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Original Article

The inhibitory effect of sodium thiocyanate and sodium percarbonate ratios on microorganism growth in raw milk samples as an effective treatment to extend milk quality during storage

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Abstract

Preservation of raw milk quality by activation of lactoperoxidase system (LPs) was studied for the inhibition of microorganism growth. The antimicrobial effects of LPs were examined by measuring thiocyanate (SCN^-) concentration, lactoperoxidase (LP) activity, milk composition, total bacterial count (TBC) and coliform count (CC). All parameters were analyzed at 0 h and at 25°C and 30°C as a control. Thus, the experiment was conducted to evaluate the effect of 2 different temperatures (25°C vs 30°C) and 4 ratios of NaSCN:$2Na_2CO_3 3H_2O_2$ (0:0, 7:15, 14:30 and 21:45 mg/L) on milk samples (both uninoculated raw milk samples and *Escherichia coli* (*E. coli*) inoculated milk samples) with 8 replicates per run using 0-12 h incubation time *in vitro* assay. The runs were conducted on the same 4 NaSCN:$2Na_2CO_3 3H_2O_2$ ratios and different temperature and time of incubation were used. The results showed that the milk SCN^- concentration and LP activity increased with increasing NaSCN:$2Na_2CO_3 3H_2O_2$ ratios. Milk compositions retained the quality of normal milk fat, protein, lactose, solid-not-fat (SNF) and total solid (TS) contents, and they were not significantly affected by the LPs activation. An obvious effect of the LP activated milk was the inhibition of TBC in uninoculated raw milk samples for 6 to 12 h both at 25°C and 30°C), and for 6 to 9 h in *E. coil* inoculated milk samples, whereas CC (6 h at 25°C and at least 3 h at 30°C for both uninoculated and *E. coil* inoculated milk samples). It is concluded that improved preservation of milk can be achieved through the addition of 14:30 and 21:45 mg/L of NaSCN:$2Na_2CO_3 3H_2O_2$ in uninoculated and *E. coil* inoculated milk samples respectively, to extend milk quality during storage.

Keywords: lactoperoxidase system, *Escherichia coli*, microorganism growth, raw milk quality

1. Introduction

It is well recognised that commonly-used methods to prevent or retard the deterioration of raw milk quality while in transit from farm to dairy factory, especially in tropical areas remote from milk collection centres where there is a lengthy duration of cooling for transportation, are not effective. Furthermore, the costs associated with these preservation processes are high. In fact, in many regions of developing countries there frequently exist poor standards in handling systems, and economic and organisational problems associated with the high temperature; all factors which can contribute to the deterioration of raw milk. Increased delivery times due to inadequate transportation with lack of available

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refrigeration systems and funding all provide considerable challenges to extending the quality of raw milk during storage. These are the fundamental problems contributing to the decline in the quality of raw milk, and the subsequent economic losses associated with it are significant. Traditionally, Thai farmers of smallholdings store the raw milk in unrefrigerated conditions in cans or tanks which would be collected later in series from each smallholder and transported (again unchilled) in bulk collection to a processing plant or larger milk collection centre. This method of collection would often result in delays between milking and the final point of delivery at dairy processing plants of may be more than six hours in Thailand which would have significant negative consequences on milk quality. These issues contribute to a higher frequency of raw milk being rejected by the dairy factories as the product would not be acceptable to consumers (Barabas, 1994). Alternative preservation techniques are clearly required to maintain the quality of raw milk long enough to ensure that it can be transported from the producer to processing plants and preferably through to final consumers without significant deterioration. These techniques are required to be simple to use, relatively inexpensive and not represent any risk to consumers (Ryoba *et al.*, 2000).

In seeking to address this problem, an alternative way to enhance the storage stability of raw milk at high ambient temperatures has been developed. The method makes use of a naturally occurring enzyme present in the antimicrobial system in milk known as LPs. It has been shown that the LPs can increase storage times of raw milk by delaying bacterial growth (Wolfson & Summer, 1993) and also can be used to inhibit bacterial growth (both Gram-positive and Gram-negative microbes) to preserve the milk quality (Marks *et al.*, 2001). Although the natural LPs loses its effect within 2 h of the milking, it can be reactivated to stimulate the antibacterial system present in milk and to significantly extend its shelf life (FAO, 1999) by the addition of small quantities of SCN^- and H_2O_2. In this study, we aim to examine the inhibitory effect of activation of the LPs on microorganism growth and survival of pathogenic bacteria related to milk using *Escherichia coli* (*E. coli*) at 25°C and 30°C as an effective treatment model to extend milk quality during storage.

2. Materials and Methods

2.1 Raw milk sampling

Approximately 1 L raw milk samples of Holstein Friesian crossbred (>87.5% HF) lactating dairy cows were collected, from the tanks of collection centres, at Suranaree University of Technology's (SUT) farm, Nakhon Ratchasima, Thailand. This experiment was carried out from August 2014 to November 2014 at an average ambient temperature of 27.5°C. The initial raw milk sampled was confirmed as sourced from healthy dairy cattle at SUT's farm as certified by the Department of Livestock Development, Thailand. Milk samples were aseptically divided into 2 main portions stored at 25°C and 30°C for examination before (as control) and after LPs activation. The first portion used was uninoculated raw milk (control) and LPs activation was subsequently introduced. Milk samples were analysed immediately for SCN^- concentration (Codex Alimentarius Commission) CAC. 1991., H_2O_2 (Allen & Wrieden, 1982), LP activity (Isobe *et al.*, 2009), milk composition using Lactostar (Art. no:3510; Funke Gerber Labortechnik GmbH, Berlin) and examined for microbiological properties including TBC nutrient agar (NA agar) powder and CC eosin methylene blue agar (EMB agar) (AOAC, 2000) with minor modification and incubated at 0, 3, 6, 9 and 12 h.

2.2 Inoculation of milk samples with *E. coli*

The milk samples of the second portion were initially inoculated with *E. coli* ATCC® 25922™ (10^6 CFU/mL) and LPs activation was subsequently introduced.

2.3 Activation of lactoperoxidase system

The LPs was activated by the addition of SCN^- (in the form of NaSCN) and H_2O_2 (in the form of sodium percarbonate ($2Na_2CO_3 3H_2O_2$). Firstly, NaSCN was prepared from 99.7% pure NaSCN powder (UNIVA, APS, NSW, Australia), then NaSCNwas added into the milk samples at 0, 7, 14 and 21 mg/L respectively, then stirred thoroughly for 1 min thereby increasing the SCN^- content. Secondly, $2Na_2CO_3 3H_2O_2$ (Eisen-Golden Laboratories) was added at 0, 15, 30 and 45 mg/L of milk to the LP activated milk. The enzymatic reactions were completed within 5 min after thorough mixing of H_2O_2 donor (Na_2CO_3) in the milk. The activation of LPs was carried within 2-3 h after milking by reactions of SCN^- and H_2O_2.

