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Quality Analysis of Raw Milk in West Guji Zone, Oromia Regional State, Ethiopia

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ABSTRACT

The purpose of this study was to determine the compositional, physicochemical, and microbiological quality of raw milk in the West Guji zone of Oromia regional state, Ethiopia. Based on accessibility, proportionality, and target population, milk samples were obtained from thirty (30) lactating cows (9 cows in early lactation, 9 cows in mid-lactation, and 12 cows in late lactation). Soon after milking, approximately 300 lm of raw milk sample was collected from each milking cow and deposited into sterilized glass bottles. The obtained milk samples were preserved in an icebox and transported to the Ethiopian Meat and Dairy Institute's laboratory for analysis of raw milk's microbiological quality of raw milk. The collected milk samples were kept in an icebox and transported to Ethiopian meat and dairy institute laboratory to analyze microbial quality of raw milk. Each milk composition obtained from the laboratory result was analyzed using SAS software 9.4. Accordingly, the overall mean ±SE of chemical composition of raw milk in the three stage of lactation were; Moisture 87.99 ± 1.10 %, Fat 3.67 ± 0.41%, Protein 3.38 ± 0.04 %, Solid Not Fat 8.18 ± 0.09 %, Lactose 4.35 ± 0.08%, Total Solid 12.01 ± 0.39% and Ash 0.67 ± 0.01 %. Whereas, the overall mean ±SE of the physicochemical quality of raw milk in the study areas were; Specific gravity 1.023+0.002 (g/cm3), titratable acidity 0.20 ± 0.00(%) and Freezing point -0.50 ± 0.03(%). On another hand, the overall mean ±SE for coliform count was 4.51±0.15 (log10cfu/ml) and Total Bacterial Counts 6.06±0.20 (log10cfu/ml) in the three stage of lactation in the study area. In general, it can be concluded that the chemical composition and microbiological quality of raw milk produced by farmers in the study areas were found to be within Ethiopian quality standards. The milk was tested and found to be safe for human consumption as well as further processing. The sampled milk is safe for human consumption as well as for further processing. Thus, the channel of the milk from producers to consumers was within quality standard and wholesale in the study area.

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1. Introduction

Milk quality refers to a mix of chemical, physical, bacteriological, and cosmetic features that improve the acceptability of the milk product, whereas milk protection refers to the absence of pathogenic organisms and other contaminants that could be harmful to the consumer's health. The specific gravity, chemical makeup, and microbiological condition of milk determine its consistency and protection. The microbiological content and chemical composition of milk products are critical variables in influencing their quality and protection (Merwan et al., 2018).

Milk hygiene handling practices such as cleaning the udder with clean water, keeping the milking barn clean, drying the udder with individual towels, washing the milker's hands and milking utensils, using low-quality, non-boiled water for udder cleaning, and insufficient post-handling practices such as poor hygiene of milk equipment and storage containers, transportation, and retailing practices predispose the milk to microbial contamination (Abunna et al., 2018). Due to limited pre-milking and post-harvest handling standards, milk hygiene practices in Ethiopia are substandard. Milk quality is a combination of qualities (chemical, physical, and microbiological quality) that contribute to the milk product's acceptability. Total Bacterial Count (TBC) or Standard Plate Count (SPC), Coliform Count (CC), Yeast and Mould Counts, and Somatic Cell Counts are some of the most often used microbiological quality tests for milk and milk products (SCC). In general, the microbiological content of milk and milk products is a good indicator of hygienic conditions during milking, utensil cleansing, storage, and transportation (Getabalew et al., 2020).

The Ethiopian standard authority has established criteria for domestic and imported dairy products; however they have yet to be applied to ensure the safety and quality of dairy products. Furthermore, throughout the previous half-century, research has mostly focused on boosting milk output rather than on the safety and quality of dairy products (Gemechu, 2017).

Some of the most important quality parameters in milk are its chemical composition and microbiological quality. The microbiological load of milk reveals the level of hygiene practiced from milking to consumption, such as cleanliness of milking utensils, storage conditions, and mode of travel at each node of milk actors. Milk from healthy animals produced under sanitary circumstances should not contain more than 5x105 bacteria/ml (O'Connor, 1994).

