The Impact of the Sodium Thiocyanate on Preserving the Raw Cow Milk

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Abstract

The present study is planned to evaluate the keeping quality of raw milk by activation of its natural lactoperoxidase system (LPS) at about 3 h of the morning milking. The raw milk samples (about one liter each) are collected from the animal field of college veterinary university of Baghdad.

The present study shows the significant increase ($p<0.05$) in the total aerobic bacterial counts and coliform counts of control milk samples under storage temperatures. The average mean log value of total aerobic bacterial count increases significantly in the control milk samples from the starting initial count of 6.757 to 9.158 and 8.259 cfu/ml after 48 h of milk storage at ambient and refrigeration temperatures respectively. There is significant reduction from 9.158±0.379 cfu/ml in the control milk sample to 6.593±0.126 cfu/ml in stabilized milk sample that storage at ambient temperature and from 8.259±0.135 cfu/ml to 6.266±0.125 cfu /ml in control milk sample storage at refrigerated temperature after 48hours. Average mean log value of coliform count is increased significantly in the control milk samples from the starting initial count of 4.852 to 7.387 and 6.933 cfu/ml after 48 hours of milk storage at ambient and refrigeration temperatures respectively . There is significant reduction from 7.387±0.053 cfu/ml in the control milk sample to 4.852±0.170 cfu/ml in stabilized milk sample that storage at ambient temperature and from 6.933±0.177 cfu/ml to 5.066±0.061 cfu/ml in control milk sample storage at refrigerated temperature after 48hours.

Keywords: lactoperoxidase System, Cows' Milk, Sodium Thiocyanate, Coliform Counts.

Introduction

Milk is a perishable food that is highly susceptible to microbial spoilage since it contains all the essential nutrients and provide a suitable physical environment (Heeschen, 1996). The time delay from milking to delivery at the processing plant often exceeds five hours that very negatively affecting the quality of mainly non-refrigerated milk, which is often rejected by dairy processing plants and is not acceptable to consumers (Barabas, 1994). The tradition thermal processing is an effective method to achieve microbial inactivation in milk, however, it causes the degradation of the sensorial and nutritional properties of milk (Wuytack, *et al.* 2002). This

situation forces producers to find methods for raw milk preservation that should be simple to use and not pose any kind of hazard for the consumer and protecting raw milk from spoilage for periods long enough to the processing plants (Barabas, 1995 and Ryoba, *et al*.2000*)*.

The lactoperoxidase system is an antimicrobial naturally found in raw milk (Reiter and Harnulv, 1984). LPS consist of three components, which are the lactoperoxidase enzyme, an oxidisable thiocyanate substrate and hydrogen peroxide (Davidson and Brannen,1993).The lactoperoxidase catalyses the oxidation of thiocyanate by hydrogen peroxide (Reiter,1985).the peroxidation reaction is important because it has antimicrobial activity and prevents accumulation of hydrogen peroxide (toxic) excreted by microorganism and the host cells (Naidu,2000 and Seifu *et al*.,2005). The lactoperoxidase system has proven to be both bacteriostatic and bactericidal to a wide variety of microorganisms, where its activity is mediated by the reaction of thiocyanate and hydrogen peroxide under lactoperoxidase catalysis and the resultant generation of short-lived intermediary oxidation hypothiocyanate ion ($OSC\bar{N}$) which is thought to be the major antibacterial substance (Barret *et al*.,1999;Kussendrage and Hooijdank,2000).

The action of the LPS against bacteria is reported to be caused by sulfhydryl (SH) oxidation in the cytoplasmic membrane result in loss of the ability to transport glucose and also, in leaking of potassium ions, amino acids and peptides , thereby , inactivating several vital metabolic bacterial enzymes, consequently blocking their metabolism and ability to multiply (Anue and Thomas ,1978). Thiocyanate and hydrogen peroxide occur naturally in milk but in insufficient amount for the LPS to be fully operational (Reiter and Harnulv, 1984). Several workers have demonstrated the efficacy of the LPS in milk preservation when the concentrations of these substrates are increased by an exogenous supply (Ridley and Shalo,1990;EL-Agamy *et al*.,1993).

