Activation of Lactoperoxidase System: Evaluation of the Acidification Rate, Microbial Quality, and Shelf Life of Camel and Cow Milk

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Abstract: Camel milk is produced in areas where there is lack of milk cooling facilities coupled with high ambient temperature that exacerbates milk spoilage before it reaches the ultimate market and consumers. To overcome this problem lactoperoxidase system (LPS) is one the methods to preserve freshness of milk until it is marketed or reaches where there is milk cooling facilities. This study was conducted with the objectives of assessing the effect LPS activation on preservation of raw camel and cow milk and to comparing acidification rate of LPS activated camel and cow milk. The effect of LPS activation on inhibition of selected pathogens (i.e. *Escherichia coli* and *Staphylococcus aureus*) was also studied. The treatments consisted of a 2 x 4 factorial experiment (LPS activated and non LPS activated with 0, 6, 12, and 24 hrs storage time at 30°C treatments) in a Completely Randomized Design (CRD) with a factorial arrangement and three replications per treatment. Twenty-four camel and cow milk samples obtained from Errer valley ago-pastoralists and Haramaya University Dairy farm, respectively were examined for titratable acidity, total bacterial count (TBC) and coliform count (CC). The result revealed that titratable acidity, CC and TBC in LPS activated milk samples were significantly lower ($P< 0.05$) than their respective values in non LPS activated milk samples for both cow and camel milk, stored for 6, 12 and 24 hrs. The percent of acidity were not significantly (P>0-05) different than that of the initial acidity level in LPS activated cow and camel milk up to 12hrs of storage. LPS activated milk showed bactericidal effect against TBC and CC both in cow and camel milk. In the current experiment, activation of LPS in camel milk reduced the growth rate of *E. coli* as compared to non LPS activated milk samples. The bactericidal effect of the LPS suggests that activation of the LPS would be of paramount importance in controlling the growth of microorganisms and improving the microbial quality of both cow and camel milk in the study area. Cow milk with activated LPS showed a slight delay in acidification rate compared to the non LPS activated cow and camel milk using a thermophilic starter culture. From the study, we can suggest that LPS activation of both cow and camel milk helps to extend the shelf life of fresh milk up to 6 and 12 hours, respectively and enables milk producers to sell fresh milk within this time frame and reduce milk wastage. LPS activation can be used in improving the microbiological quality and the shelf-life of raw camel and cow milk where milk cooling facilities are not available. LPS activated milk could also be used for manufacturing of fermented milk products.

Keyword: Camel milk; cow milk; hydrogen peroxide; lactoperoxidase system; thiocyanate

1. Introduction

In developing countries milk is usually transported without cooling facilities for up to 20 km a day and as a result its quality deteriorates (FAO/WHO, 2005). Due to the highly perishable nature of milk and mishandling, the milk produced in the farm is subjected to high postharvest loses; up to 20-35% was reported for Ethiopia (UNDP/MOA, 1993, cited by Getachew, 2003). Therefore, temporary preservatives that are safe and easy to use would be employed to extend the shelf life of milk and to ensure safety of the consumers (Firew *et al*., 2013). In countries with an advanced dairy industry the use of cooling technology to control the bacterial quality of milk during collection, storage and transportation is commonly practiced (Lambert, 2001). However in Ethiopia, cooling milk by refrigerator is difficult due to several factors such as lack of capital, inadequate supply of electricity, less developed road systems and high operational cost of equipment especially for smallholder farmers who live in distant rural areas (Getachew, 2003). This problem is much worse for camel milk as camels are found in hot arid and semi-arid areas where milk can easily be spoiled due to rapid microbial growth. Prevention of quality loss through inhibition of bacterial growth during storage and transportation of raw camel milk is, therefore, of paramount importance as it enhances its utilization (Farah *et al*., 2007).

The International Dairy Federation (1988) and FAO/WHO (2000) proposed the use of lactoperoxidase system (LPS) for temporary preservation of raw milk during collection and transportation of milk to processing plants especially in areas with hot climate/weather condition. Lactoperoxidase (LP) is an enzyme naturally present in milk. The LPS consists of three components: lactoperoxidase (LP), thiocyanate (SCN^-) and hydrogen peroxide (H_2O_2). LP is naturally present in milk. It catalyzes the oxidation of thiocyanate by hydrogen peroxide and generates an intermediate oxidation

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products hypothiocyanite ion ($OSCN^-$), which has antimicrobial property. The hypothiocyanite ion reacts specifically with free sulfphydryl groups of bacterial proteins, thereby inactivating several vital bacterial metabolic enzymes and consequently blocks their metabolism and ability to multiply (IDF, 1988).

In Ethiopia, little effort has been made on the use of the LPS for preservation of cow milk. There are no literatures available on the use of LP system to preserve camel milk in the country. There is also scarce information on the possibility of making fermented milk from LPS treated cow and camel milk. Therefore, this study was conducted to determine the effect of LPS activation for preservation of raw camel and cow milk and evaluation of the effect of LPS activation on the shelf-life of the raw milk and its effect on acidification of milk using commercial cultures.

