

## EFFECT OF COLD STORAGE AND HEATING OF CAMEL'S MILK ON FUNCTIONAL PROPERTIES AND MICROSTRUCTURE IN COMPARISON WITH COW'S AND BUFFALO'S MILK

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### ABSTRACT

The effect of cold storage (4°C/48h.) and heating (85°C/5min.) on some properties of camel's, cow's and buffalo's milks were studied. These included acidity, pH, nitrogen distribution, rennet coagulation time (RCT), surface tension (ST), foam expansion (FE), foam volume stability (FVS), buffer intensity, the electrophoretic properties and microstructure. Cold storage of all milk samples increased acidity, and decreased pH, ST, RCT (except camel's milk) and FE (except cow's milk). No significant effect on CN, NCN and WPN was found. Heating all milk samples increased acidity, casein nitrogen (CN) and RCT (except camel's milk), decreased non-casein nitrogen (NCN), whey protein nitrogen (WPN) and non significant effect on ST. Also, heating decreased only FE of buffalo's milk and increased the others, while FVS of camel's milk was not recorded (after 15 min.). In all cases, buffer intensity curves showed same peaks and were the highest at pH( 7-8 ) and (6.5-7.5) in buffalo's and cow's milk respectively. However, camel's milk had less buffering capacity compared with either buffalo's or cow's milk. Concerning, the electrophoretic patterns, some whey proteins, especially  $\beta$ -lactoglobulin ( $\beta$ -Lg) disappeared on heating, whereas cold storage slightly decreased  $\beta$ -casein. In general, camel's milk showed different

protein patterns. There was little microstructure difference between raw and cooled (4°C/48h.) camel's, cow's and buffalo's milk; while heat treatment increased the size of casein micelles in all samples.

### INTRODUCTION

Milk is heated to improve its keeping quality by deactivation microorganisms and to achieve desirable properties in the final products (Vasbinder and Kruif, 2003). In addition, heat treatment of milk is widely used to alter the functional properties of milk and milk products for a variety of applications (Oldfield *et al* 2000) as well as such treatment is one of the most important processing parameters affecting the texture and consistency of some dairy products like yoghurt (Lucey *et al* 1998). However, it is well known that milk is a heat-labile material in which all the major components may be altered physically and/or chemically by heat. This depends on severity of heat treatment. It has been long recognized that heat treatment of milk above 70°C causes denaturation of whey proteins and some of which complex with the casein micelles (Singh & Creamer, 1992 and Singh, 1995).

Denaturation of whey proteins has been previously described as a two-step process, protein unfolding followed by aggregation (Roefs and de Kruif, 1994). The main heat-induced complex is formed between  $\beta$ -lactoglobulin and K-casein at

the micelle surface via sulphahydri-disulphide interchange reactions and hydrophobic interaction (Haque and Kinsella, 1988). On the other hand, it is common practice in the developed countries-also in Egypt- to store milk on the farm up to 2 days at low temperature (4-6°C) in order to reduce collecting costs. Such treatments of heating and cold storage improve the bacteriological quality of milk but modify several of milk properties. Composition and type of milk is quite important in this respect. For example, camel's milk was characterized by high proportion of  $\alpha$ -lactalbumin and immunoglobulin, whilst,  $\beta$ -lactoglobulin was the major whey protein in cow's and buffalo's milks (Abd El-Salam *et al* 1992). Farah and Farah (1985) reported that no protein bands homologues for bovine K-casein could be clearly detected in the electrophoretic pattern of camel's milk protein. Also, the whey proteins of camel's milk were generally more heat-stable than cow's milk (Farah, 1986). Such importance of milk proteins especially for some functional properties in dairy products as well as the nutritive value of the liquid milk give more attention for studying impact of heating and cold storage on properties of the treated milk of different species.

Therefore, the aim of this study was to apply some functional properties, nitrogen distribution, surface tension, electrophoresis and microstructure for distinguishes the changing in proteins of heat treatment or cold storage of camel's milk in comparison with cow's and buffalo's milk. This is reflected the facility of using milk to improve the quality properties of final products.

