African Journal of Food Science and Technology (ISSN: 2141-5455) Vol. 4(5) pp. 116-121, May, 2013
Available Online http://www.interesjournals.org/AJFST

Full Length Research Paper

Quality assessment of raw camel milk using dye reduction tests

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Abstract

The composition of camel milk is different from cows' milk and this has a bearing on the quality assessment parameters. To date the quality control tests used on camel milk are those established for cow milk despite the compositional differences especially the high antimicrobial substances in camel milk. The objective of this study was to establish whether the reported high indigenous antimicrobial substances in camel milk have any effect on the reductase activity of dye reduction tests in assessing raw camel milk quality. The 10-minutes resazurin and total viable bacterial count tests were carried out on pooled fresh camel milk and pooled fresh cow milk. The same samples were inoculated with 3 % cow milk with zero (0) resazurin disc reading and further subjected to 10-minutes resazurin, titratable acidity and total viable bacterial count tests. The study established that the reported high antimicrobial substances in camel milk do not inhibit its bacterial spoilage. This was shown by the high bacterial load and high developed acidity in the inoculated camel and cow milk samples. However, the reductase activity for the 10-minutes resazurin test was much lower in camel milk compared to cow milk at ($\alpha \leq 0.05$) which is an indication that the former has more inherent hydrogen acceptors responsible for the observed delayed reductase activity. The results of this study indicated that dye reduction tests are not appropriate for assessing the quality of raw camel milk.

Keywords: Indigenous antibacterial substances, reductase activity, bacterial spoilage, resazurin, inherent hydrogen acceptors.

INTRODUCTION

Milk hygiene and quality control are an important part of milk collection. At present it is not known to what extent the methods used for quality control normally applied to cows' milk can be used for camel milk (Farah, 1996). Bachmann (1992) recommended the use of quality tests generally used for cow's milk but adapted for conditions in warm developing countries. Processing and marketing of milk requires it to be fresh and of high hygienic quality. Milk which is not fresh may curdle when heated (Bachmann, 1990). To ensure that the milk is of desirable quality for processing it is subjected to quick quality assessment tests referred to as "platform" tests. These are methods of assessing milk quality at the reception in the presence of the milk producers and which do not require elaborate laboratory facilities. They are carried out with the help of simple, readily available means. They include among other tests, dye reduction tests that assess the freshness and hygienic quality of milk by measuring the biochemical activity of microorganisms in milk. Methylene blue and resazurin dyes change or lose their colour when reduced. Quick reduction or colour change of a given quantity of dye means high microbiological activity and vice versa. The colour of the dyes is sensitive to presence or absence of oxygen.

Milk not only provides the newborn with an appropriate mixture of nutrients for optimal growth but has also an array of antimicrobial factors to give protection to the young during neonate period, before the individual's own defense mechanisms are fully developed. Similar to the difference in nutritive composition of the milk between species, the composition of the antibacterial factors in milk differ in different species (Bjorck, 1991). These

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constitute the endogenous antimicrobial substances which include; lysozyme, transferrin, lactoferrin lactoperoxidase system and immunoglobulins.

There are reports that camel milk could have medicinal properties, which suggests that it contains antibacterial components (Farah, 1996). Work by Reiter (1984) demonstrated the lytic and antibacterial properties of lysozyme. According to (IDF, 1991) lysozyme which is the most abundant in camel milk is a relatively small protein and is defined as a 1,4-B-N-acetylmuramidase and classified according to enzyme nomenclature as EC. 3.2.1.17. It occurs widely in nature. Two main types of lysozyme have been described. These are Lysozyme c and lysozyme g from chicken and goose egg white. Both types consists of a single polypeptide chain. Lysozyme c has 129-130 amino acid residues of molecular weight 14,000 while lysozyme g has approximately 185 amino acid residues and hence a higher molecular weight of 19,000-21,000. Its reported high level in camel milk (648ug/100ml) is very significant and needs further elucidation, Farah (1996).

