

## 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 thiocyanate 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 thiocyanate 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 Thiocyanate, 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 *et al.*, 2008**). **Boulares *et al.*,(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°C for 72 h, that concentration of 28 mg/L (SCN<sup>-</sup>) and 60 mg/L (H<sub>2</sub>O<sub>2</sub>) would be adequate for preserving milks of different mammals at 4°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°C for several days. **Jooyandeh *et al.*,(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 *et al.* 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 *et al.* 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 *et al.*2005**). **Fonteh *et al.* (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 15<sup>th</sup> hour. **Kumar and Muthur (1989)** proved that H<sub>2</sub>O<sub>2</sub> up to 50 mg/L for the LPs activation does not affect the milk components or nutritive value. Exploitation of LP - thiocyanate - H<sub>2</sub>O<sub>2</sub> system as an effective agent against many of the disease causing organisms in plants and animals, (**Jacob *et al.* 2000**). Several studies (**Wolfson, *et al.*, 1993; Naidu, 2000; Zapico *et al.*, 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 *et al.* (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°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 thiocyanate 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, Germany**) 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 ( $2\text{Na}_2\text{CO}_3 \cdot 3\text{H}_2\text{O}$ ) respectively, and kept at  $4^\circ\text{C}$  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 ( $\text{SCN}^-$ ) 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<sub>1</sub>, LP<sub>2</sub> and LP<sub>3</sub>. The chemical and microbiological analyses were carried out in triplicate during storage period of 7 days at  $4^\circ\text{C}$ .

**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 milliliter 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<sup>0</sup>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<sup>-1</sup>) 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 thiocyanate 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<sup>o</sup> 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<sup>o</sup>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<sup>th</sup> (0.195±0.01%) while the titratable acidity was slightly high at day 7<sup>th</sup> (0.21±0.01%) 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 ( $0.199 \pm 0.00\%$ ) while the lowest titratable acidity was for the milk sample of Lp2 and Lp3 ( $0.170 \pm 0.00\%$ ).

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

Treatment	Storage Period (days)								sig.
	0	1	2	3	4	5	6	7	
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 <sup>h</sup>	0.157±.01 <sup>g</sup>	0.169±.01 <sup>f</sup>	0.175±.01 <sup>e</sup>	0.181±.01 <sup>d</sup>	0.188±.01 <sup>c</sup>	0.195±.01 <sup>b</sup>	0.21±.01 <sup>a</sup>	
Treatment	Lp <sub>1</sub>	Lp <sub>2</sub>	Lp <sub>3</sub>	C					
TA	0.171±.00 <sup>b</sup>	0.170±.00 <sup>c</sup>	0.170±.00 <sup>c</sup>	0.199±.00 <sup>a</sup>					**

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 lactoperoxidase system level one; Lp2: level two; Lp3: level three C: control.

Data 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 ( $7.8 \pm 0.02$  log cfu/ml) at the day 7<sup>th</sup> while the lowest one was ( $5.36 \pm 0.02$  log cfu/ml) for the treated 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 ( $6.94 \pm 0.01$  log cfu/ml) at day 7<sup>th</sup>. On the other hand the treated milk samples recorded lower TBC ( $6.20 \pm 0.06$  log cfu/ml) in comparison with the control samples ( $7.21 \pm 0.01$  log 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 <sub>1</sub>		Lp <sub>2</sub>	Lp <sub>3</sub>	C				
TBC(cfu/ml)	6.23±.01 <sup>b</sup>		6.20±.01 <sup>c</sup>	6.20±.01 <sup>c</sup>	7.21±.01 <sup>a</sup>	**			
Time/day	0	1	2	3	4	5	6	7	
TBC(cfu/ml)	5.38±.01 <sup>h</sup>	5.81±.01 <sup>g</sup>	6.61±.01 <sup>f</sup>	6.65±.01 <sup>e</sup>	6.72±.01 <sup>d</sup>	6.75±.01 <sup>c</sup>	6.81±.01 <sup>b</sup>	6.94±.01 <sup>a</sup>	**

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 lactoperoxidase 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°C) 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 concentrations 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 *et al.*, (2011).

The results in Table (2) showed that there were significant ( $P<0.01$ ) differences in TBC among the treatments, although Lp<sub>2</sub> and Lp<sub>3</sub> 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<sub>2</sub>O<sub>2</sub>, thiocyanate and lactoperoxidase enzyme which showed high antimicrobial activity and these results coincided with those of Haddadin *et al.* (1996), El-Sherbini *et al.*, (2000), Lin and Chow (2000) and Abdullah (2003).

The results in Table (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°C, 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).

