

A NEW TRIAL FOR PRESERVATION OF RAW COW'S MILK AT 30°C AND 5°C BY ACTIVATION OF THE NATURAL LACTOPEROXIDASE SYSTEM

By

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ABSTRACT

Preservation of the quality of raw milk by activation (stabilization) of its natural lactoperoxidase system (LP-s) was studied. Raw cow's milk samples were obtained from some collection centers and farms in villages at Damietta province. The samples were activated at about 3 hours after morning milking then stored at 30°C and 5°C. hygienic quality tests, titratable acidity % (TA), methylene blue reduction test (MBRT) and clot-on-boiling (COB), in addition to total bacterial counts (TBC) and psychrotrophic counts (PC) as a bacteriological tests were made. In each case, the keeping quality of stabilized sample was compared with that of a control. The results showed a pronounced differences between the activated and control samples. Whereas, at 30°C and 5°C stabilized milk samples were clearly spoiled at about the 12th hour and the 7th day while, control milk samples were clearly spoiled at about the 6th hour and the 3rd day of storage, respectively. This method of preservation may be used to encourage dairy farming by making possible the collection of more milk of high quality which in turn is prerequisite for increased manufacture of high quality dairy products. The public health importance of lactoperoxidase system was declared.

INTRODUCTION

Lactoperoxidase (Lp), which is one of non-immunoglobulin glycoprotein, the enzyme contains a haeme structure, with one iron molecule per mole, accounting for about 1% of the total whey protein content. 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 is destroyed at 80°C /2.5 seconds (**Limsowtin, 1992** and **Wolfson, 1993**). Lp activity is found in all cow's milk, but its content varies over a wide range from almost 13 up to 30 mg/L, several factors affecting its concentration such as breed; age; lactation stage of the cow as well as the cow's nutrition; health state and daily variations can also be considerable (**Korhonen, 1977** and **Korhonen, et al., 1977**).

Lp itself has no antibacterial activity. However, together with thiocyanate (SCN⁻) and hydrogen peroxide (H₂O₂), Lp forms a potent natural antibacterial system the so called lactoperoxidase system (LP-s), **Karen et al., (1996)**. Lp-s has proven to be both bacteriostatic and bactericidal to wide variety of microorganisms, where its activity is mediated by the reaction of SCN⁻ and H₂O₂ under Lp catalysis and the resultant generation of short-lived intermediary oxidation hypothiocyanate (OSCN⁻) which is though to be the major antibacterial substance (**Kussendrage and Hooijdkank, 2000**), but is also destroyed by heat-treatment processes (**Björck et al., 1975**), while the end products of the oxidation process (CO₂, NH₄⁻, SO₄⁻) are inert and harmless (**Oram and Reiter, 1966 & Hoogendoorn et al., 1977**). The action of Lp against bacteria is reported to be caused by sulfhydryl (-SH) oxidation in the bacterial cytoplasmic membrane result, in loss of the ability to transport glucose and also, in leaking of potassium ions, amino

acids and peptides (Anue and Thomas, 1978). The LP-s is however, far more specific because it fails to affect the mammalian cell membranes and can only affect bacterial membranes (Klebanoff and Rosen, 1979) and (Reiter, 1979).

In developing countries, most milk is produced by a large of smallholders with small quantities of milk, making milk collection and delivery time-consuming are complicated. The milk later transported usually by bicycle or donkey, to a larger collection centre where, if possible it is chilled, the collected milk is subsequently sent in bulk to a processing plant by truck. The time delay from milk to delivery at the processing plant often exceeds five hours very negatively affecting the quality of mainly non refrigerated milk, which is often rejected by dairy processing plants and is also not acceptable to consumers (Barabas, 1994). This situation forces producers to find methods for raw milk preservation, the addition of products that represent a potential public health hazard, for example, formaldehyde, antibiotics and others (Korhonen, 1980). Due to the fact in the short run it is possible to foresee an immediate technification of the milk handling procedures, it becomes necessary to look for alternative preservation technologies. These technologies should be capable of protecting raw milk from spoilage for periods long enough to the processing plants and/or consumers. They must be simple to use and not pose any kind of hazard for the consumer (Barabas, 1995) and (Ryoba et al., 2000).

For this reason, the use of healthy and suitable preservatives in milk should not only be authorized by local legislation but also encouraged in those areas where local conditions do not permit, economically or technically, the use of refrigeration in milk collection/ storage/ delivery schemes. It has been proved that small additions of naturally occurring substances in the LP-s would stimulate this indigenous antibacterial system in milk and considerably extend its shelf-life (IDF, 1988); (FDA/WHO, 1991) and (FSANZ, 2002).

This study was conducted to evaluate the effect of activation (stabilization) of lactoperoxidase system (LP-s) on the keeping quality of cow's raw milk at ambient (30°C) and refrigeration (5°C) temperature.

MATERIAL AND METHODS

Sampling technique:

Twenty, random freshly drawn morning cow's raw milk samples were obtained from some collecting centers and farms in villages at Damietta province. Samples were collected in labeled sterile polyethylene sacs, kept in insulated ice box (5°C) and transferred to laboratory without delay. At the laboratory each sample (about, 1L) was separated into two main portions (each, 500 ml): One to be stabilized by activation of LP-s and subdivided into two parts (each, 250 ml) which are stored at 30°C (ambient temperature) and at 5°C (refrigeration). The other main portion subdivided into two parts (each, 250 ml) to be kept as a control.

Activation (stabilization) of lactoperoxidase system (LP-s):

First, 0.017 gm of potassium thiocyanate (KSCN) (Merck 66271) per /L of milk was added, thereby increasing the thiocyanate content to about 15 ppm. Then, 0.034 gm of sodium percarbonate-H₂O₂ donor (Na₂ CO₃ · 1.5 H₂O₂) (Peroxid-Chemie GmbH, Munich) per /L of milk was added and the milk stirred thoroughly. In contact with water, the percarbonate decomposes into carbonate and hydrogen peroxide that corresponds to about 10 ppm of H₂O₂ (Zajac et al., 1983a & 1983b) and recommended by IDF, 1988 code of practice.

Detection of heat-treated milk:

Each sample of raw milk was subjected to Guaiac test (Schonberg, 1956) to prove that the milk samples were raw.

Determination of hygienic quality tests:**1- Determination of titratable acidity (TA):**

Titratable acidity was carried out according to (James, 1995).

2- Methylene blue reduction test (MBR):

The technique was conducted according to (Longree and Armbruster, 1996).

3- Clot-on-boiling test (COB):

The test was performed according to (Yadav et al., 1993).

Bacteriological analysis:**1-Total bacterial count (TBC):**

The technique recommended by Richardson, (1985) was applied.

2-Psychrotrophic count (PC):

According to the technique described by Biolife, (1991).

RESULTS AND DISCUSSION

Results of various hygienic quality and bacterial counts tests made, all demonstrate that activation of the LP-s in raw cow's milk samples at 30°C and 5°C were lead to an improvement of the keeping quality (tables, from 1 to 6). Clot-on-boiling test (COB) apparently indicates spoilage slightly later than the other tests used, this finding was in agreement with that recorded by (Björck et al., 1975); (Härnolv and Kandasamy, 1982) and (Härnolv and Hamid, 1984).

Further, since the effectiveness of the system is to be dependent upon the initial microbiological quality of milk (Härnolv and Kandasamy, 1982). The best effect of LP-s is obtained if the activation is slightly later when bacterial multiplication would normally have started where the indigenous antibacterial systems still had a considerable effect (Reiter, 1978). Moreover, activation of the LP-s could be considered the base of a method for suppressing the growth of lactic-producing organisms in uncooled milk (Swedish, 1975). At 10th hour the mean total bacterial counts (TBC) of stabilized samples at 30°C have definitely increased to be 4×10^6 /ml, where the initial mean TBC at 0 hour was 2.5×10^5 /ml (table, 5). Since, the controls have passed this level already at 4th hour. An extension about 6 hours is indicated. Slightly higher findings were recorded by Björck et al., (1979); Patel and Sannabhadti, (1993); Vivek-Sharma et al., (1999) & Bennett, (2000). While, nearly similar results obtained by Kamau and Kroger, (1984); El-Agamy et al., (1993); Barabas, (1995) & Lin and Chow, (2000).

