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Studies on Rennet Coagulation of Skim Camel Milk Concentrated by Ultrafiltration

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Abstract. Skim camel milk was concentrated by ultrafiltration (UF) to volume concentration ratios (VCR) of 1.0, 1.7, 2.0, 2.7, 3.3 and 4.3. Chemical analyses of skim milk and retentates were done to determine the changes in chemical composition that occurred during UF. Rennet coagulation times (RCT) of skim milk and retentates were studied using three levels of rennet, calcium chloride and pH at 35°C. Milk proteins were concentrated by UF from an average 2.77% in skim milk to 4.69, 5.63, 7.66, 9.20 and 11.2% in 1.7, 2, 2.7, 3.3, and 4.3 fold, respectively. The corresponding figures for total solids were 8.5% in skim milk and 10.87, 11.81, 14.35, 15.81 and 18.15%, respectively. RCT decreased as the protein concentration increased, but the precise influence of the latter decreased as the rennet or calcium concentration increased or as the pH decreased. However, at the highest rennet (15 mg/100 ml), or calcium chloride (0.045%) concentration or at the lowest pH (6.2) the RCT was nearly independent of the protein content.

Introduction

Ultrafiltration (UF) is a sieving process that employs a membrane with definite pores that are large enough to permit the passage of water and small molecules. When pressure is applied to a fluid, the semi-permeable membrane allows small species to pass through as permeate and larger species are retained and concentrated as retentate. In UF of milk, non-protein nitrogen and soluble components such as lactose, salts, and some vitamins pass through the membrane, whereas milk fat, proteins and insoluble salts are retained by the membrane [1, pp.1-25; 2, pp.60-95].

The process of UF offers the attractive prospect of concentrating milk at ambient temperature and so of avoiding chemical damage and flavor changes caused by the heating employed in other methods of concentration. So far the application of these methods of dairy idustry has been focused mainly on concentration of cheese whey and production of soft cheeses [3, pp. 105-170; 4;5]. Several advantages have been associated with cheesemaking by UF, including: (1) increases in the cheese yield, (2) lower overall energy consumption, (3) enzyme usage is reduced, and (4) whey disposal problems are reduced [2].

The growing use of ultrafiltration in the dairy industry, especially in the area of cheesemaking, promises to dramatically change the technology of cheese manufacture [3,5]. Since the UF retentate has higher dry matter and protein contents, as well as an increased ratio of protein to dry matter compared to native milk, its properties obviously differ from those of the milk from which it was prepared [6]. In the case of cow milk, several workers [6-10] reported that the concentration of milk by UF caused a reduction of the coagulation time. Rennet concentration has a similar strong influence. On the other hand, other workers [11,12] reported that the coagulation time increased with protein content and decreased with rennet concentration. Dalgleish [13] conclusions are even more contradictory. He observed that the coagulation time is relatively unaffected by the concentration of milk by UF. Recently, Sharma *et al.* [14] reported that the coagulation time decreased with an increase in milk concentration when milk pH was unadjusted, but remained unaffected when pH was adjusted (6.8-6.0) by adding lactic acid.

In the case of camel milk, however, no information is available in the literature on enzymatic coagulation of UF-concentrated camel milk. However, our previous studies on enzymatic coagulation and cheesemaking of camel milk [15-21] indicated that the mechanism of camel milk coagulation is similar to that of cow milk, but the coagulum obtained from camel milk was very soft compared to that of cow milk. Therefore, the objective of this work was to study the effects of milk, rennet and calcium concentrations on coagulation time of ultrafiltrated camel milk.

Materials and Methods

Preparation of milk for ultrafiltration

For each UF run, 15 kg of raw camel (Majaheim) milk was obtained from Mansour Al-Hawas farm, Al-Methneb, Qassim area. The milk was warmed to 41-45°C in a water bath and then was skimmed, weighed, and pasteurized at 72°C for 20 sec. Samples for chemical analyses were taken and refrigerated for subsequent analysis. The pasteurized skim milk was cooled to 50°C before UF.