## CONCLUSION

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

## REFERENCES

- Abdallah, M.I.M. (2003).** A new trial for preservation of raw cow's milk at 30°C and 5°C by activation of the natural lactoperoxidase system. Researcher of Food Inspection Lab., Damietta Sea' Port, Animal Health Research Institute, Agricultural Research Center, Giza, Egypt.
- AOAC (1990).** Official Methods of Analysis. 15th edn. Association of Official Analytical Chemists, (AOAC) Washington, D.C.U.S.A.
- Boulares M; Mankai M and Hassoun M. (2011).** Effect of thiocyanate and hydrogen peroxide on the keeping quality of ovine, bovine and caprine raw milk. International Journal of Dairy Technology , **64**: 52-56.
- Dufour, M.; Simmonds, R.S. and Bremer, P.J. (2004).** Development of laboratory scale clean in place system to test the effectiveness of "Natural" antimicrobial against dairy biofilms. J. Food Protection, **67**: 1438- 1443.
- Dajanta, K.; Chukeatirote, E. and Apichartsrangkoon, A. (2003).** Effect of Lactoperoxidase System on Keeping Quality of Raw Cow's Milk in Thailand. International Journal of Dairy Science, **3**: 112-116
- EL-Neemer, M.T. (2007).** Encyclopedia techniques in the dairy industry and its products. First edition, Bustan elmarefa library - Aleskandria. (In Arabic).
- El-Sherbini, M.; El-Dweny, Y.A. and Gnan, S.O. (2000):** Shelf-life of raw and pasteurized cow, camel, goat and ewe milk stored at 5°C by activation of lactoperoxidase system.. The Six Arabic Conference on Food and Technology., 16th – 18th March, Cairo, Egypt
- FAO/HWO (2005).** Benefits and potential application of lactoperoxidase system of raw milk preservation. Report of an FAO/HWO technical meeting.
- Floris, R.; Recio, I.; Berkhout, B. and Visser, S. (2003).** Antibacterial and antiviral effect of milk proteins and their derivatives . Current pharmaceutical Design, **9**: 1257- 1275.
- Foley Et Al. v. Shanahan Et Al.; and v. ce v. rsa., 133 Ga. App. 262, 211 S.E.2d 367 (1974).** Commercial Testing and product control in the Dairy Industry. Univ. college cork. Georgia Court Of Appeals. Docket Number: 49852 <http://georgia-court-appeals.vlex.com/vid/foley-et-v-shanahan-and-rsa-20479608>.

- Fonteh F A; Grandison A S and Lewis M J (2005).** Variation of lactoperoxidase activity and thiocyanate content in goats milk throughout lactation. *Journal of Dairy Research* **69** 401-409.
- Fonteh F A; Grandison A S; Lewis M J and Niba A T (2003).** The keeping quality of LPS-activated milk in the western highlands of Cameroon.
- Fonteh F A; Grandison A S and Lewis M J (2004).** Variation of lactoperoxidase activity and thiocyanate content in goats milk throughout lactation. *Journal of Dairy Research* **69** 401-409.
- Gardea A A; Carvajal-Millan E; Higuera-Ciapara I; Figueroa C; Molina-Corral J; Rascon Orozco A and Inda A (2002).** Calorimetric assessment of microbial growth in milk as affected by different conditions. *Thermochimica Acta* **394** 179-184.
- Haddadin, M.S.,A. Ibrahim and R.K. Robbinson (1996).** Preservation of raw milk by activation of the natural lactoperoxidase systems. *Food Control* **7**, [149-152].
- IDF (1988).** "International Dairy Federation", code practice for preservation of raw milk by lactoperoxidase system. Brussels (Belgium). No. 234.
- ISO (1999). International Standard Organization** .Microbiology of food and animal feeding stuffs- preparation of test samples, initial suspension and decimal dilution for microbiological examination. Part 1: General rules for the preparation of the initial suspension and decimal dilution.
- Jacob, B.M.; Essy Antony K.; Sreekumar B. and Haridas M. (2000).** Thiocyanate mediated antifungal and antibacterial property of goat milk lactoperoxidase. *Life Sci.*, 66: 2433-2439.
- Jooyandeh H; Aberoumand A and Nasehi B.( 2011).** Application of Lactoperoxidase System in Fish and Food Products. *American-Eurasian J. Agric. & Environ. Sci.*, 10 (1): 89-96, 2011 ISSN 1818-6769.© IDOSI Publications,
- Kumar, S. and Mathur, B.N. (1989):** Incidence of LP- system on the nutritional quality of milk proteins. *Indian J. Dairy Sci.* 42: 198-202.
- Lin, G.C. and Chow, C.F. (2000):** Studies on the lactoperoxidase system and its use in the extending the storage period of cows raw milk. *J. of Chinese Society of Animal Sci.*, 29 (1): 89-99.
- Naidu, A.S. (2000).** Lactoperoxidase in Natural food Antimicrobial system, Naidu, A.S. (Ed.). CRC press, Boca Raton, p: 103-132.
- Ndambi O A, Kamga P B, Imele´ H, Mendi S D and Fonteh F A (2008).** Effects of milk preservation using the Lactoperoxidase system on processed yogurt and cheese quality. *African Journal of Food Agriculture Nutrition and Development* **8** 358–374.
- Omer, R. H. and A. H. Eltinay (2008).** Microbial quality of camel's raw milk in central & southern regions of United Arab Emirates. *Emir. J. Food Agric.* **20** (1): 76-83.
- Prince, A. and Ratner A.J. (2000).** Lactoperoxidase. *Am. J. Respir. Cell Mol., Biol.*, **22**: 642-644.
- Seifu E, Buys E. M and Donkin E. F (2004b).** Quality aspects of Gouda cheese made from goat milk preserved by the lactoperoxidase system. *International Dairy Journal* **14**: 581-589.
- SPSS, (2008).** Statistical Package for the Social Sciences,( Advanced Models- base system in version 14). SPSS Statistical 17 .0.1 – December 2008.

**Wolfson, L.M. and Sumner S.A. (1993).** Antibacterial activity of the lactoperoxidase system: A review. *J. Food Prot.*, 56: 887-892.

**Zapico, P.; Gaya P.; Nuñez M. and Medina M. (1995).** Activity of Goat's milk lactoperoxidase system on *Pseudomonas fluorescens* and *Escherichia coli* at refrigeration temperatures. *J. Food Prot.*, 58:1136-1138.