Microbial Quality and Chemical Composition of Cow Milk in Adea Berga and Ejerie Districts of West Shoa Zone, Ethiopia

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Abstract
The objective of the study was to evaluate the microbial as well as chemical composition of fluid milk in Adea Berga and Ejerie districts. Preliminary quality test and laboratory analysis were carried out to determine the P^H level, Microbial quality and chemical composition. A total of 90 milk samples were collected and analyzed. About 32.2% of milk samples were checked with alcohol test positive; while 18.8% of the samples were positive to clot-on-boiling test. The specific gravity of milk samples were in the range of 1.024 to 1.032 in Ejerie and 1.022 to 1.031 in Adea Berga. The overall mean value of fat, protein and Total solid (TS) were 3.52, 3.09 and 12.19, respectively. Fat percent was significantly different ($P<0.05$) among different source of sampling points. The highest milk fat content value was recorded at Adea Berga district (3.94). Overall mean total bacterial counts and coliform counts were 6.98±0.17, 4.84±0.10 log cfu/ml and significantly different b/n sites ($P<0.05$). The highest coliform (6.64 cfu/ml) and total bacteria counts (10.69 cfu/ml) were observed at consumers level. In general the result indicated that milk samples collected from smallholder milk producers, dairy cooperatives, dairy cooperative union, milk processors and consumers were subjected to microbial contamination and does not meet the international milk quality standard. Therefore, adequate sanitary measures should be taken at all stages from production to consumer level.
Keywords: Total Bacteria Count, Coliform Count, Fat, Protein, Total Solid (TS).

INTRODUCTION

Milk is the most popular food for human consumption and contains numerous nutrients such as water, fat, protein, lactose, minerals and vitamins (Walstra *et al*., 2006). It is the major source of regular income for Smallholder milk producers because it is produced and sold daily (Dugdill *et al,* 2013*)*. Besides its benefit, it is serves as an excellent growth medium for a wide range of microorganisms (Walstra *et al*., 2006). On the other hand, the Chemical composition, particularly milk fat content is used as quality test (Zelalem, 2010). The nutritional as well as the economic value of milk is directly associated with its solids content. The higher the solids content better its nutritional value and more of a milk product can be made (Pandy and Voskull, 2011). Protein content being one of main quality determining criteria applied to milk payment to producers in many countries where others are priced according to fat and solids-non-fat composition (FAO, 2004). Information on the microbial and chemical composition of milk was essential to understand the quality of marketed milk supply. Previous research works mainly focused on microbial quality of fluid milk and very few studies were reported in both microbial and chemical composition at smallholder milk producer and dairy cooperatives. Therefore, the objective of this research study was conducted to evaluate the quality of fluid milk in terms of its microbial and chemical compositions from smallholder producer up to consumer level in the study areas.

MATERIALS AND METHODS

Description of the Study Areas

The study was conducted in Adea Berga and Ejerie districts of west Shoa zone. Adea Berga and Ejerie districts altitude the range of 1166 -3238 and 1872-2631 meters above sea level, rain fall condition ranges 887-1107mm and 991-1194mm and temperature ranges 11-21^{0}C and 14-18^{0}C, respectively.

Milk Sample Collection

Milk samples were collected from individual smallholder farmer's storage container at farm gate and primary dairy cooperatives before added to pool milk and from the bulked milk of primary dairy cooperatives; dairy cooperative union; dairy processors and consumer's storage container. A Total of 90 milk samples were collected in pre sterilized bottle, properly labeled, stoppered and transported to the laboratory in an ice packed cooler box. Microbial analysis was performed within 24 hours after sampling (HPA, 2003).

Chemical Composition

Physico-chemical properties of milk samples fat content, total solid (TS), protein, and density were determined with calibrated milk analyzer (lactoscan).

Coliform Counts (CC)

1 ml of milk sample was added into sterile test tube having 9 ml peptone water. Appropriate decimal dilutions of milk samples were pour-plated on 15-20 ml Violet Red Bile Agar solution (VRBA). After thoroughly mixing, the plated sample was allowed to solidify. Then Petri dishes were incubated at 30°C for 24 hours and counts were made on typical dark red colonies normally measuring at least 0.5 mm in diameter on uncrowned plates

(Marth, 1978).

