Assessment of Safety and Quality of Raw Whole Cow Milk Produced and Marketed by Smallholders in Central Highlands of Ethiopia

Alganesh Tola Gemechu
Jimma University

Abstract

A study aimed to assess safety and quality of raw whole cow milk through determination of chemical and bacteriological quality was conducted in Ejere, Walmera, Selale and Debre-Birhan districts of central highlands of Ethiopia. Purposive random sampling technique was used for collection of 108 raw milk samples from producers in the study areas. The samples were analyzed in Holeta dairy research laboratory using standard procedures. The overall means for fat, protein, total solids, ash, lactose, SNF, total aerobic mesophilic bacteria, total coliforms, Entrobacteriaceae and titratable acidity, were 3.76 %, 3.10 %, 12.24 %, 0.61%, 5.08 %, 8.56%, 8.2 log10 cfu/ml, 8.58 log 10 cfu/ml, 11.36 log 10 cfu/ml, 0.27 respectively. All chemical components across locations showed significant (P<0.01) difference except that of ash. The microbial qualities also showed highly significant difference at P<0.01 for all locations. The average composition of protein, total solids and ash were below the standard set by the Ethiopian Standard Agency. The lower average total solids might be due to the practice of adulteration and fat skimming before taking milk to collection points. The lower protein content might be due to deficiency of crude protein in the cow ration. All the bacteriological parameters tested and titratable acidity were substandard. The higher bacteriological count in the present study could be attributable to unhygienic milking practices which features to milking without cleaning the udder and lower abdominal body part of cows. Use of local unsanitized containers for milking, dirty milking areas, poor personnel hygiene and lack of milk cooling systems are the major factors contributing to the poor bacteriological quality and titratable acidity of the milk samples. respectively. Hence, regulatory bodies should take strict monitoring and quality control measures at all levels from production to consumption. There should also be sustainable awareness on the good manufacturing practices. This would rectify the shortcomings in both properties of milk to assure the delivery of safe and quality raw whole milk to the end users.

Keywords: Safety, quality, raw, whole, milk, smallholders, marketed, central highlands, Ethiopia

Introduction

Milk from a healthy udder contains very few numbers of bacteria as low as less than $3x10^4$ cfu/ml but may become contaminated by microorganisms from the surrounding environment during milking and milk handling (Robinson R. K., 2002). Dairy products quality defects have been accredited to poor microbiological quality of raw milk (Berg J C T., 1988). The production of high quality milk should therefore be priority for good quality end products of long shelf life and for marketing of value added products. This is very complicated in general to attain in developing countries because of poor hygiene and cleanliness during milking and subsequent milk handling, contaminated water sources for cleaning milk utensils, lack of cooling amenities, high ambient temperature and insufficient infrastructures for milk transportation to the market (Bille P G *et al*., 2000).

The daily production of a perishable commodity such as milk with high water content demands special consideration to ensure its arrival to market in an acceptable condition. If the hygienic standards of production and handling are poor, the keeping quality of milk would be very poor due to the high ambient temperatures and there will be a very high risk of spoilage. So milk should have normal composition, not adulterated and produced under hygienic conditions. (Chamberlain, 1990). The safety of dairy products with respect to food-borne diseases is a great concern around the world. This is especially true in developing countries where production of milk and various dairy products take place under unsanitary conditions and poor production practices (Mogessie, 1990; Zelalem et al, 2007).

A total of 5167 small, medium and large scale dairy farmers exist in and around Addis Ababa which encompasses the current study areas. Total milk production from these dairy farms amounts to 34.649 million litres/annum. The milk being marketed under this system is of questionable quality, it is not pasteurized and there is a possibility of adulteration. Although, few farmers produce good quality milk, hygienic quality and composition of most milk marketed in such production system is poor. Moreover, price is high even when quality of milk is low. No quality control mechanisms exist to safeguard consumers (Addis Ababa Agricultural Bureau survey report quoted by Azage and Alemu 1998).

Zelalem et al (2007) also reported that milk produced in Ethiopia in general is marketed without any form of pasteurization or quality control measures. Besides, there is inadequate information on the microbial properties and chemical composition of raw whole milk in the central highlands areas. However, huge amount of

raw whole milk is channeled to the Addis Ababa market from the smallholders in the central highlands through cooperative unions and processors. Thus, this paper reports the assessment of the safety and quality in terms of bacteriological quality and chemical composition of raw whole milk from Selale, Debre Birhan, Walmera and Ejere areas.

Materials and methods
The study areas
The study was carried out in peri-urban areas of Ejere, Walmera, Selale and Debre Birhan Districts of the central highlands of Ethiopia.

Ejere is situated at latitude and longitude of 9°2′North 38°24′East with an elevation of about 2360 meters above sea level. The average annual temperature in Ejere is 16.1 °C. The average annual rainfall is 1122 mm. It is situated in Oromia region at 49 kilometers to the West of Addis Ababa.

