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Impact of the Lactoperoxidase System on Activity of Selected Lactic Starter Cultures in Camel Milk

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ABSTRACT

Sensitivity of lactic starter cultures to the lactoperoxidase (LP)-system (LPS) was investigated by monitoring acid production by mesophilic, thermophilic and *suusac* (a traditional Kenyan fermented camel milk product) starter cultures in both LPS-treated and -untreated camel milk. Camel milk samples from three different production sites in Kenya – Kajiado, Isiolo and Nanyuki Districts – were analysed for their thiocyanate concentration. Inoculation with starter was done after 0, 4 and 8 hours of storage of LP-activated samples. Natural concentration of thiocyanate occurring in the camel milk from the three sites ranged from 9.7 to 36.4 mg/l. No addition of thiocyanate was therefore necessary to activate the LPS. There was a significant slowdown in acid development in the raw camel milk inoculated immediately after activation in all three starter cultures. Holding of LPS-activated milk for 8 hours prior to inoculation reduced inhibition of lactic acid production by the starter cultures. Camel milk preserved using this method could therefore support satisfactory mesophilic and thermophilic starter culture activity if the raw camel milk is held for up to 8 hours during storage at 30°C prior to processing. Heat treatment reduced starter inhibition by the LPS for the mesophilic and thermophilic starter cultures when LPS activation, heat treatment and inoculation followed each other sequentially. However, for *suusac* starter inhibition by the LPS for non-activated samples, those activated and inoculated after 0 and 4 hours was significant but non-significant for those inoculated after 8 hours of storage. The inhibitory effect of the LPS on mesophilic and thermophilic starter culture activity in heat-treated camel milk is apparently reactivated and increases with time of preservation of the raw camel milk by the LPS.

Keywords: lactic acid production, mesophillic, *suusac*, thermophillic, thiocyanate
Abbreviations: HTST, high temperature short time; **LPS**, lactoperoxidase system

INTRODUCTION

In fermented product manufacture, the time taken to reach the desired pH in the vat is critical for the manufacturer. Milk contains a number of antimicrobial systems designed to confer passive immunity to the calf and to provide protection to the interior of the mammary gland. These include immunoglobulins, lysozymes, and lactoferrin among other enzymes that bind vitamin B_{12}, folate and riboflavin. Their inhibitory effects on starter cultures are however eliminated if the milk is pasteurized. Of great importance is lactoperoxidase (LP; EC 1.11.1.7), which does survive pasteurization and is known to be bactericidal (Hoogendoom *et al*. 1977). The LP enzyme has been shown to be active after 30 min of pasteurization at 95°C in bovine milk (Stadhouders and Beumer 1992). LP also retains about 70% activity after HTST (72°C for 15 sec) pasteurization (Barret *et al*. 1999). Thus the availability of LP is not a limiting factor for the system to function in the fermentation vat.

The LP-thiocyanate-hydrogen peroxide system (LP-system, or LPS) has been reported to be a feasible method for the temporary preservation of raw milk (Gaya *et al*. 1991). LP catalyses the oxidation of thiocyanate by hydrogen peroxide to intermediate reaction products that have an antimicrobial effect (Reiter 1985). The activity of this system, however, varies from species to species (Wolfson and Sumner 1993). Elagamy *et al.* (1996) estimated the molecular weights of purified camel milk lysozyme, lactoferrin and LP at 14.4, 79.5 and 78 Kda, respectively while for bovine milk corresponding values were 14.4, 76 and 72.5 Kda, respectively. The concentration of camel milk lysozyme (15 µg 100 mL^{-1}) was higher than that in bovine milk (7 µg 100 mL^{-1}).

Bovine LPS for temporary preservation of raw bovine milk has been widely studied and documented (Pruit and Tenovuo 1981). However, the impact of preservation of camel milk by the LPS prior to manufacture of products from such preserved milk has not been studied.

