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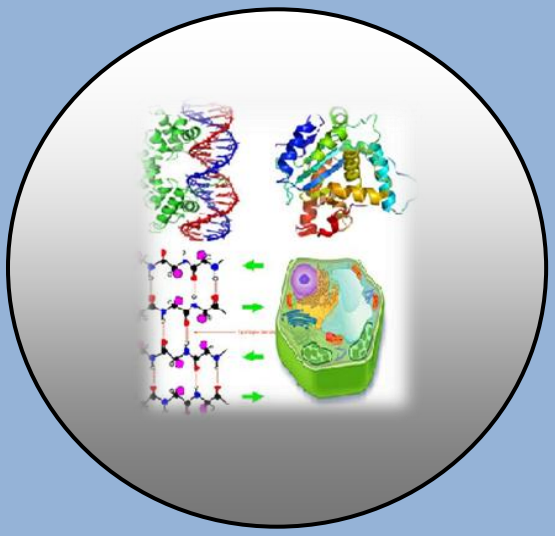

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# Physicochemical Properties and Microbial Quality of Raw Cow Milk Collected from Harar Milkshed, Eastern Ethiopia

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## ABSTRACT

***This study was aimed at determining the physicochemical properties and microbial quality of raw milk of cows collected from Harar milkshed in eastern Ethiopia. A total of 36 samples of raw milk of cows were collected randomly from four districts along the dairy value chain (i.e. milk producers, collectors and retailers from Haramaya, Kersa, Kulubi and Babile). Physicochemical properties and microbiological quality of each sample were analyzed using standard procedures. The physicochemical test included titratable acidity (TA), total solids (TS), total ash, solids-not-fat (SNF), protein and fat contents; and the microbiological analysis comprised total bacterial count (TBC), total coliforms (TC), Escherichia coli, Staphylococcus aureus, and Salmonella. The result obtained revealed a significant difference ($p<0.05$) in protein, fat, TS, SNF, TA, TBC and S.aureus count between the selected districts. Highest protein (4.31%), TS (13.88%) and SNF (8.62%) were recorded from Babile and highest fat (5.87%) and TA (0.28 %lactic acid) were recorded from Kersa and Haramaya, respectively. Microbial load of 6.25, 5.45, 5.51, 5.45 and 5.13log$_{10}$cfu/ml was recorded for TBC, CC, E.coli, S.aureus, and Salmonella, respectively. High microbial counts were found in all study areas.***

***The high microbial counts as well as detection of pathogenic bacteria in the milk samples are of major public health concerns and this calls for scrupulous hygienic measures during collection and handling of milk along the value chain in the study areas.***
***Keywords: Dairy Value Chain, Microbial Quality, Physicochemical Properties and Milk Safety.***

## INTRODUCTION

Milk is a major component in human diets all over the world. According to Ramesh (2006), 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 genetic in terms of breed and individual cow and variability among cows within a breed and environment in terms of interval between milking, stage of lactation, age, feeding regime, disease and completeness of milking.

Milk is also an important vehicle for transmission of pathogenic microorganisms to human beings unless it is produced and handled under good hygienic conditions. Thus, hygienic production of milk has to get due attention in order to provide more and better quality milk for the general public. All cases of dairy illness continued to be of bacterial origin, pathogens that have involved in communicable diseases associated with the consumption of milk include *Salmonella, Listeriamonocytogenes, Staphylococcus aureus, Campylobacter, Yersinia,* pathogenic *Escherichia coli* and *Clostridiumbotulinum* (Adesiyun*et al.,* 1995; Hahn, 1996).

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 (Spreer, 1998; Gandiya, 2001). Milk from a healthy udder contains few bacteria but it picks up many bacteria from the time it leaves the teat of the cow until it is used for further processing. Coliforms are indicators of both the manner of handling milk from milking till consumption and the quality of the milk. Milk produced under hygienic conditions from healthy animals should not contain more than $5x10^5$cfu/ml (O'Connor, 1994).

