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The Quality of Raw Cow`s Milk Preserved By Activation Of Lactoperoxidase System

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ABSTRACT

The study was conducted to investigate the keeping quality of raw cow`s milk preserved by activation of the lactoperoxidase system in Tsaeda-Christian, found in the sub zone Berik, Zoba Maekel. Three levels of sodium thiocyanate 10, 14, 18 and sodium percarbonate 20, 30 and 40 mg/L were respectively used to activate the raw milk samples and stored at incubation temperature (30^0C). Chemical composition, pH, total bacterial counts (TBC) were evaluated. Moreover, alcohol test and clot- on- boiling tests were performed. Activation of LPs in raw milk leads to improve the keeping quality. There were significant variations ($P<0.001$) in pH and TBC among the treatments throughout the storage period. The milk in all treatments remained fresh during the first 12 hours but the control became spoiled by the 14^{th} hour. LPs treatment extended milk shelf life by 6, 8 and 8 hours, for 10+20mg/L (LP1), 18+30mg/L (LP2) and 20+40mg/L (LP3) sodium thiocyanate and sodium percarbonate, respectively as compared to control under storage temperature conditions (30°C). From all parameters measured, LP2 and LP3 were the last treatments to spoil. Clot-on-boiling test detected milk deterioration 2 hours later than the other quality tests. The results revealed that no difference in milk composition (fat, protein, lactose, SNF, TS, and density) among the control and treatment samples. Therefore, this extension in the shelf-life of milk provided sufficient time for milk to be stored, transported and sold in its fresh state in the local markets without refrigeration and may encourage dairy farming in collection of more milk of high quality.

INTRODUCTION

Livestock plays a significant role in the Eritrean economy both at the household and national levels. Their role is one of the fundamental elements of family and national food security. The dairy sector in Eritrea is underdeveloped and most of the milk is produced by small scale holders adopting traditional production systems mainly from local breeds with poor productivity and low milk yield. This makes collection and delivery of milk a complicated process and time-consuming. With the present low level of domestic production and the increase in demand, there is an urgent need to develop strategies towards improving domestic milk production. However, it is imperative that before increasing production, the current milk produced does not spoil before it reaches the consumer. Preservation of raw milk is a problem to smallholder dairy farmers in rural areas, due to lack of cooling facilities which could be a major obstacle to the development of a dairy industry.

Milk is typically transported unrefrigerated for a longer distance but after a certain period will begin to deteriorate because of delay which often leads to rejection of the milk. This situation forces producers to find methods for raw milk preservation. Therefore, approaches for enhancing the availability of safe milk and dairy products are important for the continued improvement of household nutrition and health without refrigeration. Thus, use of alternative milk preservation methods that are safe, cheap and easily applicable is important. The lactoperoxidase system (LPs) addresses this limitation. The LPs is one of such methods that help to minimize microbial proliferation and extend the shelf life of milk. The system comprises the enzyme LP and two substrates: thiocyanate and hydrogen peroxide (Barrett *et al.*, 1999). These substrates are naturally present in milk but in limited quantities thereby reducing the efficiency of the LPs (Reiter and Harnulv, 1984). As it is harmless to mammalian cells, the LPs is found in other body fluids such as tears, saliva and gastric juice (Kussendrager and Hooijdonk, 2000). The use of the LPs method does not exclude the necessity of pasteurization of milk before human consumption neither exclude the normal precautions and handling routines applied to ensure a high hygienic standard of the raw milk (CAC, 2011a). The application of the system basically requires no complicated techniques, no energy consuming facilities or heavy equipment. It is cheap, easy, effective and safe to use and readily applicable in developing countries with a minimum of training requirements. Though the effects of the LPs have been broadly studied in some countries; no such study has so far been conducted in Eritrea. Therefore this study aimed to evaluate the keeping quality of raw cow`s milk preserved by activation of lactoperoxidase system under Eritrean conditions.

MATERIALS AND METHODS

Description of study area: The study was conducted in Tsaeda-Christian, found in the sub zone Berik, Zoba Maekel. The area lies at an altitude of about 2300 meters above sea level and receives an annual rainfall of about 500 mm with an average annual temperature of 25^0C.The criteria used for selecting this sub zone and the village Tsaeda-Chrstian included accessibility, number of dairy farms and dairy cattle in the area.

Milk collection and sampling technique: Freshly-drawn morning cow's raw milk samples were collected from three dairy farms. Hand milking was used to collect milk directly into lean sterilized buckets and filtered for any extraneous matters and samples were obtained aseptically in labeled sterile containers. A total of 6 liters, 2 liters from each farm were collected. The samples were immediately placed in an ice box with ice packs and transported to the National Animal and Plant Health Laboratory (NAPHL).

Samples from different farms were mixed together in a clean and sterilized stainless steel bucket and allocated to 4 treatments with 3 replicates each. Each replicate was sampled and tested at a day's interval. The bulk milk (6 litres) after thoroughly mixing was divided into four parts (each 1.5 L). The first part was set as control (cont) while the other three parts were activated by addition of three combinations of sodium thiocyanate (NaSCN) as a source of thiocyanate ion (SCN^-) and sodium percarbonate ($2Na_2CO_3.3H_2O$) as a source of hydrogen peroxide in three treatments after about 3 hours after milking and kept at 30^0C**.** The three combinations LP1, LP2 and LP3 contained 10 and 20 mg/L, 14 and 30 mg/L and 18 and 40 mg/L of NaSCN and $2Na_2CO_3.3H_2O$, in the three treatments, respectively. After about one minute of thorough mixing, three levels of sodium percarbonate were added. The milk samples were then stirred with a clean stirrer for another 3 minutes to ensure that the sodium percarbonate was completely dissolved and the hydrogen peroxide was evenly distributed in the milk (IDF, 1988). The chemical and bacteriological analyses were carried out during the storage period at 30^0C. All the above procedures were carried out under a safety cabinet to avoid contaminations**.**

Chemical analysis: Pre-treatment analysis of the initial bulk milk quality was made after thoroughly mixing to get homogenous and representative samples (IDF, 1987), to test the milk samples for the required standard quality**.** Both the control and activated milk were sampled at interval of two hours for the quality and safety tests (pH, COB, TBC and alcohol tests). The density, chemical composition and sensory analysis were also carried out during the storage time. In all cases milk coagulation indicated quality deterioration or onset of spoilage and was considered to be a break point for shelf life. The pH was determined according to the method described by Davis (1959). Total bacterial count was estimated by miles misra technique (Quinn et al., 2002). For the alcohol stability test, an equal volume of 5 ml of milk was mixed with 5 ml of 68% alcohol (Hammer and Babel, 1957 and FAO, 1999).The chemical composition of milk samples was determined using Milk Lacto Scan (Lacto star Funk Gerber Firmware,) (IDF, 1987).

Physical test: The clot-on-boiling test was conducted by boiling 10 ml of milk in sterile aluminium petridish (Harding, 1995 and O` Connor 1995). Density and temperature was measured using Lacto density meter with the capacity of measuring the density from 1.015 to1.040 g/cm^3 (Pearson, 1972). Organoleptic test was also performed throughout the analysis or during the storage period. The samples were assessed for colour, flavour and appearance.

Statistical analysis: The data of the study were analyzed statistically using GEN-STAT software VSN international, 4^{th} edition using RCBD. Least

Significant Difference (LSD) was used to determine the differences between means. Coefficient of variation (CV) was also determined

RESULTS AND DISCUSSION

Pre treatment analysis of the quality of initial milk was made to test the milk samples for the required standard quality (Table 1). The samples were tested for pH, alcohol test, COB test and chemical composition. The result showed that milk samples had acceptable quality. Moreover, chemical composition and pH values were within the range reviewed by different authors (Pandey and Voskuil, 2011 and O'Connor, 1995).

Table1: Physico-chemical properties of cow`s raw milk samples

Parameter	Average values
PH	6.61 ±0.015
Alcohol test	-
COB test	-
Fat (%)	2.88 ± 0.085
Protein (%)	3.75 ± 0.035
Lactose (%)	5.34 ± 0.055
SNF (%)	9.88 ± 0.055
TS(%)	12.77± 0.031
Density	1.031 ± 0.001

SNF: solids not fat
TS: total solids
COB: Clot-on-boiling

The pH of raw cow's milk treated with sodium thiocyanate and sodium percarbonate at concentration of 10, 14 and 18 mg/L and 20, 30 and 40 mg/L respectively and kept at incubator (30^0C) are presented in Table 2. The activity of LP system was tested every 2 hours during the storage time. Highly significant ($P<0.001$) differences in pH values among different treatments was obtained. This could be due to the effect of the lactoperoxidase system. However, there was no significant difference for LP2 and LP3 during the entire storage period. This might be due to an increase in concentration for the effect of the system hence increases the keeping quality of the milk.

Table 2: pH variation among the different samples treated by LP activation and stored at 30^0C

Treatments	Time(hrs) after activation												
	0	2	4	6	8	10	12	14	16	18	20	22	24
LP1	6.60^{al}	6.59 al	6.58 alm	6.57 amn	6.56 amn	6.55 anop	6.54 anop	6.53 aop	6.52 bp	6.49 bq	6.32 br	6.12 bs	-
LP2	6.60^{al}	6.60 alm	6.59 almn	6.57 amno	6.57 ano	6.56 anop	6.55 aop	6.55 aop	6.54 abp	6.54 ap	6.50 aq	6.36 ar	-
LP3	6.61^{al}	6.61 al	6.59 alm	6.58 amn	6.57 amno	6.57 amno	6.56 anop	6.56 anop	6.55 aop	6.54 ap	6.51 aq	6.37 ar	-
Cont	6.60^{al}	6.59 alm	6.57 amn	6.56 am	6.55 an	6.55 an	6.50 bo	6.32 bp	6.10 cq	5.89 cr	5.70 cs	5.46 ct	-
	LSD=0.02555 ***								CV%=0.2				

Means followed by different superscript within the same column (a-c) and row (l-r)) are significantly ($P< 0.05$) different

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Treatments	LP1	LP2	LP3	Cont									
pH	6.50[b]	6.54[a]	6.55[a]	6.28[c]									
Time/hrs	0	2	4	6	8	10	12	14	16	18	20	22	24
pH	6.60[a]	6.60[a]	6.58[b]	6.57[bc]	6.56[c]	6.56[c]	6.54[d]	6.49[e]	6.43[f]	6.37[g]	6.26[h]	6.08[i]	-

Main Effect

LSD for treatment= 0.00738*** LSD for time =0.01278***

Means within the same row (a-i) followed by different superscript are significantly (P< 0.05) different

LP1: samples treated by activation of LP system level one (10+ 20 mg/l of sodium thiocyanate and percarbonate).
LP2: samples treated by activation of LP system level two (14+30 mg/l of sodium thiocyanate and percarbonate)
LP3: samples treated by activation of LP system level three (18+40 mg/l of sodium thiocyanate and percarbonate)
C: control ***: highly significant difference (P< 0.001) LSD: least significant difference CV: Coefficient of variation

The result revealed little change (no significant difference (P>0.005) in the pH values among the four treatments during the first 10 hours of storage. This could be due to the quality of initial milk. However, significant (P<0.05) variations were observed between the control and the activated samples, the fastest change being observed in the control. The result showed that the control remained fresh until the 12th hour of storage. There was an extension of 6 hours for the LP1 and 8 hours for LP2 and LP3. Similar results were reported by Kassa *et al*. (2013) and Fonteh *et al*. (2005). Shorter time were recorded by Saad *et al*. (2013) and Hamid and Musa (2013). This might be due to the difference in substrate concentrations and/ or storage temperatures.

In general, the LPs treated milk showed a slower drop of pH while the pH values of the LPs untreated milk dropped fast. This indicated that the LPs untreated milk showed quicker quality deterioration in terms of shelf life than the LPs treated milk. This is in line with the findings of Fonteh *et al*. (2005) who reported quicker pH drop of LPs untreated milk kept at ambient temperatures of 21-23^{o}C. The data showed a significant improvement (P<0.05) in the quality of the activated milk samples at 30^{0}C in comparison to the control during the storage period. This is in agreement with Hamid and Mussa (2013); Kassa*et al*. (2013) and Abdullah (2003) who reported that LPs treatment resulted in increased milk shelf-life. The control raw milk sample at the 12th hour had a mean pH of 6.50. However, significant (P<0.05) fall (5.46) was observed at the end of the storage time in the control. However, the pH of treated cow's milk at the end of the storage time was significantly higher than the control (6.32, 6.36 and 6.37 for LP1, LP2 and LP3, respectively). Although both the LP2 and LP3 were the best samples that produced similar results throughout the entire storage period, The LP2 (14+30 mg/l of sodium thiocyanate and percarbonate) was more preferable because increasing the level of the substrates concentration beyond this level did not bring about a significant improvement in the shelf life of raw cow`s milk.

Statistical analyses revealed highly significant (P<0.001) differences in total bacterial count (TBC) between different treatments (Table 3). However, no significant difference (P>0.05) were obtained between different treatments up to the 6th hour of storage moreover, no variation between LP2 and LP3 throughout the entire storage period. This might be due to the usefulness of applying LP system for preservation of raw milk, because of the bactericidal and bacteriostatic effect of lactoperoxidase system.

Table 3: The effect of LP activation on TBC (cfu/ml) of cow's raw milk stored at 30 ˚C

	Treatments			
Time/hrs.	LP1	LP2	LP3	Cont
0	1.3×10^{3am}	1.3×10^{3am}	1.1×10^{3am}	1.6×10^{3an}
2	$1.6\times10^{3\ am}$	$1.5\times10^{3\ am}$	$1.5\times10^{3\ am}$	$1.6\times10^{3\ an}$
4	$2.5\times10^{3\ am}$	$1.6\times10^{3\ am}$	$1.6\times10^{3\ am}$	$3.1\times10^{3\ an}$
6	4.1×10^{3bcm}	2.5×10^{3cm}	2.5×10^{3cm}	$1.3\times10^{4\ am}$
8	3.1×10^{4bl}	1.6×10^{4cl}	$1.5\times10^{4\ cl}$	1.6×10^{5al}
	LSD=$3.7669\text{x}10^3$ ***CV%=17.2			

Means followed by different superscript within the same column (l-n) and row (a-c) are significantly (P< 0.05) different

Main effect

Treatment	LP1	LP2	LP3	Cont	
TBC(cfu/ml)	8.1×10^{3b}	4.5×10^{3c}	4.4×10^{3c}	3.6×10^{4a}	
Time (hrs)	0	2	4	6	8
TBC(cfu/ml)	1.3×10^{3c}	1.6×10^{3c}	2.2×10^{3c}	5.6×10^{3b}	5.6×10^{3a}
	LSD for treatments=$1.6846\text{x}10^3$ ***LSD for time=$1.8834\ \text{x}10^3$***				

Means within the same row (a-c) followed by different superscript are significantly (P< 0.05) different
Legend as in table (2) above

From the microbiological point of view, results of the study showed that the average initial TBC in the milk was low, namely, $1.3\text{x}10^3$, $1.3\text{x}10^3$, $1.1\text{x}10^3$ and $1.6\text{x}\ 10^3$cfu/ml for LP1, LP2, LP3and C, respectively. This implied that the initial bacterial population (load) was low, reflecting the good hygienic conditions or practices under which the milk was collected. Whereas after 8 hours of storage the mean TBC values were $3.1\text{x}10^4$, $1.6\text{x}10^4$, $1.5\text{x}10^4$, and $1.6\text{x}10^5$cfu/ml for Lp1, Lp2, Lp3 and C, respectively. A considerable variation in the initial bacteriological quality of the milk samples, from $\leq10^3$/mL to 10^6/ ml was observed by Harnulv and Hamid, (1984). Higher microbial load was recorded by Abdullah (2003) where the initial mean TBC at 0 hour was 2.5×105/ml. Various reports, (Saad*et al*., 2013; Kassa*et al*., 2013 and CAC, 2011a) have also showed that good hygienic practices in milk production are critical to the efficacy of the LPs and to the microbiological quality of the milk. Moreover, Fonteh *et al*. (2005) concluded that the length of LPs treated milk shelf-life and its acid developing rate depended on its initial quality. Similarly, Harnulv and Hamid (1984) cited that if the bacteriological quality of the milk was good when it was stabilized, a considerable extension of its keeping quality could be achieved. LP system treated milk under the storage condition of incubator (30^oC) remained fresh for more hours than LPs untreated milk under alcohol test as quality indicator. The control sample was the first to get spoiled for the alcohol test at the 14^{th} hour of storage (Table 4). Thereafter, the treated samples progressively get spoiled with increasing concentration, the LP2 and LP3samples being the last to test positive. Both the LP2 and LP3 samples produced similar results during the entire storage period. The data showed that LP1 remained fresh for an additional 6 hours and the LP2 and LP3 samples both were the last to spoil after the 20^{th} hour of storage. All the treatments were spoiled by the 22^{nd} hour of storage. Nearly similar results were reported by Fonteh *et al*. (2005) whostated that during activation of raw

milk with different levels of thiocyanate and percarbonate the milk in all treatments remained fresh during the first 12 hours but the control was spoiled by the 15th hour. The LPs treated milkkept fresh for 6 more hours than LPs untreated milk. Alcohol stability test indicated spoilage slightly earlier than with the clot-on-boiling test. These observations support the view that the alcohol stability test is more sensitive than the clot-on-boiling test in detecting milk acidity. This finding was in agreement with that recorded by Kassa*et al*. (2013), Fonteh*et al*. (2005), Kumar and Mathur (1989) and Harnulv and Kandasamy (1982).

Table 4: **The average response to alcohol stability test of LPs activated samples at 30^0C**

Treatments	Time/hrs after activation												
	0	2	4	6	8	10	12	14	16	18	20	22	24
LP1	-	-	-	-	-	-	-	-	-	-	+	+	+
LP2	-	-	-	-	-	-	-	-	-	-	-	+	+
LP3	-	-	-	-	-	-	-	-	-	-	-	+	+
Cont	-	-	-	-	-	-	-	+	+	+	+	+	+

A positive test is recorded if the sample clots up on mixing with alcohol
LP1: samples treated by activation of LP system level one (10+ 20 mg/l of sodium thiocyanate and percarbonate)
LP2: samples treated by activation of LP system level two (14+30 mg/l of sodium thiocyanate and percarbonate)
LP3: samples treated by activation of LP system level three (18+40 mg/l of sodium thiocyanate and percarbonate) C: Control:

The study showed that the samples of cow's milk treated by LPs and stored at 30^0C showed longer shelf life than untreated (control) milk. Clot-on-boiling test (COB) detected milk deterioration 2 hours later than the other tests used, for both LPs treated and untreated milk samples (Table 5). A similar observation was reported by Abdullah (2003) and Bjorck *et al*. (1975). Likewise, Harnulv and Hamid (1984) also stated that on average, spoilage according to the COB test was recorded more than two hours later than spoilage according to the 10 minute resazurin test. The control sample remained fresh for 14 hours. However, the treated milk samples remained fresh for additional 6 to 8 hours, this is in line with Kassa *et al*. (2013) and Fonteh *et al*. (2005) that under room temperature storage condition (21.5-22.5^{o}C) the LPs treated milk remained fresh for an additional 7.5 and 8 hours than the LPs untreated milk using alcohol test and clot-on-boiling test as a quality indicator, respectively. This implied that LPs treatment improved the keeping quality of raw uncooled milk. A progressive increase in shelf life with increasing substrate concentration was observed in the treated samples when the clot-on-boiling test was employed and this agreed with Fonteh (2005). The results presented in (Table 6) showed that the longest shelf life was observed in Lp2 and Lp3 samples which remained fresh beyond 22 hours of storage at incubation temperature of 30^0C. Using the same test, Kumar and Mathur (1989) and Harnulv and Kandasamy (1982) showed that by activating with 25:15 ppm (thiocyanate: peroxide), buffalo milk could be preserved for 13 hours at 18^{o}C whereas the shelf life could be extended to 19 hours if the substrate concentration was increased to 70:30 ppm.

Table 5: The average response to COB test of LPs activated cow`s raw milk samples at 30^0C

Treatments	Time/hrs after activation												
	0	2	4	6	8	10	12	14	16	18	20	22	24
LP1	-	-	-	-	-	-	-	-	-	-	-	+	+
LP2	-	-	-	-	-	-	-	-	-	-	-	-	+
LP3	-	-	-	-	-	-	-	-	-	-	-	-	+
Cont	-	-	-	-	-	-	-	-	+	+	+	+	+

A positive test is recorded if the sample coagulates up on boiling

LP1: samples treated by activation of LP system level one (10+ 20 mg/l of sodium thiocyanate and percarbonate).

LP2: samples treated by activation of LP system level two (14+30 mg/l of sodium thiocyanate and percarbonate) LP3: samples treated by activation of LP system level three (18+40 mg/l of sodium thiocyanate and percarbonate)

C: Control

The chemical composition of cow's raw milk samples treated by activation of LP system and stored at incubation (30^oC) was evaluated (Table 6). Statistical analyses showed that there were no significant ($P>0.05$) differences in fat, protein, lactose, solids not fat, total solids and density contents of all milk samples. These results are in agreement with Hamid and Musa (2013) who found out that raw milk activated by LP system showed no significant difference in the density, fat, total solid and protein of all the milk samples stored at 37^oC. Similarly Seifu *et al*. (2004) and Ndambi *et al*. (2008) concluded that the preservation of cheese milk by the LP system can be used to improve the microbiological quality and flavour of cheese without any detrimental effect on the gross chemical composition of the cheese.

Table 6: Effect of LPs on the chemical composition of cow`s raw milk samples at 30^0C

Treatments	Chemical composition (%)					
	Fat%	Protein%	Lactose%	SNF%	T. S%	Density
LP1	2.87	3.75	5.33	9.88	12.74	1.031
LP2	2.86	3.77	5.37	9.94	12.81	1.031
LP3	2.87	3.76	5.35	9.92	12.79	1.031
Cont	2.87	3.74	5.32	9.84	12.72	1.031
LSD	0.1630 ^{NS}	0.0659 ^{NS}	0.1262 ^{NS}	0.1964 ^{NS}	0.1403 ^{NS}	0.001087 ^{NS}
CV%	3	0.9	1.3	1.1	0.6	0.1

No significant difference was observed among the samples

LP1: samples treated by activation of LP system level one (10+ 20 mg/l of sodium thiocyanate and percarbonate)

LP2: samples treated by activation of LP system level two (14+30 mg/l of sodium thiocyanate and ercarbonate)

LP3: samples treated by activation of LP system level three (18+40 mg/l of sodium thiocyanate and percarbonate)

Cont: Control SNF: solid not fat T. S: total solids NS: no significant differences

Likewise, Ponce (2010), FAO/WHO (2006), Fernandez (2005) and Jooyandeh *et al*. (2011) also reported that the LP system did not induce any significant adverse effects on the chemical, physical or sensory characteristics of raw milk and processed dairy products. This might indicate that the lactoperoxidase system was an acceptable chemical method for raw milk preservation, hence the present study supported the previous recommendations given by El Zubeir *et al*. (2006), Saad *et al*. (2013) and Ndambi *et al.* (2008) that LPs could be a method of milk preservation under tropical, hot environments, especially for rural farmers that lack the refrigeration facilities.

CONCLUSION

Activation of LP system by adding different concentrations of sodium thiocyanate and sodium percarbonate enhanced the keeping quality and the shelf life of raw cow`s milk. The LP2 (18+30mg/L sodium thiocyanate and 20+40mg/L and sodium percarbonate) was the best concentration. Therefore it can be a practical application in preservation of milk. It has no significant effect on milk chemical constituents and this would support its application in dairy industry. This method of milk preservation may be used to encourage dairy farming by increasing collection of high quality milk which in return is a prerequisite for increasing of high quality dairy products manufacturing.

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