Walmera has an altitude of 2400 meters above sea level. It's annual rain fall is 1100mm; The minimum temperature is 6^0C and its maximum temperature is 24^0C. Walmera district has a latitude and longitude of 9°3′North 38°30′East, respectively. It is situated in the Oromia region at 38.7 kilometers to the West of Addis Addis Ababa.

Selale (altitude: 2500 to greater than 3000 meters above sea level; annual rain fall:1200mm; average temperature minimum 6^0C and maximum: 21^0C) . It is situated at 9.6833300^0 North latitude and $38,650000^0$ East longitude. Selale is situated in Oromia region at about 85 kilometers to the North of Addis Ababa.

Debre Birhan is located at 9.67954^0 North latitude and 39.5326^0 East longitude at an elevation of 2830 meters above sea level. The annual average annual temperature and rain fall in Debre Birhan are 14.4^0 c and 964 milimeter, respecively. Debre Birhan is located at 132.4 kilometers in the North East from Addis Ababa in Amhara region.

The seasons of the year in these areas can be broadly categorized in to wet season that covers the period from June to September. The dry season covers the time from October to May. The milk samples were collected from smallholder producers at the collection points while the milk samples were being delivered to the dairy cooperatives unions and private collectors.

Sampling and Data collection
Purposive random sampling technique was used to collect milk samples from the producers at respective sites. Sterile sample bottles were used to collect milk samples. Before sampling, the milk in the farmers containers was homogenized and samples were aseptically drawn. Before drawing milk samples, flames of local lump ('kuraz') were used to create relatively sterile surrounding while opening sample bottles. While milk sampling, sample collectors sterilized their hands and outer surfaces of sample bottles using 78% alcohol. About 200 ml of milk samples were collected from each producer. Altogether 108 samples were collected and analyzed from four locations. The samples were kept in an ice box and transported to Holeta dairy research laboratory within four hours of collection depending on the distances of the sites.

Chemical Composition
Determination of fat content
Gerber method was used to determine the milk fat content. Milk samples were kept at 37^0C for 30 minutes in a water bath to warm back the milk samples to normal body temperature of the cow. Ten ml of concentrated sulphuric acid was pipetted into a butyrometer. Then 11 ml of milk was added using milk pipette into a butyrometer having the sulphuric acid and then one ml of amyl alcohol was added. The butyrometer stopper was put on and the sample was shaken and inverted several times until all the milk was digested by the acid. Then the butyrometer was placed in a water bath at 65^0C for five minutes. The sample was placed in a Gerber centrifuge for four minutes at 1100 rpm (rotations per minute). Finally, the sample was placed in to water bath for 5 minutes at 65^0 C and fat percentage was read and from the butyrometer. The average of duplicate readings was computed and recorded (O'Connor C.B. 1994).

Determination of protein content
Formaldehyde titration method was used to determine the total protein content. 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. Then, the sample was titrated using digital dispenser/burette with 0.1N Sodium Hydroxide solution. The titration was continued until pink color becomes intense (O'Connor C.B., 1994). Finally, the burette reading was recorded. The reading was multiplied by a factor 1.74 (Foley et al., 1974).

Determination of total solids

To determine the total solids, three grams of milk sample was pipette in a pre-weighed and dried duplicate of crucibles. The crucibles were placed on a boiling water bath for 30 minutes. The samples were dried in the drying oven at 102± 2°C for 24 hours. The samples were kept at $102^{0}C \pm 2°C$ for 24 hours. Then, the dried samples were taken out of the oven and placed in a desiccator to cool and weighed. Again, the samples were dried in the oven for 1 hour as before. Cooled and reweighed again. The drying was repeated until the difference in weight between two successive weighing was not more than 1 mg (O'Connor C. B. 1994). Then the total solids was calculated as $\underline{M_2 - M_0}$ x 100

$\qquad\qquad\qquad M_1$

Where: M_0 = the mass in grams, of the crucible

M_1 = the mass in grams of the test portion

M_2 = the mass in grams of dried crucible and test portion

2. 3. 5. Solids- not –fat

The solids not fat (SNF %) was determined by subtracting the percent fat from total solids (O'Mahoney, 1988).

Determination of ash content

The total ash was determined gravimetrically by igniting the dried milk samples used for total solids determination in a muffle furnace in which the temperature was slowly raised to $550^{0}C$ until five hours. The sample was ignited until carbon (black color) disappears or until the ash residue becomes white. Finally, the ash was removed from the furnace and cooled in desiccators. The ash content was calculated as

% Ash = $\underline{\text{Burnt crucible} + \text{sample weight} - \text{Oven dry crucible weight}}$ X 100

$\qquad\qquad\qquad\qquad$ Sample weight

Finally, the result was recorded (H. Michael W. and Joseph F. Frank 2004).

Determination of lactose

To determine percent lactose of the whole milk, percent fat, protein and ash were subtracted from the total solids content. % Lactose =Percent total solids-(% fat+ % protein+ % total ash).