The camel (*Camelus dromedarius*, one–humped camel, dromedary) is uniquely adapted to hot and arid environments. It is most popular in the arid areas of Africa, particularly in the arid lowlands of Eastern Africa, i.e. in Somalia, Sudan, Ethiopia, Kenya and Djibouti for milk production. There are approximately 11.4 million camels in this region and these represent over 60% of the world's population of camels (Farah *et al*. 2007).

Traditional camel milk is consumed in a fresh or sour state. Sour milk production involves the use of mesophilic, thermophilic starters or spontaneous fermentation to produce *suusac* (Farah *et al*. 2007). The milk is either inoculated directly without heat treatment in *suusac* for spontaneous fermentation, or heat treated at 85°C for 30 minutes prior to inoculation for other starter cultures (Farah *et al.* 1990).

The combination of poor hygienic standards, high ambient temperatures and lack of refrigeration facilities render camel milk very much susceptible to spoilage due to common lactic acid bacteria (Farah *et al*. 2007). The introduction of the LPS to the camel milk trade may prolong the keeping quality of camel milk (Farah *et al*. 2007).

As a prerequisite to this, it is of importance to evaluate the effect of this preservation method on fermented camel milk products. This study was therefore conducted to investigate the effect of the LPS on the activity of selected starter cultures in camel milk.

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MATERIALS AND METHODS

Sampling and materials

Camel milk

Morning pooled camel milk samples were obtained from either Isiolo, Kajiado or Nanyuki Districts in Kenya. The milk was transported to the laboratory within 4 hours in an insulated cool box containing ice packs. After delivery to the laboratory, initial chemical and microbial analysis was done and experimentation began immediately thereafter.

Starter cultures and their propagation

Starter cultures

Thermophilic (YC-X11 YO-FLEX) and mesophilic (CHN-22-aromatic) starter cultures were obtained from Chris Hansen Laboratorium, Denmark. *Suusac* culture was obtained from samples of fermented milk collected from Isiolo District in sterile sample bottles and transported to the laboratory in an insulated cool box containing ice packs for propagation.

Propagation of pure cultures

The cultures were propagated in 10% (w/v) high heat skim milk reconstituted in distilled water. Fifty milliliters of this substrate in 100 ml conical flasks plugged with cotton wool was heat treated at 90°C for 15 min. Sufficient number of such units were prepared and stored in a refrigerator (5-6°C) until needed. Cultures were propagated using 2% inoculum of 18 h culture and incubated at 32°C until they were able to produce the desired acidity.

Analytical reagents

Technical sulphuric acid, amyl alcohol, resazurin, *p*-phenylenediamine, hydrogen peroxide, sodium thiocyanate, silver nitrate, sodium hydroxide and phenolphthalein were obtained from E. Merck, Darmstadt (Germany). Ferric nitrate reagent and trichloroacetic acid were procured from SORBO® Laboratories and BDH Chemicals Ltd., Poole, Dorset (England), respectively.

Analytical methods

Butter fat, titratable acidity, viable counts, resazurin test, total solids and LP were determined according to standard methods of the American Public Health Association (APHA 1992).

Thiocyanate content

Milk samples were analysed for thiocyanate as outlined by Partanen *et al*. (1998), and modified as suggested by the Codex Alimentarius Commission (1990).

Effect of residual LPS on production of lactic acid by starter cultures in raw and heat-treated camel milk

The LPS was activated by adding 1 ml of a freshly prepared solution of 850 ppm H_2O_2, to 100 ml of raw camel milk in screw-capped tubes. Controls consisted of untreated camel milk samples. Two milliliters of the lactic starter culture was added followed by incubation at 32°C. Acidity was determined at 2-h intervals for up to 12, 18 and 22 h for thermophilic, mesophilic and *Suusac* cultures, respectively in both LPS-activated and control samples.

To observe the effect of heat treatment prior to inoculation, LPS-activated samples were first heated at 85°C for 30 min and cooled to 30°C. The samples (100 ml) were then inoculated with 2 ml of the lactic starter cultures. Incubation and acidity determination followed a similar protocol as for raw milk trials.

