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Research Article

A Determination of Raw Milk Quality and the Most Suitable Microbiological Test at the Milk Collection Level in Two Regions of Kenya

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ABSTRACT

A study was conducted in two high dairy potential areas of Kenya to determine the bacteriological and compositional quality of milk produced by small scale farmers and the best microbiological test that could be applied at the milk collection level. A total of 297 milk samples were collected from both study sites. Direct and indirect bacteriological analysis of the milk was done using the total count, coliform count, titratable acidity and resazurin tests. Compositional analysis was done by testing for the fat content, solids not fat (SNF), density, protein and added water using a milk analyzer (Lactoscan).

The results obtained from the assessment of the bacteriological and compositional quality were judged against the Kenya Bureau of Standards (KeBS) bench marks.

Both regions had most of the samples analysed (≥77%) within the acceptable bacteriological quality levels. Test for correlation was done between the direct and indirect microbiological tests. Significant positive correlation ($P<0.05$) was found between the Resazurin test and the Total count and Coliform count test in both study areas. No significant correlation was found between the Titratable acidity and the Total count and Coliform count tests.

On compositional analysis, the average pH, fat and the freezing point were within the recommended ranges while the SNF, protein and density were below the recommended ranges. Added water was above the limit set indicating presence of adulteration.

Most of the milk collected from the study area was within the established bench marks in Kenya. However, there was need to strengthen milk testing at the collection centre level. The resazurin test was found to be a good indicator test for milk quality that could be applied at the collection level.

Key words: Bacteriological, Coliform count, Composition, Milk, Total count

INTRODUCTION

Milk is one of the oldest foods known to man and is defined as the normal, clean and fresh secretion, without any addition or subtraction, extracted from the udder of a healthy cow, and free from colostrum (Draft East African standards on raw milk, 2010). It is a complex mixture of fats, proteins, carbohydrates, minerals, vitamins and other miscellaneous constituents dispersed in water (Harding, 1999). The composition of milk varies between species, breeds and individual animals depending on the management system.

Most of the milk produced in Kenya, which is estimated to be more than 5 billion litres (FAO STAT, 2012), is from small holder dairy farmers who are estimated to be more than 1.8 million (National Dairy Master Plan, 2010) and scattered in high and medium potential crop-livestock production systems. Optimal use of resources in urbam milk production system is considered to be the key factor influencing the milk yield (Pandian *et al*., 2013). Most of this milk is informally marketed and public health concerns have been raised about its quality (Mwangi *et al*., 2000). The safety and quality of the milk produced by small scale dairy farmers who are the predominant players in the dairy industry has to be guaranteed in order to enable them retain and access convectional markets; putting into consideration that consumers were found willing to pay more for improved

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milk safety and quality attributes (Makokha and Fadiga, 2009).

It is in this regard that a study was conducted in two known high dairy potential areas; Limuru and Eldoret to determine the microbiological and compositional quality of milk produced and the most practicable milk microbiological quality test that can be applied at the milk collection level as a first step in the implementation of a quality based payment system in Kenya.

MATERIALS AND METHODS

Study area

The study was carried out in milk collection centres that supply Limuru Dairy Cooperative and Metkei Multipurpose Dairy Limited in Limuru and Eldoret regions of Kenya respectively. The collection centers in both areas were selected based on the number of farmers they served and accessibility.

A total of 9 milk collection centers participated in the study in Limuru namely: Muguga, Ngecha, Rironi, Gatimu, Tiekunu, Kabuku, Murengeti Kwambira and Nyathuna while in Eldoret, milk was collected from two main centres; Metkei and Kipsaos.

Study design

This was a cross sectional study where milk samples were collected from systematically selected dairy farmers at the dairy collection centers.

Sample size

The calculated sample size was based on a study done by Ombui *et al* (1994) on Total counts and Coliform counts in dairy farmers' cans in selected dairies in Kiambu district which found that 89.5% of samples from farmers' milk cans were considered to be of good bacteriological quality. In total, 297 milk samples were collected from collection centers scattered in the two study sites.