2. Materials and Methods

2.1. Milk Sample Collection

The study was conducted from January to April 2016 at Haramaya University and milk samples were collected from Errer valley, Babile District, Eastern Ethiopia. Raw milk samples were randomly collected from 10 lactating animals. Twenty four samples of raw morning milk were collected by directly milking into sterile containers and transported within 2-3 hours to dairy technology laboratory of Haramaya University using icebox. The collected milk samples of each cow and camel were pooled and thoroughly mixed to get representative sample. After thoroughly mixing, 200ml of milk samples were used for the determination of milk composition and detection of the naturally existing thiocyanate concentration and LP content of the milk. The pooled milk samples were divided into three portions for the analysis of microbial quality (i.e. the first portion of milk was a control and kept in sterilized bottle, the second portion was subjected to activation of the LPS and kept in sterilized bottle, and the third portion of milk was used to determine the initial microbial load of milk samples).

2.2 Experimental Design

A Complete Randomized Design (CRD) with 2 x 4 factorial was used for the experiment. The two factors studied were LPS activated and non-activated on both cow and camel milk. To determine the shelf life of raw milk, the antibacterial activity was assessed by monitoring changes in TBC, CC and titratable acidity at a time intervals of 0, 6, 12 and 24 hrs of storage at 30°C. Bacteriocidal effect of LPS activation on pathogenic bacteria was made for *E. coli* and S. *aureus*. Each treatments were assigned as; T1 (camel raw milk without LPS activation), T2 (Camel raw milk with LPS activation), T3 (Cow raw milk without LPS activation), and T4 (Cow raw milk with LPS activation).

2.3. Determination of Thiocyanate Concentration

Thiocyanate concentration in both camel and cow raw milk was determined spectrophotometrically as described by IDF (1988). Four ml of milk was mixed with 2 ml of 20% (w/ v) trichloroacetic acid solution. The mixture was blended well and allowed to stand for 30 min and filtered through Whatman No. 40 filter paper. The clear filtrate of 1.5 ml was mixed with 1.5ml of ferric nitrate reagent and the absorbance was measured by spectrophotometer at absorbance of 460 nm.

2.4. Lactoperoxidase Activity Analysis in Raw Milk

Lactoperoxidase activity of the milk samples were measured with one-step ABTS (2, 2-azino-bis-(3-ethylbenzthiazoline ethylbenzthiazoline- 6-sulphonic) acid solution (Sigma Aldrich^R, Germany) as substrate. The assay mixture consisted of 1 ml of 0.1 M phosphate buffer (pH 6.0), 5 micro liter of milk sample and 1 ml of ABTS solution. Absorbance was measured at 412 nm as a function of time for 2 min at 10s intervals using spectrophotometer adjusted at 25°C.The activity expressed in units/ml was calculated according to Kumar and Bhatia (1999).

2.5. Lactoperoxidase System Activation

Prior to activation of LPS, the natural thiocyanate concentration and LP activity in both cow and camel milk samples were determined according to IDF (1988) and Kumar and Bhatia (1999), respectively. Activation of the LPS for both cow and camel milk was done by addition of 7 mg/0.5l sodium thiocyanate as a source of SCN^- (thiocyanate) and then after 1 minute of thorough mixing, 15 mg/0.5l sodium percarbonate was added as a source of hydrogen peroxide (H_2O_2) as recommended by IDF (1988).

2.6. Chemical Composition Analysis of Raw Milk

The chemical composition of milk samples such as fat, protein, total solids, solids-not-fat and lactose content of camel and cow milk samples were determined using the MilkoScan FT1 (Foss, Hilleroed, Denmark).

2.7. Microbial Test

2.7.1 Total Bacterial Count

The TBC and CC were considered for the determination of microbial properties of both cow and camel milk samples. The sterility of diluents (peptone water) and medium used was checked by plating the medium without a sample and incubating it in the same way (Richardson, 1985). The total viable bacterial count was preformed according to the procedure of Richardson (1985). TBC was made by adding 1 ml of milk sample into sterile test tube having 9 ml of peptone water (Qualigens Fire Chemicals Private Ltd., India). After thorough mixing, samples were serially diluted up to $1:10^{-8}$. Ten to 15 ml of standard plate count agar (Don Whitley Scientific Equipment Private Ltd., India) heated at a temperature of

45°C was pour plated into duplicate and incubated at 30°C for 48 hours.