Consumers demand clean, healthy, and nutritious food that has been produced and prepared in a safe, sanitary, and pathogen-free environment. It is vital to produce high-quality milk in order to meet consumer demand. Milk that is devoid of pathogenic bacteria and hazardous poisonous compounds, free of silt and extraneous elements, of good flavor, with normal composition, appropriate in maintaining quality, and low in bacterial counts is considered to be of high quality (Ahmed et al., 2004).

Normal cow's milk comprises roughly 87.8% water and 12.2 percent milk solids, 3.3 percent fat, 3.3 percent protein, 4.7 percent lactose, and 0.7 percent minerals, vitamins, and nitrogen, all of which have an impact on milk quality. However, across the same and different breeds, milk composition varies significantly but within acceptable ranges. Human milk is higher in protein and minerals, particularly calcium and phosphorus, than cow milk. The majority of milk today has a standardized fat level of roughly 3.5g/100g (FAO, 2013).

To be effective, initiatives to boost smallholder dairy productivity and increase market orientation must be backed up and informed by a thorough understanding of the current and changing conditions of milk and dairy product production, marketing, processing, and consumption (Asfaw, 2009). As a result, the southern part of the country, in general, and the West Guji Zone in particular, are well suited to dairying because the area is a key asset with enormous cattle resources and strong indigenous knowledge for dairy cattle production, the society's socio-economic system is heavily reliant on crop-livestock production, and there is a high demand for dairy and dairy products.

The current state of chemical composition and microbiological quality of raw milk, on the other hand, has not been thoroughly investigated and documented. As a result, the current research is being carried out to determine the chemical composition and microbiological quality of milk in a number of districts in the west Guji zone.

2. Objective

To evaluate chemical composition and microbial quality of raw milk in the study area.

3. Materials and Methods

The study was conducted in west Guji zone of Oromia regional state, Ethiopia. Bule Hora is located 467km from capital city of Ethiopia Addis Ababa. West Guji Zone is also found in southern part of the Oromia Regional State and south eastern part of the country. West Guji zone has a total population of 141,579 of which 78,030 are males and 63, 549 are females. The zone was located between 38°_40° East longitude and latitude 4°_5° on the North and the altitude ranges from 500m up to 3500m above sea level. The climatic condition of West Guji zone is characterized by three agro-climatic zones, namely; Dega, Woina Dega and Kola. The coverage of each climatic zone is Dega 33%, Woina Dega 47% and kola 20%. The mean annual rain fall of the study area is about 900mm and the annual temperature of the district 25 ^{0}C (WGLEPO, 2012).

The nature of the rain fall is bi-modal: 59% of annual precipitation occurs during March to May (main rain season) and 27% from September to November (short rain season), while the period between June and August was termed as cold dry season and December to February was the long dry season.

3.1 Analysis of Quality of Raw Milk

For physicochemical composition and microbial quality analysis raw milk were collected from local cows having different lactation stages. The milk sample was collected from thirty (30) lactating cows having early, mid and late lactation stages. Accordingly, 9 lactating cows which have early lactation stages and again another 9 lactating cows which have mid-lactation stages and 12 lactating cows having late lactation stages was used. Totally, nine thousand milliliters of milk (three hundred milliliter of milk from each cow) was collected. The milk sample was collected in sterile glass bottles and transported to Ethiopian meat and dairy institute laboratory within 24 hours of milking by placing in an icebox. From the sampled milk the following physiochemical composition and microbial quality of milk were analyzed.

3.1.1 Specific Gravity

Specific gravity is the relation between the mass of a given volume of any substance and that of an equal volume of water at the same temperature was used to evaluate adulteration. According to ILRI (1995), normal cows' milk should have a specific gravity between 1.028 and 1.032 g/cm^3.