Sample collection

The milk samples were collected from randomly selected individual farmers who brought milk to the collection centers. The milk was aseptically sampled using an aluminum ladle that was first sterilized by flaming and then cooled before being used to thoroughly mix the milk. Approximately 100 ml of milk was collected and then distributed into two sample bottles. One bottle was for milk designated for microbiological analysis while the other bottle was for milk designated for compositional analysis. All the sample bottles were properly stoppered, labeled and transported to the laboratory in an ice packed cooler box.

Compositional analysis

The compositional analysis was carried out at the Central Veterinary Laboratories, Kabete and Metkei Multipurpose Dairy Limited laboratory for the samples from Limuru and Eldoret respectively. A Lactoscan milk analyzer, (Model Lactoscan SL, Milkotronic Ltd, Bulgaria) calibrated to the Kenya Bureau of standards acceptable levels was used to analyse milk for compositional parameters including the fat, SNF, density, protein, freezing point and presence of added water. The analyser did not have the option of measuring the pH so that was done separately using a pH meter (model 3320, Jenway, Felsted, UK).

Bacteriological analysis

Sample dilution for enumeration

A milk sample was used to determine dilutions to be used for the Total count and Coliform count. One milliliter of milk was diluted in physiological saline (0.85% NaCl) to obtain 10^{-1} to 10^{-7} dilutions. The dilutions were plated and incubated in plate count agar at 32^{o} C for 48 hours and violet red bile agar at 37^{o}C for 24 hours for Total viable counts and Coliform counts respectively. Dilutions with counts between 30-300 for total viable counts and 15-150 for Coliform counts were used to determine appropriate milk dilutions to be used in the analysis.

Total plate count

The samples were examined for Total plate count as per Houghtby *et al.* (1992) where 1ml of dilutions 10^{-3} to 10^{-7} was placed into labeled sterile petridishes and molten standard plate count agar (PCA) (Oxoid®) which had been prepared and maintained in a water bath at 50^{o}C was added, mixed well and left to solidify. The cultures were then incubated at 32^{o}C for 48 hours. Cultures with colonies ranging from 30-300 were selected for counting using a colony counter (model 114203, K. Schneider and Co. AG, Zurich).

The results were interpreted as per the Kenya Bureau of Standards (KeBS) / proposed East African guidelines on Total counts.

Coliform count

The coliform count was done as per Christen *et al* (1992) where 1 ml of dilutions10^{-1} to 10^{-4} was placed into sterile labeled petridishes and molten sterile violet red bile agar (VRBA) (Oxoid®) which had been maintained in a water bath at 50^{o}C was added. Mixing was then done and the media left to solidify at room temperature. Incubation was done at 37^{o}C for 24 hours after which typical red coliform colonies were enumerated. The results were interpreted as per the Kenya Bureau of Standards (KeBS)/ proposed East African guidelines on Coliform counts.

One hour resazurin test

The resazurin test was done as per Draaiyer *et al.* (2009) where a resazurin tablet (Surechem) was completely dissolved in 50 ml of sterile distilled water according to the manufacturer's instructions. One milliliter of the resulting solution was added into 10 ml of the milk sample in a test tube, mixed and then incubated at 37^{o} C for 1 hour in a water bath. The samples were then read using a Lovibond comparator (model 2000+ Tintometer, ltd Salisbury, USA) for colour change and designated numerical score value ranging from 1-6, assigned. A milk sample without the resazurin dye was similarly treated and used as the blank in the comparator. Samples with comparator readings ranging from 4-6 were taken to be within the acceptable levels based on the Kenya Bureau of Standards (KeBS) / proposed East African guidelines on milk quality.

Titratable acidity

The titratable acidity test was done as per Draaiyer *et al* (2009) where ten milliliters of the milk sample were put in a beaker on a white tile. Four drops of 1.6% phenolphthalein pH indicator were added into the milk sample and mixed. Titration was done using 0.9 N sodium hydroxide with constant shaking of the milk until an observable permanent colour change (pink) was noticed. The amount of sodium hydroxide used was then recorded and the acidity of the milk calculated by dividing the amount of base used by the volume of the milk sample. This was then expressed as lactic acid percent.