## MATERIALS AND METHODS

### Materials

Camel's milk of Dromedary was obtained from Maryout Research Station Desert Research Center. Whereas, Buffalo's milk was obtained from the herd of the Fac. Agric., Ain Shams Univ., Cairo, Egypt; cow's milk was obtained from Dina farm at the 80<sup>th</sup> Km of Alex. desert high way. Chemicals for analysis were obtained from Sigma, USA.

### Methods

#### Preparation of milk samples

Each type of milk (camel, cow and Buffalo) was divided, into three parts. The first part was raw (control); the second one was kept at 4°C for 48h.

and the third part was heated at 85°C for 5 min. then cooling. Three replicates were carried out for each treatment.

### Analytical methods

All milk samples were tested for total solids (T.S), fat, protein, lactose, ash, acidity and pH using Metter Toledo pH meter as reported by AOAC (2007).

The concentrations of total nitrogen (TN), soluble nitrogen at pH 4.6 (NCN), non-protein nitrogen (NPN) and proteose-peptone (PP) were quantified in all milk samples by the micro-kjeldahl method (AOAC, 2007). The whey proteins nitrogen (WPN) and the casein nitrogen (CN) contents were calculated respectively as follows:

$$\text{WPN} = \text{NCN} - (\text{NPN} + \text{PP}).$$

$$\text{CN} = \text{TN} - \text{NCN}.$$

#### Measurement of rennet coagulation time (RCT)

Rennet coagulation time (RCT) was carried out as given by Berridge (1945).

#### Surface tension determination

Surface tension was measured as reported by Salaün *et al* (2005). This was done by placing 100 ml of milk sample in 150 ml plastic cup, then warmed to 25°C in a controlled water bath and stirred for 1/2 min. just before measurement. A ring tensiometer (DuNouy ring Tensiometer, Krüss-Instrument, No. 8158, Germany) was used to measure the interfacial surface tension. The DuNouy ring used for determination was cleaned prior to each measurement by dipping the ring in dilute nitric acid then flaming until the ring was "red" hot in the oxidizing portion of Bunsen burner flame to remove organic materials. After cooling, the ring was then hung from the load cell and lowered to the base of sample container. The ring was pulled from the surface of sample and the force required to do so was recorded as surface tension values which read directly from the instrument scale as  $\text{N/m}^{-1}$ . Duplicate samples were prepared for measurements of each treatment.

#### Measurement of foaming properties

Foam ability and foam stability were measured (at  $5 \pm 2^\circ\text{C}$ ) as described by Closs *et al* (1990). Samples of raw, heated or cold stored milks

(200 ml) were whipped at maximum speed for 5 min. using blender (Brun, Germany). Foams were carefully transferred to a 1 L graduated glass measuring cylinder. The foaming capacity was measured according to Poole *et al* (1984) and expressed as:

$$\text{Volume increase \%} = \frac{\text{Final volume} - \text{Initial volume}}{\text{Initial volume (200 ml)}} \times 100$$

The foam stability was expressed as time (in min.) required for the foam to breakdown (time required for return to initial sample volume (200 ml)).

#### Buffer capacity

Buffer intensity curves were determined according to Morr *et al* (1973). Buffering indices (dB/dpH) were calculated for each addition of acid and buffering curves prepared by plotting these indices as a function of pH. Areas under buffering curves were integrated to estimate the intensity of buffering capacity.

#### Polyacrylamide-gel electrophoresis

A method described by Laemmli (1970) was adapted for the qualitative study. The obtained SDS-PAGE patterns were identified as described by Basch *et al* (1985) and Farrell *et al* (2004).

#### Microstructure examination of milk samples

Transmission Electron Microscopy (TEM-ZEISS, West Germany) (National Research Center, Dokki, Giza, Egypt) milk samples were examined by TEM microscope at 80 Kv and magnification (63,000X) using the method described by McMahon *et al* (1993).

#### Statistical analysis

The experimental data was analyzed using the general linear models procedure of the Statistical Analysis System (SAS, 1996). Significant differences were defined at  $p < 0.05$ .