2.7.2 Coliform Count (CC)

The CC was performed according to the procedure of Richardson (1985). One ml of milk samples were serially diluted up to 1: 10^{-6} using peptone water (Qualigens Fire Chemicals, India) and transferred into sterile plates. Ten to 15 ml Violet Red Bile Agar (VRBA) (Micro Master Laboratories Private Ltd., India) heated to a temperature of 45°C was added into duplicate petri-dishes and incubated at 30°C for 24 hours. Sterility of the medium was checked by plating the medium without milk and incubating it in the same way like that of the sample (Richardson, 1985). Colony count was calculated by using the following formula for both TBC and CC (IDF, 1991).

$$\text{Count} = \frac{S_k \, x \, d}{n1+0.1n2} \quad (1)$$

Where: S_k= sum of all colonies counted (between 10 and 300); n_1 = number of plates from the lowest dilution used for computing the count; n_2 = number of plates in the next dilution factor used for computing the count; d = reciprocal of the dilution factor of the lowest dilution used for computing the count corresponding to n_1.

2.7.3 Titratable acidity of Raw Milk

Milk acidity was measured by titrating ten milliliter of raw milk samples with 0.1N NaOH (BDH Chemicals Ltd., Poole, England) to phenolphthalein end point as described by O'Connor (1995). Ten ml of raw milk samples was pipetted into a beaker, and then three to five drops of 1% phenolphthalein (Fluka A G., Switzerland) indicator was added into the milk. The sample was titrated with 0.1N NaOH until faint pink color persists. Percent of lactic acid was calculated as:

$$\%\ Lactic\ acid = \frac{ml\ of\ 0.1N\ NaOH\ used\ x\ 0.009*100}{ml\ of\ milk\ sample\ used} \quad (2)$$

2.8. Effect of Lactoperoxidase System on *E. coli* and *S. aureus*

2.8.1. Inoculation of Milk Samples with Pathogens

The isolates of the pathogens were obtained from pathology laboratory of the College of Agricultural and Environmental Sciences at Haramaya University, Ethiopia. Milk samples were brought to room temperature (25°C) before inoculation with the specific pathogens. Two batches of 400 ml of milk sample, which have been pasteurized at 65°C for 30 min, were inoculated with 2 ml of *E-coli* and similarly two batches of 400 ml of milk samples, which have been pasteurized at 65°C for 30 min, were inoculated with 2 ml of *S. aureus*. After inoculation of the milk samples with the pathogens, 1ml samples was drawn from each inoculated milk sample and transferred into 9 ml of sterile quarter strength Ringers solution. Serial dilutions were made to determine the initial number of each pathogen in the milk samples. After a period of 1 h, to enable the bacteria to adapt, *E. coli* inoculated sample was subjected to activation of the LPS whereas the other sample was used as an untreated control, *E. coli* was enumerated using MacConkey agar after incubation at 32°C for 24 h. However, *S. aureus* was enumerated on Manitol Salt agar after incubation at 37°C for 48 h (Eyassu *et al*., 2004).

2.8.2. Activation of Lactoperoxidase System for Milk Pathogen Test

Activation of the LPS was done by adding 5.6mg/0.4l sodium thiocyanate as a source of SCN^-. After 1 minute of thorough mixing, 12mg/0.4l sodium per carbonate was added as a source of hydrogen peroxide (H_2O_2) as recommended by (IDF, 1988). The milk samples were then incubated at 30°C for 6 hr in a thermostatically controlled waterbath. After 6 hr, samples were drawn from each milk sample to determine viable bacteria both in the LPS-treated and the control milk samples and compared with the initial number of pathogens in the milk samples.

2.9. Manufacturing of Fermented Milk from LPS Activated Camel and Cow Milk.

To determine the acidification activity of starter culture for the manufacture of fermented milk from LPS activated camel and cow milk, four milk samples (100ml) were pasteurized at 85°C for 30 minutes and inoculated with 0.1U/L of the YoFlexR Mild 1.0 starter cultures (i.e. *Lactobacillus delbrueckii* subsp. *Bulgaricus* and *Streptococcus thermophilus*.) and incubated at 42°C. The acidification of the cultures were monitored using an iCinac instrument (Alliance Instruments, Frepillon, France) that measures the pH, oxidation reduction potential and temperature of the culture simultaneously.

2.10. Statistical Analysis

The analysis of microbiological data were performed using SAS 9.4 version (SAS, Institute, USA) first by converting the colony forming units per milliliter (CFU/ml) in to natural logarithms. PROC GLM procedure of SAS was used to determine statistical significance differences ($P< 0.05$). The least square means of significantly different ($P < 0.05$) treatments were computed using Tuckey test. The experiment was done in triplicate.

