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Research Article

Effect of Different Levels of Sodium Thiocyanate and Percarbonate for Activation of Lactoperoxidase on the Keeping Quality of Raw Milk

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ABSTRACT

Effect of different levels of sodium thiocynate and sodium percarbonate for activation of lactoperoxidase system on keeping quality of raw cow's milk was investigated. The milk was collected directly from the dairy farm, Faculty of Animal Production Science and Technology, the collection and examination of samples were done during February-march 2012. Three levels of sodium thiocynate and sodium percarbonate (12, 16, 20 and 20, 30 40 mg/L) were used respectively. The samples were activated at about 30 min after morning milking then stored at 37°C for 8 hours. Titratable acidity % (TA), milk composition and total bacterial counts were evaluated. In each case, the keeping quality of activated samples was compared with that of control. The results showed that there were significant variations in the titratable acidity and total bacterial counts between the activated and control milk samplesthrought the storage period. However, the control milk samples were clearly spoiled at about 4th hour of storage,. Also the results revealed that no significant differences in milk composition (fat, protein, density and total solid) was observed among control and activated milk sample.

Keywords: Milk, Preservation, Sodium Thiocynate, Percarbonate, Storage

1. INTRODUCTION

Milk is an ideal habitat for the growth and multiplication of microorganisms due to its nutritional constitution which it is the nutrient-rich liquid contains protein, carbohydrate, unsaturated fat, mineral and vitamins.Several methods were found, other than refrigeration, for retarding bacterial growth in raw milk during collection and transportation. The use of chemical preservatives such as hydrogen peroxide (H_2O_2), alkaline solutions etc, and activation of natural antibacterial enzymes are common practices in developing countries under tropical and subtropical conditions, where refrigeration is not practical [1]. The antibacterial effect of milk proteins is generally mediated by the reaction of hydrogen peroxide (H_2O_2), which is thought to be a major antibacterial substance. Lactoperoxidase, a known milk peroxidase, when combined together with H_2O_2 and iodide, produce a potent anti-bacterial system known as the Lactoperoxidase system [2]. The lactoperoxidase/ thiocyanate/ hydrogen peroxide system is an indigenous antibacterial system in milk, human saliva [3]. Activation of the LP-system has been proposed to be a useful method in preserving raw milk quality. Although the natural LP system in raw milk loses its effect within 2 h of the milking, its antimicrobial activity can be reactivated by addition of thiocyanate and hydrogen peroxide [4]. The LP system is an acceptable chemical method for raw milk preservation, especially in rural areas where refrigeration facilities are absent to farmers [5]. The LP system has been recognized as critical in the dairy industry for the preservation of raw milk, pasteurized milk, cheese and yogurt [6]. Activation of the LP system is amongst the most cost effective approaches to extend the stability of pasteurized and raw milk [7]. In the presence of low levels of thiocyanat (SCN) and H_2O_2, LP exhibits very potent bactericidal activity; this system is 50-500 times more effective than H_2O_2 alone [8]. Several workers have demonstrated the effectiveness of this system in preserving milk when the concentration of these substrates is increased by an exogenous supply [9]. Adolphe *et al*. [10] showed that the lactoperoxidase system's antimicrobial efficiency can be enhanced by better concentration ratis of the LP system components. The objective of this study is to evaluate the activation of lactoperoxidase using different levels of sodium thiocynate and sodium percarbonate and hence its effect on keeping quality of raw cow's milk at 37°C.

2. MATERIAL AND METHODS

Raw cow`s milk samples were collected from the dairy farm, College of Animal Science and Technology, Sudan University of Science and Technology, Hillat Kuku, this study was carried out between February and march 2012. Sodium thiocyanate (Fluka, Switzerland), sodium percarbonate (Riedel-de Haen, Garmany).

2.1. Experimental design

In this study 4 lit of milk were divided into four parts (each, 1L). The first part was the control without activation of Lactoperoxidase while the other three parts were activated with 12, 16 and 20 mg/L and 20, 30 and 40 mg/L of sodium thiocyanate (NaSCN) and sodium percarbonate ($2Na_2CO_3.3H_2O$) respectively, and kept at 37°C (Incubator) for 8 hr.

2.2. Method of activation of lactoperoxidase system

The LPS of the milk samples were activated by the addition of three levels (12, 16, 20 mg/L) of sodium thiocyanate as a source of thiocyanate ion (SCN^-) and after one minute of thorough mixing, three levels (20, 30, 40 mg/L) of sodium percarbonate as a source of hydrogen peroxide were added [11, 12]. The three levels were denoted as LP_1, LP_2 and LP_3. The chemical and microbiological analysises were carried out in triplicate during storage period of 8 hr for samples stored at 37°C and 7 days for those stored in the refrigeration (4°C).