Statistical analysis

A database was created in Microsoft Excel (2007) for data analysis. The data was then exported to STATA 12 ® where summary descriptive statistics and correlation analysis was done.

RESULTS

The compositional analysis from Limuru showed that the average pH was 6.63, fat 3.8%, density 1.027 g/ml, protein 3.1%, freezing point -0.541^{o}C, added water 3.42% and solids not fat 8.2% as shown in Table 1.

The average pH was within the recommended range of between 6.6-6.8, the fat was higher than the minimum recommended percentage of 3.25%, the density was slightly below the recommended range of 1.028-1.036 g/ml, the freezing point was within the recommended range of -0.525^{o}C to -0.550^{o}C while the SNF and the protein were below the recommended limit of 8.5% and 3.5% respectively. Added water was above the limit set indicating presence of adulteration.

The analysis done in Eldoret showed that the average pH was 6.64, fat was 4.28%, density was 1.028, protein was 3.64%, freezing point was -0.556, added water was 1.88% and solids not fat was 9.23% as shown in Table 2.

The overall compositional quality of milk from Eldoret was better than that from Limuru. The average pH, fat, density, protein and freezing point were higher than the recommended levels while added water was above the limit due to adulteration.

Of the milk samples collected in Limuru and Eldoret, 78% had total counts equal or less than 2,000,000 colony forming units (cfu) per ml as shown in Table 3 and 4 respectively.

Ninety one percent and 92% of the samples from Limuru and Eldoret respectively had coliform counts less than 50,000 cfu/ml as shown in Table 5 and 6 respectively.

Analysis using the resazurin test indicated that 77% and 83% of the samples from Eldoret and Limuru respectively had readings ranging from 4-6 on the Lovibond comparator indicating that the milk was of good acceptable quality. Eighty percent of the samples from

Table 1: Average milk compositional parameters in Limuru

Location	pH	Fat (%)	Solids not fat (%)	Density (g/ml)	Added water (%)	Freezing point (^{o}C)	Protein (%)
Muguga	6.63	3.83	8.28	1.027	3.42	-0.54	3.13
Ngecha	6.63	3.9	8.29	1.027	2.89	-0.54	3.16
Kwambira	6.64	3.76	7.97	1.026	6.79	-0.52	3.02
Murengeti	6.6	3.77	8.24	1.027	3.32	-0.54	3.11
Tiekunu	6.61	3.84	8.36	1.027	2.19	-0.55	3.16
Nyathuna	6.7	3.81	8.38	1.028	2.56	-0.55	3.17
Kabuku	6.60	3.84	8.33	1.027	2.31	-0.55	3.15
Rironi	6.71	3.75	8.26	1.027	2.61	-0.54	3.12
Gatimu	6.71	3.51	8.10	1.026	4.87	-0.53	3.06
Mean	6.63	3.80	8.25	1.027	3.42	-0.54	3.12

Table 2: Average milk compositional parameters in Eldoret

Location	pH	Fat (%)	Solids Non Fat (%)	Density (g/ml)	Added Water (%)	Freezing Point (^{o}C)	Protein (%)
Metkei	6.64	4.32	9.23	1.028	2.25	-0.54	3.64
Kipsaos	6.64	4.23	9.24	1.028	1.43	-0.58	3.64
Mean	6.64	4.28	9.235	1.028	1.88	-0.56	3.64

Kenya Bureau of Standards (KeBS) recommended compositional parameters for raw milk.