Results tabulated in (tables, 1&3) showed that a significance improvement in the quality of the stabilized milk samples at 30°C storage. Since 75% of control raw milk samples at the 6th hour have mean titratable acidity (TA) % lactic acid of 0.19 and 70% have methylene blue reduction time (MBRT) of 2.0 hours while, 65% have clot-on-boiling (COB) accepted samples. As, nearly similar levels demonstrated for stabilized examined milk samples by activation of its LP-s at extended time after about 6 hours. Lower finding was recorded by (Härnolv and Kandasamy, 1982).

It was now well established that the psychrotrophs have become important "spoilage organisms", while the organisms are readily killed by heat treatment, their lipases and proteases are exceptionally heat resistant so, the manufactured products from such milk can be spoiled during prolonged storage (butter, UHT milk) or during maturation (cheese), Reiter and Härnolv, (1982). Furthermore, LP activity suppresses acid production in yogurt during refrigerated storage and produces product having a softer texture (Nakada et al., 1996 and Hirano et al., 1998). Moreover, activation of the

LP-s could be considered the base of a method to prevent undue multiplication of psychrotrophs in cooled milk (Swedish, 1975).

Results recorded in **table (6)** declared that mean psychrotrophic counts (PC) of examined raw cow's milk samples at 5°C was 2.6×10^6 /ml at the 6th day show definitely increased to be at the 7th day of storage 1.3×10^7 /ml, where the initial mean (PC) was 5.5×10^3 /ml. Since, the controls have passed this levels at the 2nd day. An extension of about 4 days is indicated. These results were substantiating that by (Björck, 1978 & 1980); (Haddadin et al., 1996); (El-Sherbini et al., 1999) and (Lin and Chow, 2000).

Concerning to results in **table (2&4)** indicated that a significance improvement in the quality of the stabilized milk samples at 5°C storage. 75% of control milk samples at the 2nd day have mean TA% lactic acid of 0.177 and 60% have MBRT of 2.5 hours, while, 65% have COB accepted samples. Nearly similar levels showed for stabilized examined milk samples at extended time after about 4 days. The observation of that psychrotrophs multiplication started after two days of experiment beginning this will coincides with those observed by Zajac et al., (1983b).

It was concluded from the obtained results that the pronounced differences in hygienic quality and bacterial counts between stabilized (activated) and control raw cow's milk samples are evidenced. The bacteriostatic effect generates from the activation of LP-s in raw cow's milk had prolonged the time of storage (shelf-life) by decreasing acidity caused by microbial flora present in raw milk (Stefano et al., 1995) at temperature 30°C and 5°C for 6 hours and 4 days, respectively. Even an increase of about 6 hours and 4 days in shelf life of Lp activated milk, with 15 ppm KSCN and 10 ppm H₂O₂, can be considered to be of practical importance in salvaging border-line milk from souring.

Finally, stabilization of raw milk through activation of LP-s is considered to be a useful method for extending the shelf-life of milk in Egypt and tropical countries. It should be emphasized that this method of milk quality preservation does not exempt milk producers from the duty of observing the general principles of hygiene when handling milk on the farm. Stabilization of milk by activation of its Lp-s is a method to keep good quality milk but not a mean of concealing or improving bad quality milk. In addition, encourage dairy farming by minimizing spoilage, cutting costs of transportation and facilitating the collection of milk from remote areas.