3. Results and Discussion

3.1. Chemical Composition of Milk

The average chemical compositions of cow milk observed in this study are indicated in Table 1. The mean protein content of cow milk (3.69 g/100g) observed in this study was similar to 3.67 g/100g reported by Helen and Eyassu (2007) for cow milk sampled from Kombolcha area, Eastern Hararghe. The mean fat content of cow milk observed (3.76 g/100g) in this study, however, was lower than the value observed by Workneh (1997), Alganesh

(2002), and Helen and Eyassu (2007) who reported mean fat content of 5.9, 6.05 and 6.08 g/100g for Boran, Horro and Kombolcha cow, respectively. The total solid of cow milk obtained in this study (13.78 g/100g) is lower than the result reported by Alganesh (2002) (14.31 g/100g) for Horro cow breed and Helen and Eyassu (2007) (16.7 g/100g) for Kombolcha area local cow.

Table 1. The average (Mean± SD) chemical composition of cow and camel milk.

Variable	Cow milk	Camel milk
Fat (g/100g)	3.76 ± 1.50	3.25± 0.84
Total protein (g/100g)	3.69 ± 0.37	2.99 ± 0.70
Solid-not-fat (SNF) (g/100g)	10.53 ± 1.76	8.24 ± 0.98
Total solids (TS) (g/100g)	13.78 ± 2.38	11.65 ± 1.50
Lactose (g/100g)	5.10 ± 0.05	4.85± 0.17

The SNF content (10.53 g/100g) of cow milk observed in the current study was higher than that reported by Alganesh (2002) for Horro cow milk (8.22 g/100g). The mean lactose content of cow milk (5.10 g/100g) obtained in this study was similar to the 3.6 to 5.5 g/100g reported by Workneh (1997). The difference in values observed between the current study and earlier reports on chemical composition of cow milk may be attributed to difference in feed, breed and lactation stage of the animals.

The average chemical compositions of camel milk are also indicated in Table 1. The average chemical composition of camel milk observed in this study was 3.25, 2.99, 8.24, 11.65 and 4.85 g/100g for fat, total protein, SNF, TS and lactose, respectively. The mean fat content of camel milk (3.25 g/100g) observed in this study was higher than the value (3.0 g/100g) that reported by Yonas *et al.* (2014). The average protein content of camel milk (2.99 g/100g) observed in this study was higher than the results reported by Yonas *et al.* (2014) that the mean protein content of camel milk to be 2.90 g/100g, however the current result is in accordance with the results reported by Kula (2016).

The TS of camel milk (11.65 g/100g) obtained in the current study is similar to the results obtained by Yonas *et al.* (2014) who reported 11.60 g/100g TS for camel milk. The SNF content of camel milk (8.24 g/100g) observed in this study is lower than the results reported by Knoess *et al.* (1986) as 8.9 to 14.3 g/100g SNF for camel milk. The mean lactose content of camel milk (4.85 g/100g) is also in the range with findings of Knoess *et al.* (1986) who reported 2.9 to 5.8 g/100g lactose content for camel milk in Punjab, India. The difference observed between the current study and earlier reports could be attributed to difference in feed, season, breed and lactation stage of the animals.

3.2. Thiocyanate Content of Raw Camel and Cow Milk

The thiocyanate content of camel milk observed in this study (6.04 ppm) (Table 2) was lower than the value reported by Njage and Wangoh (2010) (i.e. 9.5-32.9 mg/l). The level of thiocyanate in pooled cow milk was 5.08 ppm. This result is also lower than the finding of Helen and Eyassu (2007) and Fonthe (2006); who reported average value of 7.38 ppm and 13.60 ppm for cow milk in Kombolcha, Eastern Ethiopia and Goudali cow in Cameroon, respectively. The thiocyanate concentration in camel milk obtained in this study was higher than that of cow milk while the lactoperoxidase activity in cow milk observed in this study was higher than that of camel milk. As stated by Korhonen (1980) several factors have been reported to affect the thiocyanate concentration in milk such as age of the animal, health of the animal, species of animal, breed, lactation stage and nutritional condition among which the kind of feed supplied plays a major role.

3.3. Lactoperoxidase Activity of Raw Camel and Cow Milk

The LP activity of camel milk found in this study (1.78 units/ml) was higher than LP activity in milk of Saanan goat (0.79 units/ml) and South African indigenous goat breed (0.26 units/ml) as reported by Eyassu *et al.* (2004). In the current study the LP activity of camel milk was lower than that of raw cow milk (Table 2). There are no previously published works on the level of lactoperoxidase activity in camel milk.

Table 2. Thiocyanate and lactoperoxidase activity content in camel and cow milk.

Variables	Camel milk	Cow milk
Thiocyanate content (ppm)	6.04±1.30	5.08±0.49
Lactoperoxidase activity (unit/ml)	1.78±0.17	2.13±0.33

Note: Values in the table are mean ± standard deviation (n=3).

The LP activity of cow milk was higher than the report of Stephens *et al* (1979) who reported 1.4units/ml. These differences might be due to differences in feed supplied, animal breed, and species. Several factors affect the LP activity in milk such as heat cycle, lactation stage, species, breed and feed of the animal (Zapico *et al.*, 1990).