Bacteriological analysis

Aerobic mesophilic plate count

Homogenized milk samples were serially diluted by adding 1ml of milk into 9 ml of peptone water. One ml of the sample from a chosen dilution was placed on the petri dish using pour plating technique. Then, plate count agar media of 15-20 ml was poured on to the petri-dish and thoroughly mixed with the sample and allowed to solidify for 15minutes and incubated for 48 $\pm$2 hours at 35°C. Finally, duplicate plates having 25-250 colonies were manually counted. The plate counts were calculated by multiplying the count on the dish by 10^{n}, in which n stands for the number of consecutive dilutions of the original sample (FAO, 1997). The result of the counts were transformed to log 10 (logarithms of 10) before the statistical analysis.

Total Coliform count

Samples were decimally diluted and plated with violet red bile agar mediua /VRBA into Petri dishes for enumeration of total coliforms bacteria as colony forming units per ml. Plates were incubated at 32 $\pm1^{0}C$ for 24±2 hours. One ml of milk sample was serially diluted using peptone water and transferred into sterile petri - dishes. 10-15 ml of violet red bile agar media tempered to a temperature of $44^{0}c$ to $46^{0}c$ was added to the milk sample and thoroughly mixed and allowed to solidify for 5-10 minutes. The mixture was then overlaid with the same plating agar media of 3 to 4 ml to inhibit surface colony formation. The medium were allowed to solidify. The plates were inverted and incubated at 32 $\pm1^{0}C$ for 24±2 hours. Counts were made manually.

$\qquad$ Finally, the plate counts were calculated as N, the number of colony forming units of coliforms per ml of milk sample using the formula N=$\sum c/(n_1+n_2)d$ where $\sum c$= Sum of all colonies on all plates counted. n_1= Number of plates in the first dilution counted

n_2= Number of plates in the second dilution counted

d= Dilution from which the first counts were obtained

(H. Michael W. and Joseph F. Frank 2004)).

Entrobacteriaceae count

Homogenized milk samples were serially diluted by adding 1ml of milk into 9 ml of peptone water to yield a dilution that gave 15-100 cfu/ml of diluted sample. Pour plating technique was used. The plates were placed on a flat leveled surface. Violet red bile glucose agar (VRBGA) medium was used to enumerate Entrobacteriaceae. The mixture was then overlaid with the same plating agar media of 3 to 4 ml. Plates were incubated aerobically

for 24 hours at 37°C and inspected for purple-red colonies surrounded by a purplish circle of light or halo color. Finally, the colonies were manually counted. The plate counts were calculated by multiplying the count on the dishes by 10^n, in which n stands for the number of consecutive dilutions of the original sample (ILSI, 2011).

Titratable acidity
Ten ml of milk was pipetted into a beaker, and then 3-5 drops of 0.5% phenolphthalein indicator was added. Then the sample was titrated with 0.1N NaOH until pink color persists. Acidity was expressed as percentage of lactic acid (O'Connor C. B. 1994). Then % lactic acid was determined as follows.

Lactic acid (%) = $\dfrac{\text{ml N/10 alkali/NaOH x 0.009 x 100}}{\text{ml of sample}}$

Data Analysis
Data was entered and cleaned using Statstical Package for Social Sciences (SPSS) version 16 (SPSS, 2011). The results of milk composition and microbial quality were analyzed using mean procedures of the SAS version 9.2. (Statistical Analysis System) (2009). Means were calculated for chemical composition and bacteriological quality of milk samples collected from producers from the four locations. The General Linear Model (GLM) procedure for least square analysis of variance in SAS (2009) was utilized. A fixed effect model was used to estimate the effects of locations for studied parameters. Mean comparison was done using DMRT (Duncan's multiple range test) for those variables whose F values appeared to be statistically significant. Differences were considered to be significant at p ≤0.001 and p ≤0.05. Possible interactions were also computed between different factors considered. Total aerobic mesophilic bacteria, Entrobacteriaceae and total coliform counts were transformed to log 10 before statistical analysis.

Results and discussion
Chemical composition of raw whole cow milk
Fat
The fat content showed highly significant difference (P<0.001) between different milk samples collected from four locations (Table 1). The fat content of milk samples collected from Debre-Birhan and Walmera differed significantly from each other. But, the fat content of milk samples collected from Ejere, Debre Birhan and Selale districts did not significantly differ from each other. The significant difference in milk fat content from Debre-Birhan and Walmera might be attributable to variation in stages of lactation, interval between milkings, age of cows, feeding regime and completeness of milking in the two locations (O'Connor C.B., 1994).

