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Assessment of Raw Cow's Milk Quality in Kombolcha, Ethiopia

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Abstract: Milk is easily digestible with high biological value containing optimum proportion and balance of food. Regardless of ethnic group and religion, most people in the world consume cow milk. Cow milk as human food is nutritious and contains fats, proteins, carbohydrates, vitamins and minerals. Documented information regarding cow milk quality in Kombolcha is scanty and very limited work has been undertaken so far. The aim of this research is to determine the quality of raw cow's milk produced at small holder dairy farms operating in Kombolcha, Ethiopia. Cross-sectional study was applied and physicochemical quality and microbial load of milk has been investigated in laboratory. Milk samples were collected using simple random sampling method. For milk quality parameters, sixty raw milk samples were proportionally taken from dairy farms in four kebeles. Using Lactoscan physicochemical properties of milk samples were determined. Descriptive statistics was applied to analyze survey data using SPSS software. Both physicochemical and microbial data were analyzed by ANOVA of SAS. Specific gravity, total solids, fat, solids not fat, protein and total mineral are within the recommended range of Ethiopian standard. The microbial quality of raw cow milk produced in urban and peri-urban dairy farms of Kombolcha is poor and capacity building of dairy farmers and their employee seems paramount in the improvement of milk quality.

Keywords: Coliform, Protein, Total Solid, Udder, Yeast and Mould

1. Introduction

As produce of cow's mammary glands, milk is easily digestible with high biological value containing optimum proportion and balance of food [1]. Regardless of ethnic group and religion, most people in the world consume cow milk [2]. The same applies to millions of Ethiopians where consumption of cow milk is high from nation's total annual milk production point of view [3]. Due to it's easily spoilage possibilities, cow milk quality deteriorates quickly within a short period of time in the absence of or faulty hygienic milk handling practices [4]. The available evidences in the existing literature revealed that cow milk is a complex biological natural fluid and convenient medium for growth of many microorganisms. This denotes the need for strict hygienic condition during milk production, preservation and distribution across the supply chain to minimize, if possible, to avoid contamination by microorganisms. In fact, physicochemical properties and microbial loads of raw cow's milk are indicators of its quality [5] and trigger an optimum need of clean milk production by urban and peri-urban small-holder dairy farmers in Ethiopia. So far, the available scanty documented information revealed that very limited work has been undertaken regarding the physicochemical and bacteriological load assessment of raw cow milk produced in Kombolcha. Therefore, this study was initiated to determine the physicochemical and microbiological quality of raw cow milk produced in the urban and peri-urban areas of Kombolcha, South Wollo zone, Ethiopia, and give/supply information for the improvement of extension delivery and support system.

2. Materials and Methods

2.1. Study Site, Design and Sample Size

The study was conducted in Kombolcha, South Wollo

Zone of Amhara Region, 376 kilometers far away from Addis Ababa (capital city of Ethiopia). Kombolcha is found 11° 1'30'' to 11^0 10'30''N latitude; 39°40'00'' to 39°46'30''E longitude; at an altitude of 1743 to 2814 meters above sea level. The annual rainfall and temperature of Kombolcha is 750 mm to 900 mm and 25°C - 30°C, respectively.

The design of this study is cross-sectional and laboratory analysis of milk samples has been undertaken. The study sites were six urban and six peri-urban *kebeles* (the smallest administrative units in Ethiopia). Among these *kebele*s two urban (*Abishager* and *Seshaber*) and two peri-urban (*Abakolba* and *Mitikolo*) were selected purposively, based on their dairy cattle population size.

Smallholder urban and peri-urban dairy farmers in Kombolcha rear local, Holstein Friesian and crossbreeds of Holstein Friesian kept under semi-intensive management system. In selected *kebeles*, households owning lactating cows are the sample frame. Simple random sampling technique was adopted to select households from the sample frame. Based on Kombolcha district livestock and fishery development office annual report (2020), 120 household in the four selected *kebeles* own 342 lactating cows. The numbers of households in the selected four *kebeles* who own lactating cows were 49, 29, 28 and 14, respectively (Table1).

