

ACTIVATION OF LACTOPEROXIDASE (LP) SYSTEM IN MILK AND ITS EFFECT ON THE QUALITY OF KAREISH CHEESE

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Abstract

In the present study, Lactoperoxidase system (LPS) was activated in fresh cow's milk. The pH and acidity of treated milk were followed during storage at room (32°C), incubator (37°C) and refrigerator (5 ±1°C) temperatures. The activation was done using SCN⁻ and H₂O₂ or SCN⁻ and Na₂CO₃.1.5 H₂O₂. The effect of activation on microbiological quality was also investigated. All the treatments greatly improved the keeping quality of milk at room or higher temperatures compared to the untreated samples. The best results were achieved by adding 14mg SCN⁻ /L: 2mL H₂O₂/L to cow's milk. In addition, slight improvement in the keeping quality of the LP-activated milk was observed when the milk was kept in a refrigerator. Kareish cheese was made from cow's milk preserved by the LPS and the effect of the LPS on the chemical, microbiological and sensory properties of kareish cheese stored for 30 days was investigated. Kareish cheese made from LP-activated cow's milk was low in total bacterial count when compared to cheese made from the untreated one (control). The LP treatment did not affect the overall chemical composition of the cheese. Activation of LP system allowed to produce of kareish cheese with high and keeping quality.

Keywords: Lactoperoxidase system, cow's milk, kareish cheese

INTRODUCTION

Kareish cheese is one of the most popular cheese varieties consumed in Egypt especially in countryside owing to its high protein, low fat and reasonable price. It has a limited shelf life of 7-11 days. This may be due to sanitary problems during manufacture cheese (uncontrolled hygiene) which increase microbial contamination. Yeasts and moulds play an important role in the spoilage of dairy products and kareish cheese particular. It also may impair public health due to the presence of mycotoxins which cause carcinogenic health problems. The microbial quality and safety of kareish cheese is the major area of concern for producers and consumers. Therefore, there is a need to develop and to improve the kareish cheese quality, and increase its shelf life.

The lactoperoxidase system (LPS) is one of several natural antibacterial systems found in bovine and other mammalian milks. The LP-system consists of: (i) lactoperoxidase (LP) enzyme, which is present in milk in sufficient amounts. It is the

most abundant enzyme in cow's milk (up to 50mg/L). It is a heat stable and can retain its normal activity after pasteurization at 63°C /30 minutes or 72°C /15 seconds, but it is destroyed at 80°C in 2.5 seconds: (ii) thiocyanate (SCN^-) that is present in varying amounts in cows' milk, depending on dietary intake, may need adjustment of its concentration to activate LP-system. The LPS has been recognized as critical in the dairy industry for the preservation of raw milk (Haddadin, *et al.*, 1996), pasteurized milk and cheese (Earnshaw, *et al.*, 1989).. The system also serves as a processing aid in the production of other dairy products (García-Graells, *et al.*, 2000). In Africa, application of the LPS was firstly tested in Kenya (Bjorck, *et al.*, 1979) as a successful means of preserving milk at ambient temperatures. Moreover, a strong effect of the LP-system against gram-negative psychrotrophs was demonstrated. It was also reported that the activation of the LPS in milk could offer an immediate and practical solution to prolong the shelf-life of milk in warm countries (Mehanna, *et al.*, 1998). The objective of the present study was to determine the effect of the activated lactoperoxidase (LP)-system on keeping quality of cow's milk as well as the manufactured kareish cheese using LP-activated cow's milk.

MATERIALS AND METHODS

Activation of the LP-Systems:-

Fresh cow's milk samples were collected from qbur center. Twenty raw milk samples, approximately 1000ml each, were collected in sterilized Pyrex bottles within one hour of milking at 6 a.m. The samples were immediately placed in a box with ice packs and transported to the laboratory. 14mg SCN^- /L and 2mL H_2O_2 (30%)/L or 14mg SCN^- /L and 30mg sodium per carbonate/L were prepared. Each of these combinations was added to 100ml milk. In addition, control cow's milk samples were kept for further analyses, without any treatment. The tested milk samples were kept at room temperature (32°C), in incubator (37°C) for 6 hours, and the cold storage of milk was done at $5 \pm 1^\circ\text{C}$ for 3 days.