Lactoperoxidase (LPS) is a glycoprotein naturally present in milk, which in itself has no antibacterial effect, but in combination with thiocyanate ($SC\bar{N}$) and hydrogen peroxide (H_2O_2) forms a potent antimicrobial system (lactoperoxidase-thiocyanatehydrogen peroxide system) against a variety of microorganisms (bacteria, fungi and viruses). The LPS has been recognized as important in the preservation of raw and pasteurized milk (Marks *et al*., 2001) by inhibiting various spoilage and pathogenic microorganisms (Seifu *et al*., 2005).

The aims of this study are planned to evaluation of the effect of activate natural Lactoperoxidase system alone on the quality of cow's raw milk under different storage temperatures and hygienic quality tests of total bacterial counts and

coliform counts duration four period 0,6 ,24 and 48 hours.

Materials and Methods:

1-Samples Collection:

Five raw cow's milk samples (about one liter each) are collected from the animal field of veterinary college university of Baghdad for activated the LPS within 3 hours after morning milking, each milk sample is divided into two equal parts (each 500ml), the first part is activated with 20mg/L strength of each sodium thiocyanate and hydrogen peroxide , where as no any activator was added to the second part of raw milk and is kept as a control (i. e without activation of its lactoperoxidase system). Each of the activated and the control milk samples is also subdivided into two part (each 250ml), the first part is stored at refrigeration temperature (5C°) and the second part was stored at room temperature (25C°).

Hygienic quality tests such as total aerobic bacterial counts and coliform counts (cfu/ml) are made for each part mentioned above at 0, 6, 24 and 48 hours of ambient and refrigeration storage temperatures. Colonies of aerobic bacteria are counted for each time point of storage using the pour plating method after 48 hours of incubation at 37C° on nutrient agar. Colonies of coliform bacteria are counted for each time point of storage using the pour plating method after 24 hours of incubation at 37C° on violet red bile agar.

2-Activation of lactoperoxidase System (LP-S):

Milk sample is preserved according to soomro *et al*.(2012) procedure with slight modification by the activation of lactoperoxidase system (LPS) with 20 mg/L strength of each sodium thiocyanate and hydrogen peroxide . Activation of LPS is after 3 hours of morning milking by addition of 20ml of freshly prepared solution of thiocyanate ion , after one minute of thorough mixing , 6.66 ml of freshly prepared solution of hydrogen peroxide is added into the milk and the mixture is thoroughly mixed for one minute to obtain the final strength of 20mg/L for the activation of (LPS). Whereas, no any activator is added to the milk sample that is kept as a control.

3-Statistical Analysis:

Data are subjected to Statistical Analysis System (SPSS, 2008) and the significant differences are determined at ($p<0.05$). The statistical analysis of the data is performed by F test analysis (ANOVA).

Results and Discussion:

The present study shows that the total aerobic bacterial counts (cfu/ml) of examined milk samples before and after activation of the LPS at room and refrigeration storage temperatures (Table 1). There is a significant increase in the total aerobic bacterial counts over the four time points in all milk samples that are not stabilized by activation of their LPS (control) at either ambient or refrigeration storage temperatures. The average mean log value of the starting initial

aerobic bacterial count in the control milk samples was 6.757±0.138 (7×10^6 cfu/ml) increases significantly ($p<0.05$) to 9.158±0.379 (39×10^8 cfu/ml) and to 8.259±0.135(21×10^7 cfu /ml) after 48 hours of milk storage at ambient and refrigeration temperature respectively (Table 1).

There is significant (*p<0.05*) reduction of the average mean log value of the starting initial aerobic bacterial count in all milk samples that are stabilized by the activation of their LPS. Activation of the natural lactoperoxidase system produced a significant ($p<0.05$) reduction of the total aerobic bacterial counts after 6 hours of ambient or refrigeration storage temperatures, where the average mean log value of total aerobic bacterial count is significantly ($p<0.05$) reduced from 7.678±0.207(68×10^6 cfu/ml) in the control milk samples to 6.193±0.036(16×10^5 cfu/ml) in the stabilized milk after 6 hours of storage at ambient temperature and from 7.192±0.112(18×10^6 cfu/ml) in the control milk samples to 6.149± 0.036(14×10^5 cfu/ml) in the stabilized milk after 6 hours of storage at refrigeration temperature . An overall conclusion based on this investigation pointed out that the activation of the LPS exhibits significantly (*p<0.05*) the highest antimicrobial effectiveness against viable bacteria in raw milk samples after 6 hours of storage at either ambient or refrigeration temperatures.