Regarding to the possible health risks for using the system, the only concern could arise from the SCN⁻, which known as a goitrogenic agent. However, an excellent review by Reiter and Härnuly, 1984 depicts the most important studies made so far on this subject. It is possible to say that even using potency level of about 42 ppm of SCN⁻, the system is not expected to indirectly pose any risk for the consumer. The H₂O₂ does not present any health hazard because it fades out a few hours after its addition, since it is consumed as substrate by the system, yielding H₂O. Moreover, the inhibitor formed by the reaction, OSCN⁻ among other, is not stable to the pasteurization treatment, so there is no inhibitor remaining; in the event there were any, the same review by Reiter and Härnuly, states that it will not affect adversely human cells and organs. Furthermore, it has been suggested by some researchers that the LP-s exists in the mouth and may play a role against the microorganisms responsible for oral cavities (Tenuovo et al., 1982). There is direct evidence that the LP-s occurs in man because one of the oxidation products of thiocyanate (hypothio cyanate) was recently detected in saliva (Ruddell et al., 1977) and (Thomas et al., 1980). The potential health risk of thiocyanate to human should be negligible (Haddadin et al., 1996).

Table (1): Analysis of raw cow's milk samples milked at 6-7 a.m. and samples were activated (stabilized) at 8-9 a.m. then stored at temperature 30 °C:

Hygienic quality tests	Treatment	% accepted samples (n=20) at (time/hours)							
		0 hr	2 hr	4 hr	6 hr	8 hr	10 hr	12 hr	14 hr
TA	Lp	100	100	85	80	80	75	65	35
	C	100	95	75	75	55	40	00	00
MBRT	Lp	100	95	85	80	80	70	60	30
	C	95	75	75	70	45	25	00	00
COB	Lp	100	90	80	80	75	65	60	35
	C	95	70	70	65	35	15	15	00

TA: titratable acidity (% lactic acid) – samples with an acidity >0.20% recorded as rejected

MBRT: methylene blue reduction test – samples with reduction time <2.0 hours recorded as rejected

COB: cold-on-boiling test – positive samples recorded as rejected

Lp: samples stabilized by activation of lactoperoxidase system

C: controls

Table (2): Analysis of raw cow's milk samples milked at 6-7 a.m. and samples were activated (stabilized) at 8-9 a.m. then stored at temperature 5 °C:

Hygienic quality tests	Treatment	% accepted samples (n=20) at (time/hours)							
		0 d	1 d	2 d	3 d	4 d	5 d	6 d	7 d
TA	Lp	100	100	95	90	90	85	75	40
	C	95	85	75	55	10	00	00	00
MBRT	Lp	100	90	85	80	70	70	65	30
	C	90	75	60	55	20	00	00	00
COB	Lp	100	95	90	85	70	70	60	20
	C	95	90	65	65	25	10	05	00

TA: titratable acidity (% lactic acid) – samples with an acidity >0.20% recorded as rejected

MBRT: methylene blue reduction test – samples with reduction time <2.0 hours recorded as rejected

COB: cold-on-boiling test – positive samples recorded as rejected

Lp: samples stabilized by activation of lactoperoxidase system

C: controls

Table (3): Titratable acidity (TA) % lactic acid and methylene blue reduction test (MBRT) of examined raw cow's milk samples (n=20), were activated (stabilized) at about 3 hours after morning milking then stored at 30 °C:

Time/hours At	Means of TA %		Means MBRT/hours	
	Lp	C	Lp	C
0 hr	0.15	0.156	14.4	10.4
2 hr	0.16	0.17	10.4	8.5
4 hr	0.16	0.185	6.7	5.0
6 hr	0.173	0.19	5.5	2.0**
8 hr	0.178	0.20*	5.5	2.0
10 hr	0.183		4.0	1.1
12 hr	0.186		2.0**	0.5
14 hr	0.20*		1.5	

Lp: samples stabilized by activation of lactoperoxidase system

C: controls

*: samples with an acidity >0.20% recorded as rejected

** : samples with MBRT <2.0 hours judged as bad grade

Table (4): Titratable acidity (TA) % lactic acid and methylene blue reduction test (MBRT) of examined raw cow's milk samples (n=20), were activated (stabilized) at about 3 hours after morning milking then stored at 5 °C:

Time/days At	Means of TA %		Means MBRT/hours	
	Lp	C	Lp	C
0 d	0.15	0.156	14.4	10.4
1 d	0.156	0.163	8.3	6.6
2 d	0.166	0.177	7.2	2.5
3 d	0.17	0.19	8.5	1.3**
4 d	0.17	0.22*	8.2	
5 d	0.177		6.4	
6 d	0.18		4.6	
7 d	0.191		1.8**	

Lp: samples stabilized by activation of lactoperoxidase system

C: controls

*: samples with an acidity >0.20% recorded as rejected

** : samples with MBRT <2.0 hours judged as bad grade

Table (5): Total bacterial counts (TBC) of examined raw cow's milk samples (n=20), were activated (stabilized) at about 3 hours after milking and stored at 30 °C:

Time/hours At	Means of TBC/ml	
	stabilized	controls
0 hr	2.5×10^5	2.6×10^5
2 hr	5×10^5	2.6×10^6
4 hr	3.0×10^6	3.8×10^6
6 hr	3.6×10^6	4.5×10^6
8 hr	3.6×10^6	5.1×10^7
10 hr	4×10^6 *	
12 hr	2.1×10^7	

* : TBC over about nearly 4×10^6 judged as bad grade

Table (6): Psychrotrophic counts (PC) of examined raw cow's milk samples (n=20), were activated (stabilized) at about 3 hours after milking then stored at 5 °C:

Time/days At	Means of PC/ml	
	stabilized	controls
0 d	5.5×10^3	5.6×10^3
1 d	9.2×10^3	8.6×10^4
2 d	1.2×10^4	1.3×10^6 *
3 d	2.8×10^5	2.5×10^7
4 d	3.1×10^5	
5 d	5.5×10^5	
6 d	2.6×10^6 *	
7 d	1.3×10^7	

* : TBC over about 4×10^6 nearly judged as bad grade

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محاولة جديدة لحفظ اللبن البقري الخام عند درجة حرارة مئوية ٣٠ و ٥ بتنشيط نظام اللاكتوبيروكسيديز الطبيعي

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باحث بمعمل فحوص الأغذية بميناء دمياط البحري – معهد بحوث صحة الحيوان – مركز البحوث الزراعية – مصر

المخلص

دراسة حفظ الكفاءة الصحية للبن الخام بتنشيط نظام اللاكتوبيروكسيديز. في هذا البحث تم جمع عينات اللبن البقري الخام من بعض مراكز تجميع اللبن و بعض المزارع في قرى محافظة دمياط. وكانت بداية التنشيط بعد حوالي ثلاث ساعات من حلبة الصباح وتمت الدراسة باستخدام كل من ثايوسيانات البوتاسيوم (KCSN) و بيركربونيت الصوديوم ($\text{Na}_2\text{CO}_3 \cdot 1.5\text{H}_2\text{O}_2$) عند درجة حرارة ٣٠ و ٥ مئوية و أجريت بعض اختبارات فحص الكفاءة الصحية للبن الخام وهى قياس النسبة المئوية للحموضة العيارية (TA) و تعين زمن اختزال المثيلين الأزرق (MBRT) وكذا اختبار التخثر عند الغليان (COB). كما تم دراسة العد الكلى للبكتيريا (TBC) عند درجة ٣٠ مئوية وعد البكتيريا المحببة للبرودة (PC) عند درجة ٥ مئوية. وأسفرت النتائج على تحسن واضح في الكفاءة الصحية للبن الخام المنشط *activated* عن العينات المراقبة *controls* بدون تنشيط حيث امتدت فترة التخزين *self-life* عند درجة ٣٠ مئوية الى حوالي ٦ ساعات بينما عند درجة ٥ مئوية الى حوالي ٤ أيام بالمقارنة بالعينات المراقبة *controls*. وتعتبر هذه الطريقة في حفظ اللبن الخام دافع للمزارع لتجميع لبن أكثر ذات كفاءة صحية ونوعية عالية وبالتالي تؤدي الى تحسين كفاءة منتجات الألبان. كما تم مناقشة النتائج و الأهمية الصحية العامة لنظام اللاكتوبيروكسيديز.