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

where,
L = corrected lactometer reading at a given temperature physical properties of milk samples

3.1.2 Titratable Acidity of Milk

Titratable acidity of the milk samples was determined according to the method of the Association of Official Analytical Chemists (AOAC, 1990). Nine ml (9) of milk sample was pipetted into a beaker and 3 to 5 drops of 1 % phenolphthalein indicator was added to it. The milk sample was titrated with 0.1N NaOH solution until a faint pink color persisted. The titratable acidity, expressed as % lactic acid, was finally calculated using the following formula;

$$\text{Titratable acidity \%} = \frac{\frac{N}{10} NaOH\ (ml)\ 0.009}{weight\ of\ milk\ sample} \text{x } 100$$

3.1.3 Total Solids (TS)

The milk samples were dried in a hot air oven at 102 ^{0}Cfor 3 hours. Finally, the dried samples were taken out of the oven and place in desiccators to cool at room temperature. Then, samples were weighed again and the total solids were calculated by the following formula according to (Richardson, 1985).

$$TS = \frac{\text{Crucible weight } + \text{Oven dry sample weight } - \text{Crucible weight}}{\text{Sample weight}} \text{x } 100$$

3.1.4 Total Protein Determination

Formaldehyde titration method was used to determine the total protein. Ten ml of milk was added into a beaker. Then, 0.5 ml of 0.5 percent phenolphthalein indicator and 0.4 ml of 0.4 percent Potassium Oxalate was added into the milk and then, the sample was titrated with 0.1N NaOH. The titration was continued until pink color becomes intense. Finally, the burette reading was recorded and reading was multiplied by a factor 1.74 (O'Connor, 1994).

3.1.5 Milk Fat Determination

Gerber method was used to determine the milk fat. Milk samples were kept at 37 ^{0}C for 30 minutes in a water bath to maintain the milk to normal body temperature of cow. Ten ml of concentrated sulfuric acid was pipetted into butyrometer. Then, 11 ml of milk was added using milk pipette into a butyrometer having the sulfuric acid and then 1ml of amyl alcohol was added. The samples were shaken and inverted several times until all the milk was absorb/digest by the acid. Finally, the sample was taken back to water bath for 5 minutes at 63 ^{0} C and fat percentage was read from the butyrometer (O'Connor, 1994).

3.1.6 Solids not Fat

The solids not fat (SNF %) was determined by subtracting the percent fat from percent total solids (O'Connor, 1994).

SNF (%) = (% TS - % fat)

3.1.7 Total Ash

The total ash was determined gravimetrically by igniting the dried milk samples in a muffle furnace in which the temperature is slowly raised to 550 ^{0}C. The sample was ignited until carbon (black color) disappears or until the ash residue becomes white.

$$\text{Percent ash} = \frac{weight\ of\ resudue}{weight\ of\ sample} \text{ x } 100$$

3.1.8 Lactose

Percent lactose was determined by subtracting the fat, protein and total ash percentages from the total solids (O'Connor, 1994).

Percent lactose = *percent total solids* - (*% fat + % protein + % total ash*)

3.2 *Microbial Quality Tests*

The microbial tests considered for determination of the bacterial load in raw milk samples. They are; Coliform Count (CC) and Total bacterial count was analyzed by using appropriate media. The estimated colony count per ml of milk was calculated by using the following formula;

$$N = \frac{\Sigma \text{C N x d}}{(1 \text{ x } n1) + (0.1 \text{ x } n2)}$$

where,
N = Number of colonies per ml of milk sample
ΣC = Sum of all colonies on plates counted
n1 = Number of plates used in lowest dilution counted
n2 = Number of plates used in highest dilution counted
d = dilution factor of the lowest dilution used.

3.3 *Data Analysis*

Chemical composition and microbial quality of raw milk was analyzed through General model of SAS software (SAS, 2014). The following model was employed for data analysis of chemical composition and microbial analysis;

Model: Yij = μ +Si + Eij

where,
Yij = individual observation
μ= Overall mean
Si = effect of the ith milk source
Eij = random error

4. Results and Discussion

4.1 *Compositional Quality of Milk*

4.1.1 Fat Content

The fat content of the milk in the study areas were presented in Table 1. The current finding revealed that, the fat contents of milk sample collected from a cow having early, mid and late lactation stages was 3.44$\pm$0.74, 3.06$\pm$0.74 and 4.52$\pm$0.64%, respectively. However, from overall the fat content of the sampled milk was 3.67 ± 0.41% in the study areas. In the current study, there were significance differences ($P<0.05$) in fat contents of the sampled milk between lactation stages. The average fat percentages of raw cow's milk collected in the study districts was lower than the fat content given for zebu breeds which is 4.9%. The entire sampled milk collected from each of the stages of lactation was fit with the Ethiopian raw milk fat content standard. However, the Food and Drug Administration (FDA) requires not less than 3.25% milk fat for fluid whole milk similarly to the U.S. Public Health Service (USPHS). Milk Ordinance and Code also recommended a minimum of 3.25%

butterfat in farm milk (Raff, 2011). So, except the milk sample collected from mid-lactation stage, the fat content of milk collected from early and late lactation stages was fulfills the criteria set by FDA, USPHS and Milk Ordinance and Code. This variation might be due to breeds of milking cow or environmental factors like feed and parity.