Manufacture of kareish cheese:-

The kareish cheese was manufactured as described by Effat, *et al.*, (2001). The cheese samples were kept in plastic pouches and stored at $5 \pm 1^\circ\text{C}$ for 30 days and analyzed at zero time, after 7, 14, 21 and 30 day of storage.

Chemical Composition:-

The pH of cow's milk samples were determined by using pH meter model CG 840, Toshiba, Japan. The titratable acidity (expressed as lactic acid percent) was estimated for different milk samples according to AOAC (2002) methods. Protein, fat,

ash and moisture contents of cheese samples were also determined according to AOAC (2002).

Microbiological Analysis:-

The microbiological analysis was carried out according to International Dairy Federation (1990).

Organoleptic properties:-

The organoleptic properties of kareish cheese were evaluated according to El-Shafei, *et al.*, (2008). The samples were presented to the panelists in a random order. The cheeses were evaluated organoleptically at zero time, after 7, 14, 21 and 30 days of storage at $5 \pm 1^\circ\text{C}$. Panelists evaluated cheese for appearance (20 points), body and texture (45 points), and flavour (35 points). Overall acceptability (100 points) scores were obtained for all sensory attributes.

Statistical analysis:-

The obtained data were statistically analyzed for analysis of variance, averages and Duncan's test according to SPSS computer program (SPSS, 1998).

RESULTS AND DISCUSSION

Table (1) presented the acidity % expressed as lactic acid and pH values of cow's milk treated with SCN^- and H_2O_2 at rate of, 14 mg/L and 2mL/L, respectively and kept at room (32°C), incubator (37°C) and refrigerator ($5 \pm 1^\circ\text{C}$). Significant ($P \leq 0.05$) changes in acidity % and pH value were recorded in both control and LP-system activated cow's milk kept at room temperature. However, the significantly ($P \leq 0.05$) increased with time reaching 0.31% at the end of the storage time in cow's milk kept at room temperature. On the other hand, the acidity of treated cow's milk at the seven hours of storage at room temperatures was recorded as 0.25%. The corresponding decrease in pH was gradual but significant. At incubator temperature, highly significant changes in acidity and pH were recorded in all control and treated milk samples. However, values of pH and acidity of control and treated milk samples kept at room temperature were slightly different from those kept in incubator and refrigerator during the same storage time. The findings indicate the importance of preserving raw milk by activation of LP-system. On contrast, these findings were agreed with Mehanna, *et al.*, (1998), who reported that LP-system improved the keeping quality of milk. Activation of the LP-system by adding SCN^- : H_2O_2 at rate of 14 mg/L : 2mL/L was more effective in cow's milk stored at both room and incubator temperatures.

Table 1. Effect of adding 14mg thiocyanate/L and 2mL of hydrogen peroxide/L on the pH and acidity (%) in cow's milk kept at room (32°C), incubator (37°C) and refrigerator (5±1°C) temperature.

