THE EFFECT OF LACTOPEROXIDASE SYSTEM ON ENHANCING THE MICROBIOLOGICAL QUALITY OF GOAT MILK AND YOGHURT

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SUMMARY

The objectives of this study were to determine the effect of activation of lactoperoxidase system (LPs) to increase the shelf life of goat milk and yoghurt. Samples of goats' milk were collected, taken under complete aseptic conditions, during December 2004 to May 2005 in North Sinai. These samples were divided to three combination groups from sodium thiocyanate and sodium percarbonate were tested for LPs activation as follows: Group 1 (G_1) (14 mg/L + 30 mg/L), Group 2 (G_2) (15 mg/L + 10 mg/L) and Group 3 (G_3) (20 mg/L + 25 mg/L), respectively. In general, Activation of LPs in goat milk caused a considerable slowing down rate of increase in titratable acidity during storage at 18–24 ^{o}C as compared to the control. The titratable acidity was increased from 0 until 72 hours in all groups; this effect was most pronounced in G1. The TABC was highly decreased significantly ($P < 0.01$) from 0 to 24 hours by 45, 40 and 26% in G1, G2, and G3, respectively. While, at 48 hours the TABC were increased by 19, 23 and 11% in G1, G 2 and G 3, respectively. This result could be occurred due to the LPs effect was decreased after 24 hours, which helping to increased TABC. The PC, SC and CC were in the same trend as TABC. The results of LPs application in yoghurt show that, the titratable acidity of yoghurt was not changed across the time from 1 to 21 days in both G1 and G2. While, in G3 was rapidly decreased after 7 days until 21 days. In G1 and G2 the TABC was decreased after 7 days of storage to 7.9 and 6 log cfu/ml, respectively. In contrast, at 14 days, the TABC was increased in G1 and G2 while, G3 was decreased in the same time. Moreover, at 21 days, the TABC was decreased in G1 and G2. The coliform was even more affected by LPs than the TABC. In all LPs groups, the yoghurt coliform was not detected throughout the different refrigeration periods.

Keywords: lactoperoxidase system, goat milk, yoghurt

Abbreviation key: LPs = lactoperoxidase system, **TABC** = Total Aerobic Bacterial Count, **PC** = Proteolytic bacterial Count, **SC**= Spore forming bacterial Count, **CC**= Coliform bacterial Count.

INTRODUCTION

The total number of goats in Egypt is about 5 millions heads. Average daily milk yield of goat in Egypt is varied and ranged from 0.2 to 1.2 kg /head/day according to different location, breeds and stage of lactation. Moreover, the average lactation period ranged from 120 to 180 days. They account for about 61% of the total number of the animals' population (MoALR, 2005). Milk is considered as the best environment to activate and grow of bacteria. Therefore it is subjected to contaminate by bacteria and (or) yeast. The major natural antimicrobial proteins of milk are

Lactoperoxidase system, Lysozyme, Lactoferrin and Immunoglobulins. The lactoperoxidase enzyme (EC 1.11.1.7) is present at concentration of 0.1–0.7 µg /ml in goat milk (Nadiu, 2000; Fonteh et. al. 2002). But the enzyme requires extra different concentrates of hydrogen peroxide and thiocyanate to activate it; in this case it is called lactoperoxidase system (LPs). The LPs has been recommended for preservation of raw milk in areas where it is not possible to use mechanical refrigeration for technical and/or economic reasons (IDF, 1988; FAO, 1999). The objectives of this study were to determine the effect of activation of lactoperoxidase (LPs) to increase the shelf life of goat milk and on the manufacture of yoghurt.

MATERIALS AND METHODS

Milk Samples: Samples of goats milk were collected form El-Arish city (North Sinai governorate) about 320 km North East of Cairo during December 2004 to May 2005. Data were collected as a part of the project sponsored by MERC, USA titled "Multinational approaches to enhance goat production in the Middle East". The milk was collected under complete aseptic conditions during the middle stage of lactation season and subjected individually to analysis by California mastitis test (CMT) to avoid the mastitis samples. The first three squirts of milk were discarded from each teat and samples were collected into sterile bottles and transmitted to the laboratory for bacteriological examination at 8°C.