The overall mean fat percentage in this study is 3.76. This result is in agreement with the average fat content of cow milk revealed by O'Connor C.B.1995. On the other hand, the average fat percentage obtained in the current study is lower than findings of Teshome Gemechu *et al (*2015) which reported an average fat content of 4.28 percent for milk samples collected from dairy cooperatives milk collection centers, hotels, small shops/kiosks and small scale milk producers. Another study by G. Dehninet *et al* (2013) revealed higher average fat content of 5.22 percent from milk samples collected from smallholder producers in Oromia and Amhara reigions of Ethiopia. A probable reason for lower average fat percent obtained in the current study might be explained in terms of the increasing trend of skimming off of fat from milk before sales in the peri-urban set up. The other reason could be due to the relatively larger population of cross bred cows in the central highlands of the country whose milk fat content is relatively lower compared to the indigenous Zebu cattle.

According to the Ethiopian standard agency, the minimum fat percent for whole milk should not be less than 3.5 percent (ESA, 2009). Hence, the average fat percent in the current study fulfills the recommended range even though it is below average for the local breeds.

Protein
Protein content of milk samples collected from different locations showed highly (P<0.001) significant differences (Table 1). The protein content of milk samples collected from Walmera differed significantly from that of milk samples collected from Debre- Birhan, Ejere and Selale areas. The average protein content obtained from this study was 3.10 percent. This result is lower than the protein content reported by several authors as 3.48 %, 3.46±0.04 %, 3.31 % and 3.42 by AbdElrahman *et al* (2009), Fikirneh *et al* (2012), Alganesh *et al* (2007) and Teklemichael (2012), from milk samples collected from local cows, cross bred cows, local Horro cows and dairy farms in Dire Dawa, respectively. According to an on-station study report by Zelalem *et al* (2003) a protein content of 3.17 percent was reported for indigenous Boran cows. Different factors such as breed of the cow and stage of lactation might have affected the protein content of milk samples in the current study. A report by Bailey, K. et al (2005) revealed that a deficiency of crude protein in the ration may depress protein in milk due to under feeding concentrates, low forage intake, poor quality forage, failure to balance the ration for protein and minerals, or inadequately grounded grains. The same study indicated that if body stores are minimal, yields of

milk and milk components will suffer . According to Ethiopian standards Agency, the minimum percent protein content of whole milk should be 3.20 percent (ESA, 2008). Hence, the average protein content for the current study is slightly below the recommended standard for the nation.

Total solids
The total solids content of milk samples obtained during the current study showed highly significant differences (P<0.001) across locations (Table 1). The total solids content of milk samples collected from Ejere and Walmera did not vary significantly from each other. Whereas the total solids content of milk samples collected from Debre-Birhan and Selale differed significantly from milk samples collected from other locations. The overall total solids content of milk obtained during the present study revealed 12.24 percent. The average total solids content in this study is lower than average result of 13.4 percent total solids obtained from similar study conducted in Shashamane on milk from dairy cooperative collection centers, small scale milk producers, kiosks and hotels (Teshome Gemechu *et al* (2015*). Another study conducted on smallholder milk samples from Horro/Zebu cows in East Wollega by Alganesh *et al* (2007) showed higher total solids content of 14.31 percent. According to the standards set by the Ethiopian standard Agency, the minimum average percent total solids content of unprocessed whole cow milk should not be less than 12.8 percent. Therefore, the current average total solids content of milk is slightly less than the minimum requirement. The present result is again less than the average cow milk total solids of 12.7 percent recommended by O'Connor C. B. (1994). This might be due to the practice of adulteration of milk by water or other solid materials.

Ash
The ash content of raw whole milk collected from the study sites did not show significant (P< 0.001) difference across collection sites (Table 1). The overall mean of the ash content of milk samples in the present study is 0.61 percent. Nevertheless, this result is lower than the average cow milk ash content of 0.7 percent revealed by O'Connor C.B. (1994). Another similar study by Teshome G. et al (2015) revealed a high average ash content of 0.74 percent for whole milk collected from different value chain actors. O' Connor C. B. (1994) revealed that the ash content of cow milk normally remains constant at 0.7 to 0.8 % even though it is affected by feeding, breed of lactating animal and stage of lactation.

Lactose
The lactose content of the raw whole milk samples collected from different locations differed significantly at P < 0.01 (Table 1). In the present study, the lactose content of milk samples obtained from Ejere and Walmera did not vary significantly from each other. But the lactose content of milk samples collected from Selale and Debre-Birhan areas significantly (P<0.01) differed from those samples collected from Ejere and Walmera districts. The overall average of lactose content in this study showed 5.08 percent. According to the European Union Quality standards for unprocessed whole milk, the lactose content should not be less than 4.2 percent (Tamime, 2009). Conversely, O'Connor C.B (1994) proved the normal range of cow milk lactose content as 4.7 to 4.9 percent . So, the lactose content of milk in the present study is higher than the average for Zebu as well as cross breeds. According to O'Connor C. B. (1994) the composition of milk can fluctuate due to interval between milking, stage of lactation, age and health of the cow, feeding regime, completeness of milking and microbial activities such as degradation of proteins and lipids of milk can also change the composition of milk.