Experimentation for both raw and heat treated camel milk was repeated after 4 and 8 h of preservation of raw milk samples using the LPS to assess changes in the effect of the residual LPS on starter culture activity with time of storage.

Statistical analysis

Acidity was expressed as percent lactic acid and analysed for variance using Genstat Statistical software (Lawes Agricultural Trust., Rothamsted Experimental Station., 7th Edn). Analysis of variance (ANOVA) was performed to establish relationships between LPS activation, time prior to inoculation, and incubation time. Independent variables included LPS and time of storage as well as treatment-time interactions in the appropriate experiments. Means and standard deviations were calculated and, when F-values were significant, mean differences were separated by the least significant difference procedure.

RESULTS

The thiocyanate content of the camel milk samples from Isiolo, Nanyuki and Kajiado were 9.74 ± 0.39, 15.88 ± 1.24 and 32.9 ± 3.54 mg/l, respectively. No additional amount of thiocyanate was therefore used to activate the LPS.

Effect of LPS on mesophilic starter culture in camel milk

Table 1 shows the effect of the activated LPS on acid production by mesophilic starter culture in raw camel milk. LPS activation prior to inoculation resulted in a significant slowdown in the rate of lactic acid development in raw camel milk activated and inoculated immediately and after 4 h of storage. However after 8 h of storage of the LPS-activated raw milk, there was a reduction in the inhibitory effect on lactic acid production by the LPS.

ANOVA showed a highly significant effect ($p<0.001$) of LPS activation and storage time at inoculation on inhibition of lactic acid production. Effect of time at inoculation on the inhibition of lactic acid production by mesophilic starter was also significant ($p<0.001$) The inhibitory effect of LPS

Table 1 Effect of LP-system on mesophillic starter culture activity in raw camel milk

Incubation time (h)	% Lactic acid[a]			
	Control	Inoculation time interval (h) after activation		
		0	4	8
0	0.12 ± 0.016	0.12 ± 0.013	0.12 ± 0.012	0.12 ± 0.013
2	0.26 ± 0.014	0.24 ± 0.017	0.23 ± 0.015	0.27 ± 0.017
4	0.32 ± 0.012	0.25 ± 0.011 b	0.25 ± 0.010	0.32 ± 0.009
6	0.38 ± 0.014	0.30 ± 0.014 b	0.31 ± 0. 013	0.38 ± 0.014
8	0.48 ± 0.015	0.41 ± 0.012 b	0.47 ± 0. 011	0.46 ± 0.012
10	0.58 ± 0.014	0.49 ± 0.014 b	0.46 ± 0.016 b	0.54 ± 0.014
12	0.67 ± 0.013	0.62 ± 0.015 b	0.49 ± 0.017 b	0.69 ± 0.015
14	0.75 ± 0.017	0.70 ± 0.014 b	0.54 ± 0.016 b	0.78 ± 0.013
16	0.83 ± 0.014	0.80 ± 0.016 b	0.61 ± 0.018 b	0.89 ± 0.020
18	0.92 ± 0.011	0.90 ± 0.014 b	0.67 ± 0.016 b	0.92 ± 0.014

[a] Each value is a mean of three replicate experiments with three titrations each (n=9).
[b] Data with different letter within a column significantly lower than the control ($p<0.001$).

activation as compared to the control was significant at inoculation time 0 h ($p<0.001$) and 4 h ($p<0.01$) and not significant ($p>0.05$) on inoculation after 8 h storage of LPS-activated raw camel milk.

Table 2 shows the inhibitory effect of LPS activation on starter culture activity in camel milk after heat treatment prior to inoculation. Heat treatment of the LPS-activated camel milk reduced inhibition of lactic acid production by mesophilic starter culture when heat treatment and inoculation followed immediately and 4 h after activation. However, there was a reduction in lactic acid production by the LPS, when heat treatment and inoculation were done after 8 h of storage of the LPS-activated raw camel milk. ANOVA showed a highly significant effect ($p<0.001$) of LPS activation and storage time at inoculation on inhibition of lactic acid production. However, the effect of LPS activation on the starter culture activity was highly influenced by the time of inoculation ($p<0.001$). There was a non-significant inhibitory effect ($p>0.05$) of LPS activation compared to the control at time 0 and 4 h and a significant effect ($p<0.001$) after 8 h of storage.