Table 1. *Households from four kebeles owning lactating cows.*

Name of *Kebele*	Households (No)	Lactating cows (No)	Sample size (No)	Milk samples (No)
Abishager (Urban)	49	181	38	20
Sheshaber (urban)	29	69	22	15
Abakolba (peri-urban)	28	57	21	13
Mitikolo (peri-urban)	14	35	11	12
Total	120	342	92	60

Sample size was determined using the formula of a study [6].

$$n = \frac{N}{(1+N(e^2))}$$

Where:

n= Sample size,

N = Total number of smallholder dairy producers in each *kebeles,*

e = Error margin (0.05), and

1 = Probability of the occurring event.

2.2. Milk Samples Collection

Sixty raw milk samples were collected from the selected four *kebeles*. Before collection of samples, sixty universal glass bottles were sterilized with steam at 121°C for 15 minutes. Five hundred milliliters of milk samples from each farm were collected early in the morning and deliver to Kombolcha Animal Disease Survey Investigation and Diagnostic laboratory within two hours of collection for physico-chemical and microbiological analysis.

2.3. Physicochemical and Microbiological Analysis

Milk specific gravity, lactose, solids not fat (SNF), protein, fat and ash were tested using Lactoscan milk analyzer. The Lactoscan was cleaned and rinsed three times with distilled water before analysis of each samples. Results of specific gravity, lactose, total solid (SNF), fat, protein and ash were displayed on the LCD screen of the Lactoscan. Milk pH was measured by digital pH meter after calibration using pH4 and pH7 buffer solutions.

Selected parameters to be investigated for microbiological quality analysis of raw milk were standard plate count, coliform count, and yeast and mould counts. For standard plate count 1 ml of raw milk was added into sterile test tube that contains 9 ml of 0.1% peptone solution. After proper mixing of milk and peptone solution, serial dilution conducted up to 1: 10^5. Then,1ml diluted sample poured plated using 15-20 ml of Standard Plate Count Agar (oxoid) prepared based on manufacturer's guideline, autoclaved at 121°C for 15 minutes to be sterilized and left plated samples to solidify. After solidification of plated samples, they were incubated at 30 ºC for 48 hours and colonies were counted using colony counter. Thirty to three hundred colonies per plates are selected to be counted. Colonies less than 30 are estimated counts and Colonies more than 300 are too numerous to count. Number of microorganisms (colony forming units) was calculated using the formula of a study [7].

$$N = \Sigma C / [(n1x1) + (0.1xn2)] * d;$$

Where:

N = Number of colonies per ml of product (total count),

Σ C = Sum of all colonies on all plates counted (between 10 and 300),

n1 = Number of plates used in lowest dilution counted,

n2 = Number of plates used in highest dilution counted,

d= Dilution from which the first counts were obtained.

2.4. Yeast and Mould Count

For yeast and mould count, 1ml of milk sample was serially diluted with 9 ml peptone water following similar methods for total bacterial count but dilutions were surface plated on Chloramphenicol agar. The agar consisting of 5 gram yeast extract, 20 gram glucose, 0.1 gram chloramphenicol, 0.01 gram bromophenicol blue and 15 gram agar per liter of distilled water at a pH of 6.0 to 6.2. The dried plates were then incubated at 25°C for 3 to 5 days. Then, colonies with a blue green color were counted as yeasts and moulds.

2.5. Statistical Analysis

Descriptive statistics was applied for the analysis of survey data using Statistical Packages for Social Sciences (SPSS) software. Physicochemical and microbial data were analyzed using Analysis of Variance (ANOVA) of Statistical Analysis System (SAS, 2008) procedure. Prior to analysis, microbial counts were transformed into logarithmic value (log_{10}) and transformed values were analyzed using the General Linear Model (GLM) of SAS. Tukey's test was applied to detected differences between locations. Differences were considered significant when ANOVA shows a 5% significant level differences between means.