Solids not fat
The solids not fat content of the whole raw cow milk samples collected from the four locations varied significantly at P<0.001(Table 1). The SNF content of milk samples obtained from Ejere, Walmera and Selale did not vary significantly from each other. While those milk samples collected from Debre Birhan differed significantly from the milk samples collected from the other three study sites. The average SNF content of milk samples for the present study proved to be 8.56 percent. The similarity of the values of chemical components obtained from the adjacent districts Ejere and Walmera could probably be due to the similarity of animal feeds and breeds, milking and feeding practices in the two districts. The average SNF content of milk samples obtained for the current study is less than 8.7, 8.96 percents of SNF content of milk samples acquired from raw milk samples described by Bille *et al* (2009) and Janstova *et al* (2010), respectively. The minimum SNF percent set by European Quality Standards for unprocessed whole milk is 8.5 percent (Tamime, 2009). Consequently, the average SNF obtained during this study is slightly equivalent to the standard.

Table 1. Mean values ± SD for the chemical components of milk samples collected from small scale milk producers in central highlandsof Ethiopia (n=108)

Variables (%)	Milk sources				
	Ejere (N=25)	D/Birhan (N=29)	Selale (N=29)	Walmara (N=24)	Overall mean
Fat	3.97[ab]	3.48±0.53[b]	3.62±0.61[ab]	3.95±0.53[a]	3.76
Protein	3.02[a]	3.19±0.42[a]	3.12±0.37[a]	3.05±0.35[b]	3.10
Total solids	12.23[b]	12.52±1.26[a]	13.17±1.89[c]	11.02±3.41[b]	12.24
Ash	0.60	0.66±0.13	0.66±0.21	0.53±0.42	0.61[NS]
Lactose	4.73[b]	5.22±0.86[a]	6.93±1.61[c]	3.42±1.52[b]	5.08
SNF	8.29[b]	8.68±1.06[a]	9.74±1.69[c]	7.53±2.49[bc]	8.56

Means followed by different superscript letters within a row are significantly different at ($P<0.01$). n = number of observations

Bacteriological quality of raw whole cow milk
Total Aerobic Mesophilic Bacterial (TAMB) count
The total aerobic mesophilic bacteria (AMBC) count of milk obtained from smallholders at different locations showed ($P<0.001$) highly significant difference. The total aerobic mesophilic bacteria enumerated from Selale (6.97±0.35log10 cfu/ml), Debre Birhan (7.11±0.33log10cfu/ml and Ejere (7.92±0.35 log10 cfu/ml) did not differ significantly ($P<0.001$) from each other. Whereas, the total aerobic mesophilic bacterial count obtained from milk samples from Walmera differed significantly from milk samples collected from the three locations. The overall mean aerobic mesophilic bacteria enumerated in the current study is 8.2 log10cfu/ ml. According to the Ethiopian standards Authority (2009) good quality milk should not contain a total bacterial count of more than 0-200,000 cfu/ml. Another report by Amistu Kuma *et al* (2015) showed a relatively less total aerobic bacterial count of $3.85x10^2$ to $7.79 x10^6$ log cfu/ml *at* Sululta and $6.86x10^5$ to $7.88x10^6$ log cfu/ml at Holeta, respectively for milk samples obtained from dairy cooperative union and retail shops. The standard plate count or aerobic mesophilic bacterial count is   useful indicator for monitoring the sanitary conditions present during the production, collection and handling of raw milk. The higher total aerobic mesophilic bacterial count observed in the current report may be due to initial contamination of milk samples either from milking cows' exterior of udder, lower abdominal body parts, mastitic milk, milkers and milk handlers, unhygienic milking areas and poorly handled milk containers. The sanitary conditions under which the sampled milk has been produced and handled were generally substandard (Robinson R.K. 2002).