Effect of LPS on thermophilic starter culture in camel milk

Table 3 shows the effect of LPS activation, in raw camel milk and on thermophilic starter culture activity. LPS activation resulted in a significant slowdown in acid development in raw camel milk when activated and inoculated after 0, 4 and 8 h of storage. The LPS therefore inhibited lactic acid production by thermophilic starter culture for all periods of storage of the LPS-activated raw camel milk prior to inoculation.

The interactive effect of LPS activation, inoculation time and incubation time on acid production by thermophilic starter culture was not significant ($p>0.05$). However, there was significant inhibition ($p<0.001$) of acid production by starter culture resulting from LPS activation at all the inoculation times ($p<0.01$) (**Table 3**). This inhibition was however not reduced by time at inoculation ($p>0.05$) though it depended on the incubation time ($p<0.001$).

The influence of LPS activation on thermophilic starter culture activity in heat-treated camel milk is shown in **Table 4**. 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 h of storage of the LPS-activated raw camel milk.

ANOVA showed a highly significant effect ($p<0.001$) of LPS activation and storage time at inoculation on inhibition of lactic acid production. The inhibition of the starter culture by LPS was also significantly influenced ($p<0.001$) by time of storage of the LPS-activated raw camel milk prior

Table 2 Effect of LP-System on mesophillic starter culture in activity heat treated camel milk.

Incubation time (h)	% Lactic acid[a]			
	Control	Inoculation time interval (h) after activation		
		0	4	8
	0.23 ± 0.036	0.23 ± 0.035	0.23 ± 0.029	0.23 ± 0.029
2	0.24 ± 0.034	0.25 ± 0.031	0.24 ± 0.025	0.23 ± 0.038 b
4	0.32 ± 0.037	0.29 ± 0.032	0.32 ± 0.026	0.30 ± 0.044 b
6	0.38 ± 0.036	0.35 ± 0.034	0.38 ± 0.026	0.31 ± 0.042 b
8	0.51 ± 0.040	0.52 ± 0.036	0.52 ± 0.028	0.31 ± 0.046 b
10	0.68 ± 0.031	0.68 ± 0.034	0.69 ± 0.029	0.46 ± 0.026 b
12	0.69 ± 0.032	0.70 ± 0.037	0.73 ± 0.044	0.48 ± 0.028 b
14	0.71 ± 0.034	0.72 ± 0.036	0.75 ± 0.042	0.52 ± 0.027 b
16	0.73 ± 0.035	0.74 ± 0.040	0.78 ± 0.046	0.55 ± 0.029 b
18	0.75 ± 0.037	0.76 ± 0.037	0.81 ± 0.038	0.58 ± 0.038 b

[a] Each value is a mean of three replicate experiments with three titrations each (n=9).
[b] Data with different letter within a column significantly lower than the control ($p<0.001$).

Table 3 Effect of LP-system on thermophillic starter culture activity in raw camel milk.

Incubation time (h)	% Lactic acid[a]			
	Control	Inoculation time interval (h) after activation		
		0	4	8
0	0.18 ± 0.016	0.18 ± 0.015	0.18 ± 0.017	0.18 ± 0.013
2	0.21 ± 0.015	0.18 ± 0.014	0.21 ± 0.016	0.15 ± 0.019 b
4	0.32 ± 0.017	0.22 ± 0.013 b	0.26 ± 0.015 b	0.29 ± 0.013 b
6	0.49 ± 0.015	0.30 ± 0.017 b	0.40 ± 0.019 b	0.40 ± 0.014 b
8	0.57 ± 0.014	0.41 ± 0.015 b	0.49 ± 0.013 b	0.51 ± 0.017 b
10	0.54 ± 0.013	0.44 ± 0.015 b	0.46 ± 0.013 b	0.51 ± 0.016 b
12	0.53 ± 0.015	0.49 ± 0.016	0.47 ± 0.014 b	0.49 ± 0.015 b

[a] Each value is a mean of three replicate experiments with three titrations each (n=9).
[b] Data with different letter within a column significantly lower than the control ($p<0.001$).