4.1.2 Protein Content

The protein content of the milk in the study areas were presented in Table 1. As the analyzed milk sample revealed, the average percentages of protein contents of milk sample taken from the cows having early, mid and late lactation stages were 3.32 ± 0.07, 3.44 ± 0.07 and 3.37 ± 0.06%, respectively with the overall mean of 3.38 ± 0.04%. The protein contents of the current sampled cow milk were insignificantly (P>0.05) different between lactation stages.

Therefore, the current finding was slightly in line with the value 3.12 % of protein contents reported by Dehinenet et al. (2013) while relatively in line with 3.67, 3.4 and 3.34% of protein contents reported by Derese et al. (2008), Haftu et al. (2013) and Ayisheshim et al. (2015) in cow milk of western Shewa, Southern Ethiopia and western Amhara region, respectively. Thus, the protein content of the sampled milk was within the quality standard of Ethiopia and minimum milk protein of 2.73% recommended by FDA (Raff et al., 2011). In general, the value of protein content obtained from the current study was fulfills the criteria developed by FDA for the consumers.

4.1.3 Total Solids

The total solid of the milk in the study areas were presented in Table 1. In the current study, there was significantly difference (P<0.05) in total solid contents among lactation stages. Higher total solid of 12.58 ± 0.61 % was recorded in late lactation stages while lower total solid content (11.19 ± 0.70%) was analyzed from mid-lactation stages. From overall, 12.01 ± 0.39% of total solid was analyzed which was relatively related with the result of 13.4% and 12.575% total solid reported by Teshome et al. (2015) and Tecklemikael et al. (2015), respectively. The result of this study revealed that, the total solid content of the sampled milk was fall within the quality standard of Ethiopia (Table 1). However, according to European Union, the recognized quality standards for total solids content of cow milk should not be less than 12.5% (FAOSTAT, 2007). Therefore, the total solid content of the current sampled milk was not fit the standard set by European Union.

4.1.4 Solid not Fat (SNF)

The SNF of the sampled milk was insignificantly (P>0.05) difference among lactation stages. As result of the current finding revealed, the mean percentage of SNF analyzed in the whole cow milk sample was 8.26 ± 0.16, 8.25 ± 0.16 and 8.01 ± 0.14% on early, mid and late lactation stages, respectively. According to Food and Drug Administration (FDA) as well as European Union (EU) quality standards, a minimum solids-not fat (SNF) content of whole milk is 8.25% (Raff, 2011). In view of that, except milk sample collected from late lactation, the result obtained in the present study areas was fulfills the range of quality standards given by FDA and EU.

4.1.5 Lactose

The average percentages of lactose contents of sampled whole cow milk was 4.80 ± 0.15, 4.16 ± 0.15 and 4.09 ± 0.13% in early, mid and late lactation stages, respectively. Statistically, there were insignificantly differences in terms of lactose content between lactation stages.

The current finding was slightly similar with the value of 4.47, 4.91 and 4.69% reported by Hamad et al. (2015), Legesse et al. (2015) and Gurmessa et al. (2015) in raw cow milk collected from producer and market, respectively. On the other hand, the present finding was in line with the value 3.6 to 5.5% of lactose content of milk reported by (O'Mahony, 1998). However, cow milk of lower fat content of 3.79% was reported by Estifanose et al. (2015) in Harar milk shade of Ethiopia. Therefore, the milk lactose content of the current study was fall within Ethiopian quality standard.

