Influence of Lactoperoxidase Enzyme Activation On The Hygienic Quality of Raw Milk

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Abstract

The efficiency of lactoperoxidase system (Lps) activation as a mean of preserving of the quality of raw buffalo milk samples was studied. The samples were obtained from some farms in village at Sharkia province. The samples were activated at about 3 hours after morning milking then stored at 7°C. Titratable acidity % (TA) and psychotropic count (PC) as hygienic quality tests were made. The results showed a differences between the activated and control samples. Whereas, at 7°C activated milk samples were spoiled at about 120 hours (5th day) while, control milk samples were spoiled at about 72 hours (3rd day) of storage. Lps was found to have bacteriostatic activity against *S. typhimurium*. Activation of Lps result in, a substantial reduction of *S. typhimurium* count and prevent its multiplication for up to 5 days. So, this method of preservation may be used to improve the quality of raw milk and to safeguard the consumers from food-poisoning microorganisms as *S. typhimurium*.

Introduction

Milk contains several natural antimicrobial constituents as lactoperoxidase system (Lps) which consists of lactoperoxidase enzyme, hydrogen peroxide and thiocyanate *(1,2)*. It is characterized by its heat stability, since it retains its activity in normal pasteurization of milk (63°C/30 minutes or 72°C/15 seconds), but it is destroyed at 80°C/2.5 seconds *(3)*. Lactoperoxidase (LP) activity is found in all bovine milk, but its content varies over a wide range from about 13 up to 30 mg/L, several factors affecting its concentration such as breed, age and lactation stage *(4)*. LP catalyzes the oxidation of thiocyanate (SCN^-) to hypothiocyanate ($OSCN^-$), which oxidizes protein saulfhydryl group (-SH) of the bacterial cytoplasmic membrane to sulfenyl-thiocyanate derivatives leading to immediate inhibition of the respiration of the cell *(5)*. It will also be shown that such treatment of milk is harmless to man when consumed and this based on: (a) thiocyanate level in human saliva

and gastric juice are far higher than in milk (50-200 and 40-50 ppm, respectively); (b) detection of one the major oxidation products ($OSCN^-$) in human saliva thus, the end products of Lps are harmless; (c) specific damage to the bacterial cytoplasmic membrane, but not to mammalian cell membranes and (d) amounts of SCN^- and H_2O_2 added to preserve the quality of milk are very small and it is rapidly consumed in the oxidation of SCN^- *(6)*. Extended refrigerated storage of milk on the farm, in transport and at the processing plants have created a unique problem for the dairy industry due to rapid growth of psychotropic bacteria and their extracellular lipolytic and proteolytic enzymes, which play an important role in the deterioration of milk and its products *(7)*. This situation forces many producers to find a preservative products that represent a potential public health hazard as well as affecting on the hygienic quality of milk, for example, formaldehyde, antibiotics and others *(8)*. To inactivate food borne pathogens, novel technologies such as bio-preservation system, non-thermal technologies, or combined treatment have been studied. Different groups of bacteria showed a varying degree of resistance to the Lps. Gram negative organisms such as salmonellae isn't only inhibited by Lps but may be killed *(9)*.

Therefore, the main purpose of the present study is to evaluate the effect of activation of lactoperoxidase system (Lps) on the hygienic quality of buffalo's raw milk as well as the antibacterial effect on *S. typhimurium* at refrigeration (7°C) temperature.

Material and Methods

1) Milk samples

Ten random freshly drawn morning buffalo raw milk samples were obtained from some farms in villages at Sharkia province. Samples were collected in labeled sterile polyethylene sacs, kept in insulated ice box (5°C) and transferred to laboratory without delay. At laboratory each sample (about 1 L) was separated into two main portions (each, 500 ml), one to stabilized by activation of lactoperoxidase system (Lps) and other kept as controls. Both controls and activated parts were examined after 0,

24, 48, 72, 96 and 120 hours from the time of activation and all samples were kept at 7°C.

2) Activation of lactoperoxidase system *(10)*

The activation of Lps was carried within 2-3 hours after milking by addition of sodium thiocyanate (NaSCN, BDH chemical, LTD, Poole, England), 20 mg/L milk. Plunging milk for about one min., followed by sodium percarbonate ($Na_2CO_3.1.5\ H_2O_2$, Peroxide-chemie Gmgh, Munich), 40 mg/L milk. The milk was then stirred for another 2-3 min. The enzymatic reactions were completed in the milk within 5 min. after addition of H_2O_2 donor (Na_2CO_3).

3) Determination of hygienic quality

a) Determination of titratable acidity *(11)*.

b) Psychotropic count *(12)*.

4) Bacteria: *Salmonella typhimurium* strain ATCC 13311 was obtained from Microbiological Resources Center, Cairo Mircen-Egypt, the Egyptian Microbial Culture Collection (EMCC). Stock cultures were maintained on nutrient agar slopes and subcultured when required at 37°C overnight in nutrient broth.

5) Activation of lactoperoxidase system (Lps) in milk against *S. typhimurium*. *S. typhimurium* was inoculated into a set of individual freshly drawn buffalo's milk samples (2 control and 2 with activated lactoperoxidase) to achieve a cell concentration of 10^6-10^7 organisms/ml. The samples with activated lactoperoxidase and control were incubated at 7°C and sub-samples removed after 0, 24, 72, 96 and 120 hours and to a spread plate count using xylose lysine desoxycholate agar (XLD) with plates being incubated at 37°C/24 hours.