### RESULTS AND DISCUSSION

Chemical composition of the camel's, cow's and buffalo's milk are shown in Table (1). The results show buffalo's, cow's milks had lower ash

and lactose contents but higher fat and protein, compared with the camel's milk. These results agree with those reported by Shahein (2006).

Data in Table (2) indicate the acidity as lactic acid (%), pH values and rennet coagulation time (RCT, min.) of raw, cold storage, and heated camel's, cow's and buffalo's milk. Cold storage and heating of each type of milk samples resulted in a significant increase in acidity and decrease of pH values, as compared with raw milk samples. The changing in the acidity and pH values were more pronounced in buffalo's and cow's milk than the camel's milk i.e. no coagulum was found. Significant differences ( $p < 0.05$ ) could be detected between raw, cold storage, heated cow's and buffalo's milk for RCT. On the other hand, no time could be recorded for RCT of camel's milk. The increase in the acidity and decrease in the pH values of heated milk could be due to the transference of calcium phosphate from the soluble phase to the colloidal phase, which would result from the liberation of hydrogen ions (Jennes and Patton, 1959). The obtained results for camel's milk were similar to the findings of Bayoumi (1990) who reported that the raw camel's milk characterized with poor rennet ability and even with the addition of  $\text{CaCl}_2$ . The changes of acidity and pH values of all cold storage milk could be due to growth of psychotropic bacteria (Cousin *et al* 1992). The surface tension (ST) was not significantly affected by the applied treatments in camel's milk. However, cold storage treatment had a significant effect in cow's and buffalo's milk. This may be due to the impact of cold storage on solubilization of some casein into milk serum (Ali *et al* 1980; Dzurec & Zall, 1985 and Lucey *et al* 1998).

Table (3) indicates the effect of the applied treatments on the nitrogen distribution (mg/100 ml) of the camel's, cow's and buffalo's milk. The significant changes due to heat treatment was an increase in the casein nitrogen (CN) with a corresponding decrease in whey protein nitrogen (WPN). The significant decrease in non-casein nitrogen (NCN) may be attributed mainly to the effect of such heat treatment on denaturation of whey protein. Increasing in the CN thereby causing denatured WPN associate with casein precipitate by the casein precipitating agents. Also, it could be noticed that, heat treatment induced no changes in non-protein nitrogen (NPN) and proteoseptone nitrogen. These results are in agreement with the finding of Stephan and Ganguli (1974). The camel's milk was generally more heat-stable than buffalo's and cow's milk, the degree of whey

Table 1. Gross composition of fresh milk samples (of the camel's, cow's and Buffalo's) milk

Content	Type of milk		
	Camel's	Cow's	Buffalo's
Total Solids (T.S %)	11.94	11.83	15.68
Fat %	3.10	3.65	6.14
Total Protein %	2.81	2.98	3.84
Lactose %	5.13	4.48	4.86
Ash %	0.90	0.72	0.80

Table 2. Effect of cold storage (4°C/48h.) or heating (85°C/5min.) treatments on the acidity, pH values, rennet coagulation time (RCT) and surface tension of the camel's, cow's and buffalo's milk

Treatments	Acidity (%)	pH values	RCT (min.)	Surface Tension (N/m <sup>-1</sup> )
<b>Camel:</b>				
Raw	0.211 <sup>b</sup>	6.44 <sup>a</sup>	N.C	53.62 <sup>a</sup>
Cold stored	0.232 <sup>a</sup>	6.10 <sup>b</sup>	N.C	52.10 <sup>a</sup>
Heated	0.215 <sup>b</sup>	6.45 <sup>a</sup>	N.C	52.50 <sup>a</sup>
<b>Cow:</b>				
Raw	0.166 <sup>c</sup>	6.64 <sup>a</sup>	2.50 <sup>b</sup>	57.00 <sup>a</sup>
Cold stored	0.196 <sup>a</sup>	6.46 <sup>c</sup>	2.00 <sup>c</sup>	51.75 <sup>b</sup>
Heated	0.185 <sup>b</sup>	6.56 <sup>b</sup>	3.52 <sup>a</sup>	55.80 <sup>ab</sup>
<b>Buffalo:</b>				
Raw	0.173 <sup>c</sup>	6.61 <sup>a</sup>	1.35 <sup>b</sup>	58.75 <sup>a</sup>
Cold stored	0.218 <sup>a</sup>	6.40 <sup>c</sup>	1.10 <sup>c</sup>	50.50 <sup>b</sup>
Heated	0.194 <sup>b</sup>	6.52 <sup>b</sup>	2.21 <sup>a</sup>	58.60 <sup>a</sup>