Considerable amount of cow milk is produced by the farmers and pastoralists in eastern Hararghe. In Ethiopia, the safety of milk with respect to food-borne diseases is of great concern especially where production of milk takes place under unsanitary conditions and poor production practices. To the best of the researchers' knowledge, there was no study conducted previously about the physico-chemical properties and microbial quality, and associated health risk factors of cow milk produced in eastern Hararghe. Therefore, the purpose of this study was to assess the physico-chemical properties and microbial quality of raw cow milk collected from different locations of Harar milkshed areas in Eastern Ethiopia.

## MATERIALS AND METHODS

### Description of the study area

The study was conducted in Harar milk shed areas, eastern Ethiopia. Harar is located on a hill top in the Eastern extension of the Ethiopian highlands, about 500 km from Addis Ababa at an elevation of 1,885 meters above sea level.

Dire Dawa, one of two chartered cities in Ethiopia, lies in the Eastern part of the nation, on the Dechatu River, at the foot of a ring of cliffs that has been described as "somewhat like a cluster of tea-leaves in the bottom of a slop-basin, at altitude and longitude of 9°36'N41°52', 9°36'N41°52'E.

**Experimental design**

The laboratory analysis was performed at Microbiology, Dairy Technology and Food Science Laboratories of Haramaya University, Ethiopia during the period from April to August, 2014. Sampling frame consisted of all milk producers, collectors and also retailers who are participated in the milk value chain. A total of 36 raw cow milk samples were collected at morning and evening from different Harar milkshed areas (i.e. Kulubi, Kersa, Haramaya, and Babile).The experiment was conducted in triplicate.

**Data collection and milk sampling**

Data on milk handling practices by milk producers, collectors and also retailers were collected during milk sampling. Other related information with the risk factor for microbial contamination was also collected. The representative milk samples for analysis were collected from all actors involved in the dairy value chain of Harar districts based on the preliminary survey conducted to identify and map the dairy value chain. The milk samples were taken in accordance to the procedure of Health Protection Agency (HPA, 2004) and the Food and Drug Administration (FDA, 2003). Samples were collected aseptically using sterile bottles and transported in an ice box for analysis. Until the analysis is performed the samples were kept at 4°C and analyzed within 48 hrs of collection (HPA, 2004).

**Analysis of physicochemical properties of milk**

The physicochemical property of cow raw milk collected from Harar milkshed areas were analyzed using standard procedures to determine titratable acidity (TA) (AOAC, 1995), total solids (TS) (AOAC, 1995), fat (Richardson,1985), solids not-fat (SNF) (Harding, 1995), ash (Pearson, 1976),and protein (AOAC, 1995).

**Microbial quality Analysis**

***Methylene blue reduction (MBR) test***

One ml of Methylene Blue and 10 ml of milk sample were added to each test tube and placed in water bath at a temperature of 35$^{o}$C. Milk samples were checked for decolorization after 30 minutes of incubation and subsequently the readings were made at hourly intervals. The time for complete color change of Methylene blue between last inversion and decolorization was recorded. The time for change in color was recorded. The dye reduction time refers to the microbial load in the milk and the total metabolic reactions of the microorganism (Ombui*et al.,* 1995).

**Table 1. The suggested classification of milk quality based on MBR test.**

| Milk grade | Decolorization time |
|---|---|
| Excellent | Not decolorized in 8 hours |
| Good | Decolorized in less than 8 hours but not less than 6 hours |
| Fair | Decolorized in less than6 hours but not less than 2 hours |
| Poor | Decolorized in less than 2 hours |

**Total bacterial count (TBC)**

Total bacterial count was performed according to Harrigan and MacCance (1976). A serial dilution of $10^{-2}$ up to $10^{-4}$ was prepared using sterile water and peptone water. One ml of sample was pour plated over standard plate count (SPC) agar (Oxoid) and incubated at 37$^{o}$C for 24 h. The count of colony was performed using colony counter according to Marth (1978).

**Enumeration of total coliforms (TC)**

Enumeration of total coliforms (TC) was performed using standard multiple tube fermentation technique (Andrews, 1992).

**Presumptive test:** The medium used for this test was MacConkey Broth (Oxoid). One mlof milk sample was added into sterile test tube having 9 ml peptone water. Appropriate decimal dilutions of milk samples were inoculated to MacConkey broth culture medium witha Durham's tube. All tubes were incubated at 37$^{o}$C for 24 h. For positive tubes, numbers of bacteria were looked out from statistically calculated, Most Probable Number (MPN) prepared tables used for MPN (Andrews, 1992).