Ultrafiltration process

The bench-scale UF system (Fig. 1) consisted of a feed tank for holding the milk, a Masterflex peristaltic pump (Cole-Parmer Instrument Co., Chicago, IL, USA) for recycling milk, two pressure gauges to monitor inlet and outlet pressures, a hollow fiber UF module with a polysulphone membrane of 30,000 molecular weight cut-off (Model UFP-30-C-4, A/G Technology, Needham, MA, USA), and a container to collect and measure the permeate.

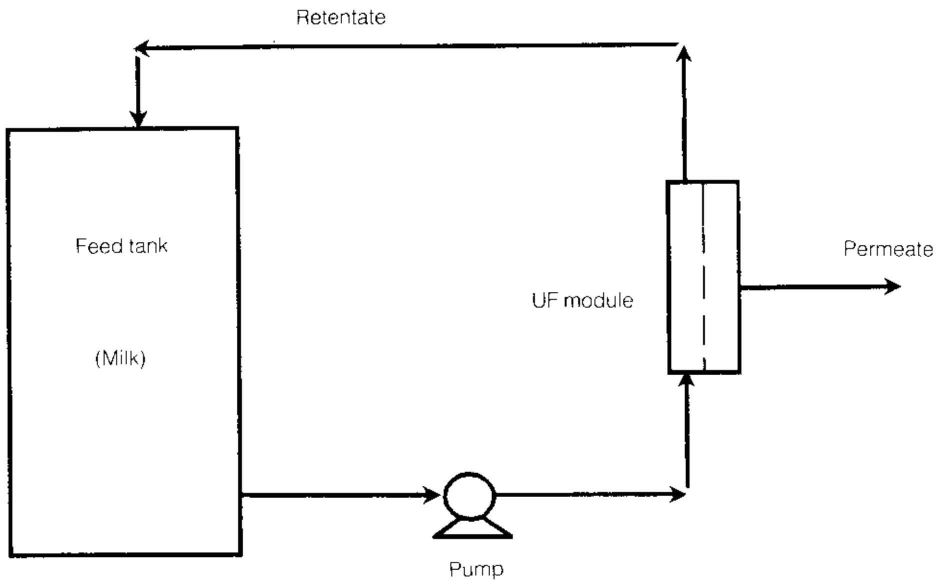

Fig. 1. Schematic of ultrafiltration system.

The UF process was started by pumping the milk at 50°C through the membrane module while maintaining inlet and outlet pressures of 20 and 5 psi, respectively. Permeate volume was monitored continuously to determine reduction in the milk volume to five different volume concentration ratios (VCR).

$$\text{Volume concentration ratio (VCR)} = \frac{\text{Initial volume of milk}}{\text{Concentrate (retentate) volume}}$$

At different VCR, retentate and permeate samples were taken and refrigerated for subsequent analysis. As the concentration reached 4.3-fold (VCR = 4.3), UF system was stopped and the remaining retentate was stored with the rest of the samples in the refrigerator. After each run, the membrane module was cleaned and sanitized according to the manufacturer's instructions and stored in a 5°C cooler.

Experimental procedures

Three levels of rennet were prepared from 3% (w/v) rennet power (Chr. Hansen's Lab. A/S, Copenhagen, Denmark). Each level of rennet was added to 10 ml of milk to give 3, 6 and 15 mg/100 ml of milk sample. The coagulation time of milk samples was measured in triplicate at three levels of pH (6.6, 6.4 and 6.2), at three levels of calcium chloride (0.015, 0.030 and 0.045% w/v) and at 35°C using a modified version of the Sommer-Matson rennet tester as described by Mehaia and Cheryan [22]. The pH of milk was adjusted to the desired levels by adding 40% (w/v) lactic acid, while stirring the samples with a magnetic stirrer.