Table 4 Effect of LP-system on thermophillic starter culture activity in heat treated camel milk.

Incubation time (h)	% Lactic acid[a]			
	Control	Inoculation time interval (h) after activation		
		0	4	8
0	0.23 ± 0.018	0.22 ± 0.020	0.23 ± 0.018	0.22 ± 0.016
2	0.30 ± 0.020	0.27 ± 0.017	0.30 ± 0.015	0.23 ± 0.017 b
4	0.36 ± 0.019	0.39 ± 0.023	0.34 ± 0.022	0.24 ± 0.023 b
6	0.50 ± 0.020	0.48 ± 0.020	0.46 ± 0.021	0.32 ± 0.019 b
8	0.56 ± 0.023	0.55 ± 0.019	0.47 ± 0.019 b	0.37 ± 0.024 b
10	0.58 ± 0.017	0.56 ± 0.018	0.42 ± 0.020 b	0.42 ± 0.017 b
12	0.60 ± 0.020	0.59 ± 0.020	0.38 ± 0.019 b	0.48 ± 0.018 b

[a] Each value is a mean of three replicate experiments with three titrations each (n=9).
[b] Data with different letter within a column significantly lower than the control ($p<0.001$).

Table 5 Effect of LP-system on *suusac* starter culture activity in raw camel milk.

Incubation time (h)	% Lactic acid[a]			
	Control	Inoculation time interval (h) after activation		
		0	4	8
0	0.21 ± 0.003	0.21 ± 0.004	0.21 ± 0.020	0.21 ± 0.025
2	0.25 ± 0.020	0.22 ± 0.025 b	0.25 ± 0.014	0.22 ± 0.013 b
4	0.30 ± 0.032	0.23 ± 0.025 b	0.26 ± 0.020 b	0.26 ± 0.002 b
6	0.38 ± 0.020	0.30 ± 0.017 b	0.32 ± 0.021 b	0.32 ± 0.026 b
8	0.45 ± 0.014	0.37 ± 0.025 b	0.40 ± 0.012 b	0.39 ± 0.011 b
10	0.55 ± 0.020	0.49 ± 0.026 b	0.51 ± 0.032 b	0.48 ± 0.009 b
12	0.64 ± 0.014	0.59 ± 0.011 b	0.61 ± 0.013 b	0.57 ± 0.016 b
14	0.83 ± 0.013	0.77 ± 0.011 b	0.83 ± 0.011	0.75 ± 0.014 b
16	0.95 ± 0.002	0.88 ± 0.002 b	0.97 ± 0.002	0.85 ± 0.014 b
18	1.06 ± 0.014	1.00 ± 0.014 b	1.12 ± 0.009	0.96 ± 0.020 b
20	1.18 ± 0.002	1.12 ± 0.002 b	1.23 ± 0.003	1.06 ± 0.012 b
22	1.29 ± 0.014	1.23 ± 0.011 b	1.37 ± 0.014	1.17 ± 0.012 b

[a] Each value is a mean of three replicate experiments with three titrations each (n=9).
[b] Data with different letter within a column significantly lower than the control ($p<0.001$).

Table 6 Effect of LP-system on *suusac* starter culture activity in heat treated camel milk.

Incubation time (h)	% Lactic acid[a]			
	Control	Inoculation time interval (h) after activation		
		0	4	8
0	0.19 ± 0.013	0.18 ± 0.013	0.18 ± 0.012	0.19 ± 0.014
2	0.22 ± 0.010	0.23 ± 0.014	0.20 ± 0.011	0.23 ± 0.012
4	0.26 ± 0.013	0.26 ± 0.013	0.25 ± 0.010	0.29 ± 0.013
6	0.32 ± 0.020	0.32 ± 0.015	0.31 ± 0.011	0.34 ± 0.013
8	0.38 ± 0.013	0.38 ± 0.010	0.38 ± 0.008	0.39 ± 0.007
12	0.54 ± 0.013	0.54 ± 0.013	0.57 ± 0.015	0.60 ± 0.014
14	0.74 ± 0.013	0.66 ± 0.020	0.73 ± 0.024 b	0.78 ± 0.022
16	0.94 ± 0.010	0.78 ± 0.011 b	0.93 ± 0.015 b	0.98 ± 0.018
18	1.13 ± 0.015	0.90 ± 0.012 b	1.05 ± 0.012 b	1.16 ± 0.013
20	1.33 ± 0.010	1.02 ± 0.013 b	1.15 ± 0.015 b	1.34 ± 0.012
22	1.53 ± 0.013	1.13 ± 0.010 b	1.25 ± 0.015 b	1.52 ± 0.011

[a] Each value is a mean of three replicate experiments with three titrations each (n=9).
[b] Data with different letter within a column significantly lower than the control ($p<0.001$).

to inoculation. The inhibition changed from insignificant ($p>0.05$) on inoculation at time zero to significant on inoculation after 4 ($p<0.05$) and 8 ($p<0.01$) h storage of the LPS-activated raw camel milk prior to heat treatment and inoculation (**Table 4**).

Effect of LPS on *suusac* starter culture in camel milk

The data in **Table 5** shows the mean of three experiments on the effect of an activated LPS on acid production by *suusac* starter culture in raw camel milk. LPS activation resulted in a significant slow down in acid development in raw camel milk when activated and inoculated after 0, 4 and 8 h of storage. The LPS therefore inhibited lactic acid production by *suusac* starter culture for all storage periods of the LPS-activated raw camel milk prior to heat treatment.

ANOVA showed that acid production was not significantly influenced ($p>0.05$) by LPS activation and time at inoculation interactively. However, LPS activation significantly lowered acid production by the starter culture ($p>0.001$) at all the inoculation times ($p<0.01$).

Table 6 shows data on the effect of LP-activation on acid production by *suusac* culture in heat-treated camel milk. In *suusac* starter, LPS activation resulted in a significant slow down in acid development in activated raw camel milk when heat treatment and inoculation followed immediately and after 4 h of storage. This inhibition was however reduced on storage of the activated raw camel milk for 8 h prior to heat treatment and inoculation.

ANOVA showed a significantly different ($p<0.05$) effect of LPS activation and time of inoculation on acid production by the culture. There was a significant influence ($p<0.001$) of time of starter culture inoculation on inhibition. Lactic acid production in samples heat treated and inoculated at zero and 4 h after LPS activation was significantly slower ($p<0.05$) and not significantly affected ($p>0.05$) by heat treatment and inoculation after 8 h of storage.

DISCUSSION

The LPS is activated when the thiocyanate concentration is between 10 and 15 mg/L and that of H_2O_2 is between 8 and 10 mg/L (Zapico *et al*. 1991; Santos *et al*. 1994). No additional amount of thiocyanate was therefore used to activate the LPS in this study.

Results of sensitivity of mesophilic, thermophilic and *suusac* lactic starter cultures to the LPS showed that in all the three types of starters, LPS activation resulted in a significant slowdown in acid development in raw camel milk when activated and inoculated immediately. Similar results by Packham (2002) in an investigation on inhibition in a range of *Lactococcus lactis* cultures by the LPS revealed a significant inhibition of all strains when grown on reconstituted skim milk in the presence of an active LPS. An initial survey by Packham (2002) demonstrated variable sensitivity among 13 strains of *L. lactis* subspp. *cremoris* and *lactis* grown in reconstituted skim milk. The present study and that by Packham (2002) contrast with findings by Oram and Reiter (1966) who noted stimulation by the LPS. None of the cultures used in our study and that by Packham (2002) were stimulated by the LPS.