2.3. Chemical analysis

The Titratable acidity of milk samples was determined according to Foley Et Al [13]. The fat content of milk samples was determined according to AOAC [14]**.** Kjeldahl method was used for protein evaluation according to AOAC [14]. The total solids contents were estimated according to method described by EL-Neemer [15]**.**

2.4. Microbiological analysis

Total bacterial count was estimated by standard method [16]. One milliter of milk was diluted with 9 ml sterile buffer peptone water then serial dilutions were made with 81 ml sterile peptone water for milk samples up to 10^{-5}-10^{-6}-10^{-7} were carried out on plate count agar plates then sterile Plate count agar were spread (Dextrose, India 2016) onto PCA plates for total bacterial count, which were then incubated at 37°C for 24 h. Every trial was conducted separately three times (each in triplicate), and the arithmetic means with the corresponding standard deviations were calculated. The resulting colonies were counted and expressed as colony forming units per milliliter (cfu/mL^{-1}) of sample, and then the microbial counts were expressed as their logarithms.

2.5. Statistical analysis

Statistical analyses were carried out with SPSS [17] Version 17. General linear model was used for data analysis (factorial design), three levels of each of sodium thiocynate and sodium percarbonate (12, 16 and 20; 20, 30 and 40 mg/L) were used and two levels of storage periods (8hrs and 7 days) were used. Duncans Multiple range test were used to compare means of the different treatments.

3. RESULTS

Results in Table 1 present the titratable acidity (expressed as lactic acid %) of cow's milk treated with sodium thiocyanate and sodium percarbonate at levels of 12, 16 and 20 mg/L and 20, 30 and 40 mg/L respectively kept at incubator (37°C) for 8 hours.

Table 1: Titratable acidity % of raw cow's milk samples treated with different levels of sodium thiocynate and sodium percarbonate during storage at 37°C (incubation)

Treatment	Storageperiods(h)					
	0	**2**	**4**	**6**	**8**	Sig
Lp_1	0.157±.01	0.163±.00	0.163±.00	0.173±.00	0.173±.00	
Lp_2	0.15±.00	0.160±.00	0.160±.00	0.170±.00	0.170±.00	**
Lp_3	0.15±.00	0.160±.00	0.160±.00	0.170±.00	0.170±.00	
C	0.16±.00	0.170±.00	0.190±.00	0.190±.00	0.22±.10	
Sig			**			
Main effect						
	Storage period(hrs)					
Treatment	0	2	4	6	8	
TA%	0.154±.01[e]	0.163±.01[d]	0.168±.01[c]	0.176±.01[b]	0.183±.01[a]	**
Treatment	Lp_1	Lp_2	Lp_3	C		
TA%	0.166±.001[b]	0.162±.001[c]	0.162±.001[c]	0.186±.001[a]		**

Means within the same row followed by different superscript are significantly ($p< 0.05$) different

TA: Titratable acidity (% lactic acid).sample with an acidity >0.20 % recorded as rejected.

Lp1: samples treated by activation of lactoperxidase system level one (12+ 20 mg/l of sodium thioyanat and percarbonate).

Lp2: level two (16+30 mg/l of sodium thioyanat and percarbonate)

Lp3: level three (20+40 mg/l of sodium thioyanat and percarbonate)

C: control

The Data showed that there was a significant ($p<0.01$) improvement in the quality of the activated milk samples at 37°C in comparison with the control during storage, therefore the LP activity of the three treated samples tended to decrease after 8 h of incubation. The control raw milk sample at the 4th hour had mean titratable acidity of 0.19%. Therefore small changes were observed in the acidity for the first 3hrs of storage of both control and LP-treated milk kept. However, significant ($p<0.01$) increase was observed in titratable acidity (0.22%) at the end of the storage time in the control samples, in contrast the acidity of treated cow's milk at the end of the storage time reached 0.173%, 0.170% and 0,170% for Lp_1, Lp_2 and Lp_3 respectively.

Table 2: Effect of LP activation on the total bacterial counts (log cfu/ml) of the cow's raw milk samples stored at 37 °C for 8 hours

	Treatments				Sig
Time/hours	Lp1	Lp2	Lp3	Control	
0	5.39±.05	5.34±.00	5.35±.02	5.41±.04	
2	5.63±.01	5.42±.02	5.42±.02	6.41±.03	
4	6.46±.08	6.38±.02	6.38±.02	6.57±.02	
6	6.52±.01	6.42±.02	6.42±.02	6.68±.01	**
8	6.56±.04	6.45±.02	6.45±.02	7.73±.03	
sig		**			

Main effect						Sig
Treatment	Lp_1	Lp_2	LP_3	C		
TBC(cfu/ml)	6.11±.01[b]	6.00±.01[c]	6.00±.01[c]	6.56±.01[a]		**
Time/hr	0	2	4	6	8	
TBC(cfu/ml)	5.38±.01[e]	5.72±.01[d]	6.45±.01[c]	6.51±.01[b]	6.80±.01[a]	**

