

Use of the Lactoperoxidase System to Enhance Keeping Quality of Pasteurised Camel Milk

Kamau Murigu Patrick Njage^{1*} • John Wangoh²

¹ ETH-Zurich, Institute of Food Science and Nutrition, Schmelzbergstrasse 7, CH-8092 Zurich, Switzerland

² University of Nairobi, College of Agriculture and Veterinary Sciences, Department of Food Science, Technology & Nutrition, P.O. Box 29053, Nairobi Kenya

Corresponding author: * kamau.patrick@gmail.com

ABSTRACT

The activity of the lactoperoxidase-thiocyanate-hydrogen peroxide system (LPS) varies between species. However, its effect on keeping quality of pasteurised camel milk has not been studied. We investigated the ability of a combination of LPS and pasteurisation to lower survival of microorganisms in pasteurised camel milk. LPS was activated in camel milk followed by pasteurisation after 0, 4, and 8 hrs of storage. Shelf life, specific growth rate and final microbial count were monitored during storage of the milk at 10 and 20°C. Shelf life was 15, 32, 17 and 17 days for non-activated control and those pasteurised after 0, 4, and 8 hrs of storage of the LPS activated raw camel milk respectively during storage at 10°C. At 20°C, the shelf life was 6, 13, 9 and 7 days for non-activated control and those pasteurised after 0, 4, and 8 hrs of storage of LPS activated raw camel milk, respectively. The number of viable bacteria in untreated samples reached 10⁸ after 45 days compared to 10⁵-10⁷ in treated samples during storage at 10°C. Viable counts were 10⁸ after 15 days in untreated compared to 10⁷-10⁹ in treated samples under storage at 20°C. The mean specific growth rates at 10°C storage temperature were 0.51, 0.2, 0.41 and 0.5 for the non-activated control, activated and pasteurised after 0, 4, and 8 hrs, respectively. At 20°C storage temperature, the mean specific growth rates were 1.46, 0.27, 0.69 and 1 for the inactivated control, activated and pasteurised after 0, 4, and 8 hrs, respectively. The LPS can therefore be used to improve the shelf life of camel milk when activation is done prior to pasteurisation.

Keywords: hurdle technology, hydrogen peroxide, shelf-life, thiocyanate

INTRODUCTION

Milk contains various species specific protective protein based systems that protect the milk and neonate from a range of microbiota (Boots and Flori 2006). One of these systems is the lactoperoxidase-thiocyanate-hydrogen peroxide system (LPS). The LPS consists of three essential components: the enzyme lactoperoxidase, thiocyanate and hydrogen peroxide (Seifu *et al.* 2005). Lactoperoxidase catalyses the oxidation of thiocyanate by hydrogen peroxide producing the antimicrobial hypothiocyanite (OSCN⁻) ion with broad spectrum of antimicrobial effects against bacteria, fungi and viruses (Seifu *et al.* 2004, 2005).

The LPS has been reported to be a feasible method for the temporary preservation of raw milk (Seifu *et al.* 2005). The activity of this system in milk, however, varies from animal species to species (Sumner and Wolfson 1993). Elagamy *et al.* (1996) estimated the molecular weights of purified camel milk lysozyme, lactoferrin and lactoperoxidase at 14.4, 79.5 and 78 Kda, respectively; for bovine milk, corresponding values were 14.4, 76 and 72.5 Kda, respectively. Njage and Wangoh (2008) found thiocyanate levels in camel milk ranging from 9.74 ± 0.39 to 32.9 ± 3.54 mg/l, which are sufficient for activation of the LPS without extraneous addition.

Whereas the most investigated and recommended application of the LPS in foods is in the dairy industry for the preservation of raw milk during storage and transportation for processing, other innovative applications are being explored (FAO/WHO 2005; Seifu *et al.* 2005). One of these is activation prior to application of approved thermal processes which extends the shelf-life of dairy products significantly. This provides possibility for replacement of high-temperature processes with more economical and nutrient and/or quality retaining lower temperature treatments (Seifu *et al.* 2005).

There has been a recent increase in consumer preference for natural foods without chemical additives while still maintaining the demand for enhanced shelf-life (Elliot *et al.* 2004). Hurdle technology advocates the deliberate combination of existing and novel preservation techniques in order to establish a series of hurdles that lowers the possibility of microorganisms present to survive (Leistner and Gorris 1995). This has resulted in the investigation of naturally occurring preservatives including the LPS, lactoferrin, lysozyme, avidin, plant extracts such as spices and their essential oils, sulfur and phenolic compounds (Elliot *et al.* 2004).

The LPS has also been shown to improve the shelf life of bovine milk when activation is done prior to pasteurisation (Kamau *et al.* 1991). This is because lactoperoxidase is the most heat stable enzyme in milk and is only inactivated above 78°C for 15 s therefore leaving sufficient activity to catalyse the reactions between thiocyanate and hydrogen peroxide in pasteurized milk (FAO/WHO 2005; Seifu *et al.* 2005; Trujillo *et al.* 2007)

The combination of poor hygienic standards, high ambient temperatures and lack of refrigeration facilities render camel milk and its products very much susceptible to microbial spoilage (Farah *et al.* 2007). However, the effect of the use of the LPS on keeping quality of pasteurized camel milk has not been investigated. Prevention of microbial spoilage of pasteurised camel milk during storage and/or transportation can help to improve and enhance milk production and utilisation of camel milk (Farah *et al.* 2007).

This study investigated effect of the LPS on keeping quality of pasteurised camel milk.

MATERIALS AND METHODS

Morning pooled camel milk samples were obtained from 3 different and far-apart production points; Isiolo, Kajiado and Nanyuki

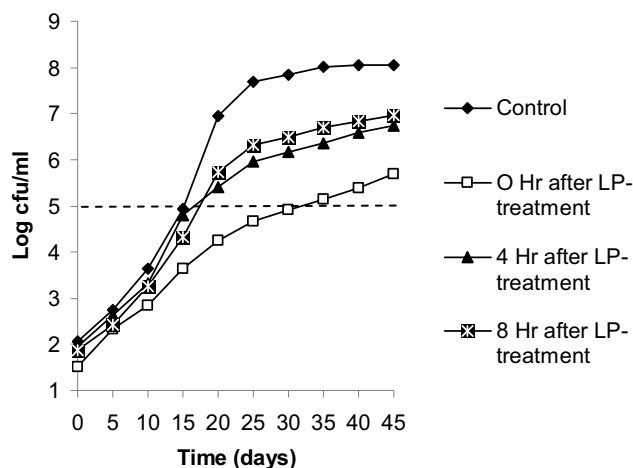


Fig. 1 Changes in viable counts of camel milk pasteurised in control after 0, 4 and 8 hrs of LP-system activation and stored at 10°C.^{a,b}
^aPlotted points are the means of four experiments. Vertical bars indicate one standard deviation. ^bHorizontal dotted grind line represents end of shelf-life: the time at which $\text{cfu} = (\text{cfu})_{\text{sl}} = 100,000 \text{ cfu/ml}$.

Districts in Kenya and delivered to the laboratory in cool boxes to maintain it below 10°C. The LPS was activated by addition of 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 untreated camel milk samples. The tubes were immersed in a water bath and heated to a final holding temperature of 63°C for 30 min. The samples were cooled in an ice bath and then stored at 10 and 20°C in environmental cabinets with constant shaking. Pasteurisation was repeated after 4 and 8 hours of storage of the LP-activated raw camel milk samples at 30°C to investigate influence of storage time at average ambient temperatures in milk production sites on effect of LPS and pasteurisation on shelf-life. Samples were drawn at 3- and 5-day intervals and viable bacteria were determined by the standard plate count method (APHA 1992) during storage at 20 and 10°C, respectively. Determinations consisted of 4 experiments in triplicate sample. Shelf-life was observed as the number of days it takes to exceed $1 \times 10^5 \text{ CFU/ml}$ (KEBS 1977).

Statistical analysis

Microbiological counts, expressed as log cfu/ml, were analysed for variance using Genstat Statistical software 7th Edn (VSN International; <http://www.vsnl.co.uk/software/genstat/>). Analysis of variance was performed to establish relationships between LPS activation, storage time prior to pasteurisation and time of storage after pasteurisation. Independent variables included LPS activation and time of storage as well as treatment-time interactions. Means and standard deviations were calculated and, when *F*-values were significant ($P < 0.05$), mean differences were separated by the least significant difference procedure.

RESULTS

The effect of activating the LPS in raw camel milk prior to pasteurisation and storage at 10 and 20°C is illustrated by **Figs. 1** and **2**, respectively. The activated LPS in conjunction with pasteurisation extended the shelf-life of camel milk. However, this extension was more when pasteurisation followed immediately than when it was done 4 and 8 hrs after activation.

For all the treatments the number of survivors was small ($< 150 \text{ cfu/ml}$) and not significantly different ($P > 0.05$). At both 10 and 20°C storage after pasteurisation, the specific growth rates were significantly lower in the LP-treated camel milk samples than in the controls ($P < 0.05$) (**Table 1**).

For the 10°C storage trial, the shelf-life in days taken for cfu/ml to reach 100,000 was 15.3, 32.5, 17 and 17 days for pasteurised untreated control samples, and those treated and then pasteurised after 0, 4 and 8 hrs storage, respectively

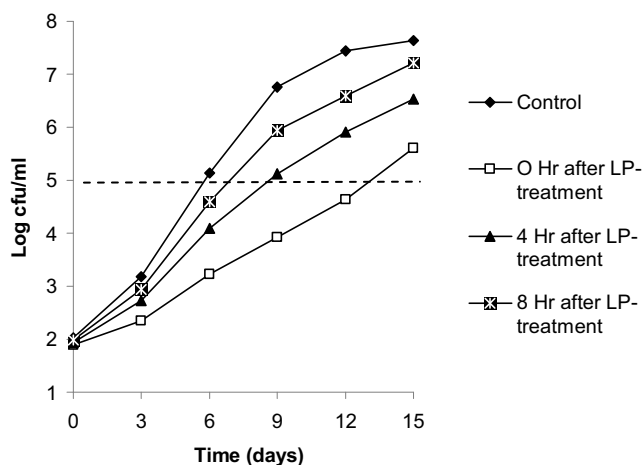


Fig. 2 Changes in viable counts of camel milk pasteurised in control after 0, 4 and 8 hrs of LP-system activation and stored at 20°C.^{a,b}
^aPlotted points are the means of four experiments. Vertical bars indicate one standard deviation. ^bHorizontal dotted grind line represents end of shelf-life: the time at which $\text{cfu} = (\text{cfu})_{\text{sl}} = 100,000 \text{ cfu/ml}$.

(**Table 1**). The number of viable bacteria in untreated control samples reached 10^8 after 45 days compared to treated samples where counts reached 10^5 - 10^7 and were significantly lower ($P < 0.05$) in the treated samples (**Table 1**).

During storage at 20°C, the shelf-life, in days taken, for cfu/ml to reach 100,000 was 6, 13, 9 and 7 days for pasteurised untreated control samples, and those treated and then pasteurised after 0, 4 and 8 hrs storage, respectively (**Table 1**). The number of viable bacteria in untreated reached 10^8 after 15 days compared to treated samples where counts reached 10^6 - 10^7 (**Table 1**) and were significantly lower ($P < 0.05$) in the treated samples.

DISCUSSION

We demonstrate extension of shelf-life of pasteurized camel milk by activation of the LPS prior to pasteurisation and the influence of subsequent storage temperature on this effect.

At 10°C storage temperature we found a shelf-life of 15.3, 32.5, 17 and 17 days for pasteurised untreated control samples, and those treated and then pasteurised after 0, 4 and 8 hrs storage, respectively. The effects of the LPS render bacteria more susceptible to thermal destruction by damaging bacterial membranes among other effects (Wit and Hoodydonk 1996; DeValdez *et al.* 1988). Activity of the LPS has also been shown to persist after pasteurisation of raw milk (Marks *et al.* 2001). Batch pasteurisation at 65°C for 30 min has little effect on lactoperoxidase activity while with HTST pasteurization at 72°C for 15 sec, approximately 70% of enzyme activity is retained (FAO/WHO 2005). However, inactivation kinetics of enzyme lactoperoxidase will vary from species to species and they have been studied for cow (Ludikhuyze *et al.* 2001; Marín *et al.* 2003) and caprine milks (Trujillo *et al.* 2007). Kamau *et al.* (1991) found out that during storage at 10°C, the shelf life in days was 16.4 for untreated pasteurised cow milk, and 38.5 for LPS treated pasteurised cow milk. The differences between shelf-life extension ability of the LPS in the results by Kamau *et al.* (1991) and those from the present work could be attributed to interspecies difference in the activity of the LPS and variation in the microbial quality of the raw milk samples. Thiocyanate concentration in milk has been reported to vary with breed, species and animal feed (Boussouel *et al.* 2000; Fonteh *et al.* 2002) while activity of lactoperoxidase enzyme depends on the sexual cycle of the cow, season, breed and feed (Kussendrager and van Hooijdonk 2000; Fonteh *et al.* 2002). Synergistic antibacterial effect has also been reported by the addition of further hurdles including nisin (Boussouel *et al.* 2000) and monolaurin in cow milk (Kussendrager and van Hooijdonk 2000;

Table 1 Effect of the LP-system on growth specific growth rate (day⁻¹), shelf-life (days) and final total viable counts (Log cfu/ml) in camel milk LP-system activated at 0, 4 and 8 hrs prior to pasteurization.

	10°C				20°C			
	Control	0 Hr	4 Hr	8 Hr	Control	0 Hr	4 Hr	8 Hr
SGR ^{a,b,c}	0.51	^d 0.2	^d 0.41	0.5	^d 1.46	^d 0.27	^d 0.69	^d 1
Shelf-life ^{b,c}	^d 15.25	^d 32.5	17	17	^d 6	^d 13	^d 9	^d 7
Maximum Count ^{b,c}	^d 8.04	^d 5.69	6.74	6.96	^d 7.63	^d 7.21	^d 6.53	^d 5.61

^aSGR- Specific Growth Rate^bMean of four replicate experiments^cMeans with similar superscript within a row are significantly different ($P < 0.001$)

McLay *et al.* 2002; Boots and Floris 2006).

Specific growth rates for the LP-treated samples were significantly lower than in the control ($P < 0.05$). The mean specific growth rates at 10°C storage temperature were 0.51, 0.2, 0.41 and 0.5 for the non-activated control, activated and pasteurised after 0, 4, and 8 hrs, respectively. At 20°C storage temperature, the mean specific growth rates were 1.46, 0.27, 0.69 and 1 for the inactivated control, activated and pasteurised after 0, 4, and 8 hrs, respectively. The slower growth rates in LPS-treated camel milk in the present study tally with the results of Kamau *et al.* (1991). Different bacteria species differ in their susceptibilities to the LPS (Seifu *et al.* 2005) and therefore their survival after such treatments. This indicates that the microbial species surviving pasteurisation in the camel milk LPS-treated prior to pasteurisation are different from those surviving in the controls.

Shelf-life extension decreased with increase in storage time of the LPS activated camel milk before pasteurisation. Kamau *et al.* (1990) demonstrated the most rapid killing and the lowest D values after exposure of *L. monocytogenes* to the LPS, for samples heated immediately following activation of the LPS. They also found out that with increasing holding time, there was a gradual increase in the D value and a decrease in the killing rate. They thus concluded that *L. monocytogenes* could recover from the effects of the LPS and eventually regain normal heat resistance (Kamau *et al.* 1990). It was also observed that after 8 hours, most of the cells may have recovered from the LPS as reflected in higher D values which approached those obtained for the untreated bacteria (Kamau *et al.* 1990). In contrast, increased shelf life of bovine milk, preserved by the LPS 4 days prior to pasteurisation has been reported (Martinez *et al.* 1988). However, this was followed by post-pasteurisation reactivation of the LPS and during the 4-day storage period, bacteria may have recovered from the LPS (Martinez *et al.* 1988). The observed increase in shelf life therefore probably resulted from the post-pasteurisation reactivation of the LPS. The LPS is also less heat stable under lower pH conditions, possibly due to release of calcium from the molecule (Boussouel *et al.* 2000). The shelf-life extension decrease detected in the present study could therefore have resulted from reduced stability of the camel milk LPS before pasteurisation.

We noted higher shelf-life extension when LPS activated camel milk was stored at 10°C than at 20°C after pasteurisation. Cell repair and subsequent recovery by bacteria from damage induced by the LPS may not be as efficient in low-temperature environment (Leistner 2000). Consequently, proliferation of bacteria is further reduced at low temperatures not permissive for growth (Elliot *et al.* 2004). Storage of LPS activated and pasteurised camel milk at lower temperatures therefore provided an additional hurdle.

The LPS can therefore be useful in improving the microbiological quality and therefore shelf-life of pasteurised camel milk. The influence of time of preservation of raw camel milk prior to pasteurisation should however be considered.

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