Chemicals: **Sodium percarbonate ($Na_2Co_3.3H_2O_2$): was obtained from BDH chemicals Ltd. Poole England.**
Sodium thiocyanate (Na SCN): LOBA chemie PVT.LTD was used as a source of SCN^-.

Activation of Lactoperoxidase System: Sodium thiocyanate (Na SCN) and sodium percarbonate (Na_2CO_3. $3H_2O_2$) were used to activate LPs, three combination groups were tested. Group 1 (G_1) was 14mg/L Na SCN + 30mg/L $Na_2CO_3.3H_2O_2$, Group 2 was (G_2) 15mg/L Na SCN+ 10mg/L $Na_2CO_3.3H_2O_2$, and Group 3 was (G_3) 20mg/L Na SCN+ 25mg/L Na_2CO_3. $3H_2O_2$.

Yoghurt manufacture: Four Yoghurt groups were made (three made from three LPs treated groups plus control made from untreated goat's milk after heat treated at 90°C for 10 min. and cooled to 42°C). Starter (Freeze dried lactic culture for Direct Vat Set (DVS) Thermophilic lactic culture, type Yoghurt) was added at the rate of 2% and incubated at the same temperature. Later, yoghurt samples within each group were incubated at 42°C during 4–6 hours, until coagulation occur. These samples were transferred to a refrigerator and storage at 10°C. Microbiological analyses were done to measure acidity (AOAC, 1990) at 0, 7, 14 and 21 days.

Microbiological analysis

1. **goat milk:** According to American Public Health Association (APHA, 1992) the following bacteria were counted in different types of specific media for control and 3 LPs treated milk groups at 0, 24, 48 and 72 h.
   Total Aerobic Mesophilic Bacterial Count (TABC) was estimated using standard plate count agar medium,
   Proteolytic Bacterial Count (PC) was estimated using Standard plate count agar with 10% skim milk,

Spore forming bacterial count (SC) was estimated using Stander plate count agar with 0.1% soluble starch and

Coliforme bacterial counts (CC) were counted using Violet Red Bile Agar medium

2. **Yoghurt:** Yoghurt samples were prepared according to Tamime and Robinson (1999). Standard Plate Count Agar was used for enumeration to total bacterial count and Mac-Conkey broth was used for enumeration coliform bacterial count: (Most Probable Number (MPN)).

Statistical Analysis: Data of the two experimental were analyzed by the General Linear Model (GLM) procedure of SAS. (1998), according to the following model:

$Y_{ijk} = \mu + G_i + T_j + e_{ijk}$

Where,

Y_{ijk} = any observation,

μ = overall mean,

G_i = the effect of i^{th} LPs groups i =1–4,

T_j = the effect of j^{th} times j =1–4,

e_{ijk} = the residual assumed to be normally and independently distributed with mean 0 and variance σ^2_e.

RESULTS AND DISCUSSIONS

Effect of LPs on titratable acidity, pH and some flora: The results presented in Table 1 show that, there were significant ($p<0.05$) differences between the LPs groups and between times on all studied traits.

Table 1. Analysis of variances of the effects of using LPs group in goat milk at different times

S.O.V	df	*MS*				
		TA	TABC	PC	SC	CC
Total	79					
Time	3	0.09	8.54	3.31	9.12	5.91
Group	3	0.02	15.79	7.17	2.52	18.23
Error	73	0.01	3.42	3.68	5.96	6.59

S.O.V = source of variation, MS = means squares, df = degree of freedom,
TA = Titratable Acidity, TABC = Total Aerobic Bacterial Count,
PC = Proteolytic Count, SC= Spore forming bacterial Count,
CC= Coliform bacterial Count.

Titratable acidity % in goat milk after LPs activation: As can observed from results present in Table 2. Activation of LPs in goat milk caused a considerable slowing down rate of increase in titratable acidity during storage at 18–24 °C as compared to the control sample. These results were confirmed with the results obtained by Kamel and El Shaer (2004). They were estimated the acidity percentages as 0.13, 0.15, 0.17 and 0.18% in Shami goat milk in North Sinai of Egypt in the same level of LPs as G1 (14 mg/L Sodium thiocyanate plus 30 mg/L Sodium percarbonate) under different 4, 20, 30 and 40°C, respectively. These results were also confirmed the results obtained by Haddadin *et. al.* (1996), which estimated the acidity percentages as 0.16, 0.21 and

0.3% in Caprine goat milk under the same level of LPs as G2 (15 mg/L Sodium thiocyanate plus 10 mg/L Sodium percarbonate) at 4, 22 and 30 °C, respectively.

