}$ cfu/ml) in milk samples collected from dairy farms of Khartoum State. However, the total bacterial count obtained from Mizan Aman and Dehub Bench were higher than that reported by Debebe (2010) who found a total bacterial count of $6.98 \pm 0.15 \log_{10}$ cfu ml⁻¹ milk samples collected from milk producers (Table 2). The mean total bacterial count of raw cow's milk ($7.091 \log_{10}$ cfu/ml) obtained in this study was lower than the earlier findings of Zelalem (2010), Haile *et al.* (2012) and Teklemichael (2012) who reported a total bacterial count of $9.10 \log_{10}$ cfu/ml for milk samples collected from different parts of Ethiopia, $10.28 \log_{10}$ cfu/ml from distribution containers (at selling point) and $9.137 \log_{10}$ cfu/ml from vendors, respectively.

Milk produced under hygienic conditions from healthy cows should not contain more than 5×10^4 bacteria per milliliter (O' Connor, 1993). Higher total bacterial count observed in the present study could probably be due to lack of knowledge about clean milk production, use of unclean milking utensils and plastic containers for collecting and keeping milk, initial contamination of the milk samples either from the udder of the cow or the milkers hand and the poor hygienic quality of milking area.

Coliform count

The mean coliform count was significantly different ($P < 0.05$) among milk samples collected from Mizan Aman, Dehub and Shei Bench *Woredas* (Table 2). On the other hand, there was no marked difference among milk samples collected from Mizan Aman and Dehub Bench *Woredas*. The coliform count obtained from Shei Bench *Woreda* was significantly lower ($P < 0.05$) than milk samples obtained from Mizan Aman and Dehub Bench *Woredas* (Table 2).

The overall mean of coliform count observed in raw cow's milk samples collected from Mizan Aman, Dehub and Shei Bench *Woredas* were 5.203 ± 0.230 , 5.187 ± 0.211 and $4.911 \pm 0.324 \log_{10}$ cfu/ml, respectively (Table 2). The coliform count obtained in the current study was greater than that reported by Abdalla and Elhagaz (2011) who found coliform counts of $2.23 \pm 0.136 \log_{10}$ cfu/ml from milk samples collected at Khartoum State dairy farms. On the other hand, Zelalem and Bernard (2006) obtained higher coliform count of $6.57 \log_{10}$ cfu/ml for raw cow's milk collected from different producers in the central highland of Ethiopia. In the current study area, some animals are kept in muddy barn and hygienic conditions were poor. This possibly has exposed the milk to high risk of contamination, which in turn increase the microbial count. The existence of coliform bacteria in high proportion is suggestive of unsanitary condition or practices during production or storage.

According to the European Union standards for total bacterial and coliform counts of raw milk should be less than 10^5 and 10^2 cfu/ml, respectively (Fernandes, 2009). The present study showed that the coliform count of all milk samples exceeds the standards given for raw milk by European Union and US regulations. Generally, the presence of high numbers of coliforms in milk indicates that the milk has been contaminated with fecal materials, unclean udder and teats of cow's, inefficient cleaning of the milking containers, poor hygiene of milking environment, contaminated water and cows with subclinical or clinical coliform mastitis can all lead to elevated coliform count in raw milk (Jayarao *et al.*, 2004).

Spore forming bacterial count

Mean spore forming bacterial count (SFBC) was significantly different ($P < 0.05$) among milk samples collected from three study areas (Table 2). However, there was no marked difference between Mizan Aman and Dehub

Bench *Woredas*. Likewise there was no significant difference between Debus Bench and Shei Bench *Woredas*. The mean SFBC of raw cow's milk obtained in this study ($6.3411 \pm 0.371 \log_{10}$ cfu/ml) was agreed with earlier finding of Teklemichael (2012) who reported a spore forming bacterial count of $6.392 \pm 0.154 \log_{10}$ cfu/ml from milk vendors in Dire Dawa town. However, the lower SFBC ($4.703 \pm 0.069 \log_{10}$ cfu/ml) was reported by Teshome *et al.* (2014) for milk sample collected from Shashemene town. Numerically higher SFBC in milk samples obtained from Mizan Aman may indicate that there was poor environmental sanitation and poor handling practice at the production and selling sites. It could also be associated to the spores which transferred from feed, feces, bedding material and soil in to milk.

Yeast and mould count (YMC)

The overall mean of YMC were 4.001 ± 0.588 , 3.944 ± 0.346 and $3.762 \pm 0.468 \log_{10}$ cfu/ml for milk samples collected from the Mizan Aman, Debus and Shei Bench *Woredas*, respectively. Mean value of yeast and mould counts was not significantly different ($P < 0.05$) among milk samples collected from three study areas (Table 2). However, numerically the YMC of Mizan Aman town was higher than the milk samples obtained from Debus and Shei Bench *Woredas*. Teshome *et al.* (2014) reported higher overall mean Yeast and mould counts of 4.206 ± 0.082 for milk sample collected from small scale milk producers, small shops, hotels and dairy cooperative milk collection centers. Numerically the higher YMC observed in milk obtained from Mizan Aman town might be attributed to contamination from air, containers or poor personal hygiene of milk handler.

CONCLUSIONS

The physical properties and chemical composition of the collected raw cow milk samples were within the recommended levels of European Union and FAO established quality standards. However, the observed microbial quality of milk produced at three study areas was poor. This might be due to the poor hygienic condition of the milking environment (absence of separate area for milking and failure to clean milking areas regularly), absence of cooling system, poor sanitary condition of the milk containers, poor udder and teats cleaning practice, failure to use separate towel for each cow, use of plastic buckets, keeping the milk at room temperature and poor personal hygiene of the milkers. In general, the microbial quality of raw cow milk produced by three areas do not meet the international standards set by regulatory agents and thus could pose health hazards to the consumers. Therefore, this suggests the need for enriched hygienic practices and educating the public on safety issues and personal hygiene in milk handling. It would be a great interest if further investigations are to be carried out to identify and isolate different species of microorganisms that might cause public health importance.

Conflict of Interests

The authors did not declare any conflict of interest.

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