Table (1): The Effect of Sodium Thiocyanate on Raw Cow's Milk Samples that were Activated at about 3 hours After Milking then Stored at Room (25C°) and Refrigerated Temperatures(5C°).

Duration of Storage (h)	Mean± SD of total bacterial counts (log cfu/ml)			
	Storage period at (5 C°)		Storage period at (25 C°)	
	Control	Activated of LPS	Control	Activated of LPS
0h	6.757±0.138 A d	6.087±0.041B b	6.757±0.138A d	6.087±0.049B b
6 h	7.192±0.112 A c	6.149± 0.036 B c	7.678±0.207A c	6.193±0.036 B b
24 h	7.713±0.204A b	6.295±0.179B c	8.299±0.303A b	6.536±0.244 B a
48 h	8.259±0.135A a	6.266±0.125B c	9.158±0.379 Aa	6.593±0.126 B a

● Different small letters in a column reveal significant differences (*p<0.05*) between hours of incubation.

● Horizontal different capital letters reveal significant differences (*p<0.05*) between the mean values.

This result indicated to the total coliform counts (cfu/ml) of examined raw milk samples before and after activation of LPS at room and refrigeration storage temperatures (Table 2).

The average mean log value of the starting initial coliform counts in the control milk samples that is 4.852±0.283(15×10^4 cfu/ml) increased significantly ($p<0.05$) to 7.387±0.053(25×10^6 cfu/ml) and to 6.933±0.177(11×10^6cfu/ml) after 48 hours of milk storage at ambient and refrigeration temperatures respectively.

Activation of the natural lactoperoxidase system producs a significant reduction of the total coliform counts after 6 hours of storage at ambient and refrigeration temperatures , where the average mean log value of total coliform counts is significantly reduced from 5.799±0.385(19×10^5 cfu/ml) in the control milk samples to 4.088±0.340 (36×10^3cfu/ml) in the stabilized milk after 6 hours of storage at ambient temperature and from 5.152±0.219(22×10^4 cfu/ml) in the control milk samples to 3.807±0.178(9×10^3 cfu/ml) in the stabilized milk after 6 hours of storage at refrigeration temperature.

An overall conclusion based on this investigation points out that the activation of the LPS exhibited significantly (*p<0.05*) the highest antimicrobial effectiveness against coliform bacteria in raw milk after 6 hours of storage at either ambient or refrigeration temperatures. Several workers have demonstrated the efficacy of the LPS in milk preservation when concentration of thiocyanate and H_2O_2 are increased by an exogenous supply (Ridley, *et al.(*1990) ; El-Agamy, *et al.(*1993) and this leads to the activation of the LPS and reduction in the total viable count (Chakraborty, *et al*.(1986); Marks, *et al*.(2001) report that after pasteurization of cow' s milk , an active LPS enhanced the keeping quality of milk inoculated with *Pseudomonas aeraginosa* and *Staphylococcus aureus* . (Abdullah, 2003) show differences in the keeping quality between the stabilized raw milk samples (Activated LPS) and the control raw milk samples stored at ambient (30C°) and refrigeration (5C°) temperatures ,where at 30C° and 5C° the stabilized raw milk samples are clearly spoiled at the 12th hour and the 7th day respectively while the control raw milk samples are clearly spoiled at the 6th hour and the 3rd day respectively .The LPS has been recommended for preservation of raw milk as an alternative to cooling by several workers from different countries (Fontch, *et al*.2005) . The activation of LPS by the addition of sodium thiocyanate and H_2O_2 to raw milk is used to prevent undue bacterial multiplication during collection and transport to the diary processing plant in countries where refrigeration may not be feasible and the use of LPS for the stabilization of milk has been approved by codex CAC/GL13-1991). According to the results obtained from the present study the keeping quality of raw milk can be improved by the activation of its natural

Lactoperoxidase system by exogenous supply since the time delay from milking to delivery at the processing dairy plant often exceeds 6 hours very negatively affecting the quality of non-refrigerated milk in countries with high ambient temperature such as Iraq.