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Total Aerobic Bacterial Count (TABC) in LPs treated milk: Influence of LPs groups (Table 2) on TABC of goat milk stored at 18–24°C. That extraneous addition of Sodium thiocyanate and Sodium percarbonate immediate highly affected significantly ($p < 0.01$) on TABC for all treated groups as compared with control at 0 time. Result showed that, in control TABC was increased by about 11% from 0 to 24 hours and by the same percent from 24 to 48 hours. While, from 48 to 72 hours was decreased strongly by 20%. This result could be explained by the level of acidity was increased, which affected on TABC at 72 hours stored. While, in three groups the TABC was highly decreased significantly ($P < 0.01$) from 0 to 24 hours by 45, 40 and 26% in G1, G2, and G3, respectively. While, at 48 hours the bacteria were increased by 19, 23 and 11% in G1, G 2 and G 3, respectively. This result could be occurred due to the LPs effect was decreased after 24 hours, which helping to increased TABC. So, it could be recommended that, the LPs treated goat raw milk should not stored more than 24 hours at 18–24°C. In spite of that, in all studied groups the TABC was decrease at 72 hours due to acidity effect. Thus bacterial cells though multiplying might have been deprived of some metabolic function to spoil the milk.

Table 2. LSM ± SE of LPs groups and four different times in goat milk

Time (hours)	C		G1		G2		G 3	
	LSM	**±SE**	**LSM**	**±SE**	**LSM**	**±SE**	**LSM**	**±SE**
Acidity %								
0	0.16±0.044		0.11±0.044		0.12±0.044		0.12±0.044	
24	0.20±0.044		0.15±0.044		0.20±0.044		0.18±0.044	
48	0.29±0.044		0.21±0.044		0.29±0.044		0.28±0.044	
72	0.35±0.044		0.29±0.044		0.31±0.044		0.35±0.044	
Overall	**0.25±0.044**		**0.19±0.044**		**0.23±0.044**		**0.23±0.044**	
TABC (log/cfu/ml)								
0	5.92 ± 0.827		5.64 ± 0.827		5.87 ± 0.827		5.88 ± 0.827	
24	6.55 ± 0.827		3.90 ± 0.827		4.20 ± 0.827		4.66 ± 0.827	
48	7.26 ± 0.827		4.66 ± 0.827		5.15 ± 0.827		5.16 ± 0.827	
72	6.06 ± 0.827		3.55 ± 0.827		3.94 ± 0.827		4.07 ± 0.827	
Overall	**6.450± 0.827**		**4.44± 0.827**		**4.79 ± 0.827**		**4.94 ± 0.827**	
PC (log cfu/ml)								
0	4.81 ± 0.858		4.71 ± 0.858		4.84 ± 0.858		4.79 ± 0.858	
24	5.52 ± 0.858		4.23 ± 0.858		4.61 ± 0.858		4.89 ± 0.858	
48	6.96 ± 0.858		4.90 ± 0.858		5.34 ± 0.858		5.45 ± 0.858	
72	6.35 ± 0.858		4.13 ± 0.858		4.74 ± 0.858		5.45 ± 0.858	
Overall	**5.91± 0.858**		**4.49 ± 0.858**		**4.88 ± 0.858**		**5.15 ± 0.858**	
SC (log cfu/ml)								
0	2.74 ± 1.092		1.70 ± 1.092		2.33 ± 1.092		2.09 ± 1.092	
24	3.24 ± 1.092		1.78 ± 1.092		2.39 ± 1.092		2.66 ± 1.092	
48	2.31 ± 1.092		1.08 ± 1.092		1.13 ± 1.092		2.04 ± 1.092	
72	0.93 ± 1.092		1.50 ± 1.092		0.64 ± 1.092		0.87 ± 1.092	
Overall	**2.31± 1.092**		**1.15 ± 1.092**		**1.62 ± 1.092**		**1.91 ± 1.092**	