**Confirmatory test:** The medium used for this test was Brilliant Green Bile(BGB) Broth a selective medium for *E. coli* (Oxoid).The tubes were inoculated with positive tubes (BGB broth), incubated at 44$^{\circ}$C for 48 h. After incubation, tubes were examined for gas production and color change(i.e. green to yellow).A loop full of suspension from positive BGB broth tubes were streaked on Eosin Methylene Blue (EMB) agar (Oxoid) and plates were incubated at 37$^{\circ}$C for 24 h (Harrigan and MacCance, 1976).

**Detection and enumeration of pathogenic bacteria**

A Portion of 1ml from each sample was aseptically homogenized with nine ml sterile enrichment broth of *E. coli*, *Salmonella* and *Staphylococcus aureus* and incubated at 37°C for 24 h.

***Escherichia coli*:** For the isolation and identification of *Escherichia coli*, the enriched sample was cultured on selective medium Levine Eosin Methylene Blue (EMB) Agar and incubated at 37°C for 24 h and morphologically typical colonies producing metallic sheen were counted

***Staphylococcus aureus:*** *Staphylococcus aureus* was isolated by using the technique given by Baird Parker (1962). Enriched samples were spreaded on Baird Parker Agar (BPA) and the plate was incubated at 37°C for 24–48 h. Appearances of jet black colonies surrounded by white halo were counted as *Staphylococcus aureus* (Marth1978).

***Salmonella spp:*** Detection of *Salmonella* was performed according to Harrigan and MacCance (1976). One ml from the enriched sample were inoculated into Selenitecystein broth (Oxoid) and incubated for 24 h at 37$^{o}$C. Positive tubes were pour plated on Bismuth Sulfite agar (Oxoid)and incubated at 37$^{o}$C for 24 h. The pure colonies were counted using colony counter (Marth, 1978).

**Statistical analysis and models**

All microbial counts were converted to the base 10 logarithm of the number of colony forming units per ml ($\log_{10}$cfu/ml) of raw cow milk samples and the means and their standard deviations were calculated. Data were analyzed using Analysis of Variance (ANOVA) through the General Linear Models (GLM) procedure of the statistical analysis system software (SAS 9.1.3). Least significant differences were used to separate means at $p<0.05$.

The physicochemical data collected during the study period were subjected to one way analysis of variance using SAS computer software of version 9.1.3. The collected data were stratified into main factor (value-chain actors and location). When F-test was found significant, means were compared using LSD. A 5% ($P<0.05$) level of significance was used to determine statistical significance.

The models used for the analysis are described below.
Model: Assessment of physicochemical and microbiological properties of milk (part I and II)
$Y_{ijk}=\mu + \alpha_i + e_{ij}$
Where; $Y_{ijk}$= the response variable
$\mu$= overall mean
$\alpha_i$= Effect of value-chain actors/location
$e_{ijk}$= random error

## RESULTS AND DISCUSSION

### *Methylene blue reduction (MBR) tests*

The mean value of methylene blue test colour disappearance time of cow raw milk samples was 2:44 h in four selected districts. Among the selected districts, shorter decolorizationtimes (1:52, 1:46 h) of raw milk were recorded for Haramaya and Kersa while longer decolorization times (2:44, 2:35h) were recorded for Babile and Kulubi (Table 3).

**Table 2. Mean value of methylene blue reduction (MBR) test of cow raw milk across the districts.**

| **Districts** | ***MBR test (h)*** | ***milk grade*** |
|---|---|---|
| Babile | 2:44±0.23$^{b}$ | Fair |
| Haramaya | 1:52±0.02$^{a}$ | Poor |
| Kersa | 1:46±0.16$^{a}$ | Poor |
| Kulubi | 2:35±0.26$^{b}$ | Fair |
| Average | 2:44±0.17 | |

*Means±SD; $^{abc}$ Means bearing different superscripts in the same column differ significantly ($p<0.05$); MBR: Methylene blue reduction test, n= 16

Bongard *et al.* (1995) and Marker *et al.* (1997) reported that blue color disappearance in short time indicates higher microbial load in the milk sample. In this study, most of cow's raw milk shows very short decolorization time of the dye. This may be due to poor milk handling practices during milking, poor animal health services, and use of poor potable water.