Total Bacteria Count (TBC)

1 ml of milk sample was added into sterile test tube having 9 ml peptone water. Appropriate decimal dilution of milk samples were pour-plated on 15-20 ml SPCA (standard plate count agar) solution and mixed thoroughly. The plated sample was allowed to solidify and then incubated at30°C for 48 h. Colony counts were made using colony counter (Marth, 1978).

Alcohol Test

Five ml of milk and 5 ml of 68 percent alcohol (ethanol) were placed in a test tube. The test tube was inverted several times with the thumb held tightly over the open end of the tube. Then the tube examined for formation of curd particles (O' Connor, 1994).

Clot-On-Boiling Test

Clot-on-boiling test was carried out by placing about five ml of milk in a test tube and then it was placed in a boiling water bath for five minutes. Finally; the test tube was carefully removed from the water bath and examined for the presence of floccules (O'Connor 1994).

Method of Data Analysis

The total bacteria and coli form count data was transformed to log values before subjected to statistical analysis. The log transformed values were analyzed using the General Linear Model (GLM) for least square mean in Statistical Analysis Software (SAS) version 9.0 (2004). Duncan multiple Range test mean (DMRT) comparisons were used to see the mean difference between sampling sources.

RESULTS AND DISCUSSION

Alcohol and Clot-on-boiling tests:

The total collected milk samples 32.2% were positive with alcohol and 18.8 % were positive with clot-on-boiling testes (Table 1). These observations support the view that the alcohol test is more sensitive than the clot-on-boiling test as reported by O'Connor (1994). Zelalem (2010) reported 21% milk samples checked with alcohol test were positive, while only 14% of the samples were positive for clot-on-boiling test in the central highland of Ethiopia.Asamnew and Eyasu (2011) also reported 51% of smallholder and dairy cooperatives milk sample clot by alcohol test and 23% clot on boiling test in Bahirdar zuria and Mecha district. Milk samples collected from dairy cooperatives, unions, processors and consumers had high value on both tests as compared to milk samples collected from individual farmers at farm and cooperative gate. Ejerie and Adea Berg districts were at farm gate negative in clot- on- boiling test and very minimum numbers of samples were clotted on alcohol test.

Table 4: Alcohol and Clot –On-Boiling Tests in the Study Areas

Milk sources	N	Positive Results in percents (%)	
		Alcohol	Clot-on-Boiling Test
Ejerie District			
At farm gate	20	10	-
At coop gate	10	20	10
Bulked milk at coop	6	33.3	16.7
Adea Berga District			
At farm gate	20	15	-
At coop gate	10	30	30
Bulked milk at coop	6	50	33.7
Dairy coop union	6	66.7	50
Processors	6	83.3	50
Consumers	6	83.3	66.7
Overall men	90	32.2	18.8

N= number of milk samples Coop= dairy cooperatives

Microbial quality of raw whole milk

The overall average total bacteria count (TBC) and coliform count (CC) of raw whole milk were 6.98 and 4.84 log cfu/ml, respectively (Table 2). The total bacteria and coliform counts were significantly different ($P<0.05$) among different milk sources. The overall mean total bacterial count of raw milk produced in the study area was 6.98 log cfu/ ml. This value is much higher than the acceptable value of 1×10^5 bacteria per ml of raw milk (O'Connor, 1994). This high level of contamination of milk might be due to initial contamination originating from the udder surface, quality of cleaning water, milking utensils. Therefore, total bacterial count is a good indicator for monitoring the sanitary conditions practiced during production and handling of raw milk (Chambers, 2002). A good instance worth mentioning was reduced total bacterial count observed in milk sampled from farmers who received training on hygienic milk production and handling, and who used recommended milk containers as compared to that produced by the traditional milk producers (Rahel, 2008; Sintayehu *et al*., 2008).