3.4. Effect of Lactoperoxidase System on Milk Titratable Acidity

Significant ($P<0.05$) difference in titratable acidity was observed in LPS activated milk than non LPS activated samples (Table 3). T4 (LPS activated milk samples) showed significantly ($P<0.05$) retarded lactic acid production as compared to T3 (non LPS activated cow milk) at 6, 12 and 24 hrs of storage. There was no increase

in acid production observed between initial level of lactic acid production until 6 h of storage in LPS activated milk samples (T4).This result suggested that under the current condition, activation of LPS could extend the shelf stability of cow milk up to 6h at temperature of 30°C (this temperature was maintained using water bath). But after 6h of storage period, there was resumption in acid production in LPS treated cow milk samples (T4).

Table 3. Effect of LP system activation on percent lactic acid production in camel and cow milk stored at 30°C over a period of 24h.

Treatment	Storage time			
	Initial	6h	12h	24h
T1	0.17 ± 0.02^{ef}	0.18 ± 0.02^{ef}	0.31 ± 0.02^{d}	0.51 ± 0.02^{b}
T2	0.17 ± 0.02^{ef}	0.16 ± 0.02^{f}	0.17 ± 0.02^{ef}	0.37 ± 0.02^{cd}
T3	0.19 ± 0.02^{ef}	0.29 ± 0.02^{de}	0.47 ± 0.02^{bc}	0.68 ± 0.02^{a}
T4	0.19 ± 0.02^{ef}	0.19 ± 0.02^{ef}	0.32 ± 0.02^{d}	0.49 ± 0.02^{b}

Note: T1=camel milk not LPS activated (camel milk control), T2=camel milk LPS activated, T3=cow milk not LPS activated (cow milk control), T4=cow milk LPS activated. Values in the table are least square means ± standard error (SE) (n=3). Means with the same superscript letter in the table are not significantly different (P>0.05).

The highest lactic acid production was observed at 24 h of storage period as compared to storage period of 12 and 6 hrs. This suggests that effect of LPS activation was decreased as storage time increased and *vice versa*. The lactic acid production for the control treatment increased significantly throughout the storage time.

In the present study, LPS treated cow milk (T4) retarded the level of lactic acid production by 0.19% as compared to the control treatment when stored for 24 hrs (Table 4). The antimicrobial effect of the LPS depends on the initial microbial load of the milk. The lower lactic acid production observed in LPS treated milk after 24 hrs of storage suggests that under good hygienic milking and handling conditions, activation of LPS might extend the shelf life of cow milk for more than 6 hrs of storage which would had a paramount importance for milk producers and collectors. The current finding was in agreement with an earlier finding of Helen and Eyassu (2007) who reported that LP activation extended the shelf life of raw cow milk up to 7 hrs. However, Taye (1998) reported that LPS activation extended the shelf life of raw cow milk up to 3 hrs in Arusi area, Oromia Regional State. The observed difference might be attributed to the difference in the initial microbial load of the samples used and prevailing ambient temperature in the area. The average initial level of lactic acid in cow milk used for this study was 0.19%. Raw cow milk has an initial acidity ranging from 0.14 to 0.18% (Moyo, 2001).

Activation of LPS was also undertaken for camel milk (Table 3) and LPS activated camel milk had significantly lower ($P<0.05$) lactic acid production than non LPS activated milk samples. Acid production was not significantly ($P> 0.05$) different between initial level of lactic acid production and at 12 hrs of storage in LPS treated camel milk samples. This result suggested that under the current condition, activation of LPS prolonged the shelf stability of camel milk for up to 12 hrs at 30°C storage temperature and retarded level of lactic acid production by 0.14% as compared to control when stored for 24 hrs. Njage and Wangoh (2010) reported that, LPS activation extended the shelf life of raw camel milk up to 16 hrs in Kenya at storage temperature of 30°C. The difference observed between the present study and earlier reports might be attributed to differences in one or more of the factors such as the natural thiocyanate content, initial microbial load of the samples used and prevailing ambient temperature in the respective area. In this study, activation of LPS activity in camel milk significantly retarded level of lactic acid production compared with cow milk throughout the storage period (Table 3). The initial acidity of camel milk observed in the present study is in agreement with the findings of Mulugojjam *et al.* (2013) who reported an initial acidity of 0.16% for camel milk in Eastern Ethiopia. Camel milk non LPS activated (T1) did not show increase in lactic acid production until 6 hrs of storage. While lactic acid production in non LPS activated cow milk increased throughout the storage period. This implies that non LPS activated camel milk has longer shelf life as compared to that of non LPS activated cow milk. This may be attributed to differences in the amount of natural inhibitors in the milk of the two different species of animals.

3.5. Effect of LPS Activation on Total Bacterial and Coliform Count

The total bacterial count in LPS activated cow milk samples (T4) was significantly lower ($P<0.05$) than that of the control milk at 6 hrs of storage period (Table 4). Total bacterial count decreased by 1.07 log unit in LPS activated milk (T4) when compared to non LPS activated cow milk (T3) at 6 hrs of storage (Table 4).