Bovine milk contains very low levels of lysozyme i.e 0.1 µg/ml (Vakil et al., 1969). Studies have however, shown that mastitic milk, contains higher concentrations in the range of 1-2 µg/ml. Human milk, on the other hand, is much richer in lysozyme and contains on average 100 µg/ml. It is also suggested that lyzozyme may have an indirect effect on the defence systems as an immunomodulator through the stimulation of the immune system by break-down products of the hydrolysis of peptidoglycan (Bjorck, 1991). According to this author, this hypothesis has been supported by the findings that feeding infants lysozyme-enriched formulas results in an increased level of secretion of IgA in faeces.

Milk lactoperoxidase (LP) system (lactoperoxidase, hydrogen peroxide and thiocyanate) can inhibit lactic acid bacteria and kill a wide range of gram-negative bacteria; it is now used to prevent contamination of both cooled and uncooled raw milk as well as the protection of neonate against intestinal infection. According to Bjorck et al. (1979) lactoperoxidase / thiocyanate / hydrogen peroxide system is activated by increasing the thiocyanate level in bovine raw milk to 15 ppm and subsequent addition of 7.5 ppm hydrogen peroxide The aim of this study was to establish whether the reported high endogenous inhibitory substances in camel milk have any effect on the resazurin dye-reduction test in assessing the quality of raw milk.

MATERIALS AND METHODS

Study site

The study was conducted in Marsabit and Moyale Districts and at Egerton University Chemeron Field Station in Baringo District. These are arid and semi-arid areas of Kenya where camels thrive.

Milk sampling

Camel milk samples were obtained from pooled camel milk in Marsabit and Moyale Districts and Egerton University Chemeron Field Station in Baringo District. Cow milk samples were obtained from pooled Kenya Agriculture Research Station (KARI) in Marsabit, Moyale farmers and Egerton University Dairy Processing Plant. The California Mastitis Test (CMT) negative milk samples were the only ones that were collected and transported in ice-boxes to the laboratories at Marsabit KARI station and Egerton University Dairy and Food Science and Technology department.

Total bacterial viable count test

The total bacterial viable count of pooled fresh and inoculated raw camel and cow milks were based on the standard plate count method described by Houghtby et al. (1992). Six serial dilutions were made and the highest four were cultured on a plate count agar and incubated at 32˚ C for 48 hours. Colonies were counted at the end of the incubation period to establish the bacterial count.

Dye reduction test

The rate of dye reduction by microorganisms was assayed according to the method described by Wango and Farah (2004). Pooled fresh cow and camel milk samples with resazurin disc reading of six (6) were inoculated with 3 % cow milk that had a resazurin disc reading of zero (0) and their rate of dye reduction determined. This was a simulation of formal raw milk marketing which is expected on reception at the processing plant to take at least three hours before undergoing processing.

Ten-minutes resazurin test was carried out by adding 1ml resazurin dye to 10 ml milk sample in a sterile test tube and incubated in a thermostatically controlled water bath at 37˚ C for 10 minutes. A Lovibond Comparator with resazurin disc 4/9 was used to check the colour change in the given milk samples.

Titratable acidity test

The developed acidity in the samples was determined according to the method described by International Dairy Federation (1990). This involved measuring 9 ml of the milk samples into the conical flasks, and adding 1 ml 0.5 % alcoholic phenolphthalein indicator then titrating with 0.1 N sodium hydroxide (NaOH) until a faint pink colour

Table 1. 10-Minutes Resazurin Test on Camel and Cow Milks (n = 4)

Incubation time (Hours)	Camel milk Disc reading	Disc reading per hour	Cow milk Disc reading	Disc reading per hour
0	6.0		6.0	
1	5.4	0.6	5.5	0.5
2	4.5	0.9	4.0	1.5
3	3.5	1.0	0.0	4.0

Table 2. Titratable Acidity expressed as % lactic acid (n = 4)

Incubation time hour	Camel milk	% lactic acid per hour	Cow milk	% lactic acid per hour
0	0.17		0.15	
1	0.19	0.02	0.17	0.02
2	0.22	0.03	0.19	0.02
3	0.23	0.01	0.20	0.01

appeared. The results were expressed as % lactic acid where 1/10 ml NaOH is equal to 0.09 % w/v lactic acid.