*a, b: means within the same row followed by different superscript are significantly ($p< 0.05$) different. TBC: total bacterial count. p1: samples stabilized by activation of Lactoperoxidase system level one. Lp2: level two. Lp3: level three. C: control. **: significant different ($P<0.05$)*

Data in Table 2 shows total bacterial counts (TBC) of raw cow's milk samples at 37°C. Total bacterial counts were log 5.39, 5.36, 5.39 cfu/ml for Lp_1, Lp_2 and Lp_3 respectively,at zero hour whereas after 8 hours of storage the mean TBC were; 6.60, 645,6.45 and 7.73 log cfu/ml for Lp1,Lp2 and Lp3 respectively.Sgnificant variations were observed in the total bacterial between the control and the activated samples.

Table 3: Effect of LP activation on the chemical composition of the cow's raw milk samples stored at 37 °c for 8 hours

Treatment	Density	Fat%	T.S%	Protein%
Lp1	1.027±.01	4.17±.12	12.58±.25	2.9±.12
Lp2	1.028±.01	4.17±.12	12.58±.25	2.9±.14
Lp3	1.028±.01	4.17±.12	12.58±.25	2.9±.14
C	1.028±.01	4.17±.12	12.58±.25	2.9±.12
Sig	NS	NS	NS	NS

Data in Table 3 present the chemical composition of the activated LP cow's milk samples stored at incubation (37°C) temperature. Statistical analysis showed that there were no significant ($p>0.05$) differences in the density, fat, total solids and protein contents of all the milk samples studied.

4. DISCUSSION

The activity of LP system was assayed every 2 hr during the incubation time (8hours) the results showed that the Lp activity of LP (the three levels) of samples tended to decrease after 8 hr of incubation.

Statistical analysis showed (Table 1) that there were significant ($p<0.01$) differences in titratable acidities among the treatments, this might be due to the action of lactoperoxidase system, however there were no significant different ($p>0.05$) for Lp2 (16:30 mg/L) & Lp3 (20:40 mg/L) this could be due to high concentration of thiocyanate and hydrogen peroxide (increase the effectiveness of this system) which decrease acid development and hence increase the keeping quality of the milk, these results are in line with the results of Fonteh [9] and Abdallah [18] who stated that during activation of raw milk with different levels of thiocynate and percarbonate the milk in all treatments remained fresh during the first 12 hours but the control was spoiled by the 5th hour. Lower finding was recorded by Harnulv [19]. Activating milk at this rate extended the shelf life of milk by at least 8 hours at incubation temperature (37°C), compared to control samples which spoiled at 4 hours. These results were in line with the results of Masud [20] and Abdallah [18], who stated that the shelf life of raw milk stabilized with 10, 20 and 30 ppm of H_2O_2 and NaSCN and stored at 40 °C, significantly ($p < 0.05$) increased up to 8 and 16 h as compared with the control which curdled within 6 h of milking but not consistent with those of [1].This could be due to the variation in the storage time and concentration. Activation of the LPS by adding SCN: H_2O_2 at level of 16:30 and 20: 40 ml/L is more effective in cow's milk stored at incubator temperatures; these are in line with the results of Sulieman [21].

The results in Table 2 showed that there were significant ($p<0.01$) differences in total bacterial count due to treatment, This might be due to the usefulness of applying LPS for preservation of raw milk, because of the killing and inhibition effect of lactoperoxidase system on the growth of organisms, but there were no significant different among Lp_1 and Lp_3, this could be due to high concentration of thiocyanate and hydrogen peroxide. These results were consistent with the results of Abdullah [18, 22, 23].

The results in Table (3) showed no significant difference in the density, fat, total solid and protein of all the milk samples stored at 37°C, these results agree with the results of other researches [24-28, 5].

5. CONCLUSION

The storage time and the concentration of thiocyanate/hydrogen peroxide added to LPS-activated milk have very significant independent and interactive effects on the keeping quality of the milk at incubation temperature (37°C). Raw milk activated with 12 mg/L thiocyanate and 20 mg/L hydrogen peroxide, 16 mg/L thiocyanate and 30 mg/L hydrogen peroxide and 20 mg/L thiocyanate and 40 mg/L percarbonate can remain fresh at incubation temperatures (37°C) for at least 8 hours as opposed to the untreated milk whose shelf life under the same conditions was only 4 hours. This increases the shelf life of milk by about 50 %, thus allowing sufficient time for it to be stored, transported and sold still in its fresh state in the local markets. The density, fat, protein and total solid content of treated milk sample were not affected by LPS treatment; this allows sufficient time for the milk to be transported from the collection point to a processing centre without refrigeration. Activation of the LP system by sodium percarbonate–NaSCN at lower concentration had less effect on the Keeping quality of milk.

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