a, b, c...: Means with the different letters within the same column and kind of milk are significantly different ( $p < 0.05$ ).

N.C = No coagulation.

**Table 3.** Effect of cold storage (4°C/48h.) and heat (85°C/5 min.) treatments on the nitrogen distribution (mg/100 ml) of the camel's, cow's and buffalo's milk

Treatments	Casein nitrogen	Non-casein nitrogen	Whey Protein nitrogen	Proteose-peptone nitrogen	Non-protein nitrogen
<b>Camel:</b>					
Raw	348 <sup>b</sup>	147 <sup>a</sup>	102 <sup>a</sup>	16 <sup>a</sup>	29 <sup>a</sup>
Cold stored	350 <sup>b</sup>	150 <sup>a</sup>	105 <sup>a</sup>	16 <sup>a</sup>	29 <sup>a</sup>
Heated	391 <sup>a</sup>	104 <sup>b</sup>	59 <sup>b</sup>	16 <sup>a</sup>	29 <sup>a</sup>
<b>Cow:</b>					
Raw	431 <sup>b</sup>	140 <sup>a</sup>	70 <sup>a</sup>	40 <sup>a</sup>	30 <sup>a</sup>
Cold stored	433 <sup>b</sup>	145 <sup>a</sup>	75 <sup>a</sup>	40 <sup>a</sup>	30 <sup>a</sup>
Heated	501 <sup>a</sup>	70 <sup>b</sup>	00	40 <sup>a</sup>	30 <sup>a</sup>
<b>Buffalo:</b>					
Raw	579 <sup>b</sup>	162 <sup>a</sup>	78 <sup>a</sup>	51 <sup>a</sup>	33 <sup>a</sup>
Cold stored	582 <sup>b</sup>	164 <sup>a</sup>	80 <sup>a</sup>	51 <sup>a</sup>	33 <sup>a</sup>
Heated	658 <sup>a</sup>	84 <sup>b</sup>	00	51 <sup>a</sup>	33 <sup>a</sup>

a, b...: Means with the different letters within the same column and kind of milk are significantly different ( $p < 0.05$ ).

proteins denaturation due to heat treatment was about 43 %. These may be due to absence or deficiency of K-casein and  $\beta$ -lactoglobulin in camel's milk might be a cause of its higher heat stability at high temperature (Farah and Atkins, 1992). There were no major differences ( $p < 0.05$ ) in nitrogen profile between raw and cold storage milk samples.

Results in Table (4) indicate that the raw cow's milk had the lowest foam expansion compared with the other milks, while camel's milk had the excellent one. This also was true with heated milks. However, cow's milk characterized with higher foam stability compared with the other types of milk. Such results may be due to the distribution of casein micelles in camel milk which is significantly broader than that of buffalo's milk with greater number of larger micelles of 350-500 nm. (Farah 1993) and higher in molecular weight of casein micelles (El Agamy and Kamal, 1998).