Statistical analysis

A completely randomized design was used in the study. The treatments for this study were pooled fresh camel milk, pooled fresh cow milk, pooled fresh camel and cow milk inoculated with 3 % cow milk with resazurin disc reading of zero (0); the responses were 10-minutes resazurin dye reduction, developed acidity and total viable bacterial count. All treatments were performed in quadruplicate. Data were analyzed using General Linear Model (GLM) statistical analysis system (SAS) version 6.12 statistical package (1989) to compute the analysis of variance (ANOVA) and means separated by least significance difference (LSD).

RESULTS AND DISCUSSION

The milk samples before inoculation had a total viable count of 10×10^5 colony forming units per milliliter (cfu/ml) and 3.6×10^5 cfu/ml for camel and cow milks respectively which according to Kenya Bureau of Standards (KEBS) (1976) standards was good quality milk. After inoculation, all milk samples had an overgrowth and could not be counted. The viable plate count levels for both camel and cow milk samples after inoculation, indicated that there was no appreciable inhibition of the introduced resazurin dye reducing microorganisms.

Table 1 show that there was no major difference in the rate of resazurin dye reduction between the camel and cow milk for the first two hours. However, the rate of dye reduction thereafter increased for cow milk reaching 0 within 3 hours while that of camel milk reduced at a lower rate reaching 3.5 disc reading after 3 hours.

The rate of acid development between the cow and camel milks for the first three hours of storage was not significant and the inherent antimicrobial substances in camel milk did not inhibit introduced spoilage microorganisms (Table 2) and (Figure 1).

Figure 1 has shown that there are no significant differences in the rate of resazurin dye reduction between the camel and cow milk for the first two hours. However, the rate of reduction increased for cow milk after 2 hours until it reaches 0 within 3 hours while that of camel reduced at a lower rate reaching 3.5 disc reading after 3 hours.

Figure 2 showed that the rate of acid development between the cow and camel milk for the first three hours of storage is not different and that the antibacterial activity in camel milk was not effective against the inoculated spoilage microorganisms.

A statistical analysis is presented in table 3. The rate of dye reduction between camel and cow milk is significantly different while the rate of acid development is not significantly different.

Dye-reduction tests for the determination of the bacteriological quality of milk are based on the ability of certain enzymes such as dehydrogenases, which are mainly flavin enzymes of the bacterial cell, to transfer hydrogen from a substrate to biological acceptors. Suitable chemical substances such as methylene blue (BM) or resazurin, can also act as acceptors. During this reaction the dyes which are the chemical substances are reduced. The rate of reduction depends on the enzyme activity or enzyme concentration and this has been used as an index for the number of bacteria present (Lück, 1991). The principle of these tests is to add the dye to the