### Physicochemical Quality

Table 3, illustrates physico-chemical properties of milk samples from different value-chain actors. There was no significant difference ($P<0.05$) in all physico-chemical properties among the value-chain actors.

**Table 3. Physicochemical quality parameters of raw cow milk across the value chain in Harar milkshed areas**

| Milk Quality parameters | Mean values of milk physicochemical quality parameters across actors | | | | |
|---|---|---|---|---|---|
| | **Producers** | **Collectors** | **Retailers** | **Overall** | **(P<0.05)** |
| Ash | 0.65±0.11 | 0.73± 0.23 | 0.64±0.10 | 0.68±0.16 | NS |
| Protein | 3.61±0.80 | 3.31 ± 0.69 | 3.61±0.30 | 3.51±0.63 | NS |
| Fat | 5.17±0.71 | 4.98± 1.01 | 5.23±0.82 | 5.12±0.84 | NS |
| TS | 13.35±1.42 | 12.79 ±1.47 | 13.17±0.91 | 13.10±1.28 | NS |
| SNF | 8.18±1.47 | 7.81 ± 0.68 | 7.94±0.60 | 7.98±0.98 | NS |
| Lactose | 3.92±0.92 | 3.76 ± 0.46 | 3.69±0.66 | 3.79±0.69 | NS |
| TA | 0.25±0.05 | 0.25 ± 0.03 | 0.25±0.05 | 0.25±0.05 | NS |

NS: non-significant, SNF: solids-not-fat, TA: titratable acidity, TS: total solids.

Table 4 illustrates physico-chemical properties of milk samples from different locations in Harar milkhed. There was a significant difference (P<0.05) in protein%, fat%, total solids%, solids-not-fat% and titratable acidity among the study areas. The study shows that the highest protein (4.31%±0.41), TS (13.88%±1.10) and SNF (8.62% ±1.11) were recorded from Babile and, highest fat (5.87%±0.43) and TA (0.28±0.06) were recorded from Kersa and Haramaya, respectively.

The overall mean value of the fat and protein (5.13%, 3.51%) in the study area is higher than the Ethiopian standard (value of 3.50%, 3.20%)ES (2009). The overall fat content recorded in this study (5.13%) is similar with the result obtained by Dehinenet*et al.,* (2013) on local cattle which is 5.22%. Fresh milk can have an initial acidity because of the buffering capacity (O' Mahoney, 1988); but in this study, milk tested was kept long at ambient temperature between milking and analysis attributing to high acidity. The higher microbial load at some localities particularly Hararmaya is due to the higher titratable acidity of milk sold in the area.

**Table 4. Physicochemical quality parameters of raw cow milk across districts in Harar milkshed areas.**

| Milk Quality parameters | Milk physicochemical quality parameters across districts | | | | | |
|---|---|---|---|---|---|---|
| | **Babile** | **Haramaya** | **Kersa** | **Kulubi** | **Overall** | **(p<0.05)** |
| Ash | 0.70±0.20 | 0.70±0.03 | 0.68±0.04 | 0.62±0.26 | 0.68±0.16 | NS |
| Protein | 4.31±0.41 | 3.12±0.29 | 3.19±0.31 | 3.42±0.60 | 3.51±0.63 | *** |
| Fat | 5.26±0.55 | 4.64±0.53 | 5.87±0.43 | 4.74±1.11 | 5.13±0.84 | ** |
| TS | 13.88±1.10 | 12.17±0.70 | 13.28±1.09 | 13.09±1.59 | 13.10±1.28 | * |
| SNF | 8.62 ±1.11 | 7.52±0.34 | 7.41±0.83 | 8.34±0.97 | 7.98±0.98 | * |
| Lactose | 3.61±0.91 | 3.71±0.24 | 3.54±0.63 | 4.30±0.63 | 3.79±0.69 | NS |
| TA | 0.24±0.02 | 0.28±0.06 | 0.26±0.02 | 0.22±0.05 | 0.25±0.05 | * |

NS: non-significant, SNF: solid not fat, TA: titratable acidity, TS: total solid

**Microbiological analysis**

The result obtained indicates all samples showed contamination with coliforms, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella.* The mean values of $\log_{10}$cfu/ml for microbial load analysis of all identified actors across districts are shown below (Tables 5 and 6).