Figure (1) shows the buffer index (BI) [dB/dpH] versus pH values of raw, cold stored (4°C/48h.) and heated (85°C/ 5 min.) milk samples as titrated with 0.1N HCl from pH 10.0 to 3.0. The maximum buffering capacity of buffalo's milk was attained at

pH range about 6-7 for heated or cold stored samples compared with raw one (7-8). The corresponding values for cow's milk were about 6.0, 6.5 and 7.5 in order. However, camel's milk had lower values i.e. less buffering capacity compared with either buffalo's or cow's milk. The little variations in the buffer capacity among raw, heated and cold stored milk may be due to the effect of such treatments on the salt distribution. In this respect, Al-saleh and Hammad (1992) reported that the shapes and positions of maximum peaks in the buffering curves for cow and camel milk are different and pasteurization had slight effect on the buffering capacity of them. Generally, the obtained results revealed that, the buffering capacity of milk from the various species studied increased in order: buffalo's > cow's > camel's milk. The different behaviour of these milks in the buffer index could be due to the descending order of their protein content as well as the form and nature of protein fractions which confirmed by electrophoresis (Fig. 2). These results are in accordance with Immam *et al* (1974); Hassan *et al* (1987) and Abd El lateef (2001) for milk from different mammals.

Table 4. Effect of cold storage (4°C/48h.) and heat (85°C /5 min.) treatments on Foam expansion (FE%) and Foam volume Stability (FVS% after 5,10,15 and 20 min.) of the camel's, cow's and buffalo's milk

Treatments	Final Vol.( ml.)	FE%	FVS %			
			5	10	15	20
<b>Camel:</b>						
Raw	725	262.5	72.4	17.2	0.0	
Cold stored	460	130	56.5	51.0	0.0	
Heated	750	275	73.3	13.5	0.0	
<b>Cow:</b>						
Raw	365	82.5	45.2	31.5	20.5	0.0
Cold stored	500	150	60.0	45.0	13.0	0.0
Heated	415	107.5	51.8	42.1	36.1	0.0
<b>Buffalo:</b>						
Raw	500	150	60	20	0.0	0.0
Cold stored	435	117.5	54	42.5	0.0	0.0
Heated	385	92.5	48	35	22	0.0

Fig. (2) shows the electrophoretic patterns of all milk samples. In general, the proteins of raw milk fractionated into 6 main fractions namely  $\kappa$ -casein,  $\beta$ -casein,  $\alpha_s$ -casein,  $\alpha$ -Lactalbumin, ( $\alpha$ -La),  $\beta$ -Lactoglobulin ( $\beta$ -Lg) and bovine serum albumin (BSA). This agrees with the well-known information given in the literature. It may be of interest to note that the mobility and intensity of the different fractions were greatly affected by milk type and the camel's milk showed the pronounced differences in this respect. This agrees with findings of El-Agamy and Kamal (1998).

Concerning, effect of heat treatments (columns 1, 2 and 3, Fig. 2), it is clear that such treatment decreased intensity of whey proteins bands including  $\alpha$ -La and  $\beta$ -Lg with corresponding increase in  $\kappa$ -casein region. This was expected since heat treatments of milk at  $>70^\circ\text{C}$  cause denaturation of whey proteins. Some of the denatured whey proteins complex with casein micelles involving  $\kappa$ -casein via hydrophobic interactions and formation of intermolecular disulphide bonds (Lucey *et al* 1998). The presence of disulphide bonds among whey proteins and between whey proteins and casein was suggested as a disulphide- bond-blocking agent prevented the complete decrease of  $\alpha$ -La and  $\beta$ -Lg electrophoretogram bands (Dzurec and Zall, 1985). However, the degree of interaction between  $\beta$ -Lg and  $\kappa$ -casein depends on the time and temperature of heating, concentration of proteins, pH and presence of salts (Singh,

1995). This may give explanation for the different behaviour of the different milks towards the applied heat treatment. Also, the increase of molecular size of the formed complex due to heating may cause less migration *via* the small pore size of the gel (Dalgleish, 1990 and Singh & Creamer, 1991) causing more intensity bands in  $\kappa$ -casein region or slightly before that region.

Fig. (2) Shows also differences in  $\beta$ -casein region, its band was more intense in case of buffalo's milk. However, in all cases cold storage of milk (columns 4, 5 and 6 of Fig. 2) caused dissociation of  $\beta$ -casein from micelles and its subsequent solubilization in milk serum. This agrees with Dzurec and Zall, (1985).