	Time (hr)	Cow's milk					
		Room		Incubator		Refrigerator	
		pH	Acidity	pH	Acidity	pH	Acidity
Control	1	6.60 ±0.08a A	0.15 ±0.02e A	6.60 ±0.08a A	0.15 ±0.02e A	6.60 ±0.08a A	0.15 ±0.02 e A
	2	6.25 ±0.02 b C	0.17 ±0.02d A	6.23 ±0.02b B	0.17 ±0.02d A	6.36 ±0.02b A	0.16 ±0.02d B
	3	6.15 ±0.02 c C	0.20 ±0.02c A	6.16 ±0.02 c B	0.20 ±0.02c A	6.28 ±0.02c A	0.18 ±0.02c B
	4	6.09 ±0.02 d C	0.24 ±0.02b A	6.10 ±0.02 d B	0.24 ±0.02 b A	6.12 ±0.02d A	0.21 ±0.02 b B
	5	5.72 ±0.02 e C	0.31 ±0.02a A	5.89 ±0.02 e B	0.26 ±0.02a B	5.96 ±0.02 e A	0.25 ±0.02a C
LP-System	1	6.62 ±0.02 a A	0.15 ±0.02 g A	6.60 ±0.02 a B	0.15 ±0.02g A	6.61 ±0.02a C	0.15 ±0.02 g A
	2	6.56 ±0.02 b B	0.17 ±0.02 f A	6.54 ±0.02b C	0.17 ±0.02 f A	6.57 ±0.02b A	0.16 ±0.02f B
	3	6.48 ±0.02c C	0.18 ±0.02e A	6.49 ±0.02c B	0.18 ±0.02 e A	6.52 ±0.02 c A	0.17 ±0.02 e B
	4	6.40 ±0.02d C	0.19 ±0.02d A	6.42 ±0.02 d B	0.19 ±0.02d A	6.45 ±0.02 d A	0.18 ±0.02 d B
	5	6.32 ±0.02 e B	0.22 ±0.02 c A	6.33 ±0.02 e A	0.22 ±0.02 c A	6.08 ±0.02 e C	0.19 ±0.02 c B
	6	6.05 ±0.02 f A	0.22 ±0.02 b A	6.03 ±0.02 f B	0.22 ±0.02 b A	5.77 ±0.02 f C	0.20 ±0.02 b B
	7	5.52 ±0.02 g C	0.25 ±0.02 a B	5.58 ±0.02 g A	0.26 ±0.02 a A	5.56 ±0.02 g B	0.26 ±0.02a A

Means ±standard error. a, b, c Means within the same column with different letters are significantly different (P ≤0.05).

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The data given in Table (2) reveal that activation of the LP-system by adding SCN^- : Na_2CO_3 at rate of 14 mg/L: 30 mg/L respectively. This was more effective in the case of cow's milk stored at both given temperatures. This means that activation of the LP-system gave the best results with cow's milk at any given storage temperature. Also, the lower the storage temperature, the higher was the effectiveness of the LP-system. Their agreed with the findings of Bjorck *et al.*, (1979) who reported that the action of the LP system was temperature dependent.

Table 2. Effect of adding 14mg thiocyanate/L and 30mg of sodium per carbonate/L on the changes in pH and acidity (%) in cow's milk kept at room (32°C), incubator (37°C) and refrigerator (5±1°C) temperatures.

	Time (hr)	Cow's milk kept					
		Room		Incubator		Refrigerator	
		pH	Acidity	pH	Acidity	pH	Acidity
Control	1	6.60 ±0.02 a A	0.15 ±0.02 e A	6.60 ±0.02 a A	0.15 ±0.02 e A	6.60 ±0.02 a A	0.15 ±0.02 e A
	2	6.55 ±0.02 b A	0.17 ±0.02 d C	5.53 ±0.02 b C	0.173 ±0.02 d B	6.36 ±0.02 b B	0.183 ±0.02 d A
	3	6.45 ±0.02 c B	0.20 ±0.02 c C	6.49 ±0.02 c A	0.224 ±0.02 c A	6.18 ±0.02 c C	0.201 ±0.02 c B
	4	6.29 ±0.02 d B	0.24 ±0.02 b B	6.42 ±0.02 d A	0.243 ±0.02 b A	5.88 ±0.02 d C	0.232 ±0.02 b C
	5	5.92 ±0.02 e B	0.31 ±0.02 a A	6.29 ±0.02 e A	0.262 ±0.02 a C	5.66 ±0.02 e C	0.273 ±0.02 a B
LP-System	1	6.95 ±0.02 a A	0.15 ±0.02 g C	6.58 ±0.02 a C	0.153 ±0.02 a B	6.61 ±0.02 a B	0.154 ±0.02 g A
	2	6.56 ±0.02 b A	0.16 ±0.02 f C	6.54 ±0.02 b A	0.164 ±0.02 a B	6.47 ±0.02 b B	0.172 ±0.02 f A
	3	6.51 ±0.02 c A	0.17± 0.02 e C	6.51 ±0.02 c A	0.183 ±0.02 a B	6.21 ±0.02 c B	0.194 ±0.02eA
	4	6.40 ±0.02 d B	0.18 ±0.02 d C	6.46 ±0.02 d A	0.222 ±0.02 a A	6.10 ±0.02 d C	0.203 ±0.02 d B
	5	6.32 ±0.02 e B	0.20 ±0.02 c B	6.43 ±0.02 e A	0.223 ±0.02 a A	5.98 ±0.02 e C	0.223 ±0.02 c A
	6	6.05 ±0.02 f B	0.22 ±0.02 b C	6.21 ±0.02 f A	0.262 ±0.02 a A	5.77 ±0.02 f C	0.242 ±0.02 b B
	7	5.92 ±0.02g B	0.25 ±0.02 a C	5.98 ±0.02 g A	0.273 ±0.02 a A	5.56 ±0.02 g C	0.262 ±0.02 a B