Milk Component: KeBS Recommended Standards; Added Water: 0% added water; Fat content: Not less than 3.25%; Solids Not Fat: Not less than 8.50%; Density: Between 1.028 g/ml – 1.036 g/ml; Freezing Point: Between -0.525^{o}C to -0.550^{o}C; Protein content: Not less than 3.5%; pH: Between 6.6-6.8

Table 3: The percentage of samples with various Total counts/ml from Limuru

Location	Collection centre	No. of samples analysed	Samples with $\leq 10^3$.cfu/ml	Samples with > 10^3 -$\leq 2*10^5$cfu/ml	Samples with > $2*10^5$ -$\leq$ 10^6cfu/ml	Samples with >10^6- $\leq$ $2*10^6$cfu /ml	Samples with > $2*10^6$cfu/ml
Limuru	Muguga	20	3 (15%)	8 (40%)	5 (25%)	0 (0%)	7(35%)
	Gatimu	10	0 (0%)	4 (40%)	3 (30%)	0 (0%)	3(30%)
	Kabuku	23	0 (0%)	14 (61%)	5 (22%)	1 (4%)	3(13%)
	Kwambira	29	0 (0%)	7 (24%)	6 (21%)	2 (7%)	14(48%)
	Rironi	10	0 (0%)	8 (80%)	1 (10%)	0 (0%)	1(10%)
	Tiekunu	42	1 (2%)	31 (74%)	6 (14%)	2 (5%)	4(10%)
	Nyathuna	10	0 (0%)	8 (80%)	0 (0%)	1 (10%)	1(10%)
	Murengeti	29	2 (7%)	19 (66%)	2 (7%)	1 (3%)	7(24%)
	Ngecha	29	1 (3%)	12 (41%)	11 (38%)	1 (3%)	5(17%)
	Muguga	202	7 (3%)	111 (55%)	38 (19%)	8 (4%)	45(22%)

Table 4: The percentage of samples with various Total counts/ml from Eldoret

Location	Collection centre	No. of samples analysed	Samples with $\leq 10^3$.cfu/ml	Samples with > 10^3 -$\leq 2*10^5$cfu/ml	Samples with > $2*10^5$ -$\leq 10^6$cfu/ml	Samples with >10^6- $\leq 2*10^6$cfu /ml	Samples with > $2*10^6$cfu/ml
Eldoret	Metkei	53	0 (0%)	31 (59%)	8 (15%)	0 (0%)	14(26%)
	Kipsaos	42	2 (5%)	31 (74%)	4 (10%)	0 (0%)	7(17%)
		95	3 (3%)	62 (65%)	12 (13%)	0 (0%)	21(22%)

KeBS recommended Total counts for raw milk; 0-1,000,000 cfu/ml : Very good quality; 1,000,000- 2,000,000 cfu/ml: Good quality; >2,000,000 cfu/ml: Bad quality

Table 5: The percentage of samples with various Coliform counts/ml from Limuru

Location	Collection centre	No. of samples analysed	Samples $\leq 10^1$ Coliform count	Samples with >10^1 $\leq 10^3$counts/ml	Samples with > 10^3 -5* 10^4coliforms counts/ml	Samples with > 5* 10^4coliforms / ml
Limuru	Muguga	20	0 (0%)	10 (50%)	6 (30%)	4 (20%)
	Gatimu	10	0 (0%)	5 (50%)	5 (50%)	0 (0%)
	Kabuku	23	1 (4%)	13 (57%)	10 (43%)	0 (0%)
	Kwambira	29	0 (0%)	17 (59%)	10 (34%)	2 (7%)
	Rironi	10	0 (0%)	8 (80%)	2 (20%)	0 (0%)
	Tiekunu	42	1 (2%)	21 (50%)	15 (36%)	6 (14%)
	Nyathuna	10	1 (10%)	9 (90%)	1 (10%)	0 (0%)
	Murengeti	29	2 (7%)	18 (62%)	6 (21%)	5 (17%)
	Ngecha	29	1 (3%)	17 (59%)	11 (38%)	1 (3%)
		202	6 (3%)	118 (58%)	66 (33%)	18 (9%)

Table 6: The percentage of samples with various Coliform counts/ml from Eldoret

Location	Collection centre	No. of samples analysed	Samples $\leq$ 10^1Coliform count	Samples with >10^1 $\leq 10^3$counts/ml	Samples with > 10^3 -5* 10^4coliforms counts/ml	Samples with > 5*10^4coliforms/ml
Eldoret	Metkei	53	7 (13%)	32 (60%)	14 (26%)	7 (13%)
	Kipsaos	42	11 (26%)	33 (79%)	8 (19%)	1 (2%)
		95	18 (19%)	65 (68%)	22 (23%)	8 (8%)