During the study of Microphotographs of all treatments by Transmission Electron Microscopy (TEM), it was observed that the casein micelles had spherical shapes with a wide range of diameters. Also, there were more small micelles in cow's milk than in camel's and buffalo's milk. Moreover, casein micelles of buffalo's milk showed discrete, clearly defined particles. Also, casein micelles of cow's and camel's milks appeared adhered to each other and consisted of more numerous branches. After heating buffalo's cow's and camel's milk increased the formation of protein were observed. This may be due to the denaturation of whey proteins precipitated on the surface of the casein micelles, which gradually became linked through whey protein bridging. The polypeptide

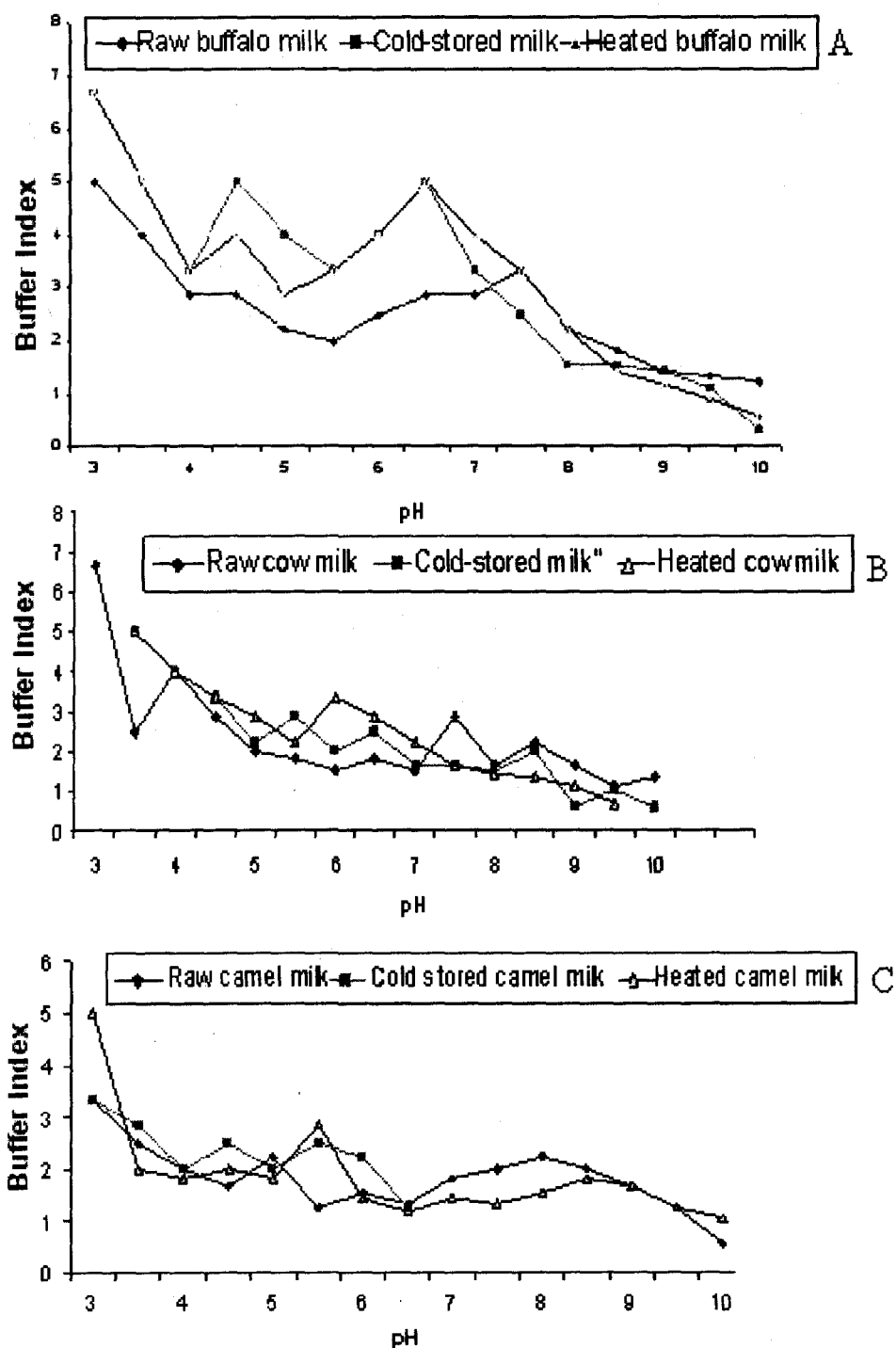


Fig. 1. Buffer intensity curves of raw, cold storage (4°C/48h.) and heat treatment (85°C/5min.) of buffalo's (A), cow's, (B) and camel's (C) milk.

Nr	Treatments										
	Heated milk			Cold stored milk			Raw milk			Orig. CN	Marker
