ORIGINAL ARTICLE

Microbial Quality and Chemical Composition of Raw Whole Milk from Horro Cattle in East Wollega, Ethiopia

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Abstract

A study of the microbial quality and chemical composition of raw milk from Horro cows raised at rural farm households was conducted in Guto Wayu and Bila Sayo districts of East Wolloga. Thirty willing households owning one or more cows were randomly selected from Guto Wayu and Bila Sayo districts of East Wollega. Fifteen samples of whole raw milk were taken twice from each household in each location within each of the two months of the study and were used for the analysis. The mean standard bacteria count (6.8×10^4 log cfu mL^{-1}) and coliform count (7.4×10^7 log cfu mL^{-1}) of milk samples from Bila Sayo was significantly higher than those of milk samples from Guto Wayu. The acidity (0.28 and 0.31) respectively of the raw milk samples from both locations was similar and the SBC, CC and acidity values were generally above acceptable standards. The means for the total solids, solids-not-fat, total protein, casein, fat, lactose, ash, and specific gravity were 14.31 ± 0.03, 8.22 ± 0.01, 3.31 ± 0.01, 2.63 ± 0.01, 6.05 ± 0.02, 4.51 ± 0.01, 0.70 ± 0.01, and 1.03 ± 0.01 respectively. The result of the microbial investigation indicated poor bacteriological quality of milk samples from the two locations, whereas the chemical composition was adequate. The milk from Horro cattle from the two locations should be pasteurised and adequate sanitary measures taken at all stages of production to consumption of the milk to ensure the production of good quality raw milk.

***Keywords**: Microbial quality, chemical composition, whole raw milk, Horro cattle*

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INTRODUCTION

Dairying provides one of the most cost effective methods of converting crude animal feed resources into high quality protein rich food for human consumption (Abaye *et al*., 1991; O' Conner, 1993; Gebre *et al*., 2000). However, the daily production of a perishable commodity 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 (FAO, 1990). Consequently, the compositional and organoleptic properties of tropical milk products may differ from similar products manufactured in cooler climates (ILCA, 1992). Dairy product quality starts at the farm as good dairy products can only be made from good quality raw milk. 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 rather unsanitary conditions and poor production practices (Mogessie, 1990; Zelalem and Faye, 2006). More so, the composition of milk makes it an optimum medium for the growth of microorganisms that may come from the interior of the udder, exterior surfaces of the animal, milk handling equipment and other miscellaneous sources such as the air of the milking environment (Richardson, 1985). The conventional procedure for measuring the sanitary quality of milk is to estimate its bacterial content. The number of bacteria in aseptically drawn milk varies from animal to animal, even from different quarters of the same animal. Aseptically drawn milk from healthy udder contains the average of 500 –1000 bacteria mL^{-1}. The initial counts of 10^5 bacteria mL^{-1} in milk are evidence of poor production hygiene (O' Conner, 1994).

Milk produced in Ethiopia is marketed without any form of pasteurisation or quality control measures (Zelalem and Faye, 2006). Furthermore, 98% of the annual milk productions are by subsistence farmers in the rural areas where dairy facilities are almost non-existent (Tsehay, 1998). This implies that dairy processions in the country are basically limited to smallholder level and are of poor hygienic qualities. There is scanty information on the microbial properties and chemical composition of raw milk in Ethiopia (Eyasu and Fekedu, 2000; Zelalem and Faye, 2006). There also seems to be no reports on the raw milk from Horro cows in Ethiopia. This paper reports the assessment of the microbial quality and chemical composition of raw whole milk from Horro cow in Guto Wayu and Bila Sayo districts of East Wolloga.

MATERIAL AND METHODS

Study area

The study was carried out in Guta Wuya (alttitude: 2100 masl; average temperature: 18.3^{o}C, East and North latitude: 36^{o} 33^{1} and 9^{o} 05^{1} respectively; annual rainfall: 1782mm) and Bila Sayo (alttitude: 1650 masl; average temperature min, max: 13.2 and 27.9^{o}C; east and North latitude: 37^{o} 09^{1}; annual rainfall: 1237mm).