Chemical analysis

Total solids and ash contents of skim milk, retentate and permeate samples were determined according to AOAC [23]. Nitrogen was determined by the standard micro-Kjeldahl method of the AOAC [23]. A nitrogen conversion factor of 6.38 used for calculating protein contents and various fraction of milk samples. Milk samples were fractionated for total nitrogen (TN) and non-protein nitrogen (NPN) by the method of Rowland [24]. pH of milk samples measured with an Orion pH meter (Orion Research Inc., Cambridge, MA, USA). Calcium in skim milk, retentate and permeate samples was analyzed by atomic absorption spectrophotometry Model 1100B (Perkin-Elmer Corp., Analytical Instruments, Norwalk, CT, USA) according to the method reported by IDF [25]. All analyses of skim milk, retentate and permeate samples were performed in duplicate.

Results and Discussion

Chemical composition of retentates

Concentrations of total solids, ash, total nitrogen, protein nitrogen and calcium are presented in Table 1. In fig. 2, the changes in the composition of the above constituents of skim milk during UF are expressed as the increase of the concentration factor of the individual components as a function of the VCR. As the VCR of skim milk was increased by approximately 1.7, 2.0, 2.7, 3.3 and 4.3-fold, protein concentration increased from an initial 2.77 in skim milk to 11.92% in the 4.3-fold retentate, indicating a 4.3-fold increase in concentrtion and about 100% retention. Total solids,

Table 1. Composition of skim camel milk and milk retentates

VCR	pH	Total solids	Ash %	Total nitrogen	Protein nitrogen	Total calcium (mg/100g)	Calcium/protein (mg/g)
1.0	6.67	8.50	0.83	3.22	2.77	108.6	39.2
1.7	6.67	10.87	0.96	5.18	4.69	180.9	38.5
2.0	6.65	11.81	1.05	6.16	5.63	216.1	38.4
2.7	6.64	14.35	1.20	8.22	7.66	290.2	37.9
3.3	6.61	15.81	1.35	10.47	9.20	350.0	38.0
4.3	6.60	18.15	1.52	12.82	11.92	482.8	38.0

total nitrogen and ash contents increased from an initial 8.5, 3.22 and 0.83% in skim milk to 18.15, 12.82 and 1.52% in the 4.3-fold retentate, respectively. This increase was proportional to the concentration factor of the retentates but to a lesser degree indicating the loss of small molecular weight components such as lactose, soluble salts and non-protein nitrogen. These observations were comparable with those presented, for cow milk, by Green *et al.* [26], Srilaorkul *et al.* [27] and Premaratne and Cousin [28].

Milk components such as minerals, that are small than the membrane bores and associated with proteins do not permeate but gradually increase in concentration [26,29]. However, the concentrations of these components increase to a lesser degree than those of proteins. Table 1 indicates the 4.1-fold higher calcium content in a 4.3-fold retentate. Premaratne and Cousin [28] observed a 4.3-fold higher calcium in a 4.3-fold retentate. Premaratne and Cousin [28] observed a 4.3-fold higher calcium content in a 5-fold retentate and Mattews *et al.* [30] noted a 1.64-fold higher calcium content in a 2-fold retentate.

Rennet coagulation time (RCT) of UF-concentrated camel milk

Coagulation time is an empirical measurement of coagulation easier to determine than the three parameters on which it depends: (1) the enzymatic rate, (2) the aggregation rate and (3) the degree of proteolysis at which the aggregation starts [31]. Coagulation time strongly affected by enzyme, calcium concentrations and by pH of the milk.

Figure 3 shows the effect of protein and rennet concentration on coagulation time of camel skim milk and milk retentates at pH 6.6 and 35°C. At any one rennet

