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EFFECT OF LACTOPEROXIDASE-THIOCYANATE-HYDROGEN PEROXIDE SYSTEM AND STORAGE TEMPERATURE ON KEEPING QUALITY OF RAW CAMEL MILK

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ABSTRACT

The use of lactoperoxidase system (LP-system) in temporary preservation of raw milk has been found useful particularly in places where refrigeration is not feasible. The activity of this system, however, varies from species to species and there are no reports on its effect in camel milk. This study was conducted to investigate the preservative effect of the LP-system on raw camel milk. Camel milk samples were obtained from Kajiado, Isiolo and Nanyuki districts, Kenya and LP-system was activated by the addition of hydrogen peroxide (H_2O_2) to a concentration of 8.5ppm Changes in total viable bacterial counts and titratable acidity in LP-activated and non-activated (control) camel milk were then determined during storage at 10, 20 and 30^oC. The combined effect of increasing levels of thiocyanate (NaSCN) and hydrogen peroxide (H_2O_2) on antibacterial activity of LP-system in raw camel milk was investigated at 30ºC by monitoring changes in total viable bacterial counts and lactic acid development in raw camel milk at NaSCN:H_2O_2 concentrations ratios of 0, 10:10, 20:20, 30:30 and 40:40ppms. Natural concentration of thiocyanate occurring in the camel milk from the three districts ranged from 9.7 to 36.4 mg/l and respective districts were significantly different ($p<0.05$) from each other. No additional amount of thiocyanate was, therefore, used to activate the LP-system. Microbial growth was halted for 15, 17 and 76 hrs at 30, 20 and 10^oC, respectively by activation of the LP-system in raw camel milk at 8.5ppm. Viable counts increased significantly ($p<0.05$) during storage at 10, 20, 30^oC conditions. Shelflife was extended by 19 hrs during storage at 10 and 20^oC and 4 hours at 30^oC. Increased levels of NaSCN and H_2O_2 significantly ($p<0.05$)delayed bacterial growth and lactic acid production. Shelflife of the camel milk as determined by lactic acid production was 4 hrs for control and increased to 6, 12, 16 and 16 hrs for NaSCN: H_2O_2 ratios of 10:10, 20:20, 30:30 and 40:40 ppm, respectively. The present investigation shows that by activating the LP-System, it is possible to extend the storage period of raw camel milk and that the effect of the LP-System on the microbes varies with temperature of storage and levels of thiocyanate and H_2O_2. Practical application would be achieved by controlled activation using commercial LP-system kits for pooled camel milk at collection centers and combined with cooling facilities where possible for further extension of keeping quality.

Key words: Lactoperoxidase system, preservation, camel milk

INTRODUCTION

The combination of high ambient temperatures and lack of refrigeration facilities are amongst the leading factors contributing to rapid spoilage of camel milk [1]. In Somalia and Kenya, camel milk production areas are often located far from target urban markets at distances which could range from 20 to 120 km [2]. During rainy seasons, cases of milk surplus are experienced, coupled with delays in milk delivery to the markets due to unreliable transportation. This leads to prolonged holding at high environmental temperatures. In most situations, storage of milk is done using unhygienic containers. Other inappropriate practices include mixing of evening and morning milk, pooling of milk from different suppliers and exposure during marketing [2].

Growth of contaminants in raw camel milk poses a threat to consumer health whereas the rapid spoilage reduces the market value of the milk, hence leading to income losses to both producers and vendors [3]. Prevention of quality loss through inhibition of bacterial growth during storage and transportation of camel raw milk is, therefore, paramount because it would enhance its utilization [2]. The lactoperoxidase-thiocyanate-hydrogen peroxide (LP) system has been reported to be a feasible method for the temporary preservation of raw milk [4]. The lactoperoxidase enzyme catalyzes the oxidation of thiocyanate by H_2O_2 to intermediate hypothiocyanate ($OSCN^-$), which has anti-microbial effect [5]. The use of the lactoperoxidase method was approved at the 19th session of the *Codex Alimentarius* Commission but appropriate stipulated guidelines need to be followed [6]. The activity of the LP-system varies depending on the milk source [7]. The use of LP-system for successful preservation of raw bovine milk has been studied [5]. There is scarce information on the use of activated LP-system in extending the keeping quality of raw camel milk. This study was, therefore, conducted to determine the effect of the activated LP-system on the viable bacterial counts and lactic acid production in raw camel milk.

MATERIALS AND METHODS

Sampling

Ten batches of one litre each of morning camel milk samples were obtained from pastoralists from , Isiolo, Kajiado and Nanyuki Districts in Kenya. Pooled milk from 3 herds in each district was collected aseptically in 1 litre screw cap Schott flasks and transported in cool boxes to maintain it at below 10^{o}C using ice packs. All the samples were delivered to the microbiology laboratory, at the Department of Food Science, Nutrition and Technology, University of Nairobi within 8 hrs. After delivery to the laboratory, experimentation began immediately thereafter while samples for analysis of thiocyanate were frozen at -20°C.

METHODS

Milk samples were analysed for thiocyanate as outlined by Partanen *et al*. [8] and modified as suggested by *Codex Alimentarius* Commission [9].

Effect of H_2O_2 activated-LP system on viable bacterial counts and lactic acid production in raw camel milk

Three pairs of 500ml milk samples were obtained from the pooled milk samples obtained by pooling all the samples together. To one of the samples in the pair, was added 1 ml of a freshly prepared solution of 4250 ppm H_2O_2 (E. Merck Darmstadt, Germany), followed by thorough mixing to achieve approximately 8.5 ppm H_2O_2. The other acted as untreated control with no H_2O_2 treatment. This was done for the 3 sets and each treated sample and its control was then incubated at either temperature of 30, 20 and 10^{o}C.