2.4 Analysis of thiocyanate concentration

SCN^- can be determined in the milk, after deproteinisation with TCA, as the ferric complex by measuring the absorbance at 460 nanometres (nm). Four millilitre of milk were mixed with trichloroacetic acid (TCA) 20% (w/v) solution for at least 30 min and then filtered through a suitable filter paper (Whatman No. 40). 1.5 mL of the clear filtrate was then mixed with 1.5 mL of the ferric nitrate reagent. Ferric nitrate reagent [$(Fe(NO_3)_3.9H_2O$], was dissolved in 50 mL 2M HNO_3* (*2M HNO_3 is obtained by diluting 138.5 mL 65% HNO_3 to 1000 mL with distilled water) and stored in darkness. The measurement was required to be carried out within 10 min from the addition of the ferric nitrate solution as the coloured complex was not stable for any length of time. SCN^- at concentrations of 0 to 25 ppm was used as standards.

2.5 Analysis of hydrogen peroxide concentration

H_2O_2 concentration in milk was performed according

to a minor modification method of Allen and Wrieden (1982). Absorbance was measured the absorbance at 436 nm by the spectrophotometer.

2.6 Measurement of lactoperoxidase activity

Milk fat was removed immediately by centrifugation at 12,000 x g for 5 min at 4°C, with the residue used for the assay. 10 µL of the defatted milk were mixed with 200 µL of tetramethyl benzidine (TMB) solution and incubated at 37°C for 30 min. After brief centrifugation at 6,000 x g for 1 min, the optical density of the supernatant was measured at 655 nm wavelength by spectrophotometer. LP from bovine milk (lyophilized powder) (E.C. 1.11.1.7) (Sigma, St. Louis, MO) at concentrations of 0 to 24.0 U were used as standards. The quantitative results were expressed in Units (U)/mL of enzyme. (1 U formed 1 mg of purpurogallin from pyrogallol in 20 s at pH 6.0 at 20°C).

2.7 Determination of total bacterial count and coliform count

For TBC, the medium was prepared by NA agar (M001; HIMEDIA) for the development of colonies for TBC by suspending 28 g of NA agar in 1000 mL of purified water. The medium was boiled until completely dissolved and then sterilized by autoclaving at 15 psi steam pressure (121°C) for 15 min. One mL of each milk sample was aseptically mixed with 9 mL of sterile deionized water. Ten-fold serial dilutions were then performed in sterile deionized water and 1 mL from the milk dilutions was poured on to a Petri plate which was then incubated at 32°C for 48±3 h. The resulting numbers of colonies were counted and recorded with a colony forming units per milliliter (CFU/mL) before and after activation of LPs samples. For CC, the medium was prepared by using EMB Agar (Levine) (M002; HIMEDIA) by suspending 37.46 g of EMB Agar in 1000 mL of purified water and then sterilized by autoclaving. The experimental procedures were the same as above described for TBC, except that medium plate was incubated for 24±2 h.

2.8 Statistical analysis

Data analyses were conducted using the General Linear Models procedure of the statistical analysis system (SAS, 1996). Data were analyzed separately as a 2x4 factorial in completely randomized designs (CRD) with temperature, the ratios of NaSCN:$2Na_2CO_3 3H_2O_2$ and their interaction included in the model as fixed factor effects. When the interaction between temperature and the NaSCN:$2Na_2CO_3 3H_2O_2$ was significant, orthogonal polynomial contrasts were performed to determine linear, quadratic and cubic responses to the temperature within the NaSCN:$2Na_2CO_3 3H_2O_2$ ratios. When the main effect of NaSCN:$2Na_2CO_3 3H_2O_2$ ratios were significant, orthogonal polynomial contrasts were performed to determine overall linear, quadratic and cubic responses to temperature. Significance was declared at $P<0.05$.

3. Results and Discussion

3.1 Effect of temperatures and NaSCN:$2Na_2CO_3 3H_2O_2$ ratios on SCN^- concentration, H_2O_2 and LP activity

The effect of temperature on the concentration of SCN^- and LP activity in uninoculated (Table 1) and *E. coli* inoculated milk samples (Table 4) depended upon the interaction of temperature x NaSCN:$2Na_2CO_3 3H_2O_2$ ratios ($P< 0.001$) after activation of LPs. The LP activity of uninoculated and *E. coli* inoculated milk samples after activation of LPs were observed to have increased cubically with increasing ratios of NaSCN:$2Na_2CO_3 3H_2O_2$, whereas the concentration of SCN^- increased quadratically with increasing ratios in only uninoculated raw milk samples. It has been reported that the natural cyclic pattern of LP activity shows alternating peaks and troughs throughout during 7 d at 4°C in winter season (Fonteh *et al.*, 2001). This experiment was carried out from August 2014 to November 2014, thus LP activity may probably show the same pattern as in the winter season. Although the highest SCN^- concentration and LP activity were observed at the highest NaSCN:$2Na_2CO_3 3H_2O_2$ ratios at 25°C, the influence of LPs activation between at 25°C and 30°C on the changes in SCN^- concentration and LP activity was negligible (Table 1 and 4). Table 1 and 4 summarize the changes in the concentration of SCN^- and LP activity; they increased significantly after activation of LPs with increasing NaSCN:$2Na_2CO_3 3H_2O_2$ ratios at the temperatures tested. Similarly, Panthanara *et al.* (2005) reported that the SCN^- concentration in raw milk increased with increasing NaSCN solution added (from 5.16 to 11.11, 15.48 and 19.89 ppm) at 0, 5, 10 and 15 ppm respectively. However, in this case at SUT, LP activity in raw milk showed an obviously low increase level at 0.03 U/mL of enzyme after the addition of $2Na_2CO_3 3H_2O_2$ at 30 mg/kg milk (Maneerate, 2006). Therefore, there was no clear confirmation that the exogenous supplies of SCN^- and/or H_2O_2 levels were sufficient to increase the concentration of SCN^- or LP activity, or to sufficiently activate the LPs to extend the quality of of the milk at various temperatures, because large variations in LP activity and SCN^- content in raw milk were observed among individual animals. The SCN^- concentration depended on the initial milk SCN^- naturally present or from feeding (Wolfson & Sumner, 1993), while LP activity is affected by individual animal species, feed and stage of lactation (Fonteh *et al.*, 2002) and concentration of LP, corroborating that the enzyme is mainly present in the serum phase, which could explain the differences observed. Otherwise, the exogenous supply of SCN^- and H_2O_2 needed to activate the LPs for raw milk preservation will vary in quantity depending on the above mentioned factors. According to The Codex Alimentarius Commission (CAC GL 13/91), recommended guidelines have been established for the use of LPs activation by the addition of 10-15 mg/L of NaSCN in raw milk to increase the overall levels in activated milk to be in the order of 20 mg/L. In general, increasing concentrations of approximately 14 mg/L of both SCN^- (in the

Table 1. Effect of temperatures (25°C vs 30°C) and NaSCN:$2Na_2CO_3 3H_2O_2$ ratios (0:0, 7:15, 14:30 and 21:45 mg/L) on thiocyanate (SCN^-) concentration, lactoperoxidase activity (LP) and milk composition in uninoculated *E. coli* raw milk samples after activation of LPs. (*N*=8)

Temperature	Ratio (mg/L)	After activation of the LPs by addition of NaSCN:$2Na_2CO_3 3H_2O_2$						
		Concentration		Milk composition (%)				
		SCN^- (ppm)	LP (U/mL)	Fat	Protein	Lactose	SNF	TS
25°C	**Mean**	**13.72**	**13.99**	**4.12**	**3.03**	**4.52**	**8.40**	**12.52**
	0:0	3.63^{d}	4.08^{d}	4.12	3.04	4.52	8.39	12.51
	7:15	10.17^{c}	10.66^{c}	4.12	3.03	4.53	8.40	12.52
	14:30	17.37^{b}	17.35^{b}	4.12	3.04	4.52	8.40	12.52
	21:45	23.72^{a}	23.89^{a}	4.12	3.03	4.52	8.40	12.52
	Contrast	*l, q, c*	*l, c*	*ns*	*ns*	*ns*	*ns*	*ns*
30°C	**Mean**	**12.66**	**13.36**	**4.12**	**3.03**	**4.52**	**8.40**	**12.52**
	0:0	3.60^{d}	4.00^{d}	4.12	3.04	4.52	8.40	12.51
	7:15	8.67^{c}	9.79^{c}	4.12	3.03	4.53	8.39	12.52
	14:30	15.50^{b}	16.96^{b}	4.12	3.04	4.52	8.40	12.52
	21:45	22.85^{a}	22.70^{a}	4.12	3.04	4.52	8.40	12.52
	Contrast	*l, q*	*l, c*	*ns*	*ns*	*ns*	*ns*	*ns*
Ratio addition	0:0	3.62^{D}	4.08^{D}	4.12	3.04	4.52	8.40	12.51
	7:15	9.42^{C}	10.22^{C}	4.12	3.03	4.53	8.39	12.52
	14:30	16.43^{B}	17.15^{B}	4.12	3.04	4.52	8.40	12.52
	21:45	23.29^{A}	23.29^{A}	4.12	3.04	4.52	8.40	12.52
	Contrast	*l, q*	*l, c*	*ns*	*ns*	*ns*	*ns*	*ns*
SEM								
Temperature		0.027	0.013	0.001	0.002	0.001	0.001	0.001
Ratio addition		0.038	0.018	0.001	0.002	0.002	0.002	0.002
Temperature*Ratio		0.054	0.026	0.002	0.003	0.003	0.003	0.002
P value								
Temperature		<0.001	<0.001	0.827	1.000	0.599	0.185	0.249
Ratio addition		<0.001	<0.001	0.821	0.683	0.353	0.181	0.187
Temperature*Ratio		<0.001	<0.001	0.912	0.888	0.780	0.647	0.717

^{a,b,c,d} Means within the same column within temperature having different superscript letters are different at P<0.05; ^{A,B,C,D} Means within the same column for the main effects of temperature or ratio having different superscript letters are different at P<0.05; *l, q, c*: Within a column, the effect of ratio for individual temperature or the main effect of ratio is linear, quadratic, and cubic, respectively, at P<0.05; SNF = solid not fat; TS = total solid; SEM = standard error of mean

form of sodium or potassium salt) and sodium percarbonate (30 mg/L) is recommended by Codex Alimentarius Commission so that the reaction with each other is sufficient to activate the LPs in raw milk. Therefore, the LP activity measured at 1.44 U/mL is sufficient to act as a catalyst for LP activity (Marshall *et al.*, 1986). Our data shows that the introduction of the LPs at different temperatures can significantly increase the SCN^- concentration and LP activity similar to that reported elsewhere.

In all the milk samples H_2O_2 was not detected either before or after activation of LPs. Neither was H_2O_2 detected in LP-treated raw milk (Maneerate, 2006). In fact, H_2O_2 is not normally detected in raw milk (Seifu *et al.*, 2005). H_2O_2 only exists temporarily, being absorbed by the substrate of the system in a few hours and does not pose any hazards. This result shows bacteria did not contaminate the milk, such as with catalase-negative bacteria or mastitis infection. These bacteria possess the ability to produce sufficient amounts of H_2O_2 under anaerobic conditions. In contrast, Schiffman *et al.* (1992) observed that values of H_2O_2 obtained ranged from 2 to 4 mg/L for sterile cow milk and gradually decreased for 6 h and continued throughout the analysis at 6, 12, 24, and 48 h after sampling in Manchega ewe milk (Althaus *et al.*, 2001).

3.2 Effect of temperatures and NaSCN:$2Na_2CO_3 3H_2O_2$ ratios on milk composition

Milk composition was not affected at any of the temperatures, NaSCN:$2Na_2CO_3 3H_2O_2$ ratios or their interactions before or after activation of LPs either with uninoculated *E.coli* or with inoculated milk samples. Maneerate (2006) reported that the milk composition obtained from the tank of raw milk and the quality of LP-treated milk at 16.781:30 mg/kg of KSCN:$2Na_2CO_3 3H_2O_2$ were similar to those of regular raw milk. They was no evidence of negative effects in the milk composition activated with the LPs. Regarding the effects of activation of the LPs with different concentrations of SCN^- and H_2O_2 on the preservation of quality in the milk samples, no effect on milk composition have found (Ndambi *et al.*, 2008; Omer Ibrahim & Zawahir, 2013; Ponce, 2010; Seifu *et al.*, 2004b). Thus, it is not unexpected that the present results derived from the samples prior to LP activation conformed quite closely to those of regular raw milk. Indeed, there has been no evidence of undesirable effects in populations consuming milk activated with the LPs for more than 10 years (Fernandez *et al.*, 2005).