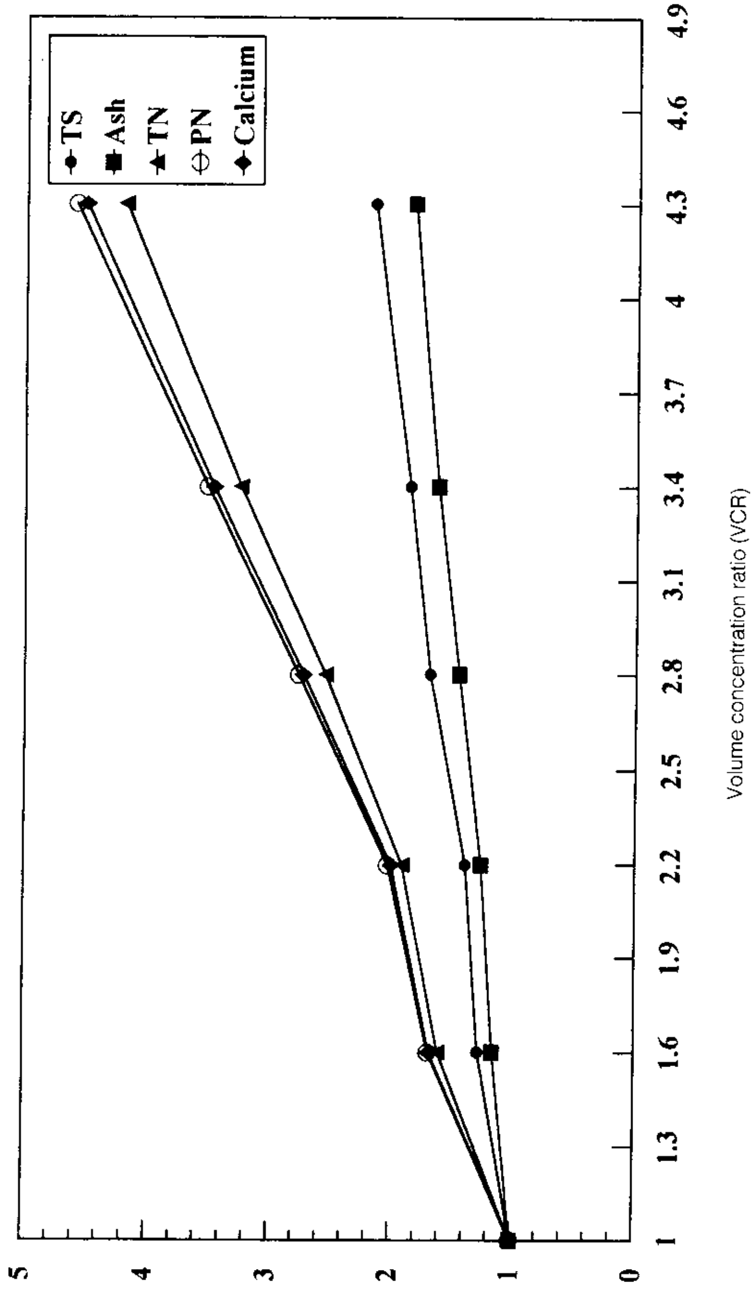

Fig. 2. **Concentration factor for some constitutents of skim camel milk as a functioin of volume concentration ratio.**

