INFLUENCE OF LACTATION STAGE AND STORAGE TEMPERATURE ON THE ACTIVITY OF LACTOPEROXIDASE ENZYME SYSTEM ON MICROBIAL LOAD OF RAW COW'S MILK

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Abstract
This study was carried out to investigate the effect of using the lactoperoxidase enzyme system (LPS) on improving the keeping quality and increasing the shelf life of raw milk. Fresh milk samples were obtained after morning milking from Butana cows at different stages of lactations. The milk samples were cooled immediately before transported to the laboratory, where they were divided into two groups (control and treated), then each group was subdivided into four sub-groups and kept at 5±2°C, 13±2°C, 25±2°C and 37±2°C temperatures. All samples were subjected to microbial examinations (total bacterial count, psychrotrophic bacterial count, coliform count and yeast and mould counts), acidity and clot-on-boiling test during the period of storage. The results showed significant ($P<0.05$) variations between the control and LPS treated milk samples on total bacterial count, psychrotrophic bacterial count, coliform count and yeast and mould counts. Moreover the data revealed that cows' milk samples from different stages of lactation treated with LPS showed longer shelf life and better keeping quality during all storage degrees of temperature compared with the controlled samples under the same conditions. The study concluded that although the LPS activity is affected by the storage temperature of the milk, it is useful in prolonging the shelf life of raw milk. Hence it could be applied in hot areas like Sudan in order to utilize the huge quantity of the milk produced in the traditional systems.

Key words: Lactoperoxidase enzyme system, cow's milk, microbial load, keeping quality, stages of lactation, storage temperature.

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1. INTRODUCTION

As milk contains all the required nutrients for growth, contaminating bacteria if once get access into the milk, could rapidly multiply and render it unsuitable for further processing and/or unfit for human consumption (Getachew, 2003). 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 (Kamau *et al*., 2010). The activity of the LP-system varies depending on the milk source (Thakar and Dave, 1986). The activated LPS is effective in raw milk of different species (CAC, 2004). Ndambi *et al*. (2008) reported that LPS is an acceptable chemical method for raw milk preservation, especially in rural areas when refrigeration facilities are absent to farmers. Seifu *et al*. (2005) and El Zubeir *et al*. (2006) reported that LPS can be used as a sufficient procedure for keeping milk in farm and areas where the cooling facilities are absent. El Zubeir (2012) evaluated the hygiene quality of raw cow milk after preservation with LPS and found that the additin of lactoperoxidase prolongs the shelf life of milk. This effect being more pronounced upon storage at 8°C than 30° C. It also caused a delay in clot on boiling test

FAO (2013) reported that adding a pre-packaged activator containing thiocyanate and a source of hydrogen peroxide such as sodium percarbonate, activates and extends the effects of the natural lactoperoxidase system in raw milk. Where refrigeration is not possible, this addition increases the acceptable quality of raw milk for about 24 hours at 15°C or between 6 and 8 hours at 30°C, allowing smallholders sufficient time to store and/or transport it to a central depot for processing. Elien *et al*. (2001) reported that preservative effect of LPS on buffalo and cow's milk at 30° C and 8°C caused a considerable slowing down in the rate of increase in titrable acidity (TA) during

storage. They added that the total bacterial counts decreased sharply after 2 hours of activation and increased slowly during the rest of storage.

Augusta and Andrea (2010) analyzed lactoperoxidase activity in individual milk samples of 14 Friesian cows at 2, 14, 30, 90 and 180 days postpartum. Enzyme activity decreases from 0.75 U/ml in samples taken 2 days post partum to 0.36 U/ml 180 days postpartum. Lactoperoxidase activity was higher when the analysis was performed 6 hours after milking (0.40 U/ml) compared to 48 hours (0.15 U/ml). Variation among cows was also large ranging from 0.54-0.94 U/ml on a specific sampling day. Such variations indicate that the efficiency of enzyme in preserving milk is affected by many factors including stage of lactation, individual animal and sampling day. Hence the present study was done to study the effect of LPS from Butana cows with different parities and stages of lactation and to correlate the preservation of LPS on the shelf life of Butana cows' raw milk in relation to storage temperature.

2. MATERIALS AND METHODS

2.1 Source of samples

Samples of milk were obtained at morning from traditional farm in Shambat area around the Faculty of Animal Production University of Khartoum. The milk samples (36 samples) were collected separately from each animal; the udder was cleaned carefully and dried with clean towels. The samples were preserved in clean, sterilized containers and the pH was determined immediately after milking.

2.2 Experimental procedure

The milk samples were divided into two equal portions, one portion was preserved with the lactoperoxidase enzyme system (LPS) that was obtained from Ministry of Animal Resources, offered by the FAO for field trail of the LPS in Sudan, while the second was kept as control. Each portion was further subdivided into four equal groups, the first was kept at 37°C, the second was kept at 25°C, the third was kept at 13°C and the fourth was kept at refrigerator (5±2° C).

2.3 Examination of the samples

Microbiological examination such as preparation of serial dilutions, preparation of the media were carried out by Houghtaby *et al*. (1992), while sterilization of equipments was done as described by Marshall (1992). The milk samples were subjected to determine the total viable bacterial count according to Houghtaby *et al*. (1992). The coliform count was done as described by Harrigan and MacCane (1996) and yeast count and psychotropic bacterial count were estimated according to Frank *et al*. (1992. Acidity was determined according to American Public Health Association (1976) while clot–on-boiling test was determined according to IDF (1990). They were examined daily to assess the shelf life of the milk samples.

3. RESULTS AND DISCUSSION

The study showed that lactoperoxidase enzyme system (LPS), degrees of temperature and stages of lactation were affected significantly (P≤0.05) microbial load (total viable bacterial count, coliform count, yeast count and psychotropic bacterial count) of Butana milk samples kept at (5±2°C, 13±2°C, 25±2°C and 37±2°C).

The effect of lactoperoxidase enzyme system (LPS), degrees of temperature and stages of lactation on the total bacterial count (TBC) of Butana cattle's milk samples were affected significantly (P≤0.05) by LPS, storage degrees of temperature and stages of lactation (Table 1).

The means and standard errors of log total bacterial count (TBC) of LPS treated cattle's milk samples at early, medium and late stages of lactation that stored at 5±2°C were 5.01±0.32, 5.01±0.26 and 5.05±0.23 respectively, while the non-treated milk samples under the same degree of temperature and stages of lactation revealed by 5.15±1.44, 5.19±0.25 and 5.17±0.24, respectively (Table 1). The result showed that samples kept at 5±2°C provide remarkable effect of decreasing

log total bacterial count (TBC), while the control samples that kept at 37±2°C contain higher load of total bacterial count compared with the LPS treated samples stored at the same degree of temperature (Table 1). These results were in line with the results of Masud *et al*. (2010) and Hamid and Mohamed (2013) who reported that activated milk samples by the LPS extended the shelf life of milk by at least 8 hours at incubation temperature (37°C). The result also agreed with Rasbawati *et al*. (2014) reported that lactoperoxidase system decreasing total bacteria from 8 log cfu/ ml to 5 log cfu/ ml.

The means and standard errors of log psychrotrophic, coliform and yeast and mould counts of LPS treated Butana cattle milk samples were affected significantly (P≤0.05) by addition of LPS, storage degrees of temperature and stages of lactation (Table 1, 2, 3 and 4). Similarly Assah *et al*. (2007) found that LPS reduced the microbial load in milk stored under ambient temperature by more than one log cycle, after 8 hours of storage.

The LPS extended the shelf life of raw milk by an average of 9-10 days at 5±2°C, 8-9 days at 13±2°C, 8-7 days at 25±2°C and 1.00-1.17 days at 37±2°C (Table 5). A maximum difference in the shelf life was noticed between treated milk stored at 5±2°C, 13±2°C, 25±2°C and 37±2°C compared to the control samples. Refrigeration alone could store fresh milk in good quality for about two days (42 - 60 hours). Meanwhile, a combination of refrigeration and LPS could preserve fresh milk for up to one week (9-10 days). This was also proven by the finding obtained by Kamau *et al*. (2010) and Baulares *et al*. (2011) who reported that by activation of the LPS in raw milk, it is possible to store ovine, bovine and caprine milk at 4°C for several days. Similarly El Zubeir (2012) reported 9 days and 3 days at refrigeration and room temperature respectively, for camel milk. Also Saad *et al*. (2013) showed highly significant (P≤ 0.001) effect of LPS on the shelf life, acidity and microbial content of sheep milk.

Table (1): Effect of LPS enzymes, temperature and lactation stages on log total bacterial count of cattle's milk

Temperature (°C)	Control milk			LPS milk		
	Lactation stages					
	1st	2nd	3rd	1st	2nd	3rd
5±2°C	5.15±1.44^{gh}	5.19±0.25^{g}	5.17±0.24^{g}	5.01±0.32^{i}	5.01±0.26^{i}	5.05±0.23^{i}
13±2°C	5.22±0.21^{f}	5.21±0.33^{f}	5.24±0.21^{e}	5.09±0.26^{h}	5.09±0.21^{h}	5.10±0.20^{h}
25±2°C	5.39±0.17^{c}	5.35±0.18^{d}	5.41±0.21^{c}	5.16±0.18^{gh}	5.16±0.21^{gh}	5.17±0.08^{g}
37±2°C	5.59±1.84^{a}	5.53±0.02^{b}	5.57±0.23^{a}	5.33±0.18^{d}	5.34±0.25^{d}	5.34±0.22^{d}
P-value	0.0341*					
SE±	0.0062					

Values are mean±SD.
The means sharing the same superscript letters are not significantly (P>0.05) different according to DMRT.
SE± ≡ Experimental standard error; P-value ≡ Level of significance (probability)

Table (2): Effect of LPS enzymes, temperature and lactation stages on log psychrotrophic bacterial count of cattle's milk

Temperature (°C)	Control milk			LPS milk		
	Lactation stages					
	1st	2nd	3rd	1st	2nd	3rd
5±2°C	4.85±0.44^{h}	4.89±0.45^{g}	4.90±0.45^{ef}	4.43±0.43^{h}	4.45±0.45^{h}	4.44±0.53^{h}
13±2°C	5.03±0.22^{bc}	4.98±0.28^{d}	5.00±0.15^{bc}	4.67±0.26^{j}	4.67±0.39^{j}	4.68±0.21^{j}
25±2°C	5.05±0.16^{a}	5.04±0.17^{b}	5.06±0.46^{b}	4.80±0.22^{i}	4.78±0.27^{ij}	4.79±0.77^{ij}
37±2°C	5.50±1.76^{a}	5.51±0.15^{a}	5.54±0.10^{a}	4.94±0.21^{e}	4.92±0.20^{e}	4.98±0.16^{d}
P-value	0.0491*					
SE±	0.0085					

Values are mean±SD.
The means sharing the same superscript letters are not significantly (P>0.05) different according to DMRT.
SE± ≡ Experimental standard error; P-value ≡ Level of significance (probability)

Table (3): Effect of LPS enzymes, temperature and lactation stages on coliform count of cattle's milk

Temperature (°C)	Control milk			LPS milk		
	Lactation stages					
	1st	2nd	3rd	1st	2nd	3rd
5±2°C	4.65±0.49^{h}	4.64±0.28^{h}	4.61±0.34^{hi}	4.41±0.23^{j}	4.43±0.25^{j}	4.48±1.39^{i}
13±2°C	5.07±0.53^{de}	5.09±0.14^{d}	5.18±0.12^{c}	4.69±0.49^{g}	4.68±0.19^{g}	4.68±0.13^{g}
25±2°C	5.27±0.21^{b}	5.24±0.23^{b}	5.25±0.24^{b}	4.88±0.55^{f}	4.91±0.16^{f}	4.89±0.16^{f}
37±2°C	5.42±0.77^{a}	5.44±0.15^{a}	5.45±0.58^{a}	5.05±0.39^{e}	5.01±0.14^{e}	5.07±0.13^{de}
P-value	0.0396*					
SE±	0.0047					

Values are mean±SD.
The means sharing the same superscript letters are not significantly (P>0.05) different according to DMRT.
SE± ≡ Experimental standard error
P-value ≡ Level of significance (probability)

Table (4): Effect of LPS enzymes, temperature and lactation stages on yeast count of cattle's milk