4.1.6 Mineral (ash) Content

The total mineral content in the sampled milk was presented in Table 1. As the current result revealed, the mean percentages of ash contents of the sampled milk were 0.68 ± 0.02, 0.68 ± 0.02 and 0.66 ± 0.02% in early, mid and late lactation stages, respectively with overall mean of 0.67 ± 0.01%. The current finding indicated that, the percentage of mineral content obtained from the sampled raw milk was insignificantly differences among lactation stages. Therefore, in all lactation stages, the mineral contents of the sampled raw cow milk were within the Ethiopia quality standard. So, the current finding was slightly similar with the value of 0.69, 0.76 and 0.82% which was reported by Hamad et al. (2015), Teshome et al. (2015) and Gurmessa et al. (2015), respectively with the milk sample collected from producers and market. The ash content of the raw milk collected from household milk producers in West Guji zone for the current finding falls within the usual ranges of 0.6 to 0.9% (O'Mahony,1998).

4.2 Physicochemical Quality of Raw Milk

4.2.1 Titratable Acidity

Acidity was an important parameter for the indication of the development of acid- producing microbes from lactose fermentation in the milk as well as an indicator of freshness of milk. The acidity content of the sampled milk was presented in Table 1. As result of the analyzed milk sample indicates, the mean acidity percentages of the sampled whole cow milk were 0.21 ± 0.01, 0.20 ± 0.01 and 0.18 ± 0.01% in early, mid and late lactation stages, respectively with overall mean of 0.20 ± 0.00. The titratable acidity of the current milk sample was not in line with the value of 0.1 to 1.17% titratable acidity of Ethiopian standard for fresh milk. The current result indicated that, the analyzed milk was not fresh milk which might be due to inappropriate storage during transportation or mishandling in laboratory which causes the development of acidity due to growth of acid-producing microorganisms. The present finding was relatively in line with the result of 0.215, 0.29 and 0.22% which were reported by Legesse et al. (2017), Teshome et al. (2015) and Gurmessa et al. (2015) with cow milk collected from producers and market, respectively. Therefore, the higher titratable acidity of raw milk samples collected from the study area might be due to bacterial growth and multiplication during transportation of milk to laboratory for analysis.

4.2.2 Freezing Point

As indicated in Table 1, the average percentages of freezing point of the sampled raw cow milk was -0.57 ± 0.06, -0.56 ± 0.06 and -0.37 ± 0.05% in early, mid and late lactation stages, respectively with the overall mean -0.50 ± 0.03%. As noticed from the current result, the freezing point of the sampled milk was insignificantly different between lactation stages. Therefore, the freezing points of milk sample collected from early and mid- lactation stages were fit with Ethiopian standard of -0.547 to-0.590% while the milk sample taken from late lactation stages was sub-standards of Ethiopian standards.

4.2.3 Specific Gravity

The specific gravity of the sampled milk was indicated in Table 1. Specific gravity is the relation between the mass of a given volume of any substance and that of an equal volume of water at the same temperature. Result of the current finding revealed, the specific gravity of the sampled raw cow milk collected from early, mid and late lactation stages were 1.029±0.001, 1.031±0.004 and 1.029±0.002g/cm^3, respectively. The statistical value of the current finding was fall within the ranges of 1.028 and 1.032 g/cm^3 value which was reported by ILRI (1995) for normal cows' milk. This value indicates as the sampled milk was free from any liquid or solid adulteration.

4.3 Microbial Quality of Raw Milk Analysis

4.3.1 Total Bacterial Count

The total bacterial count analyzed in the sampled milk was presented in Table 1. The total bacterial count analyzed from the current sampled milk was 5.60 ± 0.36 log10 cfu/ml, 5.57 ± 0.36 log10 cfu/ml and 7.00 ± 0.31 $\log_{10}$ cfu/ml in early, mid and late lactation stages, respectively. The overall total bacterial count analyzed in the study areas was 6.06 ± 0.20. The total bacterial count was significantly $P<0.05$ different between the three stage of lactation. The current finding of the total bacterial count was less than the value 3x10^5/ml of the maximum limits given for Commingled milk. Sherikar et al. (2004) reported that milk samples were graded as very good when the bacterial count did not exceed 2x10^{5}cfu/mL which is in line with the present finding. The result of the present study indicated that, the collected sampled milk had proper hygienic practices during milking and collected from health and hygienic cows.