	Camel	Buffalo	Cow	Camel	Buffalo	Cow	Camel	Buffalo	Cow		
1	2	3	4	5	6	7	8	9	10	11	

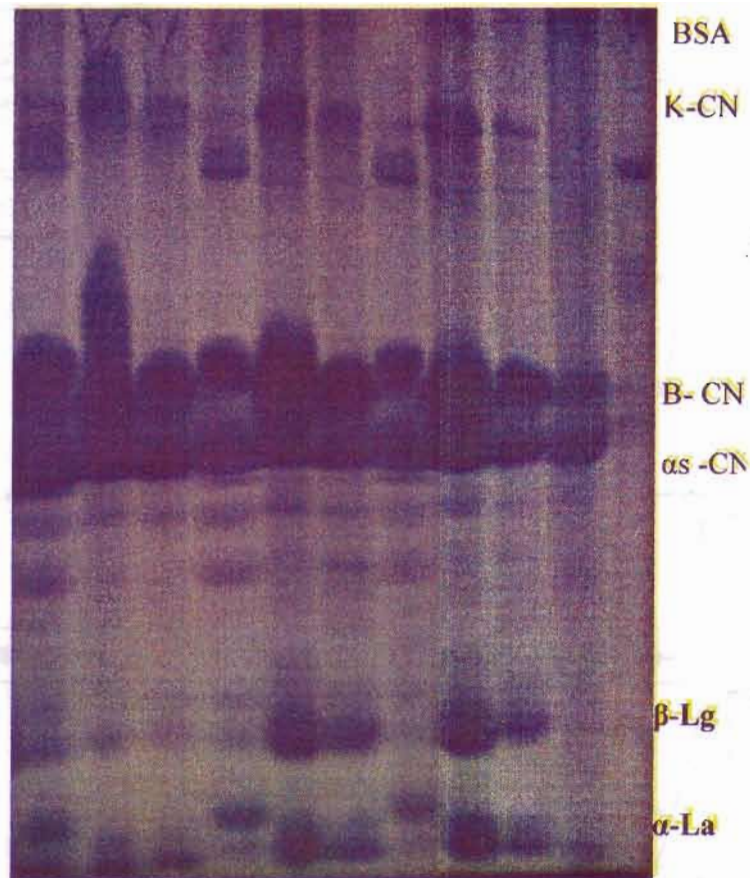


Fig. 2. The electrophoretic patterns of heated (85°C/5 min) and cold stored (4°C/48h) and raw camel's, buffalo's and cow's milks. The lanes were (1, 2, 3) heated samples, (4, 5, 6) cold samples, (7, 8, 9) raw samples, (10) casein standard, (11) molecular weight markers

chains may also be linked through disulphide bond, especially between K-casein and  $\beta$ -lactoglobulin as the result of the electrophoretic patterns. This indicated that the cross-linking capacity of denatured whey protein plays a key role in milk structure after heating; it contributes to an increase in the degree of bridging between protein

particles. Buffalo's milk appeared spherical in shape with smooth surface and to be composed of a large number of small subunits. This could be explained by the reported by Omar, (1985) who observed that heating of milk caused an increase in the number of free submicelles from disintegrated casein micelles.