Table 2. Continuation

Time (hours)	C		G1		G2		G 3	
	LSM	**±SE**	**LSM**	**±SE**	**LSM**	**±SE**	**LSM**	**±SE**
CC (log cfu/ml)								
0	4.24 ± 1.148		3.82 ± 1.148		3.95 ±1.148		4.04 ± 1.148	
24	5.54 ± 1.148		2.27 ± 1.148		2.99 ± 1.148		4.16 ± 1.148	
48	6.23 ± 1.148		3.70 ± 1.148		4.43 ± 1.148		4.96 ± 1.148	
72	5.27 ± 1.148		2.61 ± 1.148		3.06 ± 1.148		3.56 ± 1.148	
Overall	**5.32 ± 1.148**		**3.10 ± 1.148**		**3.61 ± 1.148**		**4.18 ± 1.148**	

C = Control group.
G1 = GROUP1 (14 MG/L SODIUM THIOCYANATE + 30 MG/L SODIUM PERCARBONATE).
G2 = Group2 (15 mg/L Sodium thiocyanate + 10 mg/L Sodium percarbonate).
G3 = Group3 (20 mg/L Sodium thiocyanate + 25 mg/L Sodium percarbonate).

Proteolytic Bacterial Count (PC) in LPs treated milk: Table 2 show the effect of LPs on PC. PC is encountered in milk are quite resistant of LPs (Patel and Sannabhadti, 1993). PC in control group was increased by about 15% from 0 to 24 hours and by 26% from 24 to 48 hours. While, from 48 to 72 hours was decreased slowly by 9%. This result could be explained by the level of acidity was increased, which affected on PC in later stage of storage (72 hours). While, in all treated groups the PC was decreased significantly ($P < 0.01$) from 0 to 24 hours by 10, 5 and 2% in G1, G2, and G3, respectively. Moreover, at 48 hours the bacteria were increased by 16, 16 and 12% in G1, G2 and G3, respectively. This result could be occurred due to the effect of LPs was decreased after 24 hours, which helping to increased PC. So, it could be confirm the recommendation of the goat raw milk should not stored more than 24 hours after LPs treated at 18–24^{o}C. In spite of that, in all studied groups the PC was decrease at 72 hours due to acidity effect. Thus bacterial cells though multiplying might have been deprived of some metabolic function to spoil the milk.

Spore forming Bacterial Count (SC) in LPs treated milk: As can observe from results present in Table 2, in control group the SC was increased by about 18% from 0 to 24 hours. While, the SC was decreased by 29 and 60% at 48 hours and at 72 hours, in the three treated groups the LPs in goat milk samples caused a considerable slowing down rate of the SC during storage periods, as compared to the control. This effect was most pronounced in G1 in all treated groups. Moreover, in all treated groups the SC was increased significantly ($P < 0.01$) from 0 to 24 hr by 5, 2 and 27% in G1, G2 and G3, respectively. Then, at 48 hours the bacteria were decreased significantly by 40, 53 and 23% in G1, G2 and G3, respectively. In spite of that, in both of G2 and G 3 the SC was continuously decreased at 72h by 43, 57%, respectively, but in G1 the SC was increased by 40%.

Coliform Bacterial Count (CC) in LPs treated milk: Influence of LPs groups on the CC of goat milk stored at room temperature. It is evident Table 2. In control group, the CC was increased by about 31 and 13% at 24 and 48 hours, respectively. While, the CC was decreased by 15% at 72 hours. In both of G1 and G2, the CC was decreased significantly ($P < 0.01$) at 24 hours by 41 and 24%, respectively. While, in G3, the CC was increased by 3% at 48 hours. Moreover, the CC was decreased significantly ($P < 0.01$) at 48 hours by 63, 48 and 19% in G1, G2, and G3, respectively. In spite of that, in all studied groups the CC was decreased at 72 hours due to acidity effect.

Production of yoghurt from LPs activated goat milk: The results presented in Table 3 show that, there were significant ($p<0.05$) differences between the LPs groups and between times on all studied traits.

Table 3. Analysis of variances of the effects of using LPs group in yoghurt goat milk

S.O.V	df	*MS*		
		TABC	*CC*	**TA**
Total	16			
Time	3	1.54	1742500	0.02
Group	3	2.30	2102500	0.14
Error	9	1.72	1742500	0.02

P<0.01, df = degrees of freedoms, MS = Mean Squares. CC= Coliform bacterial Count.
TA = Titratable Acidity, TABC = Total Aerobic Bacterial Count,

Titratable acidity % of LPs treated yoghurt in goat milk: The results presented in Table 4 revealed that titratable acidity of yoghurt after 1 day from storage at 10 °C in G1 and G2 was 1.19 and 0.97% respectively. These values were less than 1.25% estimated in control sample and less than 1.35% obtained in G3. These results were the same as reported by Mehanna & Hefnawy (1988) and Nokuda *et. al.* (1996) which found that the Titratable acidity was decreased due to LPs activated in milk before manufactured the yoghurt. In general, the acidity was not changed across the time in both G1 and G2. This might be due to malfunction of starter culture as it is sensitive to antimicrobial agents. While, the acidity in G3 was rapidly decreased after 7 days until 21 days. This result shows that G3 with more effected on acidity than G1 and G2 due to different concentration of activated LP. These results imply that the addition of LPs suppressed the rate of acid production but has not effected on the bacterial growth which confirmed the same result obtained by Nokuda *et. al.* (1996).

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TABC in LPs treated yogurt: As shows in Table 4, the TABC in yoghurt in G1 and G3 was higher than G 2 (refrigerated for 24 hours). The result showed that TABC in control groups was increased to 9.42 log cfu/ml after 14 days of storage. In G 1 and G2 the TABC was decreased after 7 days of storage to 7.98 and 6 log cfu/ml, respectively. While, in G3, the TABC was increased to 9.37 log cfu/ml. This result could be occurred due to the effect of LPs in TBAC within the different groups. In contrast, the TABC was increased in G1 and G2 after 14 days of storage, and G3 was decreased in the same time. While, the TABC was decreased in G1 and G2 after 21 days of storage. This result was confirmed the result obtained by Nokuda *et. al.* (1996), which the bacteriostatic effect was not exerted, but acid production was partially inhibited. This indicates that the starter cultures examined in this experiment showed varying degrees of sensitivity to the LPs, which confirmed the same result obtained by Seifu *et. al.* (2003). These results suggested storage time should not longer than 14 days in all treated groups accept in G3 which not longer than 7 day.

Coliform Bacterial Count in LPs treated yoghurt: As can be observed from results present in Table 4, the coliform was even more affected by LPs than the TABC with the effect begin most pronounced all treatments. The coliform continued to increase in the control samples through storage until 14 days. The coliform counts in control group in yoghurt was increased from 0 to 5400 MPN /ml then declined at 21days to reach 0 MPN /ml. However, in all LPs groups in yoghurt coliform was not detected throughout different storage times. This refer to the positive effect of LPs on the coliform bacteria

CONCLUSIONS

The LPs activation naturally increased the shelf life of milk to 48 hours after the system activated in goat milk at North Sinai of Egypt. The best LPs group, which naturally increases the shelf life of goat milk, was 14mg/L Sodium thiocyanate + 30mg/L Sodium percarbonate (G1). The best LPs group, which increases the shelf life of yoghurt, was 15mg/L Sodium thiocyanate + 10mg/L Sodium percarbonate (G2). The starter cultures showed varying degrees of sensitivity to the LPs. Group 3 showed more effect on acidity of yoghurt than G1 and G2. The LPs groups positively affect the TABC and CC of yoghurt. The yoghurt refrigeration time should not be longer than 14 days in all LPs treated groups except in case of G3 which not longer than 7 day.

Table 4. Average of LPs activated yoghurt in goat milk at different times

Storage Time (d)	Acidity %			
	C	**G1**	**G2**	**G3**
1	1.25	1.19	0.97	1.35
7	1.30	1.20	1.15	1.09
14	1.62	1.15	1.00	1.20
21	1.80	1.20	1.07	1.20
Overall	**1.49**	**1.19**	**1.05**	**1.21**
	TABC (log cfu/ml)			
1	7.67	9.36	7.40	9.12
7	8.62	7.98	6.00	9.37
14	9.42	8.94	8.93	6.00
21	8.06	7.41	5.00	7.70
overall	**8.45**	**8.42**	**6.83**	**8.05**
	Coliform bacterial count (MPN/ml)			
1	0	0	0	0
7	400	0	0	0
14	5400	0	0	0
21	0	0	0	0
overall	**1450**	**0**	**0**	**0**

C, G1, G2, and G3 as defied before

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