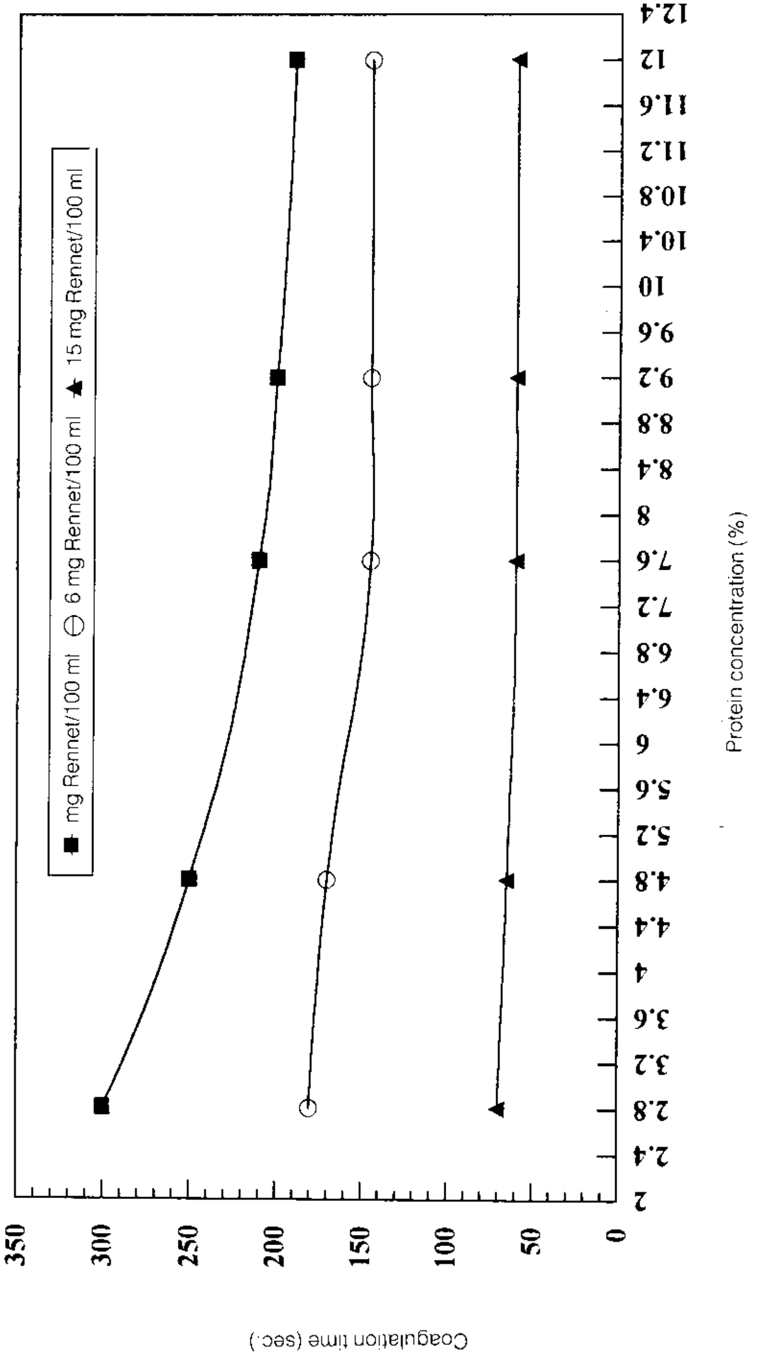

Fig. 3. The effect of protein and rennet concentrations on coagulation time of UF skim camel milk at pH 6.6 and 35°C.

concentration the coagulation time decreased as the protein concentration increased, but the precise influence of the latter decreased as the rennet concentration increased. However, at the highest rennet concentration (15 mg/100 ml) the coagulation time was nearly independent of the protein content. Similar observations were reported, for cow milk, by some workers [6, 8-10]. On the other hand, other workers [11,12] reported that the coagulation time increased with protein content and decreased with rennet concentration.

The effects of protein concentration and pH on coagulation time of skim camel milk and milk retentates using 6 mg rennet/100 ml of milk samples at 35°C are shown in Fig. 4. RCT was highly pH-dependent. Enzymatic coagulation of milk is inversely related to its pH [19]. However, samples of pH 6.4 and 6.2 showed slightly reduction in coagulation time, as protein concentration increased up to 5.63%, while the curves flattened out at higher protein concentration. Lucisano *et al.* [10] reported that a marked reduction in coagulation time was observed when pH of cow milk retentate was reduced from 6.7 to 6.3. Whereas Sharma *et al.* [14] reported that the coagulation time of UF cow milk decreased with a decrease in pH from 6.8 to 6.0 and the coagulation time decreased with an increase in milk concentration when milk pH was unadjusted (7.0), but remained unaffected when pH was adjusted to 6.8, 6.4 and 6.0 by adding lactic acid.

Figure 5 shows the effects of protein and calcium concentrations on coagulation time of skim camel milk and retentates using 6 mg rennet/100 ml of sample at pH 6.6 and 35°C. Addition of calcium chloride to the milk prior to renneting not only increases the calcium concentration but also decreases the pH of the milk. Both factors accelerate the renneting process [32, p. 662]. Mