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Full Length Research Paper

Effect of the lactoperoxidase system and container smoking on the microbial quality of goats' milk during storage at an ambient temperature

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The effect of the lactoperoxidase (LP) system in combination with container smoking on the microbial quality of goats' milk was assessed during storage at ambient temperature. The titratable acidity, coliform count (CC) and total bacterial count (TBC) in LP activated milk samples (T_2) decreased by 0.13%, 1.6 and 1.33 log_{10} units, respectively as compared to their respective values in the control (T_1) at 7 h of storage. Coliform count and TBC in T_2 decreased by 0.33 and 0.20 log_{10} units, respectively at 7 h of storage as compared to the initial count. When container smoking was combined with the LP system (T_4), no acid development was observed in the milk samples. It can be concluded that container smoking combined with the LP system could effectively control microbial growth and extend the shelf life of goats' milk stored at an ambient temperature by at least 7 h.

Key words: Goat milk, lactoperoxidase system, shelf life, smoking.

INTRODUCTION

Kombolcha woreda (an administrative district that consists of many peasant associations depending on its size) is found in eastern Ethiopia at a distance of 14 km west of Harar town. The woreda has good potential for dairy production and milk is produced in all rural districts of the woreda. Cows and goats are the major dairy animals that produce milk in the woreda. Goats are important milk producers in eastern Hararghe and the majority of the people in this region consume either fresh or boiled goat milk due to its nutritional and alleged medicinal values. Goats' milk is particularly valued in Kombolcha woreda because of its suitability for "Hoja" (a traditional beverage prepared by mixing milk, water and dried coffee leaves and boiling the mixture) making (Helen, 2007). Unlike other parts of Ethiopia, there is huge demand for fluid milk on the market in and around Kombolcha and as a result processing of milk and consumption of fermented dairy products is not as such common in the woreda. The high demand for fluid milk in the area created marketing opportunity to the farmers and milk is often a regular source of income for the farmers in Kombolcha woreda. However, milk is often produced and handled under poor sanitary conditions. As a result, large volume of milk is spoiled in the area due to lack of appropriate preservation methods.

Although goats' milk is a very nutritious food, it is also an important vehicle for transmission of pathogenic microorganisms to human beings unless it is produced and handled under good hygienic conditions. Thus, hygienic production of milk has to get due attention to provide more and better quality milk for the general public. The major methods used to safeguard the bacteriological quality of raw milk in industrialized countries are pasteurization and cooling. However, these methods are often not practical in most developing countries for technical and/or economic reasons. Thus, use of alternative milk preservation methods that are safe, cheap and easily applicable under farm conditions is of paramount importance. The lactoperoxidase (LP) system is one such method that helps to minimize microbial load and extend shelf life of milk. The use of the LP system for preservation of raw milk has been reported by several authors from different countries (Björck et al., 1979; Thakar and

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Dave, 1986; Fontheh et al., 2005). These authors, however, suggested that the effectiveness of the system depends on the conditions that prevail in a given area particularly the microbial load of the milk before treatment and the prevailing ambient temperature.

In many parts of Ethiopia, milk vessels are usually smoked using wood splinters of *Olea africana* to impart desirable aroma to the milk. Smoking of milk containers was also found to lower the microbial load of milk (Mogessie and Fekadu, 1993). Smoking of milk vessels is the major method that is traditionally used to preserve raw milk in the study area. In Kombolcha woreda, it is hardly possible to find a farmer who delivers milk to the market in unsmoked container. Due to lack of road and transportation systems, some farmers in the study area walk more than 10 km to deliver their milk to the market. Even after arrival at the market, the milk may be kept for several hours at the high ambient temperature in the open market until it is sold. Under these circumstances, the chance of spoilage of milk is very high. Thus, development of a practical method that could help farmers to preserve their milk during storage and transportation to the market would help minimize spoilage of milk at the prevailing high ambient temperature.

The lactoperoxidase system acts in synergy with other food preservation methods to control the growth of microorganisms in food systems (Zapico et al., 1998; García-Graells et al., 2002; McLay et al., 2002). Thus, the LP system combined with container smoking may inhibit the growth of microorganisms in goats' milk and thereby extend its shelf life. Use of the LP system in combination with container smoking to control microbial growth in goats' milk would create a practical and promising opportunity to preserve raw milk at farm level in areas where there are no milk cooling facilities.

Most of the studies conducted so far on the use of the LP system for preservation of raw milk were conducted at laboratory scale under controlled temperature. However, only few studies have been reported on the use of the LP system at farm level and its effectiveness on raw milk preservation under real life situation during storage at field conditions (Björck et al., 1979; Härnulv and Kandasamy, 1982; Kumar and Mathur, 1989). The objective of this study was, therefore, to assess the effect of the lactoperoxidase system combined with container smoking on the microbial quality of goats' milk produced in Kombolcha woreda in eastern Ethiopia during storage at an ambient temperature.

MATERIALS AND METHODS

The study area

The study was conducted in Kombolcha woreda which is located in eastern Ethiopia at a distance of 14 km from Harar town. The woreda comprises 19 Peasant Associations (PAs) and has a total. area of about 46,461 hectares of which 74% is mid highland and 26% is lowland (MoARD, 2004). Kombolcha woreda receives an average annual rainfall ranging from 600 - 900 mm and its altitude ranges from 1600 to 2400 m above sea level (MoARD, 2004). The mean minimum and maximum annual temperatures of the woreda were 14 to 25°C, respectively (MoARD, 2004) and the average ambient temperature of the study area at the time of the experiment was 23°C.

Raw milk sampling and arrangement of treatments

Out of the 19 PAs in the woreda, four PAs (21%) were purposively selected based on their distance (located at a distance of 2, 5, 7 and 9 km) from the main market. Among 120 households who were previously interviewed to assess goat milk handling and preservation practices in the area, twenty willing households who had two or more lactating does were randomly selected from the four PAs to provide the goat milk samples. Training was given to each participant on hygienic milk handling and stainless steel milking cans were provided to each participant to minimize variation in the quality of milk samples collected.

Goat milk sampling was done within 2 to 3 h after milking and directly from the owners' milk vessels. About 100 to 250 ml of milk samples were collected three times from the 20 households each at two weeks interval and these samples were pooled and thoroughly mixed. The average composition of the milk samples was 6.9% w/v fat, 4.5% w/v protein, 19.3% w/v total solids, 12.2% w/v solids-not-fat and 0.93% w/v ash. The goat milk samples had an average thiocyanate content of 3.89 ppm.

Representative milk samples (5 liters) collected each time were divided into five portions (of 1 L each), labeled as T_1, T_2, T_3, T_4 and T_5 and kept in screw capped sterile bottles for microbial quality tests. The first portion of milk (T_1) was used as a control, that is, milk without any added preservative. The second portion (T_2) of milk was subjected to activation of the LP system. The third portion of milk (T_3) was kept in smoked container in order to determine the effect of container smoking on microbial quality of milk. Container smoking was done using wood splinters of *Olea africana* at the Haramaya University (HU) dairy laboratory. The milk bottles were inverted over the smoking wood until the smoke dies out (≈ 30 min). The fourth portion of milk sample (T_4) was subjected to activation of the LP system and kept in smoked bottles to determine the combined effect of the LP system and container smoking on microbial quality of the milk. All the four milk samples were delivered to the laboratory at an ambient temperature (22 - 23°C). The fifth portion of milk (T_5) was put in a sterile bottle and kept in an ice box and delivered to HU dairy laboratory to determine the initial microbial load of the milk samples used. Additional separate milk samples were taken for chemical analysis.

All samples were transported to HU dairy laboratory (located 34 km away from Kombolcha) for determination of titratable acidity, total bacterial count and coliform count. Up on arrival at the laboratory, these tests were conducted on the milk samples delivered in an ice box (T_5) to determine the initial acidity and microbial load of the milk. The control milk sample and milk samples subjected to the different treatments were stored at an ambient temperature for a period of 24 h. Samples were taken from these milk samples at 7 and 24 h of storage for determination of titratable acidity, total plate count and coliform count. All tests were done in duplicate.

Activation of the lactoperoxidase system

Activation of the lactoperoxidase (LP) system was done on farm after 2 - 3 h of milking by addition of 14 ml of freshly prepared solution

Table 1. Effect of different treatments on acidity (% lactic acid) (mean ± SD) in goats' milk stored at an ambient temperature (22 - 23°C) over a period of 24 h (n = 3).

Treatments	Storage time		
	Initial	7 h	24 h
T_1	$0.21^{ap} \pm 0.03$	$0.34^{aq} \pm 0.01$	$0.65^{ar} \pm 0.08$
T_2	$0.21^{ap} \pm 0.03$	$0.21^{bp} \pm 0.02$	$0.41^{bq} \pm 0.12$
T_3	$0.21^{ap} \pm 0.03$	$0.26^{cq} \pm 0.05$	$0.54^{ar} \pm 0.10$
T_4	$0.21^{ap} \pm 0.03$	$0.21^{bp} \pm 0.02$	$0.39^{bq} \pm 0.09$

T_1 = control (without preservative), T_2 = Lactoperoxidase (LP) system, T_3 = container smoking and T_4 = LP system plus smoking. Means bearing different superscript letters within the same column (a – c) or row (p - r) differ significantly ($P < 0.05$), SD = standard deviation.

of 1 mg/ml sodium thiocyanate (General Chemical Division, New York) per liter of milk as a source of thiocyanate (SCN^-) ion. After 1 min of thorough mixing, 10 ml of freshly prepared solution of 1 mg/ml hydrogen peroxide (BDH Chemicals Ltd., Poole, England) was added into the milk and the mixture was thoroughly mixed for 1 min (IDF, 1988).

Titratable acidity of milk

Milk acidity was measured by titrating milk samples with 0.1 N sodium hydroxide (NaOH) (BDH Chemicals Ltd., Poole, England) to a phenolphthalein end point as described by Richardson (1985). 10 ml of milk sample was pipetted into a beaker and then 3 to 5 drops of 1% phenolphthalein (Fluka AG, Buchs, Switzerland) indicator was added into the milk. The milk sample was titrated with the NaOH solution until faint pink color persists. The titratable acidity of the milk was expressed as percent lactic acid and calculated as reported by Richardson (1985).

Microbiological tests

1 ml of milk sample was added into sterile test tube having 9 ml of peptone water (Qualigens Fine Chemicals, Private Ltd., India). After thorough mixing, the sample was serially diluted up to 1: 10^{-8} dilution level. Total viable bacterial count was determined using Standard Plate Count Agar (Don Whitley Scientific Equipment, Private Ltd., India). 10 to 15 ml of standard plate count agar heated to a temperature of 45°C was pour plated onto duplicate Petri dishes having 1 ml of milk sample and the Petri dishes were rotated to evenly distribute the sample in the agar medium. The plated sample was solidified for 15 minutes in a safety cabinet and incubated for 48 h at 30°C (Richardson, 1985). Coliform count was determined following similar procedure as indicated above for total bacterial count except that Violet Red Bile Agar (Micro Master Laboratories, Private Ltd., India) was used as growth medium. The inoculated plates were inverted and incubated at 30°C for 24 h (Richardson, 1985). The estimated count was computed by the formula described by IDF (1991).

Statistical analysis

Data for total bacterial count and coliform count were $\log_{10}$ transformed before subjected to statistical analysis. The differences in microbial counts and titratable acidity of milk samples subjected to the different treatments at a particular storage period were analyzed by the analysis of variance technique using the General Linear Model (GLM) procedure of SAS (2000). Mean comparison was done using least significant difference (LSD) for variables whose F values were found to be significant. Significant differences were calculated at 5% significance level.

RESULTS

Effect of the lactoperoxidase system and container smoking on acid development in goat milk

The effect of the lactoperoxidase system on titratable acidity of goats' milk is shown in Table 1. Treatment two (activation of the LP system) significantly ($P < 0.05$) retarded lactic acid production as compared to the control (T_1) (0.13%) and milk samples kept in smoked containers (T_3) (0.05%) at 7 h of storage (Table 1). Milk samples treated with T_2 and T_4 had the same level of acidity at 7 h of storage (Table 1). While the titratable acidity increased by 0.13% in the control milk (T_1), it remained the same as that of the initial level for 7 h both in LP activated milk samples and in milk samples treated with T_4 (Table 1).

No significant difference in acid production was observed between the control and T_3 at 24 h of storage (Table 1). However, T_4 resulted in significant reduction in acid production as compared to the control at 24 h of storage (Table 1). The level of lactic acid in milk samples stored in smoked containers was significantly higher ($P < 0.05$) than in milk samples treated with the LP system combined with smoking (T_4) both at 7 and 24 h of storage (Table 1).

Effect of the lactoperoxidase system and container smoking on microbial quality of goat milk

The effect of the LP system (T_2) on coliform count (CC) in goats' milk kept at an ambient temperature is shown in Table 2. The CC in LP activated goat milk samples (T_2) was significantly ($P < 0.05$) lower (1.6 log units) than CC in the control (T_1) and in milk samples kept in smoked containers (T_3) (0.73 log units) at 7 h of storage (Table 2). However, no significant difference in CC was observed between T_2 and T_4 at the same storage period. While the CC increased by 1.27 log units in the control, it decreased by 0.33 log units in the LP-activated goat milk (T_2) at 7 h of storage as compared to the initial count (Table 2). There was no significant difference in the coliform count (CC) between milk samples stored in smoked containers (T_3) and the control (T_1) both at 7 h and 24 h of storage (Table 2). However, significant ($P < 0.05$) reduction in CC was observed in T_4 (smoking combined with the LP system) as compared to the control (T_1) both at 7 h (1.37 log units) and 24 h (1.04 log units) of storage (Table 2).

The effect of the LP system on total bacteria l count

Table 2. Effect of different treatments on coliform count (log_{10} cfu/ml) (mean ± SD) in goats' milk stored at an ambient temperature (22 - 23°C) over a period of 24 h (n = 3).

Treatments	Storage time		
	Initial	7 h	24 h
T_1	$5.63^{ap} \pm 0.65$	$6.90^{aq} \pm 0.10$	$7.57^{ar} \pm 0.49$
T_2	$5.63^{ap} \pm 0.65$	$5.30^{bq} \pm 0.82$	$6.60^{br} \pm 0.78$
T_3	$5.63^{ap} \pm 0.65$	$6.03^{abq} \pm 0.42$	$6.90^{ar} \pm 0.44$
T_4	$5.63^{ap} \pm 0.65$	$5.53^{bp} \pm 0.80$	$6.53^{bq} \pm 0.72$

T_1 = control (without preservative), T_2 = Lactoperoxidase (LP) system, T_3 = container smoking and T_4 = LP system plus smoking. Means bearing different superscript letters within the same column (a – c) or row (p - r) differ significantly ($P < 0.05$), SD = standard deviation.

Table 3. Effect of different treatments on total bacterial count (log_{10} cfu/ml) (mean ± SD) in goats' milk stored at an ambient temperature (22 - 23°C) over a period of 24 h (n = 3).

Treatments	Storage time		
	Initial	7 h	24 h
T_1	$7.07^{ap} \pm 0.22$	$8.20^{aq} \pm 0.17$	$9.50^{ar} \pm 0.10$
T_2	$7.07^{ap} \pm 0.22$	$6.87^{bq} \pm 0.55$	$9.07^{abr} \pm 0.12$
T_3	$7.07^{ap} \pm 0.22$	$7.97^{cq} \pm 0.23$	$9.20^{ar} \pm 0.17$
T_4	$7.07^{ap} \pm 0.22$	$7.43^{bcq} \pm 0.64$	$8.77^{br} \pm 0.06$

T_1 = control (without preservative), T_2 = Lactoperoxidase (LP) system, T_3 = container smoking and T_4 = LP system plus smoking. Means bearing different superscript letters within the same column (a – c) or row (p - r) differ significantly ($P < 0.05$), SD = standard deviation.

(TBC) in goats' milk stored at an ambient temperature is shown in Table 3. The TBC in LP activated goat milk samples (T_2) was significantly ($P < 0.05$) lower (1.33 log units) than TBC in the control (T_1) and in milk samples treated with T_3 (1.10 log units) at 7 h of storage (Table 3). While the TBC increased by 1.13 log units in the control (T_1), it decreased by 0.20 log units in LP-activated goat milk samples (T_2) at 7 h of storage as compared to the initial count (Table 3).

A decrease (0.23 log units) in TBC was observed in milk samples stored in smoked containers (T_3) at 7 h of storage as compared to the control (Table 3). However, when container smoking was used together with the LP system (T_4) the TBC decreased by 0.77 log units as compared to the control. At 24 h of storage there was no significant difference in TBC between T_3 and the control (T_1); however, a significant reduction in TBC was observed in T_4 as compared to T_1 at 24 h of storage (Table 3).

DISCUSSION

The delay in acid development observed until 7 h of storage in LP activated goat milk samples indicates that under the current condition, activation of the LP system can keep goats' milk fresh for up to 7 h during storage at an ambient temperature. This result is inline with the findings of Gürsel et al. (1999) who reported that the quality of raw goat milk could be maintained at ambient temperature (20 - 30°C) for at least 6 h by activation of the LP system. Similarly, Haddadin et al. (1996) reported that acidity of LP-treated goat milk samples held at 22°C is stable for 9 - 12 h. Most of the farmers in the study area do not have cooling facilities and they usually travel more than 10 km to deliver their milk to the market. During this time, large volume of milk is spoiled at the prevailing high ambient temperature in the area. Extension of the shelf life of goats' milk by 7 h will have practical importance to the farmers in the study area and will enable safe delivery of their milk to the market.

Smoking is the most common method that is traditionally used to preserve milk in the study area and that it is difficult to find farmers who deliver milk to the market in unsmoked containers. In the present study, smoking alone did not show improvement on the microbial quality of goats' milk; however, when combined with the LP system it significantly improved the shelf life of goats' milk as determined by level of acid production and microbial count. This suggests that container smoking and the LP system do not antagonize each other and could be used together to inhibit acid production and prolong the shelf life of goats' milk under field conditions. Research reports indicate that the LP system acts in synergy with conventional food preservation methods to control the growth of microorganisms in food (Seifu et al., 2005).

The decrease in coliform count (0.33 log units) observed in T_2 at 7 h of storage as compared to the initial count suggests that activation of the LP system can extend the shelf life of goats' milk during storage at an ambient temperature up to 7 h by inhibiting the proliferation of coliforms. This result agrees with the findings of Gürsel et al. (1999) who reported that coliform counts decreased during the first 6 h of storage in goat milk samples preserved by the LP system and stored at 20 or 30°C. Unlike container smoking (T_3), T_4 (smoking combined with the LP system) significantly reduced CC as compared to the control at 7 h of storage. This suggests that the effectiveness of container smoking in controlling the growth of coliforms in goats' milk increases when it is combined with the LP system.

The decrease (0.20 log units) in total bacterial count observed in LP-activated goat milk (T_2) at 7 h of storage as compared to the initial count suggests that activation of the LP system can significantly decrease the total bacterial flora in goats' milk even during storage of milk at an ambient temperature for 7 h. The present result is in agreement with the findings of Gürsel et al. (1999) who reported that mesophilic aerobic counts decreased during the first 6 h of storage in goat milk samples preserved by the LP system and stored at 20 or 30°C.

The results of the acidity test and microbial counts support each other and indicate that activation of the LP system in addition to container smoking can maintain the quality of goats' milk for at least 7 h during storage at an ambient temperature.

Conclusion

On-farm activation of the LP system combined with container smoking can significantly improve the keeping quality of goats' milk during storage and transportation at an ambient temperature. It should be noted that since the LP system involves use of some chemicals, activation of the system should be done by experienced individuals. In view of the absence of milk cooling facilities in the area and in order to ensure delivery of safe goat milk to the consumers by activating the LP system, establishment of milk collection and distribution centers in the woreda could be one strategy that concerned agencies may opt for.

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