4.3.2 Coliform Count (CC)

The total coliform count analyzed in the sampled milk was presented in Table 1. The total coliform count analyzed from the current sampled milk was 3.56±+0.12log cfu/ml 5.63±0.24 log cfu/ml and 4.36 ±0.11 log cfu/ml in early, mid and late lactation stages, respectively. The overall Mean ±SE of coliform count of milk produced in the study area was 4.51±0.15log cfu/ml. Coliform counts in the current study were higher when compared with the acceptable limit given by the American public health service < 2log cfu/ml for grade A milk and 2-2.3 log cfu /ml for Grade B milk (APHA, 1992). The coliform count obtained in the present study is slightly similar with the value reported by Asmaninew and Eyassu (2011) who found coliform count of 4.49 log cfu/ml in Bahir Dar Zuria and Mecha Districts. Coliform count can indicate fecal contamination from equipment that has not been properly cleaned and sanitized (Bintsis et al., 2008). It is not practical to produce milk that is always free of coliform, even at high level of hygiene condition; their presence in raw milk to a certain extent may be tolerated. But their presence in large numbers in dairy products is an indication that the products are potentially hazardous to the consumer's health (Godefay and Molla, 2000).

Table 1. Physicochemical composition and Microbial quality of raw milk

Dependent Variable	Lactation Stage			Overall (N=30)		
	Early Lactation (N=9)	Mid-Lactation (N=9)	Late Lactation (N=12)			
	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Sig.	ES
Chemical composition of raw milk						
Moisture %	87.76 ± 0.07	88.81±0.64	87.42±0.36	87.99 ± 1.10	0.001	
Fat %	3.44 ± 0.74	3.06 ± 0.74	4.52 ± 0.64	3.67 ± 0.41	0.006	2.5-7
Protein %	3.32 ± 0.07	3.44 ± 0.07	3.37 ± 0.06	3.38 ± 0.04	0.301	2.9-5.0
Solid Not Fat %	8.26 ± 0.16	8.25 ± 0.16	8.01 ± 0.14	8.18 ± 0.09	0.516	>8.0
Lactose %	4.80 ± 0.15	4.16 ± 0.15	4.09 ± 0.13	4.35 ± 0.08	0.400	1-10
Total Solid %	12.24 ± 0.70	11.19 ± 0.70	12.58 ± 0.61	12.01 ± 0.39	0.003	10.5-14.50
Ash %	0.68 ± 0.02	0.68 ± 0.02	0.66 ± 0.02	0.67 ± 0.01	0.326	0.60-0.90
Physicochemical quality of milk						
Specific gravity (g/cm^3)	1.029±0.001	1.031±0.004	1.029±0.002	1.029±0.002	0.402	1.028-1.032
Titratable acidity (%)	0.21 ± 0.01	0.20 ± 0.01	0.18 ± 0.01	0.20 ± 0.00	0.020	0.1-0.17
Freezing point	-0.57 ± 0.06	-0.56 ± 0.06	-0.37 ± 0.05	-0.50 ± 0.03	0.615	-0.547 to -0.59
Microbial quality of raw milk						
Coliform count (log10 cfu/ml)	3.56±0.12	5.63±0.24	4.36 ±0.11	4.51±0.15	0.23	
Total Bacterial Counts (log10cfu/ml)	5.60 ± 0.36	5.57 ± 0.36	7.00 ± 0.31	6.06 ± 0.20	0.007	

ES: Ethiopian Standard

5. Conclusion

The goal of this study was to determine the physicochemical quality, chemical composition, and microbiological quality of raw milk in the West Guji zone of Oromia regional state, Ethiopia. The chemical composition of the milk examined was within the acceptable range for Ethiopian raw milk quality. The microbial concentration of the sampled milk, on the other hand, was lower than the maximum bacterial limit set for a milliliter of raw milk. This low microbial concentration in the milk implies effective dairy cow management and hygienic methods during milking at the farm level. The chemical composition and microbiological content of the milk analyzed were also within the recommended ranges set by the European Union, FAO, and the Food and Drug Administration (FDA). Finally, the chemical composition and microbial quality of raw milk in the

West Guji zone at the farmer's level met Ethiopian quality standards and was safe for human consumption and subsequent processing. As a result, the milk supply chain from producers to consumers in the research area should meet quality standards and be wholesale. The following recommendation was madebased on the above conclusion:

- Chemical composition and microbial quality of milk should always meet Ethiopian, FAO, FDA, and European Union (EU) quality standards at farm and market level.
- In the West Guji zone, the milk distribution channel from producers to consumers or processors should be wholesale or safe.

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Declaration of Conflict

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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