Total bacterial count in LPS activated cow and camel milk after 6 h of storage decreased by 0.09 log units compared to their respective initial count. In the current study, LPS activated milk retarded level of total bacterial count in cow milk by 1.01 log units as compared to non-activated cow milk at 12 hrs of storage period. Similarly, LPS activated cow milk decreased total bacterial count by

0.87 log units as compared to non activated cow milk at 24 hrs of storage period (Table 5). The LPS exhibited a bacteriostatic effect against a mixed raw milk flora dominated by mesophilic bacteria and also against some gram-negative bacteria such as *Pseudomonas* spp. and *Escherichia coli* (FAO/WHO, 2000). Different groups of bacteria show varying degree of sensitivity to the LPS (Eyassu *et al.*, 2005). The current result was in agreement with the finding of Helen and Eyassu (2007) who reported that activation of LPS in cow milk to have decreased level of total bacteria count compared to non LPS activated cow milk.

Table 4. Effect of LPS activation and storage time on mean TBC (log10 cfu/ml) in cow and camel milk samples at storage temperature of 30°C.

	Storage time			
Treatment	0h	6h	12h	24h
T1	5.07 ± 0.14^{de}	5.09 ± 0.14^{de}	6.48 ± 0.14^{c}	7.81 ± 0.14^{b}
T2	5.07 ± 0.14^{de}	4.94 ± 0.14^{e}	5.08 ± 0.14^{de}	6.51 ± 0.14^{c}
T3	6.20 ± 0.14^{c}	7.18 ± 0.14^{bc}	8.21 ± 0.14^{ab}	8.96 ± 0.14^{a}
T4	6.20 ± 0.14^{c}	6.11 ± 0.14^{cd}	7.20 ± 0.14^{bc}	8.10 ± 0.14^{ab}

Note: T1=camel milk not LPS activated (camel milk control), T2=camel milk LPS activated, T3=cow milk not LPS activated (cow milk control), T4=cow milk LPS activated. Values in the table are least square means ± standard error (SE) (n=3). Means with the same superscript letter in the table are not significantly different (P>0.05).

The TBC of LPS activated camel milk samples was significantly (P<0.05) lower than camel milk samples that was not LPS activated at storage period of 12 hrs and 24 hrs (Table 5). In non LPS activated camel milk samples (T1) the growth of TBC increased by 1.3 log units as compared to LPS activated camel milk (T2) after 24 hrs of storage. TBC in LPS activated camel milk did not show increase up to 12 hrs of storage period as compared to that in initial camel milk samples. Njage and Wangoh (2010) also reported a similar result where activation of LPS in camel milk decreased the multiplication of total bacteria for more than 12 hrs of storage.

These result indicate that activation of LPS in camel milk extend the shelf life of camel milk for 12 hrs, and that of cow milk for 6 hrs. Non LPS activated camel milk (T1) did not show increase until 6 hrs of storage period. The coliform count (CC) in LPS activated cow milk samples (T4) was significantly lower than that of non LPS activated cow milk (T3) at 6 hrs of storage period (Table 5). In this study it was observed that the CC decreased by 1.28 log units in LPS activated cow milk as compared to non LPS activated cow milk samples at 6 hrs of storage. Coliform count in LPS activated cow milk observed to be significantly (P<0.05) lower than that in the control treatment at 12 hrs of storage.

Coliform count (CC) was decreased by 1.0 log unit in LPS activated cow milk as compared to control (T3) after 12 hrs of storage. CC in LPS activated milk samples (T4) decreased by 0.07 log units as compared to their initial number after 6 hrs of storage. This indicates that the LPS exhibited a bactericidal effect against CC in cow milk up to 6 hrs of storage. The current finding was in agreement with the finding of Helen and Eyassu (2007) who reported a decrease of CC in cow milk after 7 hrs of activation of LPS as compared to the control treatment. In the present study, the initial CC observed in cow milk was higher than the standard level of CC in cow milk. Different researchers indicated on their reports that initial milk quality and the environment under which a given experiment conducted determine the effectiveness of the LPS in raw milk preservation (Zapico *et al.*, 1993).

Table 5. Effect of LPS activation on mean coliform bacterial count (log10 cfu m L^{-1}) (±SD) in cow and camel milk samples stored at 30°C over a period of 24h.