3.3 Effect of temperatures and NaSCN:$2Na_2CO_3 3H_2O_2$ ratios on total bacterial count and coliform count

The present data showed that the potential of LPs activation by the addition of NaSCN:$2Na_2CO_3 3H_2O_2$ ratios to raw milk samples has improved the keeping of milk quality during storage as confirmed by TBC and CC. Undesirable microbes that can cause spoilage of milk products include Gram-negative psychrotrophs, coliforms and lactic acid bacteria etc., (Ibrahim *et al.*, 2015). In addition, various bacteria of public health concern with well known food pathogenic bacteria as pathogenic strains of *E. coli* may also be found in milk and dairy products due to their rich nutrients. For this reason, increased emphasis should be placed on the microbiological examination of sterile milk when contaminated by *E. coli*. To explore this relationship further, the possible use of the LPs was investigated in order to find out whether it could serve as an alternative method of preservation of milk quality by increasing the storage stability for extended periods at high ambient temperatures, by using a microbial-contaminated experiment in which the bacterium *E. coli* was used as a spoilage model. Therefore, apart from normal milk samples, we also inoculated *E. coli* in sterile milk samples, which is the main cause of the deterioration of milk over time, in order to accelerate the spoilage rate. The effect of activation of LPs on microorganism growth was then monitored at 0-12 h for uninoculated and *E. coil* inoculated milk samples. The results of the initial mean TBC of uninoculated milk samples were 1.59×10^5 CFU/mL (Table 2) whereas those of samples which were inoculated with *E. coli* at the beginning were 1.81×10^5 CFU/mL (Table 5).

As shown in Table 2, the responses of TBC to the ratio additions depended upon the interaction of temperatures after LP activation at 0, 3, 6 and 12 h. TBC decreased with increasing ratio additions at a lower temperature (25°C). Regardless of the temperature effect, the response of TBC to additional ratios depended on the time of incubation as TBC increased with increasing time of incubation throughout the period as expected (Tables 2 and 5). The TBC of uninoculated milk samples showed a significant difference for the extension of shelf life at 7:15 mg/L NaSCN:$2Na_2CO_3 3H_2O_2$ at 25°C and 30°C for 6 h, compared with at 14:30 and 21:45 mg/L NaSCN:$2Na_2CO_3 3H_2O_2$ for 12 h (Table 2). Furthermore, the TBC of the *E. coli* inoculated milk samples revealed a similar difference in the extension of shelf life at 7:15 mg/L NaSCN:$2Na_2CO_3 3H_2O_2$ at 25°C and 30°C for 6 h, but not at 14:30 and 21:45 mg/L NaSCN:$2Na_2CO_3 3H_2O_2$ for 9 h (Table 5). The TBC of both the uninoculated and *E. coli* inoculated milk samples after LPs activation of the milk tended to decrease lower from 0 to 12 h. This suggests that the activation of LP by the addition of NaSCN:$2Na_2CO_3 3H_2O_2$ could be used as effective treatment to extend milk quality during storage by reducing the microbial population caused by the spoilage present in milk. Therefore, even if the mean TBC of both uninoculated and *E. coli* inoculated milk samples during the storage reached their peak at the end of the experiment (12 h), their pattern was slightly different from the beginning to the end of the experiment due to the narrow range of temperatures. Similarly, Dajanta *et al.* (2008) reported that the effect of the LPs on both samples of untreated raw milk (control) and *E. coli* inoculated raw milk decreased slightly with differences in the total viable counts from 0 to 8 h at 37°C, which also peaked at the end of 8 h. Although these results seem to be subject to some slight fluctuation during the different temperatures and incubation times tested, the inhibitory effect of LPs of uninoculated and *E. coli* inoculated milk samples tended to decrease after 12 h of incubation. From the results obtained in this study of the differences in bacterial counts between uninoculated and *E. coli* inoculated milk samples after activation of LPs, TBC was found to be lower than those of control throughout the experiment. It can therefore be concluded that the benefits of activation of the LPs in milk preservation are evident.

The LPs has been reported to be effective against a broad spectrum of microorganisms, the bacteriostatic and bactericidal effect was increased by LPs (Pitt *et al.*, 2000). The differences in responses to inhibitory microbial growth of LPs of Gram-positive and Gram-negative bacteria may be due to variations in species, strains of contaminating bacteria and environment conditions (Naidu, 2000). According to El-Agamy *et al.* (1992), the antibacterial system of LP is bacteriostatic to Gram-positive bacteria and bactericidal to Gram-negative bacteria, whereas the bactericidal activity against *E. coli* O157:H7 at 4°C could inhibit *E. coli* and *L. innocua* at 20°C for 24 h, but did not cause any inactivation after this period (Garcia-Graells *et al.*, 2000). Additionally, Seifu *et al.* (2004b) showed that the LPs was bactericidal and bacteriostatic against *S. aureus* ATCC 25923, *L. monocytogenes* and *E.coli* in Saanen and South African Indigenous

Table 2. Effect of temperatures (25°C vs 30°C) and NaSCN:$2Na_2CO_3 3H_2O_2$ ratios (0:0, 7:15, 14:30 and 21:45 mg/L) on total bacterial count (TBC) in uninoculated *E. coli* raw milk samples after activation of LPs at incubation time (0, 3, 6, 9 and 12 h). (*N*=8)

Temperature	Ratio (mg/L)	Number of TBC after activation of the LPs				
		Time (h)				
		0	3	6	9	12
25°C	**Mean**	**$1.56x10^{5A}$**	**$3.37x10^{5B}$**	**$2.10x10^{5B}$**	**$1.05x10^{6A}$**	**$4.53x10^{7B}$**
	0:0	$1.52x10^{5b}$	$3.53x10^{5a}$	$4.97x10^{5a}$	$3.40x10^{6a}$	$1.62x10^{8a}$
	7:15	$1.75x10^{5a}$	$3.27x10^{5b}$	$2.44x10^{5b}$	$6.67x10^{5b}$	$1.80x10^{7b}$
	14:30	$1.53x10^{5b}$	$3.67x10^{5a}$	$3.98x10^{4d}$	$5.40x10^{4c}$	$4.00x10^{5c}$
	21:45	$1.45x10^{5b}$	$3.01x10^{5c}$	$5.96x10^{4c}$	$7.12x10^{4c}$	$4.57x10^{6c}$
	Contrast	*q, c*	*l, q, c*	*l, q, c*	*l, q, c*	*l, q, c*
30°C	**Mean**	**$1.62x10^{5A}$**	**$3.51x10^{5A}$**	**$4.11x10^{5A}$**	**$5.33x10^{6A}$**	**$4.74x10^{7A}$**
	0:0	$1.79x10^{5a}$	$3.82x10^{5a}$	$7.26x10^{5a}$	$3.26x10^{6a}$	$1.71x10^{8a}$
	7:15	$1.57x10^{5b}$	$3.62x10^{5b}$	$2.67x10^{5b}$	$7.93x10^{5b}$	$1.86x10^{7b}$
	14:30	$1.56x10^{5b}$	$3.46x10^{5c}$	$1.46x10^{4d}$	$3.17x10^{4c}$	$4.14x10^{4c}$
	21:45	$1.57x10^{5b}$	$3.16x10^{5d}$	$6.29x10^{4c}$	$7.56x10^{4c}$	$2.49x10^{5c}$
	Contrast	*l, q*	*l*	*l, q, c*	*l, q, c*	*l, q, c*
Ratio addition	**Mean***	**$1.59x10^{5D}$**	**$3.44x10^{5C}$**	**$2.39x10^{5D}$**	**$1.04x10^{6B}$**	**$4.64x10^{7A}$**
	0:0	$1.66x10^{5A}$	$3.68x10^{5A}$	$6.12x10^{5A}$	$3.33x10^{6A}$	$1.67x10^{8A}$
	7:15	$1.66x10^{5A}$	$3.44x10^{5C}$	$2.56x10^{5B}$	$7.31x10^{5B}$	$1.83x10^{7B}$
	14:30	$1.54x10^{5AB}$	$3.56x10^{5B}$	$2.74x10^{4D}$	$4.29x10^{4C}$	$2.21x10^{5C}$
	21:45	$1.51x10^{5B}$	$3.09x10^{5D}$	$6.12x10^{4C}$	$7.34x10^{4C}$	$3.53x10^{5C}$
	Contrast	*l, q*	*l, q, c*	*l, q, c*	*l, q, c*	*l, q, c*
SEM						
Temperature		0.282	0.273	0.360	0.332	0.322
Ratio addition		0.399	0.387	0.509	0.469	0.456
Temperature*Ratio		0.564	0.547	0.720	0.663	0.644
P value						
Temperature		0.145	<0.001	<0.001	0.891	<0.001
Ratio addition		0.015	<0.001	<0.001	<0.001	<0.001
Temperature*Ratio		0.002	<0.001	<0.001	0.287	<0.001

[a,b,c,d] Means within the same column within temperature having different superscript letters are different at P<0.05; [A,B] Means within the same column within time having different superscript letters are different at P<0.05; [A,B,C,D] Means* within the same row within time having different superscript letters are different at P<0.05; [A,B,C,D] Means within the same column for the main effects of temperature or ratio having different superscript letters are different at P<0.05; *l, q, c*: Within a column, the effect of ratio for individual temperature or the main effect of ratio is linear, quadratic, and cubic, respectively, at P<0.05; SEM = standard error of mean

goat milk respectively, at 30°C. Furthermore, different bacteria groups show varying degrees of sensitivity to the LPs (Seifu *et al.*, 2005) it can also probably be explained by the differences in cell wall structure and their different barrier properties (de Wit and van Hooydonk, 1996). For bacteria that survive the initial bactericidal activity of the LPs, there is an extended lag phase or recovery period. The length of this lag period is highly temperature dependent, being much longer at cold storage than at high temperatures (Kamau & Kroger, 1984). Most bacteria resume regular growth after recovery. Therefore, the length of the antibacterial effect achieved by the LPs activation is inversely related to the storage temperature of the milk (IDF, 1988). The antibacterial effect of the LPs lasts for 7 to 8, 11 to 12, 16 to 17 and 24 to 26 h, when milk is stored at 30, 25, 20 and 15°C, respectively (IDF, 1988).

The effect of temperature depended on the NaSCN: $2Na_2CO_3 3H_2O_2$ ratio (temperature x ratio interaction) after activation of LPs on CC (at 3, 6, 9 and 12 h) (Table 3). CC revealed that the difference in elevated temperature had an effect on the quality of milk subject to LPs activation, as it was extended less at 30°C than at 25°C. The results further confirm the effectiveness of lower temperatures on LPs in milk preservation. These results are in conformity with those obtained by other authors who also noticed bacterial inhibi-

Table 3. Effect of temperatures (25°C vs 30°C) and NaSCN:$2Na_2CO_3 3H_2O_2$ ratios (0:0, 7:15, 14:30 and 21:45 mg/L) on coliform count (CC) in uninoculated *E. coli* raw milk samples after activation of LPs at incubation time (0, 3, 6, 9 and 12 h). (*N*=8)

Temperature	Ratio (mg/L)	Number of CC after activation of the LPs				
		Time (h)				
		0	3	6	9	12
25°C	**Mean**	**$1.77x10^{3B}$**	**$5.46x10^{3B}$**	**$9.85x10^{3B}$**	**$6.64x10^{4B}$**	**$9.77x10^{5B}$**
	0:0	$1.86x10^{3}$	$5.82x10^{3}$	$1.63x10^{4a}$	$1.83x10^{5a}$	$3.23x10^{6a}$
	7:15	$1.72x10^{3}$	$5.18x10^{3}$	$1.30x10^{4b}$	$5.27x10^{4b}$	$3.12x10^{5b}$
	14:30	$1.78x10^{3}$	$5.86x10^{3}$	$3.02x10^{3d}$	$1.10x10^{4d}$	$1.52x10^{5d}$
	21:45	$1.71x10^{3}$	$4.98x10^{3}$	$7.11x10^{3c}$	$1.90x10^{4c}$	$2.15x10^{5c}$
	Contrast	*ns*	*ns*	*l*	*l, q*	*l, q, c*
30°C	**Mean**	**$3.82x10^{3A}$**	**$1.38x10^{4A}$**	**$2.44x10^{4A}$**	**$2.87x10^{5A}$**	**$2.42x10^{6A}$**
	0:0	$4.04x10^{3a}$	$2.16x10^{4a}$	$4.29x10^{4a}$	$7.11x10^{5a}$	$7.13x10^{6a}$
	7:15	$3.89x10^{3ab}$	$1.19x10^{4b}$	$2.76x10^{4b}$	$3.60x10^{5b}$	$1.40x10^{6b}$
	14:30	$3.80x10^{3ab}$	$1.18x10^{4b}$	$1.30x10^{4c}$	$3.61x10^{4c}$	$4.16x10^{5d}$
	21:45	$3.57x10^{3b}$	$9.76x10^{3b}$	$1.40x10^{4c}$	$3.99x10^{4c}$	$7.57x10^{5c}$
	Contrast	*l*	*l, q, c*	*l, q*	*l, q, c*	*l, q, c*
Ratio addition	**Mean***	**$2.80x10^{3C}$**	**$9.62x10^{3C}$**	**$1.71x10^{4C}$**	**$1.76x10^{5B}$**	**$1.70x10^{6A}$**
	0:0	$2.95x10^{3A}$	$1.37x10^{4A}$	$2.96x10^{4A}$	$4.47x10^{5A}$	$5.18x10^{6A}$
	7:15	$2.81x10^{3AB}$	$8.55x10^{3B}$	$2.03x10^{4B}$	$2.06x10^{5B}$	$8.56x10^{5B}$
	14:30	$2.79x10^{3AB}$	$8.85x10^{3B}$	$8.02x10^{3C}$	$2.35x10^{4C}$	$2.83x10^{5D}$
	21:45	$2.64x10^{3B}$	$7.37x10^{3B}$	$1.06x10^{4C}$	$2.94x10^{4C}$	$4.86x10^{5C}$
	Contrast	*l*	*l, q, c*	*l*	*l, q*	*l, q, c*
SEM						
Temperature		0.405	0.372	0.408	0.257	0.416
Ratio addition		0.573	0.526	0.576	0.364	0.589
Temperature*Ratio		0.810	0.744	0.815	0.515	0.832
P value						
Temperature		<0.001	<0.001	<0.001	<0.001	<0.001
Ratio addition		0.004	<0.001	<0.001	<0.001	<0.001
Temperature*Ratio		0.178	<0.001	<0.001	<0.001	<0.001

[a,b,c,d] Means within the same column within temperature having different superscript letters are different at P<0.05; [A,B] Means within the same column within time having different superscript letters are different at P<0.05; [A,B,C] Means* within the same row within time having different superscript letters are different at P<0.05; [A,B,C,D] Means within the same column for the main effects of temperature or ratio having different superscript letters are different at P<0.05; *l, q, c*: Within a column, the effect of ratio for individual temperature or the main effect of ratio is linear, quadratic, and cubic, respectively, at P<0.05; SEM = standard error of mean

tion by the LPs (Eyassu *et al.*, 2004; Kangumba *et al.*, 1997; Mark *et al.*, 2001). The efficacy of the LPs persists for a limited period of time, which decreases as the ambient temperature increases; it is defined only in a range between 15 and 30°C in the original Codex guidelines (FAO, 2005). Similarly, Panthanara *et al.* (2005) reported that the activation of LPs increased SCN^- concentrations in their milk to improve the efficiency of the LPs to a decreased total plate count and CC (P<0.01) at 25°C less than at 4°C. These results confirm those of the present study that also show a decrease in the number of CC after activation of LPs which is lower when the temperature is higher. In addition to the above factors affecting LPs, there were also the storage temperatures and length of incubation (Gay & Amar, 2005; Lin & Chow, 2000). Zapico *et al.* (1995) observed that *the Pseudomonas fluorescens* counts were lower than the initial level in activated LPs goats' milk for 5 d at 4°C and 3 d at 8°C. The *E. coli* counts were not grown in raw goats' milk at 4°C, but were able to grow in the control milk with no apparent lag phase at 8°C. Consequently, the response for *E. coli* was negligible from the influence of LPs activation at 4°C. In contrast, a lag phase of 2 d was observed in activated LPs milk at 8°C, resulting in *E. coli* being lower than the control milk during the first 5 d. Stefano *et al.* (1995) reported that LPs prolonged

Table 4. Effect of temperatures (25°C vs 30°C) and NaSCN:$2Na_2CO_3 3H_2O_2$ ratios (0:0, 7:15, 14:30 and 21:45 mg/L) on thiocyanate (SCN^-) concentration, lactoperoxidase activity (LP) and milk composition in *E. coli* inoculated milk samples after activation of LPs. (*N*=8)

Temperature	Ratio (mg/L)	After activation of the LPs by addition of NaSCN:$2Na_2CO_3 3H_2O_2$						
		Concentration		Milk composition (%)				
		SCN^- (ppm)	LP (U/mL)	Fat	Protein	Lactose	SNF	TS
25°C	**Mean**	**13.18**	**13.07**	**4.12**	**3.03**	**4.52**	**8.40**	**12.52**
	0:0	3.43^{d}	4.08^{d}	4.11	3.04	4.53	8.39	12.51
	7:15	10.04^{c}	10.00^{c}	4.12	3.03	4.53	8.40	12.52
	14:30	16.91^{b}	16.03^{b}	4.12	3.04	4.52	8.39	12.51
	21:45	22.35^{a}	22.06^{a}	4.12	3.03	4.52	8.40	12.52
	Contrast	*l, q, c*	*l, c*	*ns*	*ns*	*ns*	*ns*	*ns*
30°C	**Mean**	**12.23**	**12.26**	**4.12**	**3.03**	**4.52**	**8.40**	**12.52**
	0:0	3.33^{d}	3.84^{d}	4.12	3.04	4.53	8.40	12.52
	7:15	8.50^{c}	9.37^{c}	4.12	3.04	4.52	8.40	12.52
	14:30	15.39^{b}	15.06^{b}	4.11	3.04	4.52	8.40	12.52
	21:45	21.69^{a}	20.75^{a}	4.12	3.03	4.52	8.39	12.51
	Contrast	*l, q, c*	*l, q, c*	*ns*	*ns*	*ns*	*ns*	*ns*
Ratio addition	0:0	3.38^{D}	3.96^{D}	4.12	3.04	4.53	8.40	12.52
	7:15	9.27^{C}	9.69^{C}	4.12	3.04	4.53	8.40	12.52
	14:30	16.15^{B}	15.55^{B}	4.12	3.04	4.52	8.40	12.52
	21:45	22.02^{A}	21.41^{A}	4.12	3.03	4.52	8.40	12.52
	Contrast	*l, q, c*	*l, q, c*	*ns*	*ns*	*ns*	*ns*	*ns*
SEM								
Temperature		0.033	0.016	0.001	0.002	0.001	0.002	0.002
Ratio addition		0.042	0.023	0.001	0.003	0.002	0.003	0.001
Temperature*Ratio		0.061	0.033	0.002	0.003	0.002	0.003	0.002
P value								
Temperature		<0.001	<0.001	0.848	1.008	0.620	0.206	0.260
Ratio addition		<0.001	<0.001	0.833	0.702	0.368	0.193	0.194
Temperature*Ratio		<0.001	<0.001	0.926	0.893	0.796	0.670	0.730

[a,b,c,d] Means within the same column within temperature having different superscript letters are different at P<0.05; [A,B,C,D] Means within the same column for the main effects of temperature or ratio having different superscript letters are different at P<0.05; *l, q, c*: Within a column, the effect of ratio for individual temperature or the main effect of ratio is linear, quadratic, and cubic, respectively, at P<0.05; SNF = solid not fat; TS = total solid; SEM = standard error of mean

the shelf-life of the LPs activated milk at 30°C and 35°C for 6 h, and for 7 h in goat milk during storage in ambient temperatures (Nigussie & Seifu, 2008). In addition, Ndambi *et al.* (2007) observed that the control milk stored in the refrigerator (6-8°C) for 4 d had a lower microbial load than milk stored under ambient temperatures (22-25°C) for 8 h. Also, treated milk samples stored under refrigeration for 3 d had a lower microbial load than the control milk samples stored in ambient temperatures for 5 h. Cold treated camel milk samples at 4°C with LPs were found to increase shelf life from 15 to 21 d, compared to from 3 to 7 d for the control (El-Demerdash & Otaibi, 2012).

On the other hand, the CC of uninoculated milk where the LPs was activated tended to be lower from 0 to 12 h than *E. coli* inoculated milk samples. Interestingly, it was clearly observed that, when the LPs was introduced to milk samples with uninoculated *E. coli* milk, the CC was lower than that of the samples *with E. coli* inoculated milk throughout the experiment. LPs may be used alone or in conjunction with processing of heat treatment to reduce or eliminate the bacterial load in milk products (Musser, 2011). However, the milk pasteurized with LP treatment can be reactivated to inhibit microbial growth to extend the shelf life of raw milk (Barrett *et al.*, 1999). In one study, it was found that the LPs greatly increased the keeping quality of milk at 37°C, which was inoculated with *P. aeruginosa, S. aureus* and *S. thermophilus* and pasteurised at 72°C (Mark *et al.*, 2001). Moreover, *E. coli* has been reported to be inhibited by LPs

Table 5. Effect of temperatures (25°C vs 30°C) and NaSCN:$2Na_2CO_3 3H_2O_2$ ratios (0:0, 7:15, 14:30 and 21:45 mg/L) on total bacterial count (TBC) in *E. coli* inoculated milk samples after activation of LPs at incubation time (0, 3, 6, 9 and 12 h). (*N*=8)

Temperature	Ratio (mg/L)	Number of TBC after activation of the LPs				
		Time (h)				
		0	3	6	9	12
25°C	**Mean**	**$1.85x10^{5A}$**	**$3.25x10^{5B}$**	**$2.20x10^{5B}$**	**$1.05x10^{6B}$**	**$3.49x10^{7A}$**
	0:0	$1.63x10^{5}$	$3.56x10^{5a}$	$4.99x10^{5a}$	$3.39x10^{6a}$	$1.20x10^{8a}$
	7:15	$1.77x10^{5}$	$3.29x10^{5b}$	$2.47x10^{5b}$	$6.71x10^{5b}$	$1.82x10^{6b}$
	14:30	$1.68x10^{5}$	$3.12x10^{5bc}$	$8.87x10^{4c}$	$7.96x10^{4c}$	$6.38x10^{5c}$
	21:45	$2.33x10^{5}$	$3.02x10^{5c}$	$4.31x10^{4d}$	$5.91x10^{4d}$	$5.14x10^{5c}$
	Contrast	*ns*	*l*	*l*, *q*	*l*, *q*, *c*	*l*, *q*, *c*
30°C	**Mean**	**$1.77x10^{5A}$**	**$3.53x10^{5A}$**	**$2.69x10^{5A}$**	**$1.11x10^{6A}$**	**$3.94x10^{7A}$**
	0:0	$1.81x10^{5}$	$3.84x10^{5a}$	$7.28x10^{5a}$	$3.54x10^{6a}$	$1.41x10^{8a}$
	7:15	$1.59x10^{5}$	$3.64x10^{5b}$	$2.69x10^{5b}$	$7.95x10^{5b}$	$1.86x10^{7b}$
	14:30	$2.11x10^{5}$	$3.48x10^{5c}$	$6.13x10^{4c}$	$7.32x10^{4c}$	$6.18x10^{5c}$
	21:45	$1.57x10^{5}$	$3.18x10^{5d}$	$1.64x10^{4d}$	$3.35x10^{4d}$	$5.67x10^{5d}$
	Contrast	*ns*	*l*	*l*, *q*, *c*	*l*, *q*, *c*	*l*, *q*, *c*
Ratio addition	**Mean***	**$1.81x10^{5B}$**	**$3.39x10^{5B}$**	**$2.44x10^{5B}$**	**$1.08x10^{5B}$**	**$3.70x10^{7A}$**
	0:0	$1.72x10^{5}$	$3.70x10^{5A}$	$6.14x10^{5A}$	$3.47x10^{6A}$	$1.29x10^{8A}$
	7:15	$1.68x10^{5}$	$3.47x10^{5B}$	$2.58x10^{5B}$	$7.33x10^{5B}$	$1.84x10^{7B}$
	14:30	$1.90x10^{5}$	$3.30x10^{5C}$	$7.50x10^{4C}$	$7.64x10^{4C}$	$6.28x10^{5C}$
	21:45	$1.95x10^{5}$	$3.10x10^{5D}$	$2.98x10^{4D}$	$4.63x10^{4D}$	$5.40x10^{5C}$
	Contrast	*ns*	*l*	*l*, *q*, *c*	*l*, *q*, *c*	*l*, *q*, *c*
SEM						
Temperature		0.501	0.257	0.425	0.210	0.266
Ratio addition		0.709	0.363	0.601	0.296	0.397
Temperature*Ratio		1.002	0.514	0.850	0.419	0.583
P value						
Temperature		0.715	<0.001	<0.001	<0.001	0.453
Ratio addition		0.788	<0.001	<0.001	<0.001	<0.001
Temperature*Ratio		0.273	0.174	<0.001	<0.001	0.340

[a,b,c,d] Means within the same column within temperature having different superscript letters are different at P<0.05; [A,B] Means within the same column within time having different superscript letters are different at P<0.05; [A,B] Means* within the same row within time having different superscript letters are different at P<0.05; [A,B,C,D] Means within the same column for the main effects of temperature or ratio having different superscript letters are different at P<0.05; *l, q, c*: Within a column, the effect of ratio for individual temperature or the main effect of ratio is linear, quadratic, and cubic, respectively, at P<0.05; SEM = standard error of mean

combined with high hydrostatic pressure in skimmed milk (Garcia-Graells *et al.*, 2003).

With respect to the conditions for raw milk testing and quality control systems in Thailand, a certain level of industrial organisation is required for dairy factories before purchasing raw milk, to enable grading of milk for processing into various dairy products. TBC of all milk samples did not exceed the acceptable standard set by the Ministry of Agriculture and Cooperatives of Thailand (MOAC, 2015) ($5x10^5$ CFU/mL) for the addition of all ratios from 0 to 6 h, while CC remained within acceptable standards at $1x10^4$ CFU/mL 0 to 3 h. However, after LP activation TBC and CC were maintained for study intervals at 6 and 3 h and showed a lower count throughout the experiment. The mean TBC decreased gradually until 12 h finally increasing to from 10^5 to10^7 CFU/mL and 10^3 to 10^6 CFU/mL for CC. Therefore, the 6 h extension of the shelf-life for milk with LPs activation does hold significant value in terms of the practicalities of transportation in some regions without refrigeration facilities. It can also have significant and positive implications for public health with respect to milk products made from milk for which the quality has been improved via the use of LPs activation.

Finally, it must be highlighted that the utilisation of

Table 6. Effect of temperatures (25°C vs 30°C) and NaSCN:$2Na_2CO_3 3H_2O_2$ ratios (0:0, 7:15, 14:30 and 21:45 mg/L) on coliform count (CC) in *E. coli* inoculated milk samples after activation of LPs at incubation time (0, 3, 6, 9 and 12 h). (*N*=8)

Temperature	Ratio (mg/L)	Number of CC after activation of the LPs				
		Time (h)				
		0	3	6	9	12
25°C	**Mean**	**$2.13x10^{3B}$**	**$6.10x10^{3B}$**	**$1.10x10^{4B}$**	**$7.38x10^{4B}$**	**$1.54x10^{6B}$**
	0:0	$2.34x10^{3a}$	$6.90x10^{3a}$	$1.65x10^{4a}$	$1.86x10^{5a}$	$5.23x10^{6a}$
	7:15	$2.26x10^{3a}$	$6.21x10^{3b}$	$1.30x10^{4b}$	$6.78x10^{4b}$	$4.70x10^{5b}$
	14:30	$1.98x10^{3b}$	$6.07x10^{3b}$	$7.31x10^{3c}$	$2.21x10^{4c}$	$2.31x10^{5c}$
	21:45	$1.92x10^{3b}$	$5.20x10^{3c}$	$7.20x10^{3d}$	$1.92x10^{4d}$	$2.32x10^{5d}$
	Contrast	*l*	*l*	*l*	*l*, *q*	*l*, *q*, *c*
30°C	**Mean**	**$3.84x10^{3A}$**	**$1.45x10^{4A}$**	**$2.74x10^{4A}$**	**$3.13x10^{5A}$**	**$3.07x10^{6A}$**
	0:0	$4.10x10^{3a}$	$2.38x10^{4a}$	$4.60x10^{4a}$	$8.05x10^{5a}$	$7.32x10^{6a}$
	7:15	$3.91x10^{3ab}$	$1.21x10^{4b}$	$2.85x10^{4b}$	$3.68x10^{5b}$	$3.42x10^{6b}$
	14:30	$3.70x10^{3b}$	$1.20x10^{4b}$	$2.01x10^{4b}$	$4.02x10^{4c}$	$7.74x10^{5c}$
	21:45	$3.67x10^{3b}$	$1.01x10^{4c}$	$1.51x10^{4c}$	$4.00x10^{4d}$	$7.70x10^{5d}$
	Contrast	*l*	*l*, *c*	*l*	*l*, *c*	*l*, *q*, *c*
Ratio addition	**Mean***	**$2.99x10^{3C}$**	**$1.03x10^{4C}$**	**$1.92x10^{4C}$**	**$1.93x10^{5B}$**	**$2.31x10^{6A}$**
	0:0	$3.22x10^{3A}$	$1.54x10^{4A}$	$3.13x10^{4A}$	$4.96x10^{5A}$	$6.28x10^{6A}$
	7:15	$3.08x10^{3A}$	$9.16x10^{3B}$	$2.08x10^{4B}$	$2.18x10^{5B}$	$1.95x10^{6B}$
	14:30	$2.85x10^{3B}$	$9.01x10^{3B}$	$1.37x10^{4C}$	$3.11x10^{4C}$	$5.03x10^{5C}$
	21:45	$2.79x10^{3B}$	$7.63x10^{3C}$	$1.12x10^{4C}$	$2.96x10^{4D}$	$5.01x10^{5D}$
	Contrast	*l*	*l*	*l*	*l*	*l*, *q*, *c*
SEM						
Temperature		0.409	0.514	0.543	0.336	0.194
Ratio addition		0.579	0.727	0.768	0.475	0.274
Temperature*Ratio		0.818	1.028	1.086	0.672	0.388
P value						
Temperature		<0.001	<0.001	<0.001	<0.001	<0.001
Ratio addition		<0.001	<0.001	<0.001	<0.001	<0.001
Temperature*Ratio		0.919	0.024	0.115	<0.001	<0.001

[a,b,c,d] Means within the same column within temperature having different superscript letters are different at P<0.05; [A,B] Means within the same column within time having different superscript letters are different at P<0.05; [A,B,C] Means* within the same row within time having different superscript letters are different at P<0.05; [A,B,C,D] Means within the same column for the main effects of temperature or ratio having different superscript letters are different at P<0.05; *l, q, c*: Within a column, the effect of ratio for individual temperature or the main effect of ratio is linear, quadratic, and cubic, respectively, at P<0.05; SEM = standard error of mean

LPs activation for the extension of the shelf-life of milk does not release producers or purchasers from the obligation of practising good farming techniques and vigilance in terms of observing the general principles of hygiene for the handling and transportation of raw milk. Emphasis should be placed on its use for the purposes of retaining the quality of high grade milk rather than improving the viability of low-grade or marginal milk. Equally, this technique for the preservation of raw milk quality can provide encouragement to the dairy farming industry in terms of minimising wastage and reducing the costs of transportation while facilitating the potential for collection of milk from more remote operations. The broader focus of this study is to provide additional rationale with respect to solutions for developing nations which have dairy farming industries concentrated around small operations and why 'this strategy to maintain raw milk quality by use of the LPs' is necessary for agriculture in tropical developing countries where milk storage temperatures may exceed 30°C during daytime, and may fall below 15°C during night-time. Dairy cattle farmers operating small holdings in Thailand will receive information on the research as well as information on how cooling is the best method for the purposes of the extension of raw milk quality during storage and transportation from farm to dairy plant. Therefore, only minimal training

will be required for the application of this technique under such conditions.

4. Conclusions

Activation of the LPs exhibited decreased microbial population both in uninoculated and *E. coli* inoculated milk samples, thus showing that it is an effective method for extending raw milk quality during storage in areas in Thailand where there is a lack of cooling facilities. All four additional NaSCN:$2Na_2CO_3 3H_2O_2$ ratios evaluated decreased TBC and CC of milk samples, with 14:30 and 21:45 mg/L of NaSCN: $2Na_2CO_3 3H_2O_2$ being more effective than the 0:0 and 7:15 mg/L of NaSCN:$2Na_2CO_3 3H_2O_2$. The NaSCN:$2Na_2CO_3 3H_2O_2$ ratio response was temperature dependent for most variables. For 25°C and 30°C, TBC and CC in uninoculated *E. coli* milk samples, the optimum ratio rate was 14:30 mg/L NaSCN: $2Na_2CO_3 3H_2O_2$, because a further addition in ratio failed to further decrease TBC which are the main causes of the spoilage of milk over time. For 25°C and 30°C, TBC and CC in *E. coli* inoculated milk samples, the maximum response was observed at the highest ratio (21:45 mg/L NaSCN: $2Na_2CO_3 3H_2O_2$).

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