**Table 5. Mean values of microbiological load of cow raw milk ($log_{10}$cfu /ml) across districts**

| Districts | TBC | Coliforms | *E.coli* | *S.aureus* | *Salmonella* |
|---|---|---|---|---|---|
| Babile (n=9) | 6.04±0.78[b] | 5.52±0.80[a] | 4.69±0.72[a] | 5.67±0.61[a] | 5.22±0.84[a] |
| Haramaya (n=9) | 6.64±0.87[a] | 5.65±0.87[a] | 7.32±0.96[b] | 5.55±0.89[a b] | 5.13±0.66[a] |
| Kersa (n=9) | 6.54±0.77[a] | 5.28±0.77[a] | 4.99±0.54[a] | 5.40±0.80[a b] | 5.03±0.76[a] |
| Kulubi (n=9) | 5.77±0.79[b] | 5.33±0.77[a] | 5.03±0.85[a] | 5.17±0.72[b] | 5.15±0.77[a] |
| Average (n=36) | 6.25±0.87 | 5.45±0.80 | 5.51±5.06 | 5.45±0.77 | 5.13±0.75 |

*Means±SD; [abc] Means bearing different superscripts in the same column differ significantly ($p<0.05$); TBC: Total Bacterial Count; n = 36

**Table 6. Mean values of microbiological load of cow raw milk ($log_{10}$cfu /ml) across value chain actors.**

| Actors | TBC | Coliforms | *E.coli* | *S.aureus* | *Salmonella* |
|---|---|---|---|---|---|
| Collector(n=12) | 6.29±0.81[a] | 5.53±0.94[a] | 6.54±0.69[a] | 5.51±0.73[a] | 4.94±0.59[a] |
| Producer (n=12) | 6.35±0.87[a] | 5.38±0.70[a] | 5.01±0.74[a] | 5.30±0.89[a] | 5.17±0.84[a] |
| Retailer (n=12) | 6.09±0.94[a] | 5.43±0.76[a] | 4.97±0.79[a] | 5.53±0.69[a] | 5.28±0.78[a] |
| Average (n=36) | 6.25±0.87 | 5.45±0.80 | 5.51±5.06 | 5.45±0.77 | 5.13±0.75 |

*Means ±SD; [a]Means bearing same superscripts in the same column shows no significant difference ($p>0.05$); TBC: Total Bacterial Count; n = 36

Raw milk collected from Haramaya, Kerssa, Babile and Kulubi districts contained a high count of TBC (i.e. 6.64, 6.54, 6.04 and 5.77log cfu/ml), respectively. Which is comparable with the values (6 to 8.8 log cfu/ml) reported by Fekadu (1994) for cow milk produced in southern region of Ethiopia. Similarly, Alganesh (2002) reported comparable TBC results ($7.4\times10^7$cfu/ml), for cows milk from Eastern Wollega. This variation was as a result of inadequate sanitary condition during milking, collection, storage and transportation. The study revealed that location significantly ($p<0.05$) affected the total bacterial count (TBC) of cow's raw milk.

Generally, the microbial qualities of milk obtained in the current study are poor compared to international bacteriological standards of dairy products. According to American and European community member states, the acceptable limit for TBC and coliform counts of raw milk is between $2x10^5$and $4x10^5$cfu/ml and 150 cfu/ml(Heeschen, 1997),respectively. However, the mean TBC of cow raw milk observed in the current study were 6.25log cfu/ml, and the count is greater than the upper acceptable limit. This implies that the sanitary conditions in which milk has been produced and handled are substandard. It is indicated that total bacterial count is a good indicator for monitoring the sanitary conditions practiced during production and handling of raw milk (Chambers, 2002).Other important contributors for milk contamination are teats soiled with manure, mud, feeds, or bedding. These practices expose teat end to organic waste sources, wet and muddy pens which increases the risk of developing mastitis and milk contamination (Ruegg*et al.,* 2002).