Milk Sampling procedure

Milk were sampled, transported and analysed according to standard methods of Richardson (1985) and Van den Berg (1988). Eighty farmers were interviewed but fifteen willing participants from each location were randomly selected and raw milk samples were collected from Horro cows. All together, sixty milk samples were analysed. The same quantity of milk (300mL) was collected from each household in each location for each month of the sampling period. The samples were collected aseptically in sterile bottles kept in an icebox (at < 5^oC) but samples used for the chemical analysis was treated with 0.2g of potassium dichromate and transported to the laboratory for analysis with 36 hours of sampling.

Analysis of milk samples

Raw milk samples (300mL) were collected from each household, location and within each month. One millilitre of raw milk samples each was homogenized in 9mL of 0.1% of peptone water (Oxoid) using a vortex mixture for 1min. before undertaking the microbial and chemical analysis. Peptone water and media prepared for each test (except violet Red Bile Agar (VRBA) for which boiling for 2min. was employed) were autoclaved for 15min. at 121 ^oC (Richardson, 1985). Media used were prepared according to the directions given by the manufacturers.

Microbial Analysis

The milk samples were assessed for standard plate counts (SPC); Coliform counts (CC) and titratable acidity. Dilutions were selected so that total number of colonies on a plate was between 30 and 300 for SPC, while for CC; dilutions were selected for plate counts between 15 and 150(Richardson, 1985; Van den Berg, 1988).

Standard plate count

Homogenized sample was serially diluted by adding 1mL into 9mL of sterile water containing 0.8% NaCL (common salt), until a solution is obtained that is expected to give a plate count between 30-300. One millilitre of the sample from a chosen dilution was placed on the petri dish with pour plated molten agar (10-15 ml) allowed to solidify for 15min and incubated for 48 hours at 37 ^0C. Finally, the counts were made using colony counter. The plate counts was 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 (Marth, 1978; Van den Berg, 1988; Richardson, 1985).

Coliform count: One ml of milk sample serially diluted as 1: 10^5 using peptone water was transferred into sterile plates. Molten violet red bile agar (15 ml) having temperature of 45^0c was added to the milk sample mixed thoroughly and allowed to solidify for 5-10 minutes. The mixture was then overlaid with a plating agar to inhibit surface colony formation and incubated at 37^0C for 24 hours. Counts were made using colony counter (Marth, 1978; Richardson, 1985).

Titratable acidity: Ten ml of milk was pipetted into a beaker, 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 lactic acid (O'Mahoney, 1988).

$$\% \text{ Lactic acid} = \frac{mlNaOH}{10}$$

Chemical analysis

Milk fat: 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 maintain the milk to normal body temperature of the cow. Ten millilitre 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 millilitre 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 from the butyrometer (ILCA, 1988; Van den Berg, 1988). The average of duplicate readings was computed and recorded.

Total solids: To determine the total solids, five grams of milk sample was placed in a preweighed and dried duplicate of crucibles. The samples were kept at 102^0C in a hot air oven overnight. Then, the dried samples were taken out of the oven and placed in a desiccator. Then the dry sample was weighed (O'Connor, 1994).

$$Total\ solids = \left(\frac{Crucible\ weight + oven\ dry\ sample\ weight - Crucible\ weight}{Sample\ weight}\right) x\ 100$$

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

$$. \ SNF = (TS - fat)\, x\, 100$$

Total Ash: The total ash was determined gravimetrically by igniting the dried milk samples in a muffle furnace in which the temperature was slowly raised to 550^0C. The sample was ignited until carbon (black color) disappears or until the ash residue becomes white (Richardson, 1985).

$$Percent ash = \left(\frac{Weight of\ residue}{Weight of\ sample}\right) x\, 100$$

Total protein: 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 with 0.1N Sodium Hydroxide solution. The titration was continued until pink color becomes intense. Finally, the burette reading was recorded. The reading was multiplied by a factor 1.74 (Foley *et al.*, 1974).

$$Percent\ protein = Burette\ reading\ \ x\, 1.74$$

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Casein: The percentage casein was determined by multiplying the burette reading used for total protein determination by 1.38 (Foley *et al.*, 1974).

$$Percent\ Casein = Burette\ reading\ \ x\, 1.38$$

Lactose: Percent lactose was determined by subtracting the fat, protein and total ash percentages from the percentage of the total solids (O'Mahoney, 1988).

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

Milk specific gravity: Fresh milk samples were filled sufficiently in a cylinder; lactometer was held by the tip and inserted into the milk. The lactometer was allowed to float freely until it

reached equilibrium. Then the reading at the bottom of the meniscus was recorded. Immediately, thermometer was inserted and the temperature of the milk was recorded. The following formula was used to calculate the milk specific gravity.