The antibacterial activity was assessed by monitoring changes in viable bacterial counts and titratable acidity at intervals of 0, 4, 10, 15, 25, 48, 72 and 76 hrs of storage. These temperatures were chosen to simulate ambient temperatures in camel milk producing areas (range 21-35^{o}C) and also refrigeration temperatures when available. Shelflife was measured in terms of time (hrs) taken for titratable acidity to increase to 0.2% [10].

Titratable acidity and viable counts were determined according to the standard methods by American Public Health Association [11].

Effect of combined NaSCN: H_2O_2 on activated LP-system on viable bacterial counts and lactic acid production in raw camel milk

Five sets of the raw camel milk (100mls each) were placed in screw cap sample bottles and 0, 0.3 ml, 0.6 ml, 0.9 ml and 1.2 ml of a freshly prepared solution of 3400-ppm H_2O_2 were added followed by thorough mixing to achieve 0 (control), 10, 20, 30 and 40ppm H_2O_2, respectively. Given that the camel milk had an inherent thiocyanate content of 10ppm, complementation of NaSCN was only done to achieve the final content ratios of 20:20 ppm, 30:30 ppm and 40:40 ppm of NaSCN: H_2O_2, respectively. The LP-system activated samples and the control with no H_2O_2 added were stored at 30°C and viable counts (cfu/ml) and titratable acidity (%LA) determined at intervals of 0, 4, 6, 12, 16, 20 and 24 hrs.

Statistical analysis

All the experiment treatments were conducted in triplicates. Total viable counts (log cfu/ml) and acidity (percent lactic acid) were analysed for variance using Genstat Statistical software (VSN international, 7th edition). Analysis of variance was performed to establish relationships between LP-system activation, incubation temperature and incubation time. Independent variables included LP-system activation quantities of H_2O_2 and NaSCN, incubation time and incubation temperature. Means and standard deviations were calculated and when F-values were

significant ($p<0.05$), mean differences were separated by the least significant difference procedure.

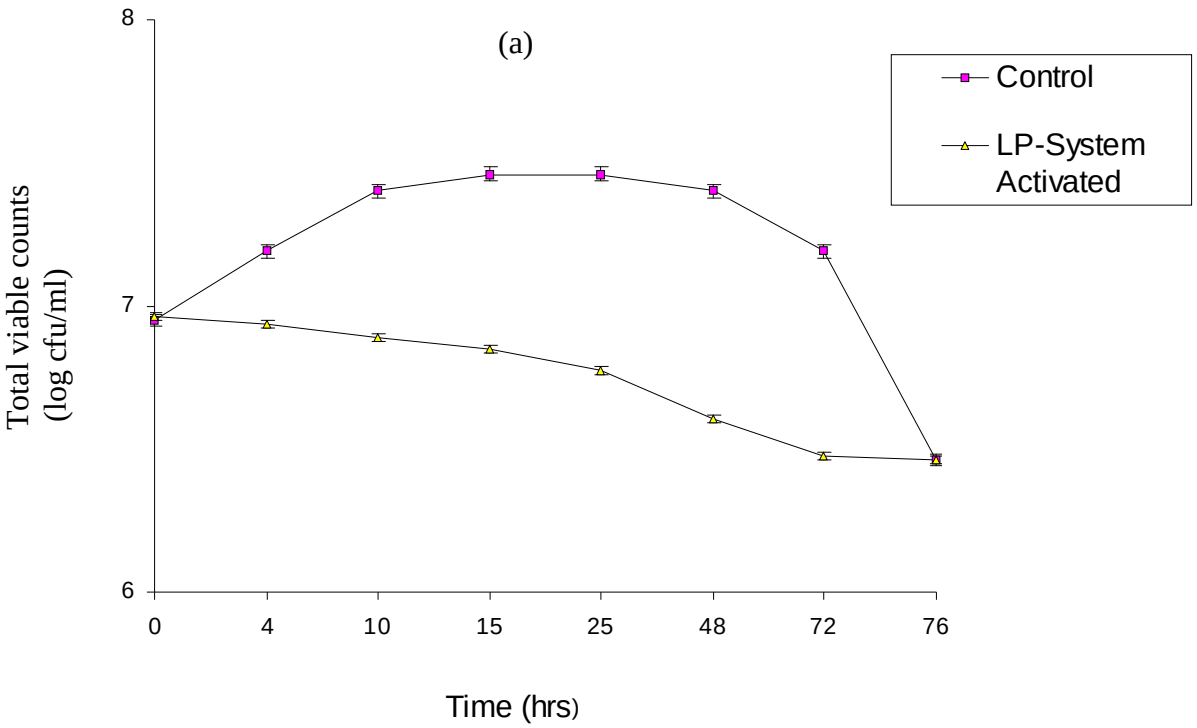
RESULTS

Thiocyanate levels in camel milk

The SCN^- content of the camel milk samples from Kajiado (32.9mg/l) were significantly ($p<0.05$) higher than samples obtained from Isiolo (9.74mg/l) and Nanyuki (15.88mg/l).

Effect of H_2O_2 activated-LP system on viable bacterial counts in raw camel milk during storage

The total viable counts for the control treatments increased ($p<0.05$) throughout the incubation period at all the three incubation temperatures of 10°C 20°C and 30°C (Fig. 1 a, b and c). At 10°C, total bacterial counts in the activated samples continued to decrease until the end of the 72 hr incubation period (Fig.1 (a)). During storage at 20°C, the total bacterial counts in the activated samples decreased ($p<0.05$) in the first 17 hrs (Fig. 1 (b)). At 30°C, the total counts in the activated samples did not increase in the first 10 hr (Fig.1 (c)).