	Storage time			
Treatment	Initial	6h	12h	24h
T1	4.35 ± 0.26^{gf}	4.38 ± 0.26^{efg}	5.47 ± 0.26^{bcdef}	6.37 ± 0.26^{abcd}
T2	4.35 ± 0.26^{gf}	3.80 ± 0.26^{g}	4.26 ± 0.26^{gfb}	5.47 ± 0.26^{bcdefg}
T3	5.12 ± 0.26^{edfefg}	6.32 ± 0.26^{bcde}	7.14 ± 0.26^{ab}	7.99 ± 0.26^{a}
T4	5.12 ± 0.22^{cdefgd}	5.04 ± 0.26^{defg}	6.13 ± 0.19^{abcdef}	7.06 ± 0.21^{abc}

Note: T1=camel milk not LPS activated (camel milk control), T2=camel milk LPS activated, T3=cow milk not LPS activated (cow milk control), T4=cow milk LPS activated. camel milk LPS activated, T3=cow milk not LPS activated (cow milk control), T4=cow milk LPS activated. Values in the table are least square means ± standard error (SE) (n=3). Means with the same superscript letter in the table are not significantly different (P>0.05).

The CC of LPS activated camel milk samples was significantly (P<0.05) lower than that of not LPS activated camel milk at storage period of 12 hrs (Table 6). CC in LPS activated camel milk samples was decreased by 0.57 log units as compared to non LPS activated control milk samples at 6 hrs of storage. At 12 hrs of storage it was observed that there is significant increase of CC in control milk sample by 1.21 log units as compared to LPS activated camel milk.

The CC in LPS activated camel milk samples was decreased by 0.55 and 0.09 log units as compared to their initial count at 6 and 12 hrs of storage, respectively. This means that the bactericidal effect of LPS against coliform bacteria increase as storage period advances from 6 to 12 hrs. The LPS exhibited a bacteriostatic effect against a mixed raw milk flora dominated by mesophilic bacteria and it exhibits bactericidal effect against some gram-negative bacteria such as *Pseudomonas* spp. and *Escherichia coli* (FAO/WHO, 2000). Different groups of bacteria show varying degree of sensitivity to the LPS (Eyassu *et al.*, 2005). Njage and Wangoh (2010) also reported that activation of LPS in camel milk retards bacterial growth until 12 hrs by using 20:20 ppm of sodium thiocyanate and sodium per-carbonate. Similarly, Firew *et al.* (2013) reported that activation of LPS in cow milk can extend the shelf life of cow milk up to 6 hrs.

3.6. Effect of LPS Activation on Microbial Pathogens

The mean *S. aureus* count increased both in the LPS activated and control camel milk samples by 0.04 and 0.86 log units, respectively after 6 hrs of incubation (Table 6). Activation of LPS in camel milk decreased the rate of growth of *S. aureus* by 0.82 log units as compared to its growth in control camel milk samples. Activation of LPS in camel milk significantly (P<0.05) decreased the growth of *S. aureus* as compared to *S. aureus* inoculated in non LPS activated camel milk after 6 hrs of storage period.

Table 6. Growth (Log10) (Mean ±SD) of selected food-borne pathogens in LPS activated and non LPS activated camel milk samples after 6 hrs of incubation at 30°C.

Variables/pathogens	Initial	6 hr
E. coli with LPS-A	6.07±0.18 ^{b}	6.25±0.07 ^{b}
E. coli non LPS-A	6.07±0.18 ^{b}	7.22±0.24 ^{a}
S. aureus with LPS-A	6.17±0.08 ^{b}	6.21±0.04 ^{b}
S. aureus non LPS-A	6.17±0.08 ^{b}	7.03±0.05 ^{a}

Note: LPS –A= LPS activated; The values in the table are least square means of log CFU/ml ± standard error (SE). (n=3). Means with the same superscript letters within a column is not significantly different (P > 0.05).

The mean *E. coli* count increased both in the LP treated and control samples of camel milk by 0.18 and 1.15 log units, respectively after 6 hrs of incubation. Activation of LPS in camel milk reduced the growth of *E. coli* by 0.97 log units as compared to that of control camel milk. Activation of LPS in camel milk sample with *E. coli* significantly (P<0.05) reduced the growth of *E. coli* as compared to not LPS activated milk at 6 hrs of storage period. According to Eyassu *et al.* (2003) the total inhibitory effect of activation of LPS on *E. coli* depends on incubation temperature employed and initial level of inoculums used. *E. coli* is a mesophilic bacterium with an optimum growth temperature between 30 and 37°C. At this temperature, *E. coli* might have been at its highest metabolic activity and thus the oxidation product of the LPS might not be able to counteract the multiplication of *E. coli.* Therefore, non-bactericidal effect of LPS activated against *E. coli* in the present study might be due to the higher temperature and initial inoculums levels used. Pruitt and Njage (1991) also reported that the variability of the bactericidal properties of milk can be caused by variations of the quantities of peroxidases contained in different milk samples.

3.7. Effect of LPS Activation on Milk Acidification

Activation of LPS in cow milk used for acidification by using thermophilic starter culture had generally delayed the acid production as compared to non-activated milk samples of cow milk (Figure 1).