KeBS recommended Coliform counts for raw milk; 0-1,000 cfu/ml: Very good quality; 1,000 – 50,000 cfu/ml: Good quality; >50,000 cfu/ml: Bad quality

Table 7: Correlation between direct and indirect microbiological test results of the milk from Limuru

Indirect tests		Direct tests	
		Total count	Coliform count
Resazurin	Pearson Correlation	0.251**	0.231**
	Significance level	.000	.001
	Number of samples	202	202
Titratable acidity	Pearson Correlation	.002*	.055*
	Significance level	.974	.433
	Number of samples	202	202

**There is a significant (P<0.05) fair correlation between the resazurin test and the total count and coliform count; *There was no significant (P>0.05) correlation between the titratable acidity test results and the total and coliform counts.

Table 8: Correlation between direct and indirect microbiological test results of the milk from Eldoret

Indirect test		Direct tests	
		Total count	Coliform count
Resazurin	Pearson Correlation	0.704**	0.552**
	Significance level	.000	.000
	Number of samples	95	95

**There is a significant (P<0.05) strong positive correlation between the resazurin test and the coliform count test; NB: The Titratable acidity test was not performed in Eldoret due to lack of appropriate equipment to conduct the test.

Limuru were found to have acidity levels within the range of 0.16±0.02 and therefore judged to be of good quality for the titratable acidity test.

Tests for correlation were conducted between the standard microbiological test (Total count and Coliform count) and the Resazurin and Titratable acidity tests. There was a significant (P<0.05) positive correlation between the Resazurin test and the Total count and Coliform count tests in Limuru and Eldoret as shown in Table 7 and 8 respectively. No significant correlation was found between the Titratable acidity and Total counts and Coliform counts.

DISCUSSION

The overall bacteriological quality of the milk supplied by farmers in Limuru and Eldoret can be termed as good based on the parameters set by the Kenya Bureau of Standards. The results were also consistent with a study done by Ombui *et al.* (1994) in Kiambu district which showed that 89.5% of samples from farmers milk cans were considered to be of good quality with no more than 50,000 cfu/ml of milk for coliform counts.

The good quality could be attributed to several practices that were observed among them being the use of aluminum containers in the transportation of milk. These containers are recommended because they don't have adhesive properties and therefore are easy to clean when compared with plastic containers (Karuga, 2009). It was also noted that most farmers lived within close proximity to milk collection centers, therefore shortening the time taken before milk reaches the factory. In Eldoret, the milk was first chilled before being collected by the processors. It has been reported that milk spoils within 3-4 hours after milking especially in hot environmental temperatures. Cooling of milk therefore is advocated to help in significantly reducing the multiplication of bacteria and in turn reducing spoilage (Hygienic milk handling and processing guide).

The environment of the animal also significantly contributes to the bacteriological quality of the milk. Most of the farmers in the two study areas used organic bedding

material (mostly wood shavings) in their cow sheds. This practice could have potentially led to the introduction of environmentally associated microorganisms in the milk. However, it has also been noted that wood products such as shavings which have a much larger particle size, do not tend to cling to teat skin and support slower growth of bacteria (Wallace, 2007).

Most of the compositional quality parameters were within the acceptable range apart from the presence of added water in both study sites and low SNF and protein content in Limuru. The adulteration of milk with water was found to be a common practice by some farmers from the study sites. This practice has been reported to not only decrease the quality of the milk but also to introduce chemical and microbial health hazards (SDP Policy Brief 4).The low SNF and protein content of the samples from Limuru could have been attributed to a variety of factors including the feed, genetics, season of the year, stage of lactation and disease (Harris and Bachman, 1988; Wattiaux, 2012).

The Plate count test has been reported to be generally accepted as the most accurate and informative method of testing the bacteriological quality of milk (Kurwijila *et al*, 1992; Godefay and Molla, 2000) while the Coliform test has been used to test for the effectiveness of cleaning procedures during milking. The fair and strong correlation between these tests and the one hour Resazurin test means that the Resazurin test could be adapted for use as an indirect indicator of the bacteriological quality of milk at the collection centres.