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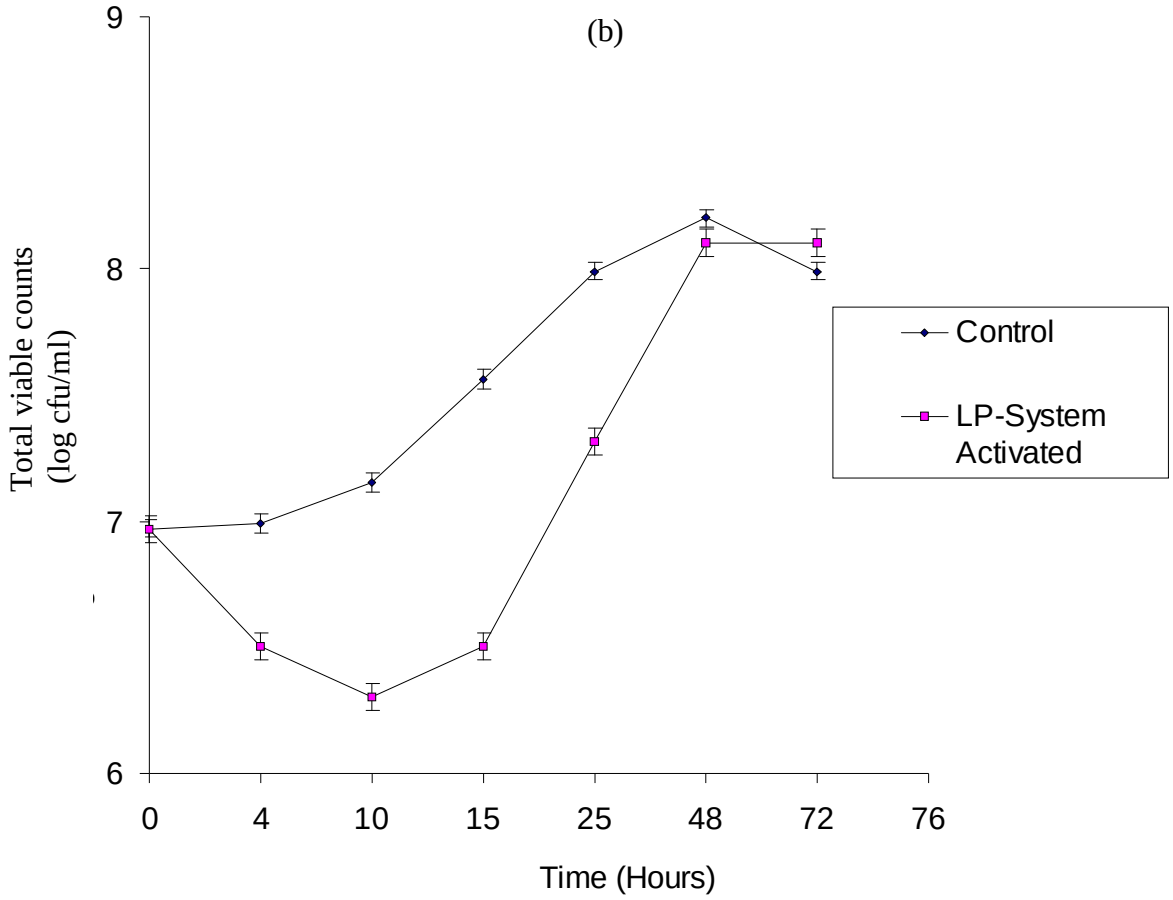


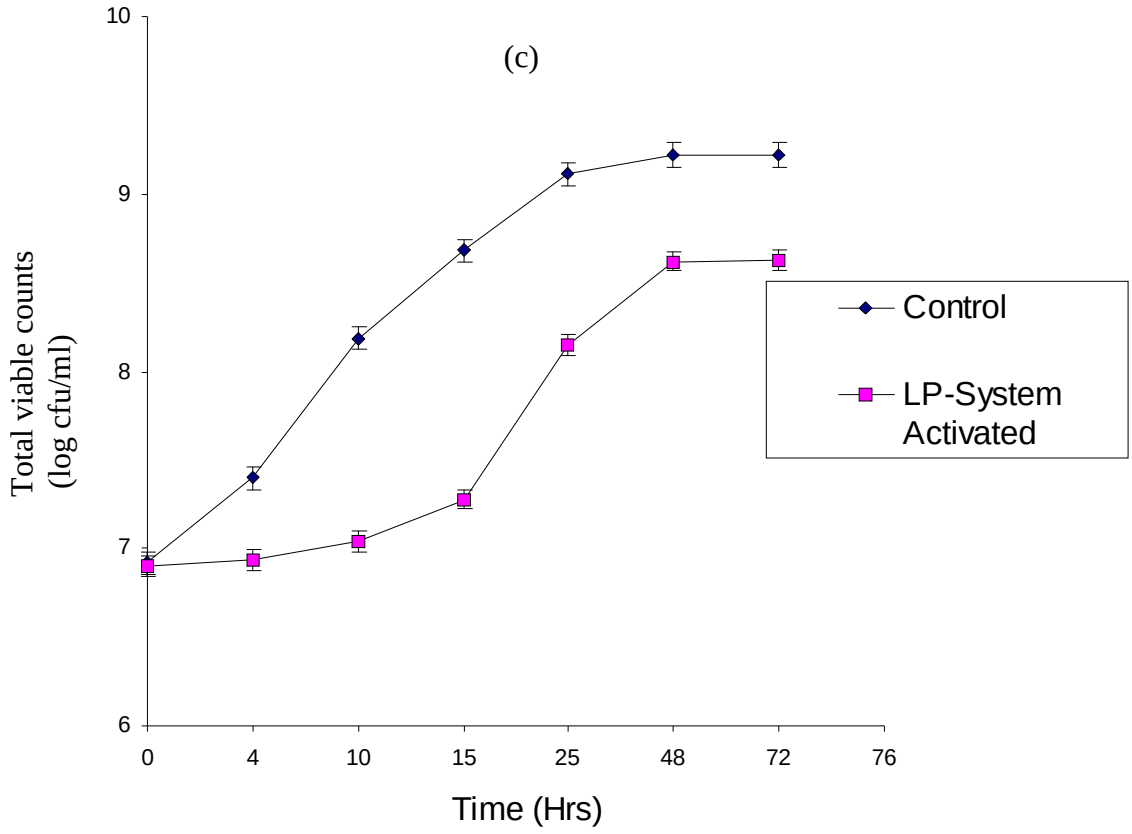
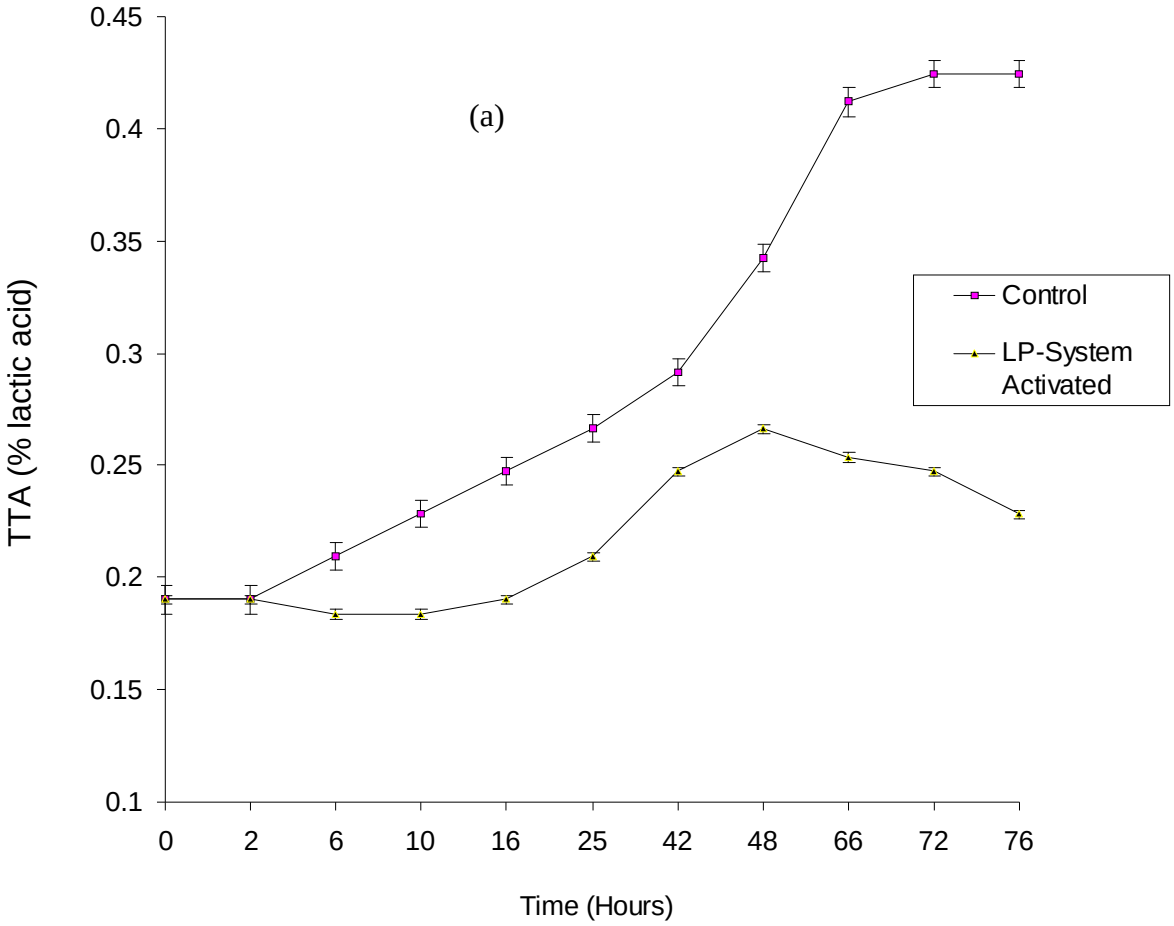


Figure 1: Effect of H_2O_2 activated-LP system on total viable counts (log cfu/ml) in raw camel milk during storage at (a) 10°C, (b) 20°C and (c) 30°C

Effect of H_2O_2 activated-LP system on lactic acid production in raw camel milk during storage

During incubation at temperatures of 10°C (Fig. 2 (a)), 20°C (Fig. 2 (b)) and 30°C (Fig. 2 (c)), there was an initial lag in acid production of 2 hrs in the control treatments followed by increase throughout the rest of the incubation period. At all the three incubation temperatures, there was resumption in acid production in the treated samples but at a much slower rate than in untreated (control) samples. There was an increase in acid production at 10hrs, 16hrs and 6hrs of storage at 10°C (Fig. 2 (a)), 20°C {Fig. 2 (b)} and 30°C (Fig. 2 (c)) respectively. Shelflife difference between LP-system activated samples and their respective controls was 19 hrs at both 10 and 20°C; and 4 hrs at 30°C.

Lactic acid production in LP-activated milk was significantly ($P<0.05$) slower at 10°C, 20°C and 30°C than in the respective controls. The lactic acid content observed after 76 hrs of storage was significantly ($p<0.05$) lower for samples with activated LP-system when compared to the control treatment at all the three incubation temperatures.



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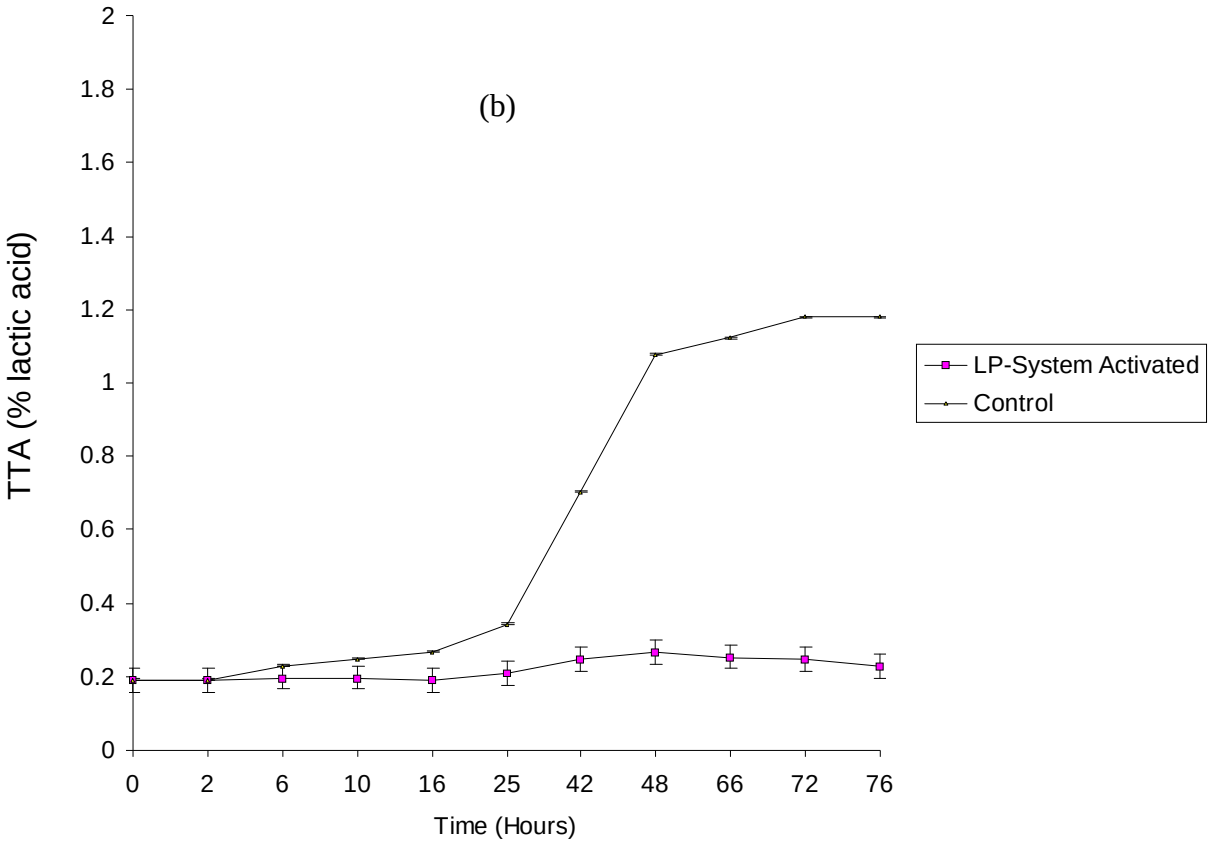