The applied model for milk quality analysis was:

$$Y_{ij} = \mu + L_i + \varepsilon_i,$$

Where;
Y_{ij} = dependent variable (milk quality parameters tests),
μ = over all mean,
L_i = location effect (Urban, Peri-urban), and
ε_i = The error term.

3. Result and Discussion

3.1. Demographic Characteristics, Feed and Breed Type

Most interviewed respondents in both urban and peri-urban areas were married and males (Table 1). The highest number of individuals engaged in urban and peri-urban dairy farming are 31-40 and 41-50 years old, respectively. With regard to education, most of urban dairy farmers' have attended formal education, namely primary, secondary and higher education (diploma level). This may have influence on farmers' awareness and attitude towards adoption of improved livestock technology compared to peri-urban farmers. The income source of peri-urban farmers was mainly emanated from mixed crop-livestock production activities (97.3%) while 58.3% of urban farmers are derived from livestock production. Urban dairy farmers used to produce different species of vegetables in their garden and generate a substantial amount of income in addition to dairy farming.

Table 2. *Demographic characteristics and income of respondents.*

Variables	Urban		Pre-urban		Total	
	N=60	%	N =32	%	N=92	%
Sex						
Male	40	66.7	23	71.8	63	68.5
Female	20	33.3	9	28.1	29	31.5
Age group						
21-30 years	1 2	20	8	25	20	21.7
31-40 years	32	53.3	6	18.7	3 8	41.3
41-50 years	12	20	11	34.4	23	25
>50 years	4	6.7	7	21.9	11	12
Marital status						
Single	10	16.7	5	15.6	15	16.3
Married	35	58.3	20	62.5	55	59.8
Widowed	5	8.3	2	6.3	7	7.6
Educational Status						
Illiterate	0	0	5	15.6	5	5.4
Informal Education	5	8.3	12	37.5	17	18.5
Primary School (1-8)	27	45	8	25	35	38
Secondary School (9-12)	13	21.7	5	15.6	18	19.5
Higher education(Diploma)	7	11.7	0	0	7	7.6
Religious Education	8	13.3	2	6.3	10	11
Income source						
Livestock	35	58.3	2	6.3	37	40.20
Crop-livestock	25	41.7	30	93.7	55	59.8

Crop residue, concentrate feed and grass hay are the main feed types supplied to dairy cows (Table 2). Concentrate feed ingredients consists of mixtures of wheat bran, Niger seed (*Guizotia abyssinica*) cake and mineral salt. Compared to urban dairy farmers, few peri-urban dairy farmers feed concentrate feed to their milking animals. This might be due to the geographical advantage of peri-urban farmers to have easy access to crop residues and hay compared to urban farmers and feed their animals so as to minimize the cost of feed.

Table 3. *Feed and breed types.*

Particulars	Urban		Pre-urban		Total	
	N=60	Percent	N=32	Percent	N=92	Percent
Feed type						
Crop residues	0	0	28	87.5	28	30.4
Concentrate & improved forage	60	100	4	12.5	64	69.2
Breed type						

Particulars	Urban		Pre-urban		Total	
	N=60	Percent	N=32	Percent	N=92	Percent
Local breed	2	3.3	5	15.6	7	7.6
Cross breed	50	83.4	24	75	74	80.4
Exotic breed	8	13.3	3	9.4	11	12

Dairy farmers in the study area do not use bedding materials in animal houses and in agreement with the findings of [8] in Northwestern Ethiopian highlands.

Also, all dairy producers don't have separate milking house and cows are milked inside animal houses. Majority of households (75%) clean animal house before milking and in disagreement with [9] where most households (83.7%) did not clean animals' house before milking.