Total coli-form counts
The total coliform bacteria enumeration of milk samples collected from smallholders showed highly ($P<0.001$) significant difference across all locations. The total coli-forms enumerated from samples collected from Ejere (8.89±1.45 log 10 cfu/ml), Debre-Birhan (7.58±1.40log 10 cfu/ml) and Selale areas (7.60±1.41log 10 cfu/ml) did not differ significantly from each other. While the samples obtained from Walmera showed the highest bacterial count of (10.26±2.41 log 10 cfu/ml. The overall mean total coli-form bacteria enumerated in the recent study revealed 8.58 log 10 cfu/ ml. According to the Ethiopian standards Agency (2009) good quality milk should not contain a total coli-form bacterial count of more than 0 –1000 cfu/ml. Similar study revealed no significant difference among total coliform counts of milk samples collected from smallholders from Hawassa (2.34±0.31log 10 cfu/ml) and Yirgalem (3.40±0.20 log 10 cfu/ml), respectively. Another study by Amistu Kuma *et al* (2015) on the assessment of raw milk microbial quality at different critical points in retail centers in Addis Ababa indicated a higher mean range of coliform bacterial counts of 5.42±1.735 to 5.78±0.985 log 10 cfu/ml of Sebeta site. Different reports by Mulugojjam Adugna et al (2013), El-Ziney and Al-Turki (2007) and Omer and Eltinay (2008) showed mean coliform bacterial counts of 2.9 log10 cfu /ml, 1.4 log10 cfu /ml and 2.83 log10 cfu/ ml for camel milk quality and safety in Eastern Ethiopia, camel milk produced in Saudi Arabia and in the United Arab Emirates, respectively. Another report by Asaminew T. and Eyasu Siefu (2011) also revealed a coliform count of 4.49 log 10 cfu/ml for milk samples collected from farmers and dairy cooperatives in Bahr Dar and Mecha districts of Amhara, Ethiopia. Therefore, the overall mean of total coliform bacteria in the recent report is by far greater than tolerable range. The higher total coliform bacterial counts observed in this study may be due to the initial contamination of milk samples during milking either from poor milker's hygiene and fecal contamination from the udder and lower abdominal parts of the body of cows because one source of these organisms is the intestinal tract of warm blooded animals (H. Michael and J.F. Frank, 2004). According to Jayarao et al (2004) contaminated water and cows with subclinical or clinical coliform mastitis can also a potential source for presence of coliform bacteria in milk.

Entrobacteriaceae count

The Entrobacteriaceae enumeration of milk samples collected from smallholders demonstrated highly (P<0.001) significant difference across all locations. The Entrobacteriaceae enumerated from samples collected from Ejere (11.59±0.37 log 10cfu/ml), Debre-Birhan (11.32±0.35 log 10cfu/ml) and Walmera (10.38±0.38 log 10 cfu/ml) did not significantly vary from each other. However, Entrobacteriaceae (12.72±0.35 log 10 cfu/ml) count from Selale was significantly different (P<0.001) from the counts obtained from all other locations. The overall average of Entrobacteriaceae in the present study was 11.36 log 10 cfu/ml. Another report on the review of Ethiopian dairy revealed a lower Entrobacteriaceae count of 5.49 log 10 cfu/ml Zelalem Y. *et al* (2011). Another report by Fulaya T. (2011) revealed a lower Entrobacteriacae count of < 10^2 to 8.0 x10^7 cfu/ml and mean cfu/ml of 3.0 x10^4 cfu/ml. The Entrobacteriacae has earned a reputation as being among the most pathogenic and most often encountered organisms in foods. The Entrobacteriacae family includes the coliform groups Eshercia, Enterobacter, Citrobacter, and Klebisella. In addition to many other genera, Salmonella, Shigella, Morganella, Providencia, Edwardseila, Proteus, Serratia and Yersinia which are isolated from animal intestines. Milk that is likely contaminated with feaces might probably have higher Entrobacteriacae counts (Hayes et al, 2001). Generally, Entrobacteriaceae are indicator bacteria that are used to provide evidence of poor hygiene, inadequate processing or post-process contamination of foods. They are often chosen because they are relatively quick and simple to detect. Their absence in food provides a degree of assurance that the hygiene and food manufacturing process has been carried out appropriately, where as their presence usually indicates a potential problem or failure in the process has occurred ((ILSI, 2011). The higher count in the present study could be related to the substandard hygienic conditions practiced during production and subsequent handling.

Titratable acidity

The titratable acidity of milk samples in the current study revealed highly significant (P<0.001) difference for samples collected from smallholder producers from the four locations (Table 1). There was no significant difference in the titratable acidity of milk samples among those from Debre Birhan and Selale districts. Never the less, the titratable acidity obtained from milk sample collected from Ejere and Walmera significantly differed from each other and from those obtained from Selale and Debre Birhan. The average titratable acidity/percent lactic acid of milk obtained during the current study showed 0.27 percent. Mulugojjam Adugna *et al* (2013) revealed a titratable acidity of 0.166 - 0.42 in the study on quality and safety of camel milk along the value chain in Eastern Ethiopia. Another study by Asaminew and Eyassu (2011) reported a titratable acidity for milk samples collected from individual farmers with (0.23 ± 0.01%) and dairy cooperatives (0.28 ± 0.01% lactic acid) in Bahir Dar District. Normal fresh milk should have an apparent acidity of 0.14 to 0.16 percent (O'Connor C. B. 1994). The production of acid in milk is normally termed as souring and the sour taste of milk is due to lactic acid production. The percentage of acid present in milk is a rough indication of its age and the manner in which it has been handled (O'Connor C. B. 1994). The current finding is an indication of inappropriate cooling and spoilage due to poor milk handling practices which might have resulted in higher bacterial growth which increased the titratable acidity.