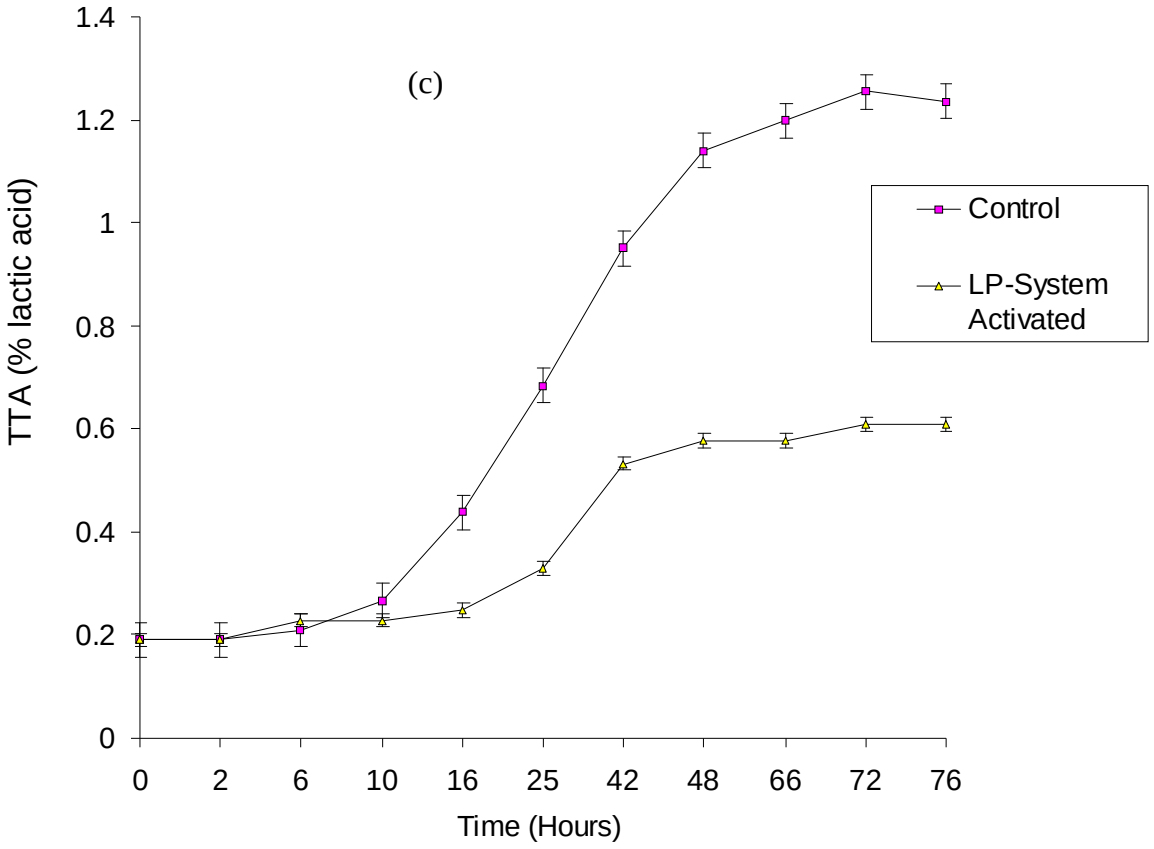

Figure 2: Effect of H_2O_2 activated-LP system on changes in total titratable acid (TTA) in raw camel milk during storage at (a) 10°C (b) 20°C and (c) 30°C

Effect of NaSCN:H_2O_2 activated LP-system on total viable counts and total titratable acidity (TTA) in raw camel milk

The total bacterial counts decreased with an increase in the doses of NaSCN:H_2O_2 upto 6 hours with the least total viable count at 5.3 log cfu/ml observed in the ratio mixture of 30:30 (NaSCN:H_2O_2) , while the highest 8.2.log cfu/ml was observed in the control (Fig. 3).

The results obtained showed a significant decrease ($p<0.05$) in total viable counts in camel milk with corresponding increase in NaSCN : H_2O_2 proportion in the milk. The

lag phase was prolonged for 4, 6, 11.5 and 9.5 hrs for 10:10, 20:20, 30:30 and 40:40 ppm ratios of NaSCN : H_2O_2 respectively (Fig. 3).

There was a significant decrease ($p<0.05$) in lactic acid production in camel milk with corresponding increase in ratios of NaSCN:H_2O_2 from 10:10 upto 30:30 in the milk (Fig. 4). The shelflife of the camel milk was 4, 6, 12, 16 and 16 hrs, for 0, 10:10, 20:20, 30:30 and 40:40 ratio mixtures of NaSCN:H_2O_2 respectively. The lag period in lactic acid production was 4.8, 6, 12 and 8 hrs for the 10:10, 20:20, 30:30 and 40:40 mixture ratios of NaSCN:H_2O_2, respectively.

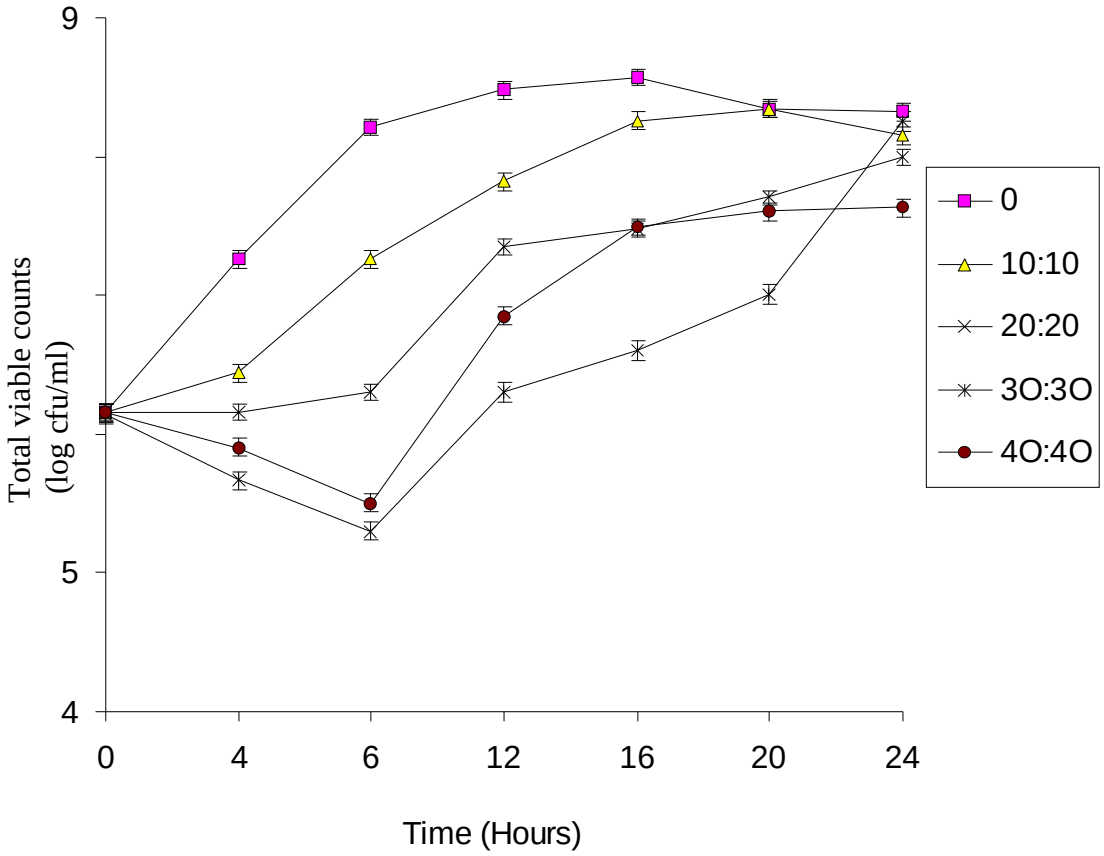

Figure 3: Effect of NaSCN:H_2O_2 activated LP system on total viable counts in raw camel milk stored at 30°C