Inhibition persisted for both the thermophilic and *suusac* starters when inoculation was done at 0, 4 and 8 h after activation of the LPS. However, for mesophilic starter culture, there was a departure with time of storage from significant inhibitory effect after 0 and 4 h to a non-significant one after 8 h of storage. The storage time dependence of the LPS activity was mentioned by Althaus *et al*. (2001) in their study on changes in LPS components during storage of cow milk, revealing a significant decrease in LPS activity over time. They recommended that studies on the effect of the

LPS to include specification on the time of storage prior to analysis. Bjorck *et al*. (1979) showed a similar trend where, at 30°C, the inhibitory effect ceased after 4 h and bacteria started to multiply. However none of the studies investigated the effect of storage on starter culture inhibition.

The investigation on the effect of the LPS on starter activity in camel milk heat-treated prior to inoculation revealed that heat treatment reduced starter inhibition by the LPS for the mesophilic and thermophilic starter cultures. This was especially noted when processing was done immediately after LPS activation. Others have noted that the post-heat treatment effect of the LPS is less in HTST-pasteurized milk than in non-heated milk (Bjorck *et al*. 1979). The mechanism by which inhibition by the LPS was markedly reduced by heating of the LPS-activated milk prior to inoculation with starter culture can be explained by the fact that the antibacterial effect of the LPS can also be reversed by various reducing agents, such as free sulphyldryl groups (Bjorck *et al*. 1979). Milk proteins are known to contain very few free SH-groups. Those present are located chiefly in β-lactoglobulin and are masked and not reactive in unheated milk. Upon heating of the milk they are unmasked due to a partial denaturation of β-lactoglobulin (Bjorck *et al*. 1979).

However, storage of the activated milk prior to heat treatment and inoculation resulted in a shift with the thermophilic and mesophilic starters from significant inhibition after 4 h of storage to highly significant inhibition after 8 h of storage at 30°C. Such slower acid production upon storage of the LPS-activated milk may be due to reactivation of the LPS by traces of H_2O_2 produced by lactic acid bacteria during milk souring (Bjorck *et al*. 1979). Martinez *et al*. (1988) discovered that the enzyme LP remains active after thermal treatments such as normal pasteurization and is responsible for subsequent reactivation of the LPS by traces of H_2O_2 produced by the starter organisms during storage of milk.

The results from thermophilic starter culture indicating significant inhibition of acid production are in accordance with those of Nakada *et al.* (1996) who studied the effect of the addition of the LPS to starter cultures used in yoghurt production from pasteurized milk. They found that at a final LPS concentration in the yoghurt of 5 ppm, which is much lower than the commonly used 8.5 ppm used in this study, the acid production in the yoghurt was suppressed almost completely during a storage period of 14 days at 10°C without affecting the viable count of the culture bacteria dramatically. They suggested that at the low H_2O_2 levels produced by the lactic acid bacteria, the resulting low activity of the LPS at the low temperature only inhibited acid production of the bacteria and did not exert a bacteriostatic effect (Nakada *et al.* 1996).

CONCLUSIONS

The LPS can therefore be useful in improving the microbiological quality and therefore shelf-life of raw camel milk meant for fermented product manufacture.

Camel milk preserved using this method still support satisfactory mesophilic and thermophilic starter culture activity if the milk is held for at least 8 h prior to processing. Storage of camel milk for 8 hours and subsequent heat treatment prior to inoculation with starter would be a useful method to overcome *suusac* starter culture inhibition by the LPS.

The use of the LPS might therefore have a significant influence on the time taken to reach the desired acidity in the vat, which is a critical factor for the manufacturer of fermented camel milk. This influence is dependent on the time of preservation of raw camel milk prior to processing of fermented products and also subsequent heat treatment prior to inoculation with starter.

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