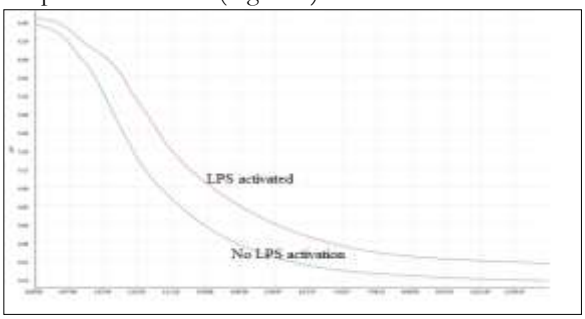

Figure 1. Effect of LPS activation on acidification activity of cow milk during 24 hrs incubation at 42°C.

As indicated from the results in Figure 1, it is possible to make fermented milk by using thermophilic starter culture from LPS preserved cow milk but the speed of acidification was slightly lower in activated milk than the non-activated cow milk. The decreased acidification rate might be due to the inhibitory compound such as hypothiocyanate formation during oxidation of thiocyanate and hydrogen peroxide during activation of lactoperoxidase system in cow milk (Njage and Wangoh, 2008). The current result is in agreement with the results reported by Eyassu (2005) who reported that there was a decline in the rate of acid production by starter cultures in LPS-activated milk. Sarkar and Misra (1992) reported that reactivation of the LPS during the manufacture of fermented milk products poses manufacturing problems. An intermediate oxidation product (i.e. OSCN) produced in LPS activated milk might causes starter culture growth inhibition and reduced lactic acid production.

Activation of LPS in camel milk used for acidification using thermophilic starter culture had generally delayed

acid production as compared to non LPS activated milk samples of camel milk (Figure 2).

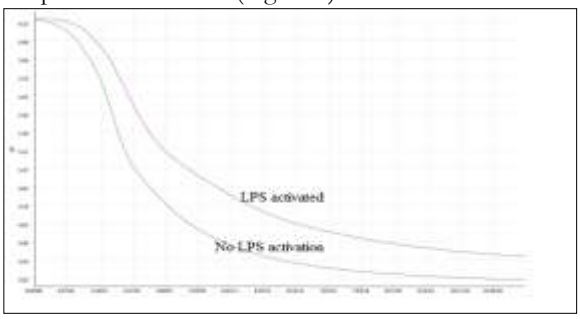

Figure 2. Effect of LPS activation on acidification activity of camel milk during 24 hrs incubation at 42°C.

As indicated in figure 2 it is possible to make fermented milk from LPS activated camel milk, however, considering the speed of acidification it was slightly lower in activated camel milk than the non LPS activated camel milk. The result observed in the current study is in agreement with that of Basaga and Dik (1994) who reported that activation of the LPS delayed the activity of starter cultures used for yoghurt production. According to Njage and Wangoh (2008) heat treatment of the LPS activated camel milk at 85°C for 30 min prior to inoculation reduced the inhibition of lactic acid production by thermophilic starter culture when heat treatment and inoculation followed immediately after activation. However, there was a reduction in lactic acid production by the LPS, when heat treatment and inoculation were done after 4 and 8 hrs of storage of the LPS activated raw camel milk. As observed from the results presented in the above figure 1 and 2, the acidification speed of the LPS activated camel milk delayed about 3 hrs as compared to that of non LPS activated camel milk to reach the desired pH value of 4.6. However, the acidification speed of the LPS activated cow milk delayed by 2 hours as compared to its non-activated cow milk to reach the desired pH value of 4.6. Comparing the acidification of LPS activated camel milk with that of cow revealed that the speed of acidification was slightly lower for camel milk than for cow milk until reaching a pH value of 4.6 (Figure 1 & 2). The final product of acidification of fermented cow milk was more viscous than camel milk. The observed variation in viscosity could be due to differences in inherent properties between the milk of the two animal species

4. Conclusion

In this study we found that activation of lactoperoxidase system prolonged the shelf life of cow and camel milk up to 6 and 12 hrs, respectively at the storage temperature of 30^0C. A significant ($P<0.05$) reduction in acid production was also observed in LPS activated milk samples compared to the control treatment at 24 hrs of storage. We also found that LPS activation also exhibited a bacteriostatic effect against CC bacteria in cow milk up to 12 hr of storage. Similarly, LPS activated camel milk exhibited bacteriostatic effect against TBC up to 12 and 24 hrs of storage. Evaluation of effect of LPS activation on selected pathogens revealed that LPS activation of camel milk significantly ($P<0.05$) decreased the rate of growth of *S. aureus* and *E. coli* count at 6 hrs of incubation. Hence, under good hygienic milking and handling conditions, activation of LPS both in camel and cow can extend the shelf life for more than 6 and 12 hrs, respectively of storage and provide opportunities for rural farmers who usually do not have milk cooling facilities. It is also possible to use the LPS activated milk for production of different fermented dairy products such as yoghurt.

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