The total bacterial count (TBC) observed in the current study is similar with the value 6.36log cfu/ml in Wolayta zone (Asrat, 2010), and Eastern Wollega (Alganesh*et al.,* 2007).However, it is lower than the reports by Ashenafi M. (1990); Solomon Mosu (2013); Godefay B and Molla B, (2000); in Hawassa and in and around Addis Ababa, respectively.

All cow raw milk samples collected from Eastern Hararghe (Haramaya, Kerssa, Babile and Kulubi) districts, showed contamination with *Staphylococcus aureus* and *Salmonella* and samples contain an average load of *S.aureus,* 5.45log cfu/ml. Tollossa *etal.,* (2012) isolated *Staphylococcus aureus, Escherichia coli, Klebsiella* spp*, Enterobacter* spp*, Pseudomonas* spp*, Salmonella, Citrobacter* spp*, Proteus* spp*,* and *Yersinia* spp from milk directly taken from udder and milk storage containers from Borana pastoral community. Fook*et al.* (2004) reported that considerably higher levels of *S.aureus* in cow milk; with 35% of samples having 4.2log cfu/ml was identified. Since *S. aureus* is potentially hazardous at $>10^4$cfu/ml (Han *et al.,* 2005), all cow milk samples in this study were not within an acceptable level. There was significant difference ($p<0.05$) in *S.aureus* count between the study areas. The contamination of the milk by *S.aureus* is often original. *S.aureus* is a poor contestant and is readily outgrown by lactic acid bacteria, so growth is limited in raw milk (Holsinger*et al.,* 1997; Asperger, 1994).On the other hand, *Salmonella* was detected in all raw milk samples that were collected from all the districts and samples contained an average load of *Salmonella* 5.13log cfu/ml. The presence of *Salmonella* as pathogenic microorganism is a health problem and this is in accordance with the finding of Gazzar*et al.,* (1992) who reported that *Salmonella* becomes a major concern for the dairy industry due to outbreaks of illness. The mean coliform and *E. coli* counts of cow raw milk samples in this study were (5.45 and 5.51log cfu/ml), respectively. The *E.coli* count in Haramaya was significantly higher ($p<0.05$) than that of Babile, Kulubi, and Kersa districts (Table 6).The total coliform counts were similar ($p>0.05$) among districts. According to a study by Ducsay (2003), the counts of *E.coli* varied from 7.1 to 12.6log cfu /ml in Pakistan. In India, 88.3% and 70% of 60 cow milk samples were contaminated by total and thermo tolerant coliforms A. (Unger, 2004). *E. coli* may be considered an indicator microorganism of feacal contamination and other enteric pathogens. In general, the coliform count observed in the current study (5.45log cfu/ml) is higher than that of4.03log cfu/ml from milk samples collected from cow kept under traditional condition in the Wolayta zone (Rahel, 2000); 4.84log cfu/ml in milk samples collected in the Bahir Dar milkshades(Derese, 2008);4.49log cfu/ml in milk samples in the West Shewa zone of Oromia region (Asaminew, 2007); and 2.76 log cfu/ml in Sudan cow milk (Asmahan*et al.,*2010).

The overall values of coliforms counts observed in the current study were much higher when compared with the acceptable limit given by the American Public Health Service: <100 cfu/ml for Grade A milk and 101-200 cfu/ml for Grade B milk (WHO, 1997). Generally, the presence of high numbers of coliforms in milk indicates that the milk has been contaminated with fecal materials and it is an index of hygienic standard used in the production of milk, as unclean udder and teats can contribute to the presence of coliforms from a variety of sources such as poor herd/farm hygiene, use of improperly washed milking equipment, unsanitary milking practices, contaminated water and cows with subclinical or clinical coliforms mastitis where all lead to elevated coliforms count in raw milk (Jayarao*et al.,* 2004).

## CONCLUSION

Milk is a highly nutritious food that serves as an excellent growth medium for a wide range of microorganisms. Based on the current microbiological analyses carried out in this study, we can provide evidence that the raw cow milk in Harar milkshed (Haramaya and Kersa)

labeled in a very poor conditions of sanitation and hygiene, and this is confirmed by the incidence of high microbial loads of coliforms bacteria, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* showed that the milk is not suitable for consumption because it exceeds the internationally recommended limits. In conclusion, raw milks collected from retailers, and collectors were seriously contaminated in the study area.

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