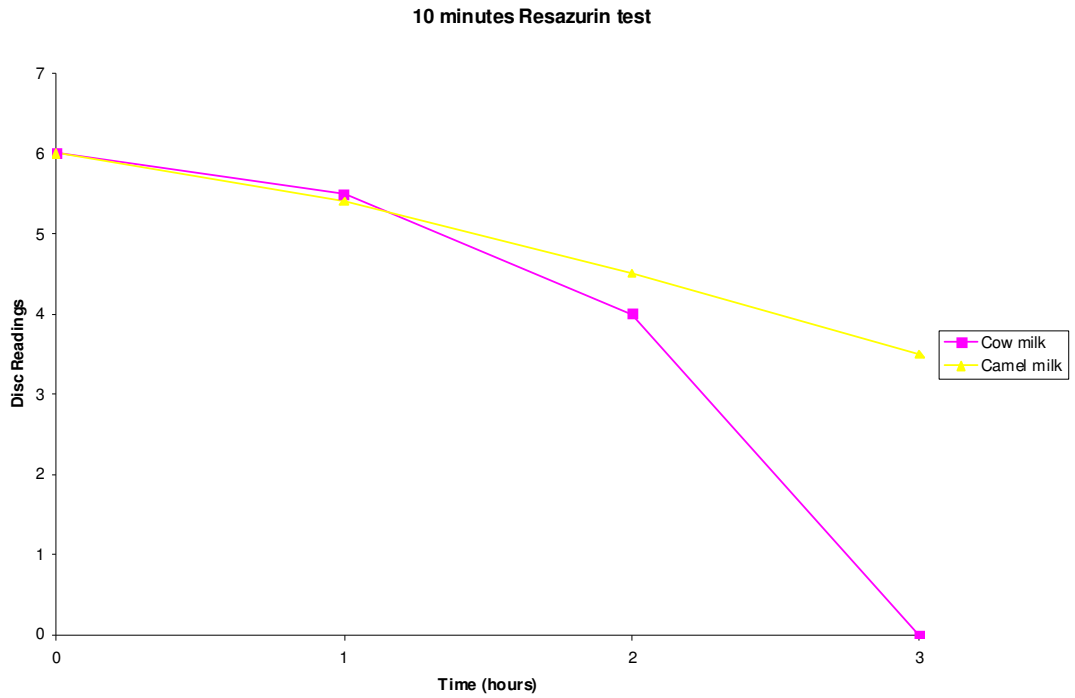

Figure 1. Rate of resazurin dye reduction for cow and camel milks

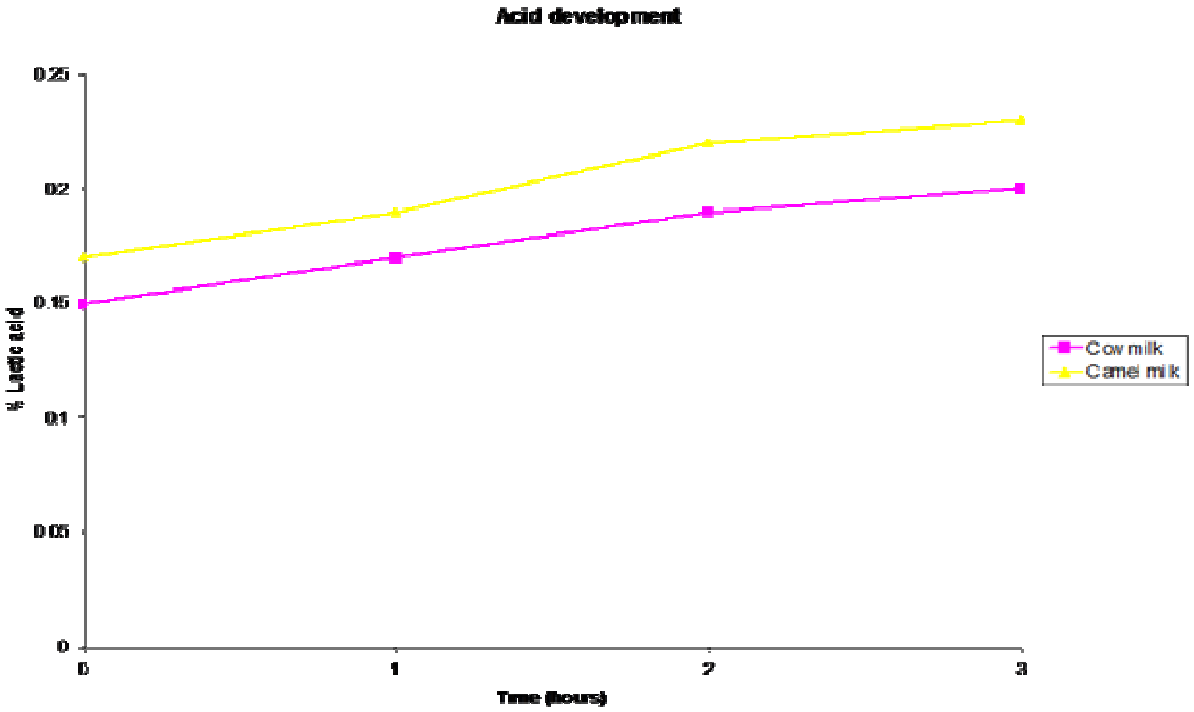

Figure 2. The curves of acid development of cow and camel milk after inoculation with resazurin dye reducing bacteria

milk followed by measuring the colour change after incubation for a specified period. The period of time needed to change or to decolourize the dye, often called reductase activity, is a measure of the bacterial activity and hence microbial content of the milk.