Conclusion

The study concludes that milk from the two study areas was generally of good quality based on the current guidelines of the Kenya Bureau of Standards and the East African standards on raw milk. It also concludes that out of the bacteriological tests used, the Resazurin test is the most applicable at the milk collection level. This may have a positive effect on the marketing of milk within Kenya and Internationally. However, milk testing structures in place at milk collection centers need to be strengthened in order to avoid adulteration. This should also include the introduction of antibiotics and aflatoxin testing.

REFERENCES

Christen GL, PM Davidson, JS McAllister and LA Roth, 1992. Coliform and other indicator bacteria. Standard Methods for the Examination of Dairy Products. 16th Edition. American Public Health Association, Washington DC, pp: 247-267.

Draaiyer J, B Dugdill, A Bennett and J Mounsey, 2009. Milk Testing and Payment Systems Resource Book; a practical guide to assist milk producer groups. Food and Agriculture Organization (FAO).

Draft COMESA/ East Africa Dairy Standards on raw milk (http://www.dairyafrica.com/EAS%2067%20raw%20milk%20cleaned).

FAO STAT, 2012 (http://faostat3.fao.org/home/index.html).

Godefay B and B Molla, 2000. Bacteriological quality of raw milk from four dairy farms and milk collection centers in and around Addis Ababa. Berliner und Münchener Tierärztliche Wochenschrift, 113: 1-3.

Harding F, 1999. Milk quality. 1st edition. Chapman and hall food science book, Aspen Publishers.

Harris B and KC Bachman, 1988. Nutritional and management factors affecting solids not fat, acidity and freezing point of milk. University of Florida, Institute of food and agricultural sciences. (www.feedbarnstore.com/animalscience/dairy/ds-25.pdf).

Houghtby GA, LJ Maturin and EK Koenig, 1992. Microbiological counts methods. Standard Methods for the Examination of Dairy Products. American Public Health Association, Washington, DC, USA.

Hygienic milk handling and processing (http:// www.fao.org/ag/gainfo/resources/documents/MPGuide/mpguide1.htm).

Karuga S, 2009. Draft report on dairy chain analysis, Timau milk shed. Micro enterprise support program trust.

Kurwijila RL, KK Hansen, IE Macha, K Abdallah and HJS Kadigi, 1992. The bacteriological quality of milk from hand and machine milked dairy herds in Morogoro, Tanzania. Afric Livest Res, 2: 59-67.

Makokha S and ML Fadiga, 2009. Exploiting markets for dairy and meat products' quality and safety: A Kenyan case study. Research report 24 on Demand for livestock products in developing countries with a focus on quality and safety attributes: Evidence from Asia and Africa, pp: 72-92.

Mwangi A, SM Arimi, S Mbugua, EK Kang'ethe and AO Omore, 2000. Assurance of marketed milk in Kenya. Faculty of Veterinary Medicine Biennial Conference. 30-31st August 2000. University of Nairobi.

National Dairy Master Plan, 2010. Action plan and implementation strategy, Volume II.

Ombui JN, HFA Kaburia, JK Macharia and G Nduhiu, 1994. Coliform counts and *Escherichia coli* in raw commercial milk from dairy farmers in Kiambu district Kenya. East Afric Med J, 71: 635-639.

Pandian ASS, JS Shree, MB Raja and D Vetrivel, 2013. Efficiency of Resources Use in Urban Milk Production in the State of Tamil Nadu, India. Inter J Vet Sci, 2: 118-120.

Smallholder Dairy Project brief 4. Public Health issues in Kenyan milk markets: (http://www.smallholderdairy.org/milk%20and%20public%20health).

Wallace RL, 2007. Bedding choices: Mastitis control and cow comfort (http:// www.livestocktrail.illinois.edu/uploads/dairynet/papers/2007%20dd%20Bedding%20Choices.pdf)

Wattiaux MA, 2012. Milk composition and nutritional value. (babcock.wisc.edu/sites/default/files/de/en/de_19.en.pdf).