The effect of activation of the lactoperoxidase system and souring on certain potential human pathogens in cows' milk

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ABSTRACT

Conventional methods of ensuring the safety and soundness of cows' milk for human consumption, such as pasteurisation, are not always practical in poor socioeconomic conditions or in rural communities that lack modern amenities. Activation of lactoperoxidase (LP) system and souring of milk were investigated as potential alternative methods to sustain the safety of milk by inhibiting certain microorganisms with known pathogenic potential. The activation of the LP-system inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* by the order of 2 log values. The inhibition of *Brucella abortus* was negligible. The replication of *Coxiella burnetti* in milk was not disturbed even after 17 h of LP-system activation at 20 °C, but the outcome of the LP-system treatment on *Mycobacterium bovis* could not be determined as the conventional culturing technique used to grow this organism did not allow full recovery. Souring inhibited the growth of *S. aureus* and *E. coli* also by the order of 2 log values. From the results obtained in this investigation are concluded that the activation of the LP-system and souring can be used to inhibit the growth of *S. aureus* and *E. coli* in cows' milk, thereby increasing its safety.

Key words: *Brucella abortus,* cows' milk, *Coxiella burnetti, Escherichia coli,* lactoperoxidase system, *Mycobacterium bovis,* souring, *Staphylococcus aureus.*

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INTRODUCTION

Cows' milk is a valuable food and source of energy, particularly for the health and well-being of infants. Its major constituents are proteins, butter-fat, milk sugar (lactose), vitamins (*e.g.* A, B1 and B2,) trace elements (*e.g.* iron, iodine, magnesium and copper[10,11,12]) and water. The proteins are of great biological value because they contain essential amino-acids such as lysine[9] that are lacking in many of the cereal varieties[25], such as maize, which form the staple diet of many people in developing countries in Africa.

Milk-borne zoonotic microorganisms are of great concern, particularly in resource-poor communities where electricity is not readily available and the control of infectious diseases is difficult due to poor socioeconomic conditions. Pasteurisation and cooling of milk are used to render and keep milk safe for human consumption in developed countries, but these methods are largely lacking in developing countries. Alternative methods have been investigated to sustain the quality of milk for human consumption. The inhibitory effect on certain microorganisms induced by activation of the lactoperoxidase system and the souring of milk, and the practicality of these 2 procedures have been studied rather extensively[1,2,30,34]. The LP-system, comprising lactoperoxidase/thiocyanate/hydrogen peroxide, is an antimicrobial system occurring naturally in milk[1] but its artificial activation has only recently been developed as a method of preserving milk. It can be activated *in vitro* to protect milk from spoilage by adding thiocyanate and hydrogen peroxide[16], and, after activation, raw milk can satisfactorily be preserved without excessive increases in bacterial counts or significant changes in flavour[17]. Souring of milk, on the other hand, has been used for thousands of years in many rural areas in Africa as a safe technique to preserve the quality of milk for human consumption[2]. To the best of our knowledge, however, data for a scientific understanding of the nutritional value of sour milk in rural areas have yet to be published. The souring process can be induced by acidifying pasteurised milk with pure starter cultures, containing *e.g.* certain streptococci[23], thermophilic bacilli[26], lactic acid bacteria[35] or yeasts[16], and bifidobacteria[22], in a continuous culturing procedure while maintaining optimum temperature, pH and culture dilution rate[23]. The antibacterial efficacy of sour milk is well documented[15,26,31]. Gandhi and Nambudripad[15] (1979) used sour milk to relieve some cases of gastrointestinal disorders in adults and children. The inhibitory effect of cultured milk products on a number of microorganisms, including some enterobacteria, clostridia and pseudomonads has been proven[21].

The aim of this study was to artificially infect cows' milk with 5 pathogens, *Staphylococcus aureus, Escherichia coli, Brucella abortus, Mycobacterium bovis* and *Coxiella burnetti*, selected on the basis of their zoonotic potential[7,8,16,18], and to investigate the inhibitory efficiency of both activation of the LP-system and souring on milk samples infected with these pathogens.

MATERIALS AND METHODS

Cows

Milk samples were collected by milking machine, using standard procedures, from 8 Friesland and 2 Jersey cows at the Onderstepoort Veterinary Institute dairy, aged between 2.5 and 6.5 years that produced between 8 and 28 kg of milk per cow per day. They were kept under the same environmental conditions and routinely tested for tuberculosis, mastitis and brucellosis.

Milk samples

After collection, the milk was immediately refrigerated and stored at 4 °C until used. Fifty milk samples, each 2 ℓ in

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volume, were then aseptically divided into 5 groups of 10 samples each. They were arranged in such a way that each cow was represented by 1 milk sample in every group (Fig. 1). The pH of the milk was between 6.7 and 6.8.

After collection of milk, a specimen of 10 mℓ was taken aseptically from each sample and divided into 2 aliquots, 1 of which was used to evaluate the concentration of thiocyanate (SCN^-) according to the method in the bulletin of the International Dairy Federation of 1988[19] and the other its chemical composition as recommended by the International Dairy Federation standard of 1990[20].

To minimise the number of bacteria other than the 5 target pathogens mentioned above, all the milk samples were heated in a thermostatic water bath at pasteurisation temperature of 63 °C for 30 min and immediately cooled at 4 °C until infection with a specific pathogen.

Pathogens

A toxin-producing *Staphylococcus aureus* ATCC 6571 strain and *Escherichia coli* vaccine strain 1021 were obtained in a freeze-dried state, aseptically reconstituted in sterile physiological saline and grown on blood tryptose agar (BTA) (Difco, Oxoid). *S. aureus* was then transferred to nutrient slopes and incubated for 24 h before being stored at 4 °C until used, while *E. coli* was transferred onto Dorset egg slants and incubated for 24 h before being stored at 4 °C. Freeze-dried *Brucella abortus* vaccine strain 19, an African isolate, was suspended in sterile distilled water, grown on BTA plates and stored on BTA and nutrient agar slants at 4 °C. *Mycobacterium bovis* ATCC strain 19210 obtained from slants of Löwenstein/Jensen medium, was reconstituted in Tween saline (1.0ℓ of 0.85 % physiological saline; 1.0 mℓ of 10 % Tween) and grown on slopes of Löwenstein/Jensen medium (pyruvate supplement) for 6 weeks at 37 °C. Slopes showing good growth were selected and stored at 4 °C until used. The suspensions with which the milk samples were infected were prepared by transferring the infectious materials from slants to appropriate fresh plates of medium, and grown for 24 h in the case of *S. aureus* and *E. coli*, 3 d for *B. abortus* and 6 weeks for *M. bovis* at 37 °C in aerobic conditions. Fresh colonies collected from the surfaces of the media were suspended in physiological saline, mixed vigorously on a whirlimixer for about 1 min and adjusted, by adding either more saline or more bacteria, to the turbidity of McFarland 0.5 standard (1.175 % $BaCl_2.2H_2O$; 0.36N H_2SO_4).

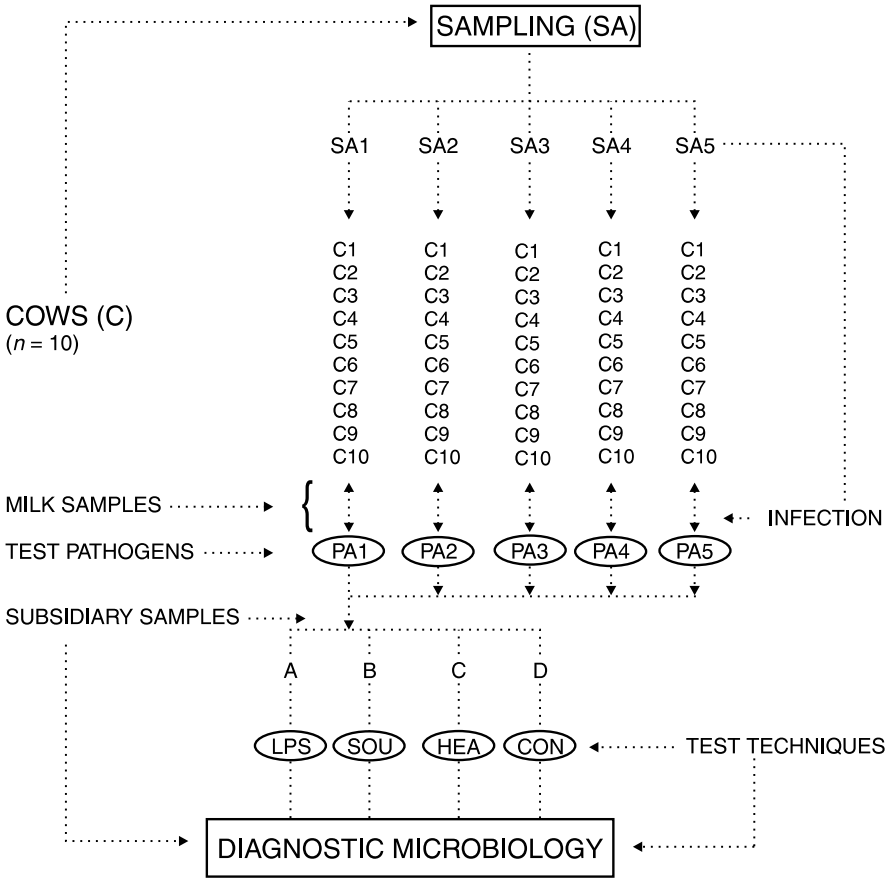

Fig. 1: **Flow diagram of the experimental design.**

A freeze-dried culture of an available Namibian strain of *C. burnetti* was aseptically reconstituted in 0.5 mℓ of sterile distilled water to which 1.9 mℓ of phosphate buffered saline was added. It was then grown under laboratory conditions in the yolk sacs of 7-day-old chicken embryos. The working antigen was obtained after 4 serial egg passages and was kept at –86 °C until used. The suspension used to infect milk samples was prepared by titrating the infected yolk and inoculating aliquots of each dilution into specific pathogen-free embryonated eggs via the yolk sac route.

Before the inoculation of milk samples with the respective suspensions of pathogenic organisms, the number of viable bacteria per millilitre was determined in suspension using a dilution method in the case of *S. aureus, E. coli, B. abortus* and *M. bovis*, and by titration for *C. burnetti*.

Infection of milk samples

Milk samples were first brought to room temperature (22 °C) and an amount of 1 ℓ was prepared for infection. The volume of the infective doses added to each litre of the 10 milk samples within a group was 2.0 mℓ of the same species of bacterium containing a known number of micro-organisms. The initial concentration of bacteria in milk samples after addition of the infective doses is given in Table 3. Each inoculated milk sample was allowed to stand for a period of time not exceeding 1 h to allow the bacteria to adapt, and then divided into 4 subsidiary samples (A–D) of equal volume, which were subjected to different treatments (Fig. 1).

Treatment of infected milk samples

Samples A from each group were subjected to the activation of the LP-system, by adding literature-recommended amounts of SCN^- and H_2O_2 to subsidiary samples in the proportion of 14 mg/ℓ and 30 mg/ℓ, respectively[1]. The LP-system for *C. burnetti* (a slow-growing organism) was activated for 17 h at 20 °C, and in the milk samples infected with the other 4 pathogens for 6 h at 30 °C. Samples B were subjected to the souring process by adding 60 mℓ of 1 % lactobacilli mother culture (prepared by adding 3 pellets of lactobacilli starter culture obtained commercially to 100 mℓ of milk and incubating at 20 °C for 24 h before use) per litre of milk and incubated at 30 °C for 24 h. Samples C were used as negative controls

and heat-treated at pasteurisation temperature for 30 min at 63 °C. Samples D were the positive controls and did not receive any of the abovementioned treatments. They were incubated for 6 h at 30 °C, after which the concentration of bacteria they contained was compared with that in the initial bacterial inoculum (Table 3) to determine whether the environmental conditions in the milk had been favourable for bacterial replication.

Quantification and confirmation of viable bacteria after treatment

At completion of each treatment, viable bacteria were quantified in both the treated and untreated samples. A dilution method was used for *S. aureus, E. coli, B. abortus* and *M. bovis*, whereas a titration method, using embryonated eggs, was performed for *C. burnetti*. Organisms present in the samples were confirmed as being the original bacterial species by using standard diagnostic microbiology.

Evaluation of the survival/killing rate of bacteria

To evaluate the survival/killing rate of bacteria after treatment, the concentration of bacteria was determined in both the original bacterial suspensions and untreated samples. The relevant concentrations were compared with one another to ascertain whether the bacteria had adjusted and replicated under the conditions prevailing in the milk samples. The concentration of bacteria in all the treated samples was determined and compared with that in the untreated samples to ascertain whether any difference in the growth pattern of bacteria occurred between the treated and untreated samples.

Evaluation of the efficiency of treatments

The effect of the LP-system on all 5 pathogens was determined, while that of souring was only determined for *S. aureus* and *E. coli*. The concentration of bacteria in all the treated samples was recorded and analysed by the Wilcoxon statistical test. The minimum, maximum and mean concentration values were determined. The mean concentration value of bacteria in the treated and untreated samples as compared with the concentration value of the original bacterial suspension, and the significance of these differences was calculated at the 5 % level of difference, after calculation of the probability value (*p*-value). The effect of the LP-system and souring on *S. aureus* and *E. coli* was evaluated by the Kruskal Wallis test of the BMDP 3D statistical package.

Table 1: **The concentration of thiocyanate in milk samples.**

Cow No.	Values of thiocyanate in milk (ppm)	
	Early	Late
1	6.0	9.0
2	5.9	4.4
3	6.1	5.2
4	4.3	5.1
5	5.8	3.8
6	8.2	2.2
7	2.1	3.1
8	2.2	2.4
9	6.6	4.4
10	7.7	4.1

Table 2: **The chemical composition of milk.**

	Average yield per cow
Milk	21.97 kg
4 % fat corrected milk	23.72 kg
Butterfat	0.99 kg
Protein	0.68 kg

Table 3: **The initial concentration of bacteria in the positive controls and the concentration of bacteria after 6 hours of incubation.**

Pathogen	Initial inoculum (org/mℓ)	Bacterial concentration (org/mℓ)
Staphylococcus aureus	3.4×10^4	1.5×10^7
Escherichia coli	3.0×10^5	3.2×10^7
Brucella abortus	3.4×10^6	7.2×10^6
Mycobacterium bovis	1.6×10^5	3.5×10^4
Coxiella burnetti	$1.0 \times 10^{1.4}$	$5 \times 10^{2.5}$

RESULTS

SCN^- concentration and chemical composition of milk

The concentration of SCN^- in the milk was determined before the 1st sampling (before infection with microorganisms) and towards the end of the experiment. The values are presented as 'early' and 'late' values in Table 1. The chemical composition of milk is given in Table 2.

Activation of the LP-system

The initial cell concentration of bacteria in the positive controls as well as the concentration of bacteria after 6 h of incubation are given in Table 3. An increase in the initial cell concentration of bacteria could be demonstrated in the positive controls for *S. aureus, E. coli, B. abortus* and *C. burnetti*, after 6 h incubation, but not for *M. bovis,* in which the cell concentration decreased from 1.6×10^5 to 3.5×10^4 org/mℓ (Table 3).

In the 10 test samples (Table 4) inoculated with *S. aureus*, 6 samples maintained the log value of 10^4 (as at the time of inoculation) and 4 of the samples increased to 10^5. In the case of *E. coli,* no sample had a log value larger than that of the initial inoculum (10^5). Three of the 5 samples had a log value of 10^5 and the other 5 samples had a log value of 10^4, which was smaller than the initial inoculum. Only 2 samples inoculated with *B. abortus* had a bacterial cell concentration slightly less than the initial one, but all had the same log value of 10^6. In *M. bovis*-inoculated samples, even the positive control did not reach the initial log value of 10^5. All samples infected with *C. burnetti* had a log value higher than $10^{1.4}$, which was the log value of the initial inoculum. No growth of any of the bacterial species occurred in the negative controls (Table 4). The mean cell concentration for each bacterial species was

calculated after activation of the LP-system and compared with the cell concentration of the corresponding positive controls. (Table 5).

Souring of milk

The mean concentrations of bacteria in the positive controls before and after incubation at 30 °C for 24 h are shown in Table 6. An increase in bacterial concentrations was noted for both *S. aureus* and *E. coli*. These bacteria did not proliferate equally in all the test units that were subjected to acidification. The concentration of *S. aureus* cells in the test samples reached 10^5 in only 4 of the 10 samples (Table 7). Four of the samples had a log value similar to that of the initial inoculum (Table 6), while 2 samples had a smaller cell concentration than the initial concentration.

In milk originally containing 3.0×10^5 org/mℓ *E. coli*, all 10 samples had, after incubation, a final log value larger than 10^5. In 2 of the 10 samples the log value of cells reached 10^7 (Table 7). The pH values of the medium in the test units varied from 3.9 to 4.1 for *S. aureus*, but were 4.1 for *E. coli* (Table 7). The mean bacterial concentrations of each pathogen in the 10 test tubes are summarised in Table 8. No growth occurred in the negative controls (Table 7).

DISCUSSION

Activation of the LP-system

In the positive controls the concentrations of *S. aureus, E. coli, B. abortus* and *C. burnetti* increased (Table 3), confirming that the environmental conditions in the milk were suitable for these bacteria to adapt and replicate. This was not the case for *M. bovis*, where the cell concentration decreased from 1.6×10^5 to 3.5×10^4 org/mℓ (Table 3).

The bacterial cell concentrations in the test units (Table 4) varied despite the fact that they were inoculated at the same time and were maintained under the same environmental conditions. This may be mainly due to technical factors such as slight differences in the bacterial inoculum size, the size of the samples streaked out on Petri dishes during the counting process, precision of counting the colony-forming units, and differences in the amount of chemicals used to activate the LP-system.

The mean concentrations calculated for all the pathogens in each group of the 10 treated samples were therefore compared with the corresponding values in the positive control samples and found to be lower for *S. aureus, E. coli* and *B. abortus*

Table 4: **The concentration of *Staphylococcus aureus, Escherichia coli, Brucella abortus, Mycobacterium bovis* and *Coxiella burnetti* in milk samples at the end of activation of the LP-system.**

Sample	Pathogen (org/mℓ)				
	S. aureus ($\times 10^5$)	*E. coli* ($\times 10^6$)	*B. abortus* ($\times 10^4$)	*M. bovis* ($\times 10^5$)	*C. burnetti* ($5 \times EID_{50}$/ mℓ)
1	2.9	1.3	4.1	3.5	$10^{3.2}$
2	0.12	0.63	6.1	2.5	$10^{2.5}$
3	2.4	3.7	5.4	3.0	$10^{2.3}$
4	0.62	0.53	5.0	5.5	$10^{2.8}$
5	0.73	1.5	7.3	4.5	$10^{2.6}$
6	0.45	0.57	5.1	4.0	$10^{2.4}$
7	0.12	5.2	3.3	3.2	$10^{1.5}$
8	3.5	0.33	4.9	2.8	$10^{2.3}$
9	0.23	0.50	3.1	3.8	$10^{1.7}$
10	2.7	2.6	4.4	3.4	$10^{1.8}$
Positive control	150	320	7.2	3.5	$10^{2.5}$
Negative control	0	0	0	0	0

Table 5: **Comparison of the bacterial cell concentration in the positive controls and the mean concentration of each bacterial species after activation of the LP-system.**

Pathogen	Cell concentration in the positive control (org/mℓ)	Mean cell concentration after activation of LP-system (org/mℓ)
Staphylococcus aureus	1.5×10^7	1.5×10^5
Escherichia coli	3.2×10^7	1.7×10^5
Brucella abortus	7.2×10^6	4.9×10^6
Mycobacterium bovis	3.5×10^4	3.6×10^4
Coxiella burnetti	$5 \times 10^{2.5}$	$5 \times 10^{2.9}$

Table 6: **Mean concentrations of bacteria in milk samples of the positive controls after initial inoculation and after 24 hours of incubation at 30 °C.**

Pathogen	Concentration after initial inoculation (org/mℓ)	Concentration after 24 h incubation (org/mℓ)
Staphylococcus aureus	3.4×10^4	3.4×10^7
Escherichia coli	3.0×10^5	8.5×10^8

Table 7: **The concentration of *Staphylococcus aureus* and *Escherichia coli* in milk samples after 24 hours of souring at 30 °C.**

Samples	*S. aureus*		*E. coli*	
	pH	Counts ($\times 10^5$ org/mℓ)	pH	Counts ($\times 10^7$ org/mℓ)
1	4.0	3.0	4.1	0.70
2	4.1	0.77	4.1	0.34
3	4.1	0.26	4.1	0.58
4	4.0	0.29	4.1	1.1
5	3.9	0.98	4.1	1.3
6	4.0	0.70	4.1	0.75
7	4.1	0.47	4.1	0.68
8	4.1	1.6	4.1	0.74
9	4.1	1.9	4.1	0.73
10	4.1	2.6	4.1	0.77
Positive control	—	340	—	85
Negative control	—	0	—	0

Table 8: **Comparison of bacterial cell concentration in the positive controls and mean concentration in the 10 test samples of *Staphylococcus aureus* and *Escherichia coli* after souring.**

Bacteria	Cell concentration in the positive control (org/mℓ)	Mean concentration (org/mℓ)
S. aureus	3.4×10^7	1.3×10^5
E. coli	8.5×10^7	7.7×10^6

(Table 5). This indicates that the LP-system in the treated samples inhibited the rate of replication of the bacteria. However, the decrease in the bacterial cell concentration in both test and control samples inoculated with *M. bovis* was not sufficient to provide proof that the loss of viability in the treated samples was caused by the inhibitory effect of the LP-system. This could have been due to the inability of the mycobacteria to replicate in the milk or the fact that the conventional technique that was used to grow *M. bovis* was not satisfactory. In samples inoculated with *C. burnetti*, the titre in the positive control was almost equal to the mean titre of the treated samples, indicating that the growth pattern of the pathogen in all samples was similar and that the LP-system did not interfere with replication of this organism.

The inhibitory effect induced by the LP-system on *E. coli* is the result of a bactericidal effect and the survival/killing rate of bacteria is strongly dependent on the initial inoculum of bacteria and the concentration of the antibacterial substance. It has been reported that *E. coli* and most other gram-negative catalase-positive organisms can be killed by activation of the LP-system[37], which was also demonstrated in this study. Eight of 10 milk samples inoculated with *E. coli* had a final cell concentration slightly lower than that at the time of the initial inoculation (Table 4), but considerably lower than the concentration in the positive control. Similar findings have been reported for some *Salmonella* serovars and *Pseudomonas* spp.[4,5,24]. It is considered that hypothiocyanate ions that result from oxidation of SCN by lactoperoxidase in the presence of H_2O_2 are responsible for the killing of bacteria, as they react specifically with their free sulphydryl groups, thereby inactivating most of their vital metabolic enzymes, consequently blocking their metabolism and ability to multiply[4,24]. In an experiment with *E. coli* 0157:H7, however, the inhibitory effect at 30 °C was limited to 12 h[13], confirming the bacterial strain dependency of the LP-system preservative effect[36].

The LP-system had a bacteriostatic effect on *S. aureus* and *B. abortus*. Eight out of 10 samples inoculated with *S. aureus* and *B. abortus* had a final cell concentration higher than that of the initial inocula, but lower than that of the positive controls (Table 4). This shows that bacteria in the treated samples recovered after a certain period of inactivation and replicated at a slower rate compared to the control samples. Generally, gram-positive bacteria are more resistant to inhibition by the LP-system and the effect is only bacteriostatic[3,6,28,32,33,37]. In our study, *B. abortus* was partially resistant to the effect of the activated LP-system. This corresponds well with its behaviour in response to certain other adverse environmental conditions[29]. *C. burnetti* was not inhibited by activation of the LP-system even after 17 h at 20 °C. The final concentration of *M. bovis* in the test samples was slightly higher than that in the positive controls. The decrease of bacterial concentration in the positive controls indicates that the mycobacteria did not adjust well to the milk within the period of 1 h that preceded incubation. The conventional culturing technique used to grow *M. bovis* might not have been sensitive enough to recover all the organisms with aliquots of the samples used for this purpose.

There are many factors that play a role in the inhibitory efficiency of the LP-system. These include the bacterial species involved, the quality and concentration of SCN^-:H_20_2, the temperature of the milk, and the duration of treatment. In an experiment with *E. coli* 0157:H7 at 1.5 and 4.5 orders of magnitude, a decrease was found at 30 °C when initial inoculums of approximately 10^4 and 10^8 respectively were used[14].

The concentration of the compounds used to initiate the LP-system also plays an important role. In this study the activation of the LP-system for evaluation of the sensitivity of *S. aureus, E. coli, B. abortus* and *M. bovis* was brought about by using 14 mg SCN^- and 30 mg H_20_2 per litre of milk for 6 h at 30 °C. Under these conditions it halted the proliferation of *S. aureus* while in the case of *E. coli* the viability of cells was affected. Growth inhibition for these bacteria was in the order of 2 log values in the treated samples after 6 h of incubation. The inhibitory effect on *B. abortus* after 6 h at 30 °C was minimal, suggesting that further investigations with other SCN^-: H_20_2 combinations should be carried out. SCN^-:H_20_2 proportions such as 30:30 or 30:45 parts per million (ppm) were reported to have a better preservative effect than activation with 10:10 or 20:20[36]. Other combinations of SCN^-:H_2O_2, such as 15:0 or 45:30 ppm, respectively, in which SCN^- is in a higher proportion than H_2O_2, were found useful for the extension of the shelf life of raw milk at 37 °C[27].

Souring of milk

The effect of the souring treatment was evaluated only for *S. aureus* and *E. coli*, because the fast-growing lactobacilli used to induce the souring process and the decrease of the pH in the medium made the counting of colony-forming units impossible for *B. abortus*, suppressed the growth of *M. bovis*, or killed the embryos before replication of *C. burnetti*.

The increase in the bacterial cell concentrations observed after 24 h in the untreated samples containing *S. aureus* and *E. coli* (Table 6) confirmed that these bacteria adapted and replicated in the milk under the conditions in which the milk samples were kept. With the addition of the lactobacilli starter culture to the test units, growth of bacteria was retarded by the order of 2 log values for a period of 24 h with the corresponding mean values for pH being 4.0 and 4.1 for *S. aureus* and *E. coli*, respectively (Table 7).The mean concentrations of cells in the 10 treated samples were 1.3×10^5 org/mℓ for *S. aureus* and 7.7×10^6 org/mℓ for *E. coli* (Table 8). These are much lower (by the order of at least 3 log values) than the concentration of the untreated samples, but higher than the concentrations present at the time of initial inoculation. This suggests that growth of bacteria in both untreated and treated samples was possible for a certain time, probably during the adaptation period.

As occurred with the LP-system, the growth pattern of bacteria in the treated samples during the souring process was not uniform. This suggests that the bacteria might have adjusted to the conditions in the milk samples in a non-synchronous manner, and that release of lactic acid and reduction of pH of the milk might also have occurred in a non-synchronous manner in the samples. The concentration of bacteria in all 10 samples inoculated with *E. coli* and in 8 of 10 samples inoculated with *S. aureus* (Table 7) was larger than the initial

concentration. This indicates that the bacteria were inhibited in the treated samples only for a certain period but then recovered and replicated again, which suggests that the inhibition was the result of a bacteriostatic effect.

An increase in the antibacterial activity of the souring process by lactobacilli has been reported for *S. aureus* and *E. coli* at 40 °C compared to 35 °C or 45 °C[15]. Using 2 % *L. acidophilus*, 40 °C was shown to be the optimum temperature for increased inhibitory activity against *S. aureus* and *E. coli*[21]. In an experiment using 2.5 % lactobacilli, the growth of *S. aureus* and *E. coli* was inhibited at 7 ± 1 °C in cow and buffalo milk for 15 days[31].

Although the same pH (4.1) was observed in all the samples inoculated with *E. coli*, the cell concentrations in the 10 test units varied. This also occurred in the samples inoculated with *S. aureus* where the pH values varied between 3.9 and 4.1 (Table 7). Therefore the drop in pH alone apparently had no positive correlation with the growth pattern of *S. aureus*, nor does it explain the retardation of the growth of *E. coli* in the milk samples. In this study only pH and growth were investigated, but we consider that the correlation between a drop in the pH, lactic acid concentration, and bacterial growth pattern should be further investigated.

Analysis of the data with the Kruskal Wallis statistical test showed that the effect of souring on *E. coli* was only bacteriostatic, as opposed to the bactericidal effect induced by activation of the LP-system. Therefore cows' milk containing *E. coli* in the order of 10^5 organisms per mℓ could be better preserved by activating the LP-system of the milk for 6 h at 30 °C than by souring it for 24 h at 30 °C. The same statistical test showed that cows' milk containing *S. aureus* in the order of 10^4 organisms per mℓ could be preserved as efficaciously either by activating the LP-system for 6 h at 30 °C or by souring for 24 h at the same temperature. Therefore in practice the LP system might be a better choice for the preservation of milk.

The replication of *B. abortus* in cows' milk was not appreciably affected by activation of the LP-system, even though a slight inhibitory effect was observed in some of the samples. The presence of sodium thiocyanate and sodium percarbonate in the milk did not interfere with the replication of *M. bovis* and *C. burnetti*. The LP-system treatment can therefore not be recommended as a safe way of preserving raw milk infected with *B. abortus*, *M. bovis* and *C. burnetti*.

Since it is not possible to provide everybody with electricity in rural areas in developing countries, the temporary use of the LP-system and souring for sustaining the quality of cows' milk should be encouraged. These methods will never replace treatment by heat or pasteurisation, and can only be successful if good quality milk is used and farmers are educated in the use of these alternative methods.

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