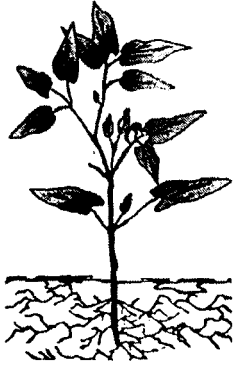


There was little microstructure difference between raw and cooled (4°C/48h.) buffalo's cow's and camel's milk. These results are in agreement with those reported by Omar, (1985) and McMahon *et al* (1993).

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## تأثير حفظ اللبن مبردا والتسخين على الخواص الوظيفية والتركيب البنائي للبن

### النوق مقارنة باللبن البقرى والجاموسى

[ ١١ ]

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### الموجز

من النيتروجين الكازينى والنيتروجين غير الكازينى ونيروجين بروتينات الشرش وحدث انخفاض فى رقم إلس الهيدروجينى ووقت التجبن بأنزيم الرنين ماعدا لبن النوق والتوتر السطحى والقابلية للخفق ماعدا اللبن البقرى. فى كل الحالات فان منحنيات السعة التنظيمية لها نفس الاتجاه ولكنها كانت مرتفعة عند رقم إلس الهيدروجينى (٧-٨) فى اللبن الجاموسى و(٦,٥-٧,٥) فى اللبن البقرى بينما أظهر لبن النوق سعة تنظيمية منخفضة .

هذا وعند فصل بروتينات اللبن فى مجال كهربائى على جل البولى اكريلاميد كانت للمعاملة الحرارية تأثير واضحا على اختفاء معظم البيتا لاكتوجلوبولين فى كل عينات اللبن المسخن. فى حين نقصت كميته البيتا كازين نتيجة حفظ اللبن مبردا. مع ملاحظه أن لبن النوق أظهر فصلا متباينا عن باقى الالبان من ناحيه عدد وقوه مناطق الفصل . اوضحت نتائج التركيب البنائى وجود فروق طفيفة بين اللبن المبرد (٤ م/م<sup>٥</sup> ٤٨ ساعة ) واللبن الخام لكل من اللبن الجاموسى والبقرى والنوق. فى حين اظهرت النتائج وجود فروق كبيرة بالمعاملة الحرارية (٨٥ م/م<sup>٥</sup> دقائق) نتيجة لحدوث دنتره بروتينات الشرش على سطح جسيمات الكازين مما أدى الى زيادة حجم الجسيمات.

يهدف هذا البحث إلى دراسة تأثير حفظ اللبن مبرد (٤ م/م<sup>٥</sup> ٤٨ ساعة) والمعاملة الحرارية (٨٥ م/م<sup>٥</sup> دقائق) على بعض خواص لبن النوق و اللبن الجاموسى والبقرى حيث اشتملت التحليلات على اللبن تقدير النسبة المئوية للحموضة ورقم الأس الهيدروجينى ، ووقت التجبن بأنزيم الرنين والتوزيع النيتروجينى والتوتر السطحى وخواص القابلية للخفق وثبات الرغوة والسعة التنظيمية والفصل الكهربى على جيل الاكريلاميد والتركيب البنائى الدقيق للبن.

أوضحت نتائج الدراسة أن معاملة اللبن حراريا زادت من قيم النسبة المئوية للحموضة والنيتروجين الكازينى ، ووقت التجبن بأنزيم الرنين فيما عدا لبن النوق، فى حين وجد انخفاض فى كل من النيتروجين غير الكازينى ، نيتروجين بروتينات الشرش ولا يوجد تأثير معنوى على التوتر السطحى. كما أن المعاملة الحرارية أدت الى انخفاض فى القابلية للخفق للبن الجاموسى بينما زادت فى الالبان الاخرى. بينما كانت ثبات الرغوة للسبن النوق منخفضة.

كان لحفظ اللبن مبردا تأثير على خواصه حيث زادت قيم النسبة المئوية للحموضة ولم يتأثر بصورة معنوية كل

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