Means ±standard error. a, b, c Means within the same column with different letters are significantly different (P <0.05). A, B, C Means within the same row with different letters are significantly different (P <0.05).

Results of hygienic quality and bacterial counts demonstrate that activation of the LP-systems in raw cow's milk samples kept at 30°C and 5°C improved the keeping quality.

Further, since the effectiveness of the LP-systems is dependent upon the initial of microbiological quality of milk (Härnolv and Kandasamy, 1982). The best effect of LP-systems 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. Moreover, activation of the LP-systems could be considered the base of a method for suppressing the growth of lactic-producing organisms in uncooled milk (El-Agamy, *et al.*, 1993). At 10th hour the mean total bacterial counts (TBC) of treated samples definitely increased to be 4×10⁶cfu/mL, while the initial mean TBC at 0 hour was 2.5×10⁵ cfu/mL (Table, 3). The controls passed that level after 4th hour. Slightly lower findings were recorded by Bennett, (2000).

Table 3. Total bacterial counts (TBC) of the examined raw cow's milk samples treated with LPS after 3 hours of milking and kept at 30 °C for 12 hours

Time (hr)	Means of TBC cfu/ /mL	
	Control	LP- system
0	2.6×10 ² a	2.5×10 ⁵ b
2	2.6×10 ⁶ a	5.0×10 ⁵ b
4	3.8×10 ⁶ a *	3.0×10 ⁶ b
6	4.5×10 ⁶ a	3.6×10 ⁶ b
8	5.1×10 ⁷ a	3.6×10 ⁶ b
10	-	4.0×10 ⁶ *
12	-	2.7×10 ⁷

a, b, c Means within the same row with different letters are significantly different (P <0.05).

Results recorded in Table (4) declared that mean psychrotrophic counts (PC) of examined raw cow's milk samples at 5°C was 2.6×10⁶ cfu/mL at the 6th day that definitely increased to 1.3×10⁷ cfu/mL at the 7th day of storage period, while the initial mean (PC) was 5.5×10³ cfu/mL. However, the controls have passed those levels at the 2nd day. An extension of about 4 days is indicated. The results were substantiating those given by Lin and Chow, (2000) for different milk types.

It was concluded from the obtained results that the pronounced differences in hygienic quality and bacterial counts between activated and control raw cow's milk samples were evidenced. The bacteriostatic effect generate from the activation of LP-systems in raw cow's milk 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, could be considered to be of practical importance in salvaging border-line milk from souring.

Finally, stabilization of raw milk through activation of LP-systems considered to be a useful method for extending the shelf-life of milk in Egypt and tropical countries. It should be emphasized that the 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-systems 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 (Miur, and Banks, 2000).

Regarding to the possible health risks of using the LP-systems, the only concern could arise from the SCN-, which known as a goitrogenic agent. However, an excellent review by Reiter and Härnolv, (1984) reported that the most important studies made so far on that 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 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, stated that it will not affect adversely human cells and organs. Furthermore, it has been suggested by some researchers that the LP-systems exists in the mouth and may play a role against the microorganisms responsible for oral cavities. There is direct evidence that the LP-system occurs in man because one of the oxidation products of thiocyanate (hypothio cyanate) was recently detected in saliva.