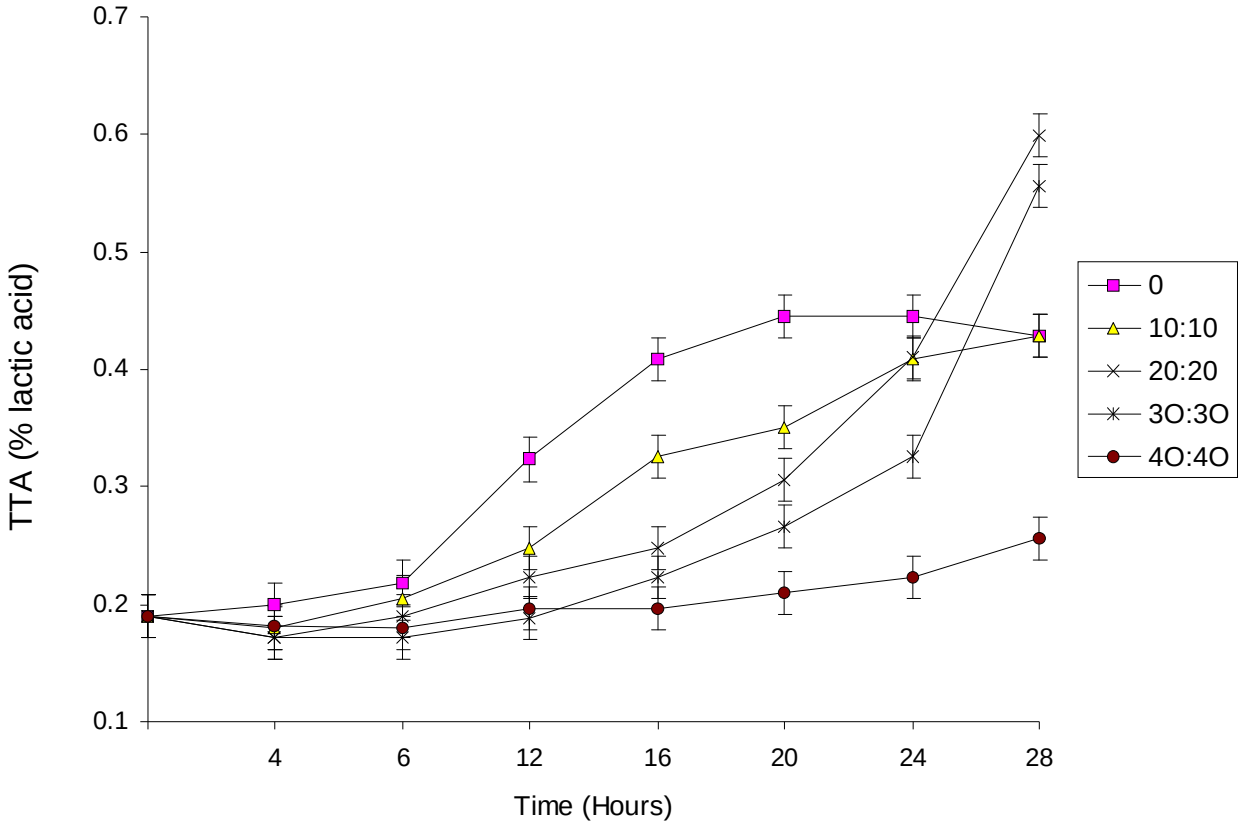

Figure 4: Effect of NaSCN:H_2O_2 activated LP-system on total titratable acidity levels of camel milk stored at 30°C

DISCUSSION

Thiocyanate levels in camel milk

The average thiocyanate values of camel milk from different sites were significantly different from each other and were also higher than levels reported in milk from other bovine species. For instance, 3.2 mg/l and 5.76 mg/l of thiocynate have been analyzed from goat milk associated with Murciano-Granadina breed and Verata breed respectively [12]. Other studies have shown thiocyanate value ranges of 3.2 to 4.5 mg/l for cow milk [13], and 5.5 to 6.0 mg/l for buffalo milk [14]. The values reported in the present work also show that thiocyanate content range (9.5 - 32.9 mg/l) of camel milk is comparably wider than those of other species investigated. According to Medina *et al*. [15], ewes in middle lactation showed values ranging from 6.98 to 12.06 mg/l of thiocyanate while Bibi and Bachman [16] reported ranges of 6.2-11.8 mg/l of thiocyanate in cow's milk. The thiocyanate level in milk is, therefore, influenced by the feeding regime of the breed, species and type of feed. Compared with other ruminants, the camel is distinguished by the high diversity of its diet. It can feed on

herbaceous plants, shrubs, shoots, cacti and date stones. During the dry season, it survives on thorny, withered plants which are low in protein but rich in fibre and cellulose content [17].