Temperature (°C)	Control milk			LPS milk		
	Lactation stages					
	1st	2nd	3rd	1st	2nd	3rd
5±2°C	2.63±2.04^{f}	2.43±2.12^{h}	2.58±2.25^{fg}	2.06±2.03^{i}	1.37±1.84^{k}	1.86±2.01^{j}
13±2°C	2.79±2.55^{e}	2.81±2.17^{e}	2.80±2.16^{e}	2.18±2.40^{h}	2.50±2.03^{g}	2.29±2.01^{j}
25±2°C	2.92±2.25^{c}	2.95±2.28^{bc}	2.90±2.23^{c}	2.27±2.49^{j}	2.60±2.02^{f}	2.38±2.61^{i}
37±2°C	2.99±2.25^{b}	3.18±1.94^{a}	2.962.22^{b}	2.85±1.88^{d}	2.86±1.89^{d}	2.88±1.91^{d}
P-value	0.0463*					
SE±	0.00527					

Values are mean±SD.
The means sharing the same superscript letters are not significantly (P>0.05) different according to DMRT.
SE± ≡ Experimental standard error
P-value ≡ Level of significance (probability)

Table (5): Mean±SD of shelf-life (days) of cattle's milk as affected by addition of LPS enzymes

Temperature (°C)		Control milk			LPS milk		
		Lactation stages					
		1st	2nd	3rd	1st	2nd	3rd
5±2°C	Range (days)	7 - 8	7 – 8	7 - 8	9 - 10	9 - 10	9 – 10
	Mean±SD	7.21±4.68	7.19±4.68	7.21±4.68	9.16±4.08	9.55±3.49	9.06±3.85
13±2°C	Range (days)	6 - 7	6 - 7	6 – 7	8 - 9	8 - 9	8 – 9
	Mean±SD	6.13±4.69	6.00±4.58	6.13±4.69	8.19±4.21	9.11±4.04	8.81±4.22
25±2°C	Range (days)	5 - 6	5 - 6	5 - 6	7 - 8	7 - 8	7 – 8
	Mean±SD	5.83 ±421	5.59 ±4.12	5.81 ±4.22	7.0 ±4.02	7.6 ±4.38	7.1 ±4.06
37±2°C	Range (days)	0.29-0.38	0.29-0.38	0.29-0.38	1.00-1.17	1.00-1.17	1.00-1.17
	Mean±SD	0.34±0.02	0.34±0.18	0.30±0.60	1.043±0.09	1.13±0.01	1.01±0.02

Values are mean±SD.
The means sharing the same superscript letters are not significantly (P>0.05) different according to DMRT.
SE± ≡ Experimental standard error
P-value ≡ Level of significance (probabili

During storage, the titratable acidity (which indicates degradation by lactic acid bacteria) of treated milk was always lower than that of control milk samples, though they had the same initial acidity (Table 6). Similarly Hamid and Mohamed (2013) demonstrated that there were significant (P<0.01) variation in titratable acidity among the treatments, which is due to the action of lactoperoxidase system. Also Kamau *et al*. (2010) reported that acidity of cow's milk treated with LPS was lower than the control samples. Moreover Elien *et al*. (2001) demonstrated that preservative effect of LPS buffalo and cow's milk at 30°C and 8°C caused a considerable slowing down in the rate

of increase in titratable acidity (TA) during This effect being more pronounced upon storage at 8°C than 30°C. It also caused a delay in clot on boiling test. Total bacterial counts decreased sharply after 2 hours of activation and increased slowly during the rest of storage as they reported. This result is supported that reported previously by Seifu *et al*. (2005) and El Zubeir *et al*. (2006), they reported that LPS can be used as a sufficient procedure for keeping milk in farm and areas where the cooling facilities are absent.

Table (6): Effect of LPS enzymes and storage temperatures on acidity of cattle's milk

Temperature (°C)	Control milk	LPS milk
5±2°C	0.52±0.67^{d}	0.23±0.06^{g}
13±2°C	0.62±2.25^{c}	0.38±0.05^{f}
25±2°C	0.71±5.88^{b}	0.47±0.02^{e}
37±2°C	1.27±8.65^{a}	0.72±0.02^{b}
P-value	0.0391*	
SE±	0.0067	

Values are mean±SD.
The means sharing the same superscript letters are not significantly (P>0.05) different according to DMRT.
SE± ≡ Experimental standard error
P-value ≡ Level of significance (probability)

4.CONCLUSION

The result encouraged the use of lactoperoxidase enzyme system (LP-system) in preservation of raw milk as it has been found useful in extending the shelf life of milk. Adequate management schemes at the level of production, processing and marketing should be applied alongside the lactoperoxidase enzyme system for a better dairy development in rural areas of Sudan.

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