The present result is also comparable with the finding of Fikrineh *et al*. (2012) who reported 7.08 log cfu/ml of TBC in mid Rift valley Ethiopia and lower than the report of Asaminew and Eyassu (2011) who reported 7.58 log cfu/ml of TBC in cow milk sampled from around Bahir Dar and Mecha district. This value is lower than total bacteria count reported by Zelalem (2010) in the central highlands of Ethiopia (9.10 log cfu/ml) and Abebe *et al.* (2012) in Southern Ethiopia (9.82 log cfu/ml). However, there was a significant microbial count difference among sampling sources of milk (Table 2). In Ejerie districts the average total bacteria count in farm gate is significantly lower than bulked milk sample at cooperatives. Moreover, milk samples collected from dairy cooperative on arrival was significantly different with bulked milk at cooperatives. Generally the trend of total bacteria count in the two districts revealed that there was increment from farm gate to milk processing plants (Table 2). This could be due to improper handling, storage and transport facilities after the milk leaves the farm. In case of Adea Berga district the average total bacteria counts of sampled milk in farm gate is significantly lower than both on arrival dairy cooperative and bulked milk at cooperatives. Bulking milk from different farmers were leads to an increased chance of milk contamination.

The overall mean coliform count (CC) of raw milk produced in the study areas were 4.84 log cfu/ml (Table 2). The coliform count obtained in the present study is higher than that reported by Asamnew (2010) who found coliform count of 4.49 logcfu/ml in Bahr dar Zuria and mecha districts. Others also reported lower values Abebe (2012) 4.03 log cfu/ml in Southern Ethiopia and Zelalem (2010) 4.58 log cfu/ml in the central Highland Ethiopia .The higher coliform count observed in the current study it might be attributed to the initial contamination of the milk through the milkers, milk containers and milking environment. Since it is not practical to produce milk that is always free of coliforms, even at high level of hygienic condition; their presence in raw milk to a certain extent may be tolerated. However, the present result was larger than the acceptable limit. Coliform (CC) count less than 100 Colony Forming Units (CFU)/ml are considered acceptable for milk intended to be pasteurized before consumption. Counts of 10 cfu/ml or less are achievable and desirable if raw milk will be consumed directly (Jones and Sumner, 1999; Ruegg, 2003). The average coliform counts of milk collected from farmer gate and upon arrival at the dairy cooperatives are significantly lower than bulked milk at cooperatives. These findings agree with Omore *et al*. (2005) who reported that bacterial counts increase and subsequently, milk quality decreases as milk passes through increasing numbers of intermediaries.

Table 5: Microbial counts of raw milk (LSM ± SE)

		Microbial quality of milk (log cfu/ml)	
Sources of milk	N	TBC	CC
Ejerie district			
Farm gate	20	5.47±0.16^{e}	3.84±0.10^{f}
Coop gate	10	6.73±0.12^{d}	4.46±0.13^{de}
Bulked milk at cooperative	6	7.25±0.27^{cd}	4.86±0.13^{d}
Adea Berga district			
Farm gate	20	6.04±0.15^{e}	4.20±0.93^{ef}
Coop gate	10	7.08±0.12^{d}	5.47±0.17^{c}
Bulked milk at coop gate	6	7.26±0.27^{cd}	5.90±0.17^{b}
Bulked milk at unions	6	7.80±0.27^{c}	5.96±0.17^{b}
Bulked milk at processors	6	9.75±0.27^{b}	6.02±0.17$^{\mathbf{b}}$
Consumers	10	10.69±0.27^{a}	6.64±0.17 ^{a}
Overall Mean	90	6.98±0.17	4.84±0.10

Means with different superscripts letters are significantly different (P<0.05)
N=Number of samples Coop=dairy cooperatives

Major chemical composition:

The overall average contents of fat, protein and total Solid contents of raw whole milk were 3.5, 3.09 and 12.19, respectively (Table 3). There was a significant difference (P<0.05) in the average fat content of raw whole milk between the two districts. The highest milk fat content value was recorded at Adea Berga district 3.9% (Table 3). The average fat content of raw whole milk observed in the current study is much less than values reported earlier. Rahel (2008), for instance, reported 5.35% fat for zebu cows in Delbo area of Wollayta zone and Alganesh (2002) indicated the value to 6.1% for Horro breed in Eastern Wollega. This might be due to the variation in milk fat content among genetically different breeds of cows and also for the different stages of lactation. The average protein and SNF content of milk as observed in the current study was 3.09 and 12.19 %, respectively (Table 3). The values obtained in the present study are consistent with that reported by Zelalem (2010) and Rahel (2008) for milk samples collected from smallholder farmers in Delbo area of Wollayta zone and central highlands of Ethiopia, respectively. Similarly, Alganesh (2002) also reported similar 3.31% protein content for milk samples collected from smallholder producers in East Wollega.