Effect of H_2O_2 activated-LP system on viable bacterial counts in raw camel milk during storage

This study showed that the LP-system, significantly ($p<0.05$) inhibited bacterial multiplication at all the three temperatures. Results of the present study on microbial growth inhibition by activation of the LP-system in raw camel milk were comparable with results obtained by Zajac *et al*. [18], which reported that at 4ºC the standard plate count in LP-activated cow milk remained basically unchanged for at least 104 hrs, whereas bacterial multiplication started after 48hrs in the controls. At 10ºC, activation resulted in a lag phase of at least 72hrs, but at 17ºC this was reduced to less than 24hrs [18]. Zajac *et al*. [18] activated the LP-system by addition of equimolar amounts of 8.5 ppm NaSCN and H_2O_2.

The results during storage at 10ºC demonstrated that the activated LP-system is able to extend the storage period of refrigerated raw camel milk to more than 76 hrs. These results concur with findings by Bjorck [19] who reported a shelf-life extension of more than 72hrs by the activation of LP-system in raw cow milk stored at 7°C. Bjorck *et al*. [13] also found that at 5ºC, where *Pseudomonas flourescens* is able to multiply, shelf-life extension is prolonged and multiplication does not occur until after 72hrs. Various workers have reported antibacterial effect of the LP-system in milk from different animal species and semi-synthetic media on different species of bacteria [5, 7, 14, 17, 20, 21, 22]. Farrag *et al*. [21] demonstrated antibacterial effect of the activated LP-system on *E. coli* 0157:H7 whose effect was inversely related to temperature. At 30ºC and 4ºC there was a lag growth of 12 hrs and 5 days, respectively. In a separate study on cow milk by Bjork [19], suppression in psychrotrophs population was observed for 5 days after activation of the LP-system. This is in contrast to the reported lag-period of about 48 hrs experienced on storage at 5ºC [19]. The difference is attributed to daily pooling and storage of milk at 5°C followed by activation of the LP-system on the second and fourth day resulted in 1000 times lower bacterial count than in untreated control after storage of milk for 6 days [19].

Effect of H_2O_2 activated-LP system on lactic acid production in raw camel milk during storage

The lag in lactic acid production in LP-activated raw camel milk compares well with results by Nakada *et al*. [23], where there was a significant decrease in lactic acid development in LP-activated raw cow milk during storage at 10°C. However, Kamau and Kroger [24] reported no significance difference between lag in lactic acid production of 8.5 ppm NaSCN and H_2O_2LP-system activated and the control cow milk samples during storage at 32ºC. Both the controls and LP-system activated samples showed a lag of 4hrs in lactic acid production [24]. The difference between results in this study and those of Kamau and Kroger [24] may be due to the higher

temperatures of storage used by the latter and also interspecies variation in the LP-system activity.

The titratable acidity test, however, did not relate well with the total viable counts results in the present work. This could be explained by the fact that some genera have a respiratory rather than a fermentative growth and hence when respiratory bacteria are predominant, lactic acid production is a poor indicator of spoilage [24].

It has been suggested that the LP-system is more active at 30ºC when the conditions are favourable for cell multiplication [21]. This may explain the reason why inhibition of acid production by the LP-system in this study was apparently more pronounced during storage at 20ºC and 30ºC compared to 10ºC. It is, therefore, possible to utilize the LP-system to increase holding time of raw camel milk for greater than 8hrs at ambient temperatures compared to untreated milk. This study, therefore, shows that by activating the LP-System in camel milk by addition of 8.5 ppm H_2O_2, it is possible to extend the storage period of raw camel milk and that the effect of the LP-System on the microbes present varies with temperature of storage.

Effect of NaSCN:H_2O_2 activated LP-system on total viable counts and total titratable acidity (TTA) in raw camel milk

The present study showed a significant reduction in total viable counts and lactic acid development with an increase in H_2O_2: NaSCN concentrations used to activate the LP-system in the milk. A study by Chakraborty *et al.* [14] on buffalo milk has reported on the possible extension of shelflife of raw milk to 15 hrs by activating the LP-System using a dose of 70:70 ppm NaSCN/H_2O_2 during storage at 37°C. This dose is much higher than the most effective dose of 30:30 ppm H_2O_2: NaSCN used in this study, which produced a shelf life of 16 hrs. Bjorck [19] recommended that a substantial increase in antibacterial effect can, however, be achieved within physiological concentrations of thiocyanate. The 30:30 ppm NaSCN:H_2O_2 levels are within physiological limits for camel milk as the levels of thiocyanate in milk samples ranged from 8.5-40 ppm, There was a lag in acid production at 4.8, 6, 12 and 8 hrs for 10:10, 20:20, 30:30 and 40:40 ppm dose of NaSCN:H_2O_2, respectively in the present study. These results compare with 0, 6, 7 and 8hrs for 0, 10:10, 20:20, 30:30 dose of NaSCN:H_2O_2 respectively reported on buffalo milk by Thakar and Dave [7]. Increasing levels of NaSCN:H_2O_2 up to 30:30 ppm during activation of the LP-system could, therefore, give the highest extension of shelf-life of camel milk.

CONCLUSION

The activated LP-system by addition of 8.5 ppm $H_2O_2$was able to enhance the shelf-life of camel milk and can therefore can be of practical application in the preservation of raw camel milk. The preservative effect can also be enhanced by increasing the concentration of H_2O_2 to match higher concentrations of physiological thiocyanate secreted in camel milk. Increasing levels of NaSCN:H_2O_2 up to 30:30 ppm during activation of the LP-System is capable of extending the shelf-life of camel milk by 16hrs. Thiocyanate ions in the form of sodium or potassium salt are available and can

be practically used in activation of the LP-system. Peroxide is also available in the form of sodium percarbonate [24]. In practical situations, sachets of thiocyanate and percarbonate are available at US$0.0025–0.01 per litre of milk [24] and can be incorporated in milk by trained personnel at collection centers.

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