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

Where, L - corrected lactometer reading at a given temperature. i.e., for every degree above 60^0F, 0.1 degree was added, but for every degree below 60^0 F, 0.1 degree was subtracted from the lactometer reading (O'Mahoney, 1988).

Statistical analysis

The number of organisms (CFU) per millilitre of milk was calculated using the following mathematical formula (1DF, 1987).

$$\sum C /(1\times n_1+0.1\times n_2)d$$

$\sum C$ = 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 factor of the lowest dilution used

The results of microbial counts were transformed to logarithmic values (log 10) and these transformed values and the chemical composition values were analysed using the General Linear Model (GLM) for least square mean in SAS (1996) using fixed effect model. The least significant Difference (LSD) test was used to separate the means and differences were considered significant at $p<0.05$

Results and Discussion

Microbiological quality of whole milk

Results of the total bacterial counts, coliform counts and titratable acidity of raw whole milk from Horro cows in Guto Wayu and Bila Sayo are presented in Table 1.

Table 1: Least square means (± s.e) of microbial counts of raw milk of Horro cow in Guto Wayo and Bila Sayo

Variables	Location				
	Bila Sayo	Guto Wayo	Mean	LSD	SL
No of observation	30	30	60		
Coliform, log cfu mL^{-1}	$6.8x10^4 \pm 0.26$	$1.4x10^4 \pm 0.26$	$2.9x10^4 \pm 0.02$	0.523	*
SPC, log cfu mL^{-1}	$7.4x10^7 \pm 0.19^a$	$2.0x10^7 \pm 0.19^b$	$4.0x10^7 \pm 0.02$	0.427	*
Titratable acidity (%)	0.28±0.02	0.31±0.02	0.30±0.01	0.523	NS

Mean with different superscripts within the same row are significantly ($p<0.05$) LSD= Least significant difference,

SL= Significance level, SPC =Standard plate count, NS= Non –significance, *=($p<0.05$)

Acidity of the milk samples did not show significant variation ($p<0.05$) by location but the total bacterial count and coliform count varied significantly by location (Table 1). Fresh milk can have an initial acidity because of the buffering capacity (O' Mahoney, 1988) but the milk tested was kept long at ambient temperature between milking and analysis attributing to high acidity. According to Richardson, (1985); O' Connor, (1994) the percentage of acid present in dairy product at any time is a rough indication of the age of milk and the manner in which it has been handled. Milk from Horro cattle from the two locations had similar acidity so the low CC and TBC from Guto Wayo as compared to Bila Sayo could not be as a result of reduced microbial load due to reduced acidity.

Total bacterial count and coliform count of the milk samples collected from Bila Sayo were significantly ($p<0.05$) higher than those from Guto Wayu. This might be due to the high environmental temperature in Bila Sayo at which the milk was held before sampling. TBC and CC were generally high and may be because the milk sampling was done in different villages, which were further apart, some samples were held in the traditional milking utensils like gourd, plastic containers, and woven grasses up to 1 and 1/2 hour after milking. Coliform bacteria could contaminate milk from manure, bedding materials, contaminated water, soil and inadequately cleaned milking utensils (Van den Ben, 1988; Kalogridou-Vassiliadau, 1991). A study conducted on raw milk from a dairy farm in Awassa indicated an initial coliform count of about 10 cfu/ml in sterile containers. This reached a level of 10^8 cfu /ml within 24 hours (Mogossie and Fekadu, 1993). In the current study area animals are kept in open muddy kraals and hygienic conditions were poor. This possibly has exposed the milk to high risk of contamination, which in turn increase the microbial count. Coliform count is especially associated with the

level of hygiene during production and subsequent handling since they are mainly of faecal origin (Van den Berg, 1988; Omore *et al*., 2001). Coliform comprises all aerobic and facultative anaerobic, gram –negative, non-spore forming rods able to ferment lactose with the production of acid and gas at 32^{o}C within 48hours (Zelalem and Faye, 2006). If coliform count of any milk is higher than a certain level, say over ten coliform organisms per millilitre of pasteurised milk, it means the milk was produced under improper procedures (Walstra *et al*., 1999). The existence of coliform bacteria in high proportion is suggestive of unsanitary condition or practices during processing or storage (Richardson, 1985)

Total plate count is the most accurate method for counting live microorganisms in raw milk and heat-treated milk (Van den Ben, 1988). Milk produced under hygienic conditions from healthy cows should not contain more than 5×10^4 bacteria per millilitre (O' Connor, 1993). Therefore, the total bacterial counts from the two locations were higher than acceptable standard, which could be associated with lack of pasteurisation, the milking utensils and plastic contains used in keeping the milk.