Table 4. Psychrotrophic counts (PC) of the examined raw cow's milk treated with LP- system after 3 hours of milking then kept at 5±1°C

Time/days	Means of PC cfu/mL	
	Control	LP- System
0	5.5×10 ³ a	4.5×10 ³ b
1	8.6×10 ⁴ a	7.2×10 ³ b
2	1.3×10 ⁶ * a	1.2×10 ⁴ b
3	2.5×10 ⁷ a	1.8×10 ⁵ b
4	5.5×10 ⁷ a	3.1×10 ⁵ b
5	-	5.5×10 ⁵
6	-	2.6×10 ⁶ *
7	-	1.3×10 ⁷

a, b, c Means within the same row with different letters are significantly different (P ≤ 0.05).

Table (5) recorded the chemical composition of kareish cheese during the storage period of all samples. The moisture content of kareish cheese manufactured by activated LP- system gradually decreased till the end of storage period, with significant differences among the samples of LP- system treated cheese. The results were in agreement with that reported by Aldo, *et al.*, (2013). Slight differences were noticed among kareish cheese in ash, titratable acidity, fat and protein contents throughout the storage period which indicating that, LP- system had slight effect on the composition of the resulted cheese. The results were in agreement with that reported by (Aldo *et al.*, 2013).

Table 5. Chemical composition of kareish cheese as affected by activation of LP-system during the storage period at 5±1°C

Treatments	Storage period days	Moisture %	Fat %	Protein %	Ash %	Titrateable acidity %
Control	Zero	76.80 ±1.32a	0.90 ±0.01d	16.50 ±0.82a	3.40 ±0.22e	1.10 ±0.02e
	7	75.54 ±1.62 b	0.90 ±0.02c	15.92 ±0.62b	3.49 ±0.32d	1.21 ±0.01d
	14	74.64 ±1.42c	0.95 ±0.04b	15.70 ±0.92c	3.62 ±0.12c	1.35 ±0.04c
	21	73.00 ±1.7d	1.00 ±0.01a	15.58 ±0.72d	3.68 ±0.42b	1.45 ±0.03b
	30	72.10 ±1.92e	1.00 ±0.05a	15.50 ±0.52e	3.75 ±0.12a	1.58 ±0.06a
LP-System	Zero	77.12 ±2.12a	1.00 ±0.12a	16.70 ±0.42a	3.10 ±0.42e	0.96 ±0.04e
	7	75.90 ±2.22b	1.00 ±0.05a	16.12 ±0.62b	3.17 ±0.52d	0.12 ±0.05d
	14	75.00 ±2.62c	1.10 ±0.08a	15.91 ±0.82c	3.22 ±0.62c	0.23 ±0.03c
	21	74.10 ±2.42d	1.10 ±0.06a	15.82 ±0.72d	3.30 ±0.72b	1.26 ±0.02b
	30	73.30 ±2.72e	1.20 ±0.09a	15.61 ±0.62e	3.36 ±0.62a	1.35 ±0.01a

Means ±standard error. a, b, c Means within the same column with different letters are significantly different (P <0.05).

Table (6) revealed average of total bacterial counts (TBC) of each treatment of kareish cheese samples. The results showed that the TBC was higher in the control sample than that made activated LP- system in milk. During the storage period, the counts decreased gradually and reached its minimum level at the end of the storage period of cold storage. The lipolytic bacteria counts showed in Table (6) were affected by activated LP- system. Lipolytic bacteria were not detected in activated LP- system but it appeared in the control samples. Proteolytic bacteria and coliform were not detected in fresh and during storage period in all kareish cheese. The results were in agreement with Hassan, *et al.*, (2004).

One of the most important parameters to determine the quality and shelf life of kareish cheese is the count of yeast and mould. In addition to quality deterioration, microbiological counts have been used as indices for the end of shelf life of dairy products (Miur and Banks 2000). The counts of mould and yeast were lower than those by Egyptian Standards (2005) being not more than 100 c6u/g. During storage period, the yeast and mould increased gradually and reached the maximum level at the end of storage period for the control.