Table (2): The Effect of Sodium Thiocyanate on Raw Cow's Milk Samples that were Activated at about 3 hours After Milking then Stored at Refrigeration (5C°) and Ambient (25C°) Temperatures.

Duration of Storage(h)	Mean± SD of coliform counts (log cfu/ml)			
	Storage period at (5 C°)		Storage period at (25 C°)	
	Control	Activated of LPS	Control	Activated of LPS
0h	4.852± 0.283 Ad	4.013±0.235B	4.852±0.283A d	4.013±0.235B
6 h	5.152±0.219A c	3.807±0.178B	5.799±0.385A c	4.088±0.340B
24 h	6.021±0.290A b	4.744±0.221B	6.849±0.196A b	5.117±0.195B
48 h	6.933±0.177A	5.066±0.061B	7.387±0.053A a	4.852±0.170B

● Different small letters in a column reveal significant differences ($p<0.05$) between hours of incubation.

● Horizontal different capital letters reveal significant differences ($p<0.05$) between the mean values.

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تأثير ثايوسينيت الصوديوم في حفظ حليب الابقار الخام

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الخلاصة

صممت الدراسة الحالية لتقييم حفظ جوده الحليب من خلال تنشيط نظام اللاكتوبيروكسيديز الطبيعي(LPS) بعد مرور ثلاث ساعات من الحلبة الصباحية. جمعت خمسة عينات الحليب لمدة خمسة اسابيع من حقل الحيواني لكلية الطب البيطري جامعة بغداد.

أظهرت الدراسة ان هناك زيادة معنوية في العدد الكلي لكل من البكتريا الهوائية وبكتريا القولون عبر الفترات الزمنية الاربعة لحفظ جميع عينات الحليب لمجموعة السيطرة سواء كان حفظها عند درجة حرارة الغرفة او الثلاجة .ازداد متوسط القيمة اللوغارتمية للعدد الكلي للبكتريا الهوائية معنويا في جميع عينات حليب مجموعة السيطرة من العدد لابتدائي لانطلاق التجربة وهو 6.757 الى 9.158 و 8.259(cfu/ml) بعد 48 ساعة من حفظ الحليب عند درجة حرارة الغرفة والتبريد بالثلاجة على التوالي,ولاحظ هبوط معنوي في اعداد البكتريا الهوائيه من 9.158±0.379 log cfu/ml من مجموعه السيطرة الى6.593± 0.126 cfu/ml من مجموعه التنشيط وفي درجه حرارة الغرفه ومن 8.259±0.135 cfu/ml من مجموعه السيطرة الى 6.266±0.125 cfu/ml من مجموعه التنشيط وفي درجه حرارة الغرفة بعد 48 ساعة من حفظ الحليب . كذلك ازداد متوسط القيمة اللوغارتمية للعدد الكلي لبكتريا القولون بصورة معنوية في جميع عينات الحليب لمجموعة السيطرة من العدد لابتدائي لانطلاق التجربة وهو4.852 الى7.387 و6.933(cfu/ml) بعد 48 ساعة من حفظ الحليب عند درجة حرارة الغرفة والتبريد بالثلاجة على التوالي. ولاحظ هبوط معنوي في اعداد البكتريا القولونية من 7.387±0.053 cfu/mlمن مجموعه السيطرة الى 4.852±0.170 cfu/mlمن مجموعه التنشيط في درجه حرارة الغرفه ومن 6.933±0.177 cfu/ml من مجموعه السيطرة الى 5.066±0.061 cfu/mlمن مجموعه التنشيط وفي درجه حرارة التبريد بعد 48 ساعة من حفظ الحليب.

الكلمات المفتاحية : ثايوسينيت الصوديوم ، حليب الابقار ، نظام اللاكتوبيروكسيديز ، العدد الكلي لبكتريا القولون.