Camel milk had low reductase activity although the rate of acid development was not different from that of cow milk as presented in table 3. According to Bachmann (1990), when adding substances such as methylene blue or resazurin, which are easily reduced, these substances

Table 3. Means of rate of dye reduction and acid development of cow and camel milks

Sample	Resazurin disc readings	Rate of dye reduction	% lactic acid developed	Rate of lactic acid development
Cow milk	4.8125[a]	2.000[a]	0.1762[a]	0.0150[a]
Camel milk	3.8438[b]	0.8333[b]	0.2025[b]	0.0183[a]

Mean values in the same column with the same letter are not siginificantly different at $\alpha \leq 0.05$.

complete with the natural hydrogen acceptors of milk. A review by Suhren and Heeschen (1991) stated that pyruvate is reduced to lactic acid in the presence of lactate dehydrogenase (LDH) with simultaneous oxidation of nicotinamide adenine dinucleotide ($NADH_2$) The decrease of $NADH_2$ is equivalent to the quantity of pyruvate used up and can be measured at 340 nm for routine purposes within a continuous flow system. Pyruvate is a central metabolic product of the pathways of the breakdown of carbohydrates, lipids via glycerol and proteins via amino acids such as alanine, serine and glycine (Suhren and Heeschen, 1991). These authors also stated that the first enzymatic steps leading to pyruvate formation proceed more rapidly than those following, since a systematic accumulation of pyruvate is observed.

The dye-reduction test with methylene blue or resazurin measures the biochemical activity of microorganisms in milk. A common feature of microbial activity is the production of hydrogen and subsequent enzymatic reduction of other substances, for example, lactic acid bacteria reduce pyruvate to lactic acid in this way (Bachmann, 1990). It is therefore evident that lactic acid production was not inhibited in the camel milk because the rate of acid development was not significantly different from that of cow milk. The low reductase activity observed in camel milk may be as a result of more inherent competing hydrogen acceptors present as compared to cow milk such that it takes longer to reduce them before the dye could be reduced with the resultant colour change. This is consistent with reviews by (El-Bahay, 1962; Rao et al.,1970; Sawaya et al., 1984;) who gave the pH range of camel milk to be from 6.50 to 6.70 with an average value of 6.56 both values being lower than those of cow milk.

The reductase activity of a specified number of bacteria depends very much on the type of bacteria present, and the biochemical characteristics or physiological conditions of the bacterial cell. With regard to these influences there is a considerable difference between refrigerated and non-refrigerated milk with the latter giving higher reductase activity than refrigerated milk. As regards type of bacteria, acid forming bacteria have a higher reductase activity than non-acid forming bacteria (Lück, 1991). The high rate of acid development observed in both milk samples indicated that the inoculated resazurin dye reducing microorganisms must have been lactic acid producers. According to Bachmann (1990), quick reduction of a given quantity of dye means high microbiological activity and vice versa. For camel milk, this is not the case because the rate of acid development is not significantly different from that of cow milk and yet the reductase activity is quite low compared to that of cow milk. It is also evident from this study that the inherent preservation effect is not quite effective against dye reducing microorganisms since the rate of acid development between the two samples was not significantly different. According to the review by Atherton and Newlander (1977), Standard Methods discourage reporting of either methylene blue or resazurin tests results in terms of bacterial numbers. It should only be interpreted in terms of bacterial activity.

CONCLUSION

It is evident from the findings of this study that dye reduction tests are not appropriate for assessing the quality of raw camel milk. As shown by other studies, dye reduction tests for assessing raw milk quality require other complementary tests especially with regard to total microbial content. The results have shown that camel milk has more inherent hydrogen acceptors than cow milk such that its rate of spoilage could not be detected early by dye reduction tests because of the delayed reductase activity.

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