Table 6. Some microbiological properties of kareish cheese made with activated LP-System during cold storage at $5\pm 1^\circ\text{C}$

Treatments	Storage period days	Total bacterial count X10 ⁶ cfu/g	Lipolytic bacterial count X10 ⁶ cfu/g	Proteolytic bacterial count X10 ³ cfu/g	Coliform cfu/g	Yeast & moulds X10 ³ cfu/g
Control	Zero	45 a	ND	ND	ND	ND
	7	39 a	ND	ND	ND	ND
	14	25 a	ND	ND	ND	2
	21	22 a	ND	ND	ND	4
	30	18 a	ND	ND	ND	7
LP-System	Zero	35 b	ND	ND	ND	ND
	7	28 b	ND	ND	ND	ND
	14	20 b	ND	ND	ND	ND
	21	15 b	ND	ND	ND	ND
	30	12 b	ND	ND	ND	ND

a, b, c Means within the same column with different letters are significantly different ($P \leq 0.05$).

The data presented in Table (7) revealed that the control kareish cheese gained the lowest score either fresh or throughout storage period. Meanwhile the cheese processed with activated LP- system had the highest score during the storage period. In general all cheese made with activated LP- system had acceptable appearance, flavour and body & texture during the storage period. The results were in agreement with those obtained by Hassan, *et al.*, (2004).

Table 7. Organoleptic assessment of kareish cheese made with activation of LP-System during the storage period at $5\pm 1^\circ\text{C}$

Treatments	Storage period days	Appearance (20)	Body & texture (45)	Flavour (35)	Total (100)
Control	Zero	18	40	32	90
	7	18	40	31	89
	14	7	18	16	51
	21	5	17	14	36
	30	4	10	10	24
LP-System	Zero	18	41	32	91
	7	18	41	31	90
	14	18	40	31	89
	21	17	40	30	87
	30	17	39	30	86

CONCLUSION

The combination of refrigeration and activation of the Lactoperoxidase system in the preparation of kareish cheese is effective to reduce the microbial deterioration during the storage period of kareish cheese.

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تنشيط نظام الاكتوبيروكسيديز في اللبن البقري واستخدامه في تحسين جوده وحفظ الجبن القريش

رشاد إبراهيم الأحول- صلا على النبي إبراهيم أبو الخير- حامد السيد حاتم

قسم بحوث كيمياء الالبان- معهد بحوث الإنتاج الحيواني- مركز البحوث الزراعية

الهدف من هذه الدراسة هو تنشيط نظام الاكتوبيروكسيديز في اللبن البقري الطازج وتأثير ذلك على كل من الأس الأيدروجيني و الحموضة للبن البقري أثناء التخزين على درجة حرارة الغرفة (32م°) و الحضان (37م°) و الثلجة (±1م°) وكذلك جوده وحفظ الجبن القريش المصنع وذلك عن طريق أضافه ثيوسيانات البوتاسيوم و فوق أكسيد الأيدروجين او بير كربونات الصوديوم كمصدر غير مباشر لفوق أكسيد الأيدروجين .كذلك دراسة تأثير التنشيط على الخواص الميكروبية للبن وكذلك الجبن القريش الناتج.

أظهرت النتائج أن كل المعاملات التي أجريت أدت إلى تحسين جوده وقوه حفظ اللبن البقري على درجات الحرارة المختلفة مقارنة بالعينات الغير معاملة.

كذلك فان استخدام فوق أكسيد الأيدروجين لتنشيط نظام الاكتوبيروكسيديز مباشرة للبن أو بطريق غير مباشره باضا فه بيركربونات الصوديوم لم يكن لها تأثير معنوي. في حين كانت النتائج أفضل عند التخزين في الثلجة مقارنة بالتخزين على درجة حرارة الغرفة أو في الحضان.

يتضح من النتائج أن تنشيط نظام الاكتوبيروكسيديز يحسن من جوده وقوه حفظ اللبن البقري وكذلك إنتاج جبن قريش ذو مواصفات جيدة وقوه حفظ عاليه.