Table 6: Chemical composition of raw milk (LSM ± SE)

Milk sampling sources		Variables		
		Fat	Protein	Total solid
Ejerie district				
Farm gate	20	3.59±0.06[b]	3.10±0.03	12.41±0.32
Coop gate	10	3.42±0.09[bc]	3.09±0.04	12.26±0.25
Bulked milk at coop	6	3.37±0.11[bc]	3.09±0.03	12.08±0.43
Adea Berga district				
Farm gate	20	3.94±0.07[a]	3.14±0.02	12.47±0.24
Cooperative gate	10	3.40±0.08[bc]	3.09±0.04	12.22±0.32
Bulked milk at coop gate	6	3.33±0.11[bc]	3.08±0.03	11.86±0.43
Bulked milk at union	6	3.30±0.11[bc]	3.08±0.02	11.99±0.43
Bulked milk at processors	6	3.30±0.11[bc]	3.07±0.04	11.75±0.45
Consumers	10	3.20±0.11[c]	3..07±0.04	11.75±0.45
Over all mean	90	3.52±0.38	3.09±0.10	12.19±0.10

Means with different superscripts letters are significantly different (P<0.05)
N= number of milk samples Coop=dairy Cooperatives

Specific gravity

The value of specific gravity of milk sample from small holder producers at cooperative before added to pool milk the values were in the range of 1.024 to 1.032 (Ejerie) and 1.022 to 1.031 (Adea Berga) districts respectively (Table 4). The normal specific gravity of milk ranges from 1.026 to 1.032 (Kurwijila, 2006). However the milk collection centers accepted 1.027 as normal parameters for specific gravity of milk. The current result indicate that about 85% of Ejerie and 65% of Adea Berga milk samples were within the acceptable range of unadulterated milk while the rest 15 % and 35 % of the samples falls below the standard and this result shows that milk was mostly adulterated with water in Adea berga district compared to Ejerie district. Milk at normal state, have unique Physico-chemical properties, which are used as quality indicators. The density of milk was commonly used for quality test mainly to check for the addition of water to milk or removal of cream. Addition of water to milk reduces milk density, while removal of cream increases it (O'Connor, 1994). Similar to current study Alehegne (2004) reported specific gravity ranging from 1.025 to 1.029 for Small holder dairy Farms in Debre Zeit. Zelalem (2010) reported that majority raw whole milk sample collected from Holetta and Selale area their specific gravity were fall within the range between 1.028 and 1.032.

Table 7 : Specific gravity test in the Study Areas

Milk sources	N	Specific gravity (g/ml)	
		Minimum	Maximum
Ejerie district			
At farm gate	20	1.028	1.032
At coop gate	10	1.024	1.032
Bulked milk at coop gate	6	1.024	1.030
Adea Berga district			
At farm gate	20	1.027	1.031
At coop gate	10	1.022	1.031
Bulked milk at coop gate	6	1.021	1.028
Dairy coop union	6	1.020	1.028
Processors	6	1.025	1.027
Consumers	10	1.020	1.027

N= number of milk samples Coop= dairy Cooperative

CONCLUSIONS AND RECOMMENDATIONS

The present study showed that the Total bacterial count (TBC), Coliform cunt (CC) and lactic acid percents were increases milk flows from producer to consumers. Microbial counts were not meeting the international acceptable limit. Therefore, awareness creations and trainings give for small holder milk producers, primary dairy cooperatives, dairy cooperative union, milk processors and individual collectors, efficient milk cooling system is required at producer and milk collectors' level and milk transportation vehicles used to transport should be equipped with cooling facilities. Finally, milk collectors should be regularly control the quality of raw milk and introduced quality based payments.

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