RESULTS

Table 1. Titratable acidity (TA) of examined raw buffalo's milk samples (n = 10), were activated at about 3 hours after morning milking then stored at 7°C

Times/hours	Means of TA%	
	Activated	Controls
0	0.14	0.14
24	0.15	0.16
48	0.16	0.19
72	0.18	0.23*
96	0.19	
120	0.21*	

*: Samples with an acidity > 0.20% recorded as rejected.

Table 2. Psychotropic counts (PC) of examined raw buffalo's milk samples (n = 10), were activated at about 3 hours after morning milking then stored at 7°C

Times/hours	Means of PC/ml	
	Activated	Controls
0	$2.3x10^4$	$3x10^4$
24	$4.5x10^4$	$7x10^4$
48	$2x10^5$	$9x10^6$*
72	$8x10^5$	
96	$5x10^6$*	
120	$1.2x10^7$	

*: Total bacterial counts over about $4x10^6$ nearly judged as bad grade.

Table 3. Influence of lactoperoxidse system (Lps) activation on *Salmonella typhimurium* in raw buffalo's milk stored at 7°C

Times/hours	Number of *Salmonella typhimurium* (cfu/ml.)	
	Activated	Controls
0	$7x10^6$	$7x10^6$
24	$3x10^5$	$7.6x10^6$
48	$8x10^4$	$8x10^6$
72	$2x10^4$	$5x10^7$
96	$5.5x10^4$	$2.2x10^8$
120	$6x10^5$	$4.5x10^9$

Discussion

Titratable acidity percentage of milk has a greater importance where it is used for assessing the keeping quality *(1)*. The results summarized in Table 1 showed that improvement in the quality of the activated milk samples at 7°C, since the control raw buffalo milk samples at 72 hours have mean titratable acidity % of 0.23. While, titratable acidity of activated milk samples were 0.21 at 120 hours. Nearly similar findings were reported *(13)*.

Lactoperoxidase system (Lps) activation controls the development of higher acidity of milk by inhibition of lactic acid bacteria *(14)*. Moreover, activation of the Lps could be considered that base of a method to prevent undue multiplication of psychrotrophs in cooled milk *(15)*.

The results presented in Table 2 declared that the mean psychotropic counts of activated milk samples at 7°C was $5x10^6$ at 96 hours while, the controls have passed more than this level at 48 hours. An extension of about 2 says (48 hours) is indicated. On contrary, an extension of about 4 days in case of activated cow milk was reported *(13)*. While, reduction of physchrotrophic bacteria for up to 5 days was reported *(16)*.

Lps activation delayed or even caused complete inhibition of psychotropic microorganisms growth in activated milk samples *(3, 17)*.

Inspection of Table 3 revealed that when Lps was activated, the growth of *S. typhimurium* monitored at 7°C. The lowest count was obtained after 72 hours ($2x10^4$) and this antibacterial effect only appeared to last for 96 hours since after that time the viable count began to increase.

Milk of control treatment showed increase in counts of $7.6x10^6$, $8x10^6$, $5x10^7$, $2.2x10^8$ and $4.5x10^9$ for storage periods 24, 48, 72, 96 and 120 hours, respectively. At the same time, treated milk showed decreases in count of $3x10^5$, $8x10^4$, $2x10^4$, $5.5x10^4$ and $6x10^5$ over the same time period of storage. Similar results were recorded *(5, 18, 19, 20, 21, 22)*.

Gram-negative bacteria are less sensitive to the inhibitory effects of Lps than for gram-positive bacteria. However, it has been reported that Gram-negative organisms can be killed by the Lps when exogenous H_2O_2 at very low non bactericidal concentrations is added or is generated enzymatically. It also was reported that lip polysaccharides of the cell envelop influence the susceptibility of Gram-negative to the system and the mutant strains of *E. coli* and *S. typhimurium* are killed by the system *(5)*.

In conclusion, our results showed that the activation of lactoperoxidase system (Lps) constitutes a useful method to improve the hygienic quality of raw milk and to safeguard the consumers and neonates against enteric infection and pathogenic microorganism as *S. typhimurium*.

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تأثير تنشيط إنزيم اللاكتوبيروكسيديز على الجودة الصحية للبن الخام

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تم إجراء دراسة فاعلية تنشيط نظام اللاكتوبيروكسيديز لحفظ الكفاءة الصحية لعينات لبن جاموس خام جمعت من بعض المزارع فى قرى محافظة الشرقية وكانت بداية التنشيط بعد حوالى ثلاث ساعات من حلبه صباحا مع حفظه عند درجة حرارة ٧ °م وأجريت بعض اختبارات فحص الكفاءة الصحية للبن الخام وهى قياس النسبة المئوية للحموضة العيارية وعد البكتريا المحبة للبرودة عند درجة حرارة ٧°م. وأسفرت النتائج على تحسن فى الكفاءة الصحية للبن الخام المنشط عن العينات الضابطة بدون تنشيط حيث امتدت فترة التخزين بدون فساد الى حوالى يومين بالمقارنة بالعينات الضابطة. تم اختيار كفاءة جهاز اللاكتوبيروكسيديز لوقف نمو السالمونيلا تيفيميوريم حيث تم اختزال العدد ثم ثبت حتى خمسة أيام. وتعتبر هذه الطريقة فى حفظ اللبن الخام يمكن استخدامها لتحسين الكفاءة الصحية للبن وحماية المستهلك من الاصابة بالتسمم الغذائى الناتج عن بعض الميكروبات مثل السالمونيلا تيفيميوريم .