Chemical composition of whole milk

The results of milk fat, total solids, solid-not-fat (SNF), total ash, total protein, casein, lactose and specific gravity of raw milk from Guto Wayu and Bila Sayo are presented in Table 2.

The milk total solids, total protein, casein, fat, lactose, ash and specific gravity did not vary significantly ($p> 0.05$) by location (Table 2). The absence of variation in these factors could be due to the similarity of breed, animal size, interval between milking, age, stage of lactation, feed regime and feeding system (Van den Ben, 1988). Milk composition varies considerably among breeds of dairy cattle (Zinash *et al.*, 1988; Chamberlain, 1990) According to O' Connor, (1994), Jersey and Guernsey breeds give milk with about 5% fat while the milk of shorthorns and Friesians contains about 3.5% fat. Horro cows can give milk containing up to 7% fat O' Connor, (1994) which implies that the fat content of the milk tested in this work from the two locations fall within acceptable range (2.5-7%) (O' Connor, 1994; Rehrahie and Yohannes, 1999). The animals sampled in both locations may have been fed with similar regime, and the milk collected at the same stage of lactation, which accounted for the close values in the fat and SNF contents of their milk (O' Mahoney, 1988).

The same specific gravity (1.03%) of milk was recorded in the two locations and the value was within the normal range of 1.028 to 1.033 (FAO, 1988), which suggests that the milk analysed were not adulterated by the addition of water. O'Connor (1993) indicated a higher milk specific gravity of about 1.035 and lower than normal value (1.020) are indicative of fat skimming off and the addition of water respectively. Similar on-farm result of specific gravity of 1.030 was reported by Zelalem and Ledin, (2001).

Table2. Least square means (± s.e) of the chemical components of raw milk of Horro cow in Guto Wayo and Bila Sayo

Location

Variables	Bila Sayo	Guto Wayo	Mean	LSD	SL
No of observation	30	30	60		NS
Total protein (%)	3.30±0.08	3.32±0.08	3.31±0.01	0.161	NS
Total Solids (%)	14.69±0.42	13.94±0.42	14.31±0.03	0.844	NS
Solids-not-fat (%)	8.30±0.12	8.14±0.12	8.22±0.01	0.241	NS
Fat (%)	6.19±0.34	6.02±0.35	6.05± 0.02	0.697	NS
Ash (%)	0.70±0.02	0.70±0.02	0.70±0.01	0.040	NS
Casein (%)	2.62±0.06	2.64±0.06	2.63±0.01	0.121	NS
Lactose (%)	4.59±0.10	4.42±0.11	4.51±0.01	0.303	NS

Mean with different superscripts within the same row are significantly ($p<0.05$), NS = not significant, LSD= Least significant difference, SL= Significance level

Table 2 shows that the total protein, total solids, ash, casein and lactose content of the milk of Horro cattle from the two locations were within the acceptable standard ranges of 2.9-5%, 10.5-14.5%, 0.6-0.9%, 2.9-5% and 3.6-5.5% respectively (O' Connor, 1994). The results of the chemical composition therefore, imply that the cattles were mature, healthy and were not underfed. The milk must have also been collected and analysed during the early lactation periods accounting for the standard protein content (O' Mahoney, 1988). It has been reported that the fat and SNF contents of cattle milk can be reduced by diseases particularly mastitis and underfeeding (Van den Berg, 1988; O' Connor, 1994).

Conclusion

The actual bacterial counts indicated poor bacteriological quality of milk samples from the two locations investigated. The use of unclean milking equipment and lack of portable water for cleaning purpose might have contributed to the poor hygienic quality of the Horro milk. The chemical composition of the milk analysed in both locations were similar and met the acceptable standards. It is recommended therefore, that adequate sanitary measures be observed at all stages of production to consumption of the milk from Horro cattles in Guto Wayo and Bila Sayo districts of East Wollega to protect the milk from spoilage.

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