Effect of Lactoperoxidase activation on the keeping quality of raw milk kept at refrigeration temperature

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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 at refrigeration temperature was studied. The milk was collected directly from the dairy farm, Faculty of Animal Production Science and Technology, the study was conducted 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 4°C for 7 days. Titratable acidity % (TA), milk composition and total bacterial counts were evaluated. The results indicated that the keeping quality of activated milk samples were improved compared with that of control. The results showed that significant variations were observed between the activated and control samples. Whereas, the control milk samples were clearly deteriorated in quality at about 5th day 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 samples.

Key words: Milk, Preservation, Sodium Thiocynate, Percarbonate, microbiological , Storage

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INTRODUCTION

Characteristics of the raw milk supply are influenced markedly by milk production and handling systems, which are associated with the followed by plants , interstate shipment of milk , and roles played by dairy plants and producer groups in obtaining and maintaining a good raw milk supply. 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. All these components support the growth of many forms of bacteria. Raw milk aseptically drawn from a healthy animal usually contains a few bacteria (**Omer *etal*., 2008**). **Boulares *etal*.,(2011)** documented that when preserved raw ovine, bovine, caprine milks by added 7, 14 and 28 mg/L of sodium thiocyanate and 15, 30 and 60 mg/L of sodium percarbonate and stored at $4^{o}C$ for 72 h, that concentration of 28 mg/L (SCN^{-}) and 60 mg/L (H_2O_2) would be adequate for preserving milks of different mammals at $4^{o}C$, the results had showed that by activation of the LP system in raw milk, it was possible to store ovine, bovine and caprine milks at $4^{o}C$ for several days. **Jooyandeh *etal*.,(2011)** said that Observations from laboratory and field studies indicated that the LPS does not induce any significant adverse effects on the chemical, physical or sensory characteristics of raw milk and processed dairy products. Activation of the LP system is amongst the most cost effective approaches to extend the stability of pasteurized and raw milk (**Gardea *etal*. 2002**). The LP system is an acceptable chemical method for raw milk preservation, especially in rural areas where refrigeration facilities are absent to farmers (**Ndambi *etal*. 2008**). Several workers have demonstrated the effectiveness of this system in preserving milk when the concentration of these substrates are increased by an exogenous supply (**Fonteh *etal*.2005**). **Fonteh *etal*. (2003)** reported that raw milk samples activated by adding various concentrations (ppm) of thiocyanate and peroxide and denoted as

0:0, 7:10 ppm, 10:10 ppm and 20:20 ppm, the results revealed that the milk in all treatments remained fresh during the first 12 hours but the control was spoiled by the 15th hour. **Kumar and Muthur (1989)** proved that H_2O_2 up to 50 mg/L for the LPs activation does not affect the milk components or nutritive value. Exploitation of LP - thiocyanate - H_2O_2 system as an effective agent against many of the disease causing organisms in plants and animals, (**Jacob *etal.* 2000**). Several studies (**Wolfson, *etal.*, 1993: Naidu, 2000; Zapico *etal.*, 1995; Prince and Ratner, 2000**) indicated that activation of the LPS can delay growth of psychrotrophic bacteria and thus delay food spoilage for several days compared to what can be achieved with refrigeration alone.**Boulares *etal*.(2011)**, noted that when activated LP system by added 14 mg/L of NaSCN and 30 mg/L of sodium percarbonate, then refrigerated at 4^{o}C for 72 h, before transformation to cheese, which had a significant effect ($p<0.05$) on the growth of APC and coliform. This study was conducted to evaluate the activation of lactoperoxidase by using different levels of sodium thiocynate and sodium percarbonate on keeping quality of cow's raw milk at refrigerator (4°C) temperatures for 7 days; and to determine the best concentration of thiocyanate/hydrogen peroxide in order to obtain maximal activation of the LP system.

MATERIALS AND METHODS

Raw cow`s milk samples were collected from the dairy farm , College of Animal Production 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**) were used.

Experimental design:

In this study 4 litres 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 4^oC for 7 days. The experiment was carried in triplicate.

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 (**IDF,1988**: **Dajanta et al,2003**). 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 7 days at 4^oC.