Table 4. *Floor type and animal house cleaning frequency.*

Floor type & cleaning frequency	Urban		Pre-urban		Total	
	N=60	%	N=32	%	N=92	%
Floor type						
Cemented floor	47	78.3	3	9.4	50	54.3
Stone slab	13	21.7	14	43.7	27	29.4
Hardened earthen floor	0	0	15	46.9	15	16.3
Cleaning frequency						
Daily	45	75	17	53.1	62	67.4
Twice per week	7	11.7	13	40.6	20	21.7
Thrice per week	8	13.3	2	6.3	10	10.9

3.2. Milking and Milking Frequency

Dairy cows are milked by hand twice per day. Before the act of milking, half of peri-urban and 88.3% of urban farmers clean animal houses. Prior to milking, calves are allowed to suckle their dams for few minutes. Udder washing before milking is the basic recommended practice in dairy farming practice to insure hygienic milk production because cows' udder may come in contact with refusals of feed, urine and dung in their house. As evidenced in this study, nearly a quarter of urban and peri-urban dairy farmers wash cow's udder before milking while the vast majority of urban (68.3%) and peri-urban (93.7%) dairy farmers did not wash their hands before milking (Table 5).

Table 5. *Milking practice.*

Variables	Category	Urban		Pre-urban		Total	
		N=60	%	N=32	%	N=92	%
Udder washing before milking	Yes	19	31.7	2	6.3	21	22.8
	No	41	68.3	30	93.7	71	77.2
Small towel to dry washed udder	Yes	19	31.7	0	0	19	20.6
	No	41	68.3	0	0	73	79.4
Number of small towel used	1towel/cow	3	5	0	0	3	15.8
	1towel for all	16	26.7	0	0	16	84.20
Animal house cleaning before milking	Yes	53	88.3	16	50	69	75
	No	7	11.7	16	50	23	25
Wash hands before milking	Yes	54	90	16	50	70	76.1
	No	6	10	16	50	22	23.9
Dry hands before milking	Yes	8	13.3	0	0	8	8.7
	No	52	86.7	32	100	84	91.3
Dressing gown during milking	Yes	2	3.3	0	0	2	2.2
	No	58	96.7	32	100	90	97.8

Respondents who don't practice udder wash before milking believed that calves could wash teats with their saliva during suckling. As opposed to the present study, the report of [10] has revealed a total absence of udder washing practice in Gurage zone of Ethiopia. Peri-urban dairy farmers totally do not dry washed udder before milking while 31.7% of urban dairy farmers use small towel to dry washed cow's udder before milking. The proportion of urban dairy farmers who use one small towel for individual cow's udder drying before milking is accounted to be only 5%. Out of the 90% urban dairy farms that wash their hands prior to milking, only 3.3% of them dry their hands before milking (86.7% of them don't). On the other hand, half of the peri-urban dairy farmers do not wash cow's udder before milking and none of did not dry their hand before milking. Except the 3.3% of urban dairy farmers, none of the milkers' dress gown (working cloth) during milking in both urban and peri-urban dairy farms.

3.3. Milk Yield and Physicochemical Property

The milk yield and lactation length of local, cross and exotic breeds in urban dairy farms were higher than the peri-urban dairy farms which might be due to differences in the supply and management of feed resources.

Table 6. *Performance of dairy cows.*

Parameters	Breed	Urban	Peri urban	Mean
Milk yield (liter/day)	Local	2	1.8	1.9
	Cross	12	6	8
	Exotic	16	8	12
Lactation length(month	Local	7	5	6
	Cross	10	6	8
	Exotic	11	9	10
Calving interval (month)	Local	23	23	23
	Cross	18	24	21
	Exotic	16	16	16
Age at first service (year)	Local	4	4	4
	Cross	3	3	3
	Exotic	2	2	2

It is an established fact that milk temperature shall be reduced to 4°C within two hours after milking. If milk not cooled within the recommended duration, conducive environment will be created for bacterial growth and multiplication during subsequent storage. Though the recommended pH value of milk is 6.6 to 6.8; higher or lower than these values denotes milk is either mastitic or acidified due to bacterial multiplication. The pH value of milk in peri-urban areas is lower ($P<0.05$) than that of urban areas (Table 7) which indicates development of acidity and milk was under fermentation before handover to customers.

Specific gravity of milk is 1.028 to 1.032 and mishandlings (manipulations) like adulteration with water and cream will decrease; while removal of milk fat and reduction of temperature (cooling) will increase its value[11].

The specific gravity of milk collected from Urban and peri urban areas of Kombolcha are in its normal range. The finding of the current study is consistent with [12, 13]. Also, result of this study is within the range of the Ethiopian Standard [14] 1.027 to 1.032 and being free from adulterations and skimming off fat.

Table 7. *Physical properties of milk.*

Parameters	Urban	Peri-urban	Overall mean	P-value
pH	6.60±0.12[a]	6.12±0.09[b]	6.36±0.10	0.005*
Specific gravity	1.028±0.001[a]	1.030±0.001[a]	1.029±0.001	0.930
Titratable acidity	0.180±0.002[b]	0.20±0.004[a]	0.19±0.003	0.001**

[ab]Means within rows with different superscripts differ significantly ($P< 0.05$)

The Titratable acidity of milk collected from peri-urban area was higher than that of urban milk producers. Generally, titratable acidity of milk in the study area is higher than the Ethiopian standard for normal fresh milk (0.10 to 0.17%). Higher values of titratable acidity denotes that milk was not fresh or milk was inappropriately stored longer before reaching to consumers leading to development of acidity due to the growth of acid-producing microorganisms. The findings of this study were lower than the report of [15] (0.215).

Total solids are residue after removal of water from milk through evaporation and consist of fat, protein, lactose and mineral matter. The total solids content value of the current study (Table 8) is within the Ethiopian quality standard limit (not less than 12.80%) [14] and comparable with that of [12] 12.87%.

However, in disagreement to this study, a little lower total solid content value of 12.33% was reported by [16]. The reported value differences in total solid contents of milk might be due to difference in breed and low energy diet in animals feed, among others.

Protein in milk can be depressed by poor quality and intake of forage, crude protein (CP) deficiency and imbalance of mineral and protein in the ration of dairy cows [19]. The milk protein content of this study is higher than the findings of [18] in Amhara region (3.1%) and [19]; and slightly lower than [20] Gurmessa et al., (2015) in Yabello district (3.9%). The milk protein content of this study is higher than the standard set for unprocessed whole cow milk for Ethiopia (not less than 3.20%) [14].

In the Ethiopian standard, fat in unprocessed whole fluid milk should not be less than 3.25%. From nation's standard unprocessed whole fluid milk fat content perspective, a little higher fat content of milk was found in peri-urban locations than in the urban ones. Nearly similar result to this study was reported by [21] in peri urban area of Sebeta (4.10%), but slightly higher result in urban area of Awash district (4.58%). Besides, the findings of [22, 15] were higher than this study. The underlying cause of variation in fluid milk fat percentage might be feed, lactation stage, season and age of animals.

Though remains relatively constant (0.7 to 0.8%), ash content of milk is affected by various factors such as breed, stage of lactation and feed of the animal. The milk Ash content of this study is higher than the report of [21] (0.59%) and lower than that of [13].

The total mineral content values of milk in Kombolcha are within the recommended range. The 9.13% solids not fat content (SNF) of his study is almost comparable to the report

of [16] in Dire Dawa and in line with the minimum quality standard of SNF set for unprocessed whole milk in Ethiopia (not less than 8.5%) [14]. The observed SNF difference between this study and [16] might be due to difference in feeding practice, season, milking method and lactation period [22].

The lactose contents of milk in the present study is in line with the findings of [27] Abi et al., (2017) (4.91%) in Somali Regional State and [20] (4.69%) in Yabelo district; but lower than the report of [12] (4.43%) in Shashemene town. The lactose content of this study is in agreement with the Ethiopian quality standard for unprocessed whole milk.

Table 8. *Milk chemical composition.*

Parameters	Urban	Peri-urban	Overall Mean	P-value
Total Solid (%)	12.72±0.10[b]	13.04±0.11[a]	12.88±0.11	0.046*
Protein (%)	3.57±0.04[a]	3.58±0.06[a]	3.58±0.05	0.826
Fat (%)	3.51±0.02[b]	4.00±0.09[a]	3.75±0.05	0.000**
Ash (%)	0.70±0.01[a]	0.72±0.01[a]	0.71±0.01	0.258
SNF (%)	9.21±0.07[a]	9.04±0.07[a]	9.13±0.07	0.10
Lactose (%)	4.94±0.12[a]	4.74±0.10[b]	4.84±0.11	0.248

[ab]Means within rows with different superscripts differ significantly (P< 0.05)

3.4. Microbial Load of Milk

In dairy farming milk can be contaminated by bacteria and common in underdeveloped countries. The microbiological quality of raw milk considered in this study is standard plate count, coliform count and yeast and mold counts. Standard plate count of raw milk gives an indication of the total number of aerobic bacteria present in milk at the time of pick up. Obviously, very clean milk will have lower bacterial counts than milk collected or handled under unsanitary conditions. Standard plate count of raw milk collected from urban *kebeles* are lower than (P<0.05) the peri-urban *Kebeles* (Table 9).

The overall mean standard plate counts of this study is almost similar to the reported value of [9] in Haramaya district (5.48 log cfu/ml) and lower than the report of [23] in Harar (6.25 log cfu/ml). Also, the standard plate count of this study is slightly higher than the acceptable standard level for raw milk (4.6 log cfu/ml) which might be due to contamination associated with either milk or cooling practice [24, 25].

Table 9. *Microbial load of milk.*

Parameters (log_{10} cfu/ml)	Urban	Peri-urban	Overall Mean	P-value
Standard plate count	5.32±0.072[b]	5.54±0.074[a]	5.43±0.07	0.036
Coliform count	3.92±0.062[b]	4.50±0.04[a]	4.21±0.05	0.000
Yeast and mould count	3.45±0.037[b]	3.54±0.04[b]	3.49±0.038	0.124

[ab]Means within rows with different superscripts differ significantly (P< 0.05)
cfu = Colony form units

Coliforms are common contaminants present in fluid milk [26]. Their presence may be tolerated for the impracticality of milk production free of them. Coliforms cause milk spoilage and directly associated with hygiene and further handling of milk [27]. Coliforms presence in raw milk beyond acceptable level triggers consumers' safety concern. The coliform count of this study (Table 9) is almost similar with the previous report of [28] in Hawassa and Yirgalem (4 log_{10}cfu/ml; 4.29 log_{10} cfu/ml); higher than the reports of [29] in Debrezeit (1.82 log_{10} cfu/ml) and [16] in Dire Dawa (4.13 log cfu/ml). The coliform count of this study is within the standards set for unprocessed whole milk in Ethiopian (4.6log10 cfu/ml) [14].

Even though raw cow's milk quality is influenced both by its compositional quality and microbial density [30], yeast and mould counts of this study are higher than [31] Bekele et al. (2015) in Dangla (0.68 log_{10} cfu/ml) and [32] in Dawa Chefa district (0.46 log10); and lower than [12] in Shasemene town (3.846 log10 cfu/ml).

4. Conclusion

Based on the findings of this study specific gravity, protein, fat and ash (total solid) contents of milk produced in urban and peri-urban dairy farms of Kombolcha is within the set ranges of the Ethiopian quality standard with the exception of titratable acidity. Even if coliform count of this study is within the Ethiopian quality standards for unprocessed whole milk, the milk handling practices of researched farms is questionable. The Standard plate count was also higher than the acceptable standard level for raw milk and denoting its poor quality.

As a long term solution, capacity building of dairy farmers and their employee on milk handling, preservation and marketing through regular extension support and services seems paramount. In this study, the number of milk samples for physicochemical and microbiological evaluation was not large enough due to financial limitations during the commencement of the study. Therefore, further research with large number of samples is recommended to generate conclusive recommendation.

Conflicts of Interest

Authors assure there is no conflict of interest.

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