Table 2. Mean values ± SD for the bacteriological quality of milk (Log10 cfu /ml) collected from producers in the central highlands of Ethiopia (n= 108)

Test organisms	Milk sources				
	Ejere (N=25)	D/Birhan (N=29)	Selale (N=29)	Walmara (N=24)	Overall mean
Total aerobic mesophilic bacteria	7.92±0.35[b]	7.11±0.33[b]	6.97±0.35[b]	11.26±0.36[a]	8.2
Total coli forms	8.89±1.45[b]	7.58±1.40[b]	7.60±1.41[b]	10.26±2.45[a]	8.58
Entrobacteriaceae	11.59±0.37[b]	11.32±0.35[b]	12.72±0.35[a]	10.38±0.38[b]	11.36
Titratable acidity (% LA)	0.16±0.02[C]	0.25±0.02[b]	0.22±0.02[b]	0.44±0.02[a]	0.27

Means followed by different superscript letters within a column are significantly different (P<0.05). LA=lactic acid, cfu/ml= colony forming units/ mililiter, N= number of observations, Log10= Logarithms of10

Conclusion and recommendation

The samples of raw whole milk collected and analyzed for their bacteriological quality and chemical properties from Ejere, Walmera, Selale and Debre-Birhan areas were substandard in terms of their bacteriological counts and titratable acidity. The chemical components such as protein, total solids and ash were also below the standard set by the Ethiopian Standard Agency and t European Standards. The poor bacteriological quality is perhaps attributable to lack of good manufacturing practices during milking and post milking handling. The practice of adulteration of milk by water from unclean sources is another increasing trend contributing to the deterioration of microbial and chemical properties of raw whole milk. The high acidity level might be due to

lack of proper cooling mechanisms. Therefore, adequate sanitary measures and strict monitoring and quality control measures should be in place at all levels from production to consumption to assure delivery of safe and quality of raw whole milk to various stakeholders.

Acknowledgements

References
ABdElrahman SM., Ahmed Am., EL Owni Ao., Ahmed MK. 2009. Microbiological and physicochemical properties of raw milk used for processing pasteurized milk in Blue Nile Dairy Company (Sudan). Australian J. Basic Appl. Sci. 3(4). 3433:3437.

Alganesh Tola, Ofodile, L.N. and Fekadu Beyene. 2007. Microbial Quality and chemical composition of raw whole milk from Horro cattle in East Wollega, Ethiopia. Ethiopian Journal of Education and Sciences. Jimma University, Ethiopia. 3 (1): 1-10.

Amistu Kuma, Degefa Tolossa and Melese Abdisa. 2015. Assessment of Raw Milk Microbial Quality at Different Critical Points of Oromia to Milk Retail Centers in Addis Ababa. Food Science and Quality Management. Volime 38.

Asaminew T, Eyassu S.2011. Microbial quality of raw cow's milk collected from farmers and dairy cooperatives in Bahir Dar Zuria and Mecha district, Ethiopia. Agric. Biol. J. North Am. 2(1):29-33.

Azage Tegegne and Alemu G/Wold, 1998. Prospects for peri-urban dairy development in Ethiopia. In: Proceedings of the fifth Annual Conference of the Ethiopian Society of Animal Production. 15-17 May, 1997. Addis Ababa, Ethiopia

Bailey, K. E., C. M. Jones, and A. J. Heinrichs. 2005. Economic returns to Holstein and Jersey herds under multiple component pricing. J. Dairy Sci. 88:2269-2280.

Berg J. C.T. 1988. Dairy Technology in the Tropics and Subtropics. Pudoc, Wageningen, The Netherlands.

Bille P G, Vovor MN, Goreseb J and EL Keya. 2000. Evaluating the possibility of adding value to goat's milk by producing yoghurt using low cost technology method for rural Namibia. The J. Food Tech in Africa. 2000;5: 130-144.

Bille P G., Haradoeb BR., Shigweda N. 2009. Evaluation of chemical and bacteriological quality of raw milk from Neudamn Dairy Farm in Namibia. African Journal of Agric. Nutrition. Dev. 9 (7):1511:1523.

Chamberlain, A. 1990. An Introduction to Animal Husbandry in the Tropics. 4th ed. John Wiley and Sons INC., New York. pp. 758.

El-Ziney and A. I. Al-Turki. 2007. Microbiological quality and safety assessment of camel milk (camelus dromedaries) in Saudi Arabia (qassim region). Applied Ecology and Environmental Research 5(2): 115-122. http://www.ecology.uni-corvinus.hu ISSN 1589 1623 2007, Penkala Bt., Budapest, Hungary.

Ethiopian Standard (ES): Unprocessed Whole/Raw Cow Milk Specification. 1st ed., 2008, ES: 3460:2008.

Ethiopian Standard (ES): Unprocessed Whole/Raw Cow Milk Specification. 2nd ed., 2009, ES: 3460:2009.

FAO (Food and Agriculture Organization of the United Nations).1997.Microbiological Analysis. Manual of Food Quality Control. FAO Food and Nutrition Paper 14/4 Rev.1.

Fikrineh Negash, Estefanos Tadesse, Esayas Aseffa*, Chali Yimamu and Feyisa Hundessa. 2012. Production, handling, processing, utilization and marketing of milk in the Mid Rift Valley of Ethiopia. Livestock Research for Rural Development (LRRD). Volume 24: 9.

Foley, Y. J., Buckley, J. and Murphy, M. F. 1974. Commercial Testing and Product Control in the Dairy Industry. University College, Cork, USA.

Fulaya T. 2011. Microbiological and chemical properties of raw milk consumed in Burdur. Journal of Animal and Veterinary Advances 10 (5): 635-641

G. Dehininet, H. Mekonen, M. Ashenafi, G. Emmanuelle. 2013. Determinants of raw milk quality under a small holder production system in selected areas of Amhara and Oromia national regional states, Ethiopia. Agriculture and Biology Journal of North America. 4 (1):84:90.

Hayes M. C. RD., Ralyea S.C., Murphy N.R. Carey J.M., Scarlett K.J. Boor. 2001. Identification and characterization of : 292- 298.

H. Michael W. and Joseph F. Frank 2004. Standard Methods for the Analysis of Dairy Products. 17th edition, American Public Health Association, Washington, D.C., USA.

ILSI (International Life Science Institute). 2011. The Enterobacteriaceae and their Significance to the Food Industry. Chris Baylis, Mieke Uyttendaele, Han Joosten and Andy Davies (eds). ILSI Europe Report Series. PP. 52.

Janstova B, Necidova L, Navratilova L. (2010). Quality of raw milk from a farm with automatic milking system

in the Czech Republic. PP. 207-2014.

Jayarao BM, Pillai SR, Sawant AA, Wolfgang DR, Hegde NV .2004. Guidelines for Monitoring Bulk Tank Milk Somatic Cell and Bacterial Counts. J. Dairy Sci., 87: 3561-73.

Mogesse Ashenafi, 1990. Microbiological quality of Ayib, a traditional Ethiopian cottage cheese. International Journal of Food Microbiology, 10:261-268.

Mulugojjam Adugnaa, Eyassu Seifu, Ameha Kebede, and Reiner Doluschitze. 2013. International Journal of Food Studies. Vol. 2.

O'Connor C.B. 1994. Rural Dairy Technology.ILRI Training Manual no.1. 1995. ILRI (International Livestock Research Institute), Addis Ababa, Ethiopia Pp. 133.

O'Mahoney, F. 1988. Rural dairy technology-Experiences in Ethiopia.ILCA Manual No. 4.Dairy Technology Unit.ILCA, Addis Ababa, Ethiopia.64 PP.M. G.

Omer, R., & Eltinay, A. 2008. Microbial quality of camel's raw milk in central and southern regions of Unitedarab Emirates Journal of Food and Agriculture, 20 (1): 76

Robinson R. K. 2002. Dairy Microbiology Hand Book. Third Edition. John Wiley and Sons Inc., New York.

SAS. 2009. Users Guide. Version 9.2. Statistical Analysis System (SAS) Institute, Inc. Carry, NC

SPSS. 2011. Statistical Packages for Social Sciences. Version 16. SPSS Corporation.

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

Tekelemikael T. 2012. Quality and Safety of Raw and Pasteurized Cow Milk Produced and Marketed in Dire Dawa Town, MSC Thesis, Haramaya University, Ethiopia.Zelalem Y, Ledin I (2001). Efficiency of smallholder butter making in the Ethiopian central highland. Pastoralism and Agropastoralism-which way forward; In: Proceedings of the eighth Annual Conference of the Ethiopian Society of Animal Production 24-26 August, 2000, Addis Ababa, Ethiopia. pp.192.

Teshome Gemechu, Fekadu Beyene and Mitiku Eshetu.2015. Physical and chemical quality of raw cow's milk produced and marketed in Shashemene Town, Southern Ethiopia. ISABB Journal of Food and Agricultural Science. Vol.5 (2), pp. 7-13.

ZelalemYilma, Yohannes Gojjam, Gizachew Bekele, AlemuGebre-Wold and Sendros Demeke. 2003. Major milk chemical composition and feed dry matter intake of multiparous Boran cows as affected by the level of milk production under bucket feeding and partial sucking calf rearing methods. Proceedings of the 11th Annual Conference of the Ethiopian Society of Animal Production (ESAP) held in Addis Ababa, Ethiopia, August 28-30, 2003. Pp. 397-411.

Zelalem Y, Bernard F, Gerard L. 2007. Occurrence and Distribution of Species of Enterobacteriaceae in selected Ethiopian Traditional Dairy Products: A Contribution to Epidemiology. Food Control,8:1397- 1404

ZelalemYilma, Emmannuelle Guernebleich and Ameha Sebsibe. 2011. Review of the Ethiopian dairy sector. FAO Sub Regional Office for Eastern Africa (FAO/SFE). Rudolf Fombad (editor).FAO/SFE. Pp. 83.per