Chemical analysis:-

The Titratable acidity of milk samples was determined according to **Foley et al (1974**). The fat content of milk samples was determined according to **AOAC (1990).** Kjeldahl method was used for protein evaluation according to **AOAC (1990).** The total solids contents were estimated according to method described by **EL-Neemer(2007).**

Microbiological analysis:

Total bacterial count was estimated by standard method (**ISO, 1999**). 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 37^0C 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.

Statistical analysis:

Statistical analyses were carried out with **SPSS (2008**) 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 7 levels of storage periods (7 days) were used. Duncans Multiple range test were used to compare means of the different treatments.

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 (4^o C) for 7 days. The results demonstrated that there was a significant ($P<0.05$) improvement in the quality of the treated milk samples at 4°C storage. The Data indicated that the storage period had significant ($P < 0.05$) effect on the titratable acidity of the milk samples of the different treatments.As the storage period progressed the titratable acidity increased but the quality of the milk samples were not deteriorated until the day 6^{th} (0.195±.01%) while the titratable acidity was slightly high at day 7^{th} (0.21±.01%) which indicated gradual deterioration in the quality of the milk samples. The results also showed that there were significant variations in the titratable acidity of the control samples and the treated

milk samples.The control milk sample had the highest titratable acidity (0.199±.00%) while the lowest titratable acidity was for the milk sample of Lp2 and Lp3 (0.170±.00%).

Table (1) Titratable acidity % of raw cow's milk samples treated with different levels of sodium thiocynate and sodium percarbonate during storage at 4°C for 7 days.

Treatment	Storage Period (days)								
	0	1	2	3	4	5	6	7	sig.
Lp1	0.15±.00	0.156±.00	0.167±.00	0.173±.00	0.173±.00	0.178±.00	0.182±.00	0.192±.00	
Lp2	0.150±.00	0.154±.00	0.166±.00	0.170±.00	0.170±.00	0.177±.00	0.179±.00	0.191±.00	
Lp3	0.150±.00	0.154±.00	0.165±.00	0.170±.00	0.170±.00	0.177±.00	0.180±.00	0.190±.00	**
C	0.155±.00	0.162±.00	0.177±.00	0.187±.00	0.21±.00	0.22±.00	0.24±.01	0.25±.01	
Sig	**								
Main effect									Sig
Day	0	1	2	3	4	5	6	7	
TA	0.151±.01^{h}	0.157±.01^{g}	0.169±.01^{f}	0.175±.01^{e}	0.181±.01^{d}	0.188±.01^{c}	0.195±.01^{b}	0.21±.01^{a}	**
Treatment	Lp_1	Lp_2	Lp_3	C					
TA	0.171±.00^{b}	0.170±.00^{c}	0.170±.00^{c}	0.199±.00^{a}					**

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

TA: Titratable acidity (% lactic acid); Lp1: samples stabilized by activation of lactoperxidase system level one: Lp2: level two: Lp3: level three C: control.

Datain in Table (2) present mean total bacterial counts (TBC) of raw cow's milk samples stored at 4°C. Results in Table (2) showed that there were significant ($p<0.05$) differences in the mean total bacterial counts (TBC) of treated raw cow's milk samples and the control through out the storage period.The highest TBC was (7.8±.02 log cfu/ml) at the day 7th while the lowest one was (5.36±.02 log cfu/ml) for the treatem milk samples(Lp1 and Lp2) at day zero. The results showed that as the storage periods advanced the TBC increased and reached the highest value (6.94±.01 log cfu/ml) at day 7th. On the other hand the treated milk samples recorded lower TBC (6.20±.06 log cfu/ml) in comparison with the control samples (7.21±.01log cfu/ml).

Table (2): Effect of LP activation on the total bacterial counts (log cfu/ml) of the cow's raw milk samples stored at 4 °C for 7 days:

Time/day	Treatments				Sig
	Lp1	Lp2	Lp3	C	
0	5.39±.01	5.36±.02	5.36±.05	5.40±.02	
1	5.61±.01	5.59±.02	5.59±.01	6.41±.03	
2	6.34±.02	6.34±.00	6.34±.01	7.4±.01	
3	6.40±.05	6.36±.03	6.34±.04	7.5±.06	**
4	6.43±.06	6.38±.02	6.38±.02	7.7±.01	
5	6.44±.03	6.41±.03	6.39±.06	7.7±.00	
6	6.5±.02	6.5±.01	6.5±.02	7.7±.02	
7	6.68±.01	6.65±.06	6.65±.05	7.8±.02	
Sig	**				

Main effect								Sig
Treatment	Lp_1		Lp_2	Lp_3	C			
TBC(cfu/ml)	$6.23±.01^b$		$6.20±.01^c$	$6.20±.01^c$	$7.21±.01^a$			**
Time/day	0	1	2	3	4	5	6	7
TBC(cfu/ml)	$5.38±.01^h$	$5.81±.01^g$	$6.61±.01^f$	$6.65±.01^e$	$6.72±.01^d$	$6.75±.01^c$	$6.81±.01^b$	$6.94±.01^a$ **

Means within the same row followed by different superscript are significantly ($p< 0.05$) different. **: significant different ($P<0.05$)

TBC: total bacterial count.Lp1: samples stabilized by activation of lactoperxidase system level one.Lp2: level two. Lp3: level three. C: control

Table (3) Effect of LP activation on the chemical composition of the cow's raw milk samples stored at 4 ˚ c for 7 days.

Treatment	Density	Fat%	T.S%	Protein%
Lp1	1.03±.00	4.2±.12	12.58±.25	2.9±.12
Lp2	1.03±.00	4.2±.12	12.58±.25	2.9±.12
Lp3	1.03±.00	4.2±.12	12.58±.25	2.9±.12
C	1.03±.00	4.2±.12	12.58±.25	2.9±.14
Sig	NS	NS	NS	NS

Sig: significant; NS: No significant; LP: samples stabilized by activation of lactoperoxidase system; C: Control

Data in Table (3) present the chemical composition of the activated LP cow's milk samples and the control stored at refrigeration (4^oC) temperature. The results indicated that the values of protein, fat, total solid, and density in all milk samples had no significant (P>0.05) differences.

DISCUSSION

The results **(**Table 1**)** showed that there were significant (P<0.01) variations in the titratable acidity between the control and the treated milk samples, this could be due to the effective concenterations of the lactoperoxidase agents.Therefore the Data demonstrated that there were no significant (P>0.05) variations in the titratable acidities of Lp2 (16:30 mg/L) and Lp3 (20:40 mg/L), this might be due to high concentrations of thiocyanate and hydrogen peroxide which inhibited the acid development and hence increase the keeping quality of the milk.The results in this study were agreed with those of Abdallah (2003), Floris *et al*. (2003), , Dufour *et al*. (2004)**,** Fonteh *et al. (*2005) and Boulares *e tal*, (2011).

The results in Table (2) showed that there were significant (P<0.01) differences in TBC among the treatments, although Lp_2 and Lp_3 treatments showed highest reduction in TBC (6.20±.01c log cfu/ml) with out significant (p>0.05) differences between them. This might be due to an optimal combination of H_2O_2 , thiocyanate and lactoperoxidase enzyme which showed high antimicrobial activity and these results coincised with those of Haddadin *et al*. (1996), El-Sherbini *et al*., (2000), Lin and Chow (2000) and Abdullah (2003).

The results inTable (3) showed no significant (P> 0.05) difference in the density, fat, total solid and protein contents of all the milk samples stored at 4^oC**,** these results were in line with those of Kumar and Mathur (1989); FAO/WHO (2005), Boulares *et al*. (2011) and Seifu *et al*. (2004 b).

CONCULSION

Its concluded that the tactivation of lactoperoxidase system with different levels of sodium thiocynate and percarbonate had significant effect on the titratable acidity and total bacterial counts of the milk samples.The best concentrations of sodium thiocynate and percarbonate were (16;20 mg/l and 30;40 mg/l) therefore the raw milk activated with the above mention levels

remained fresh at refrigeration temperatures (4°C) for at least 7 days as opposed to the untreated milk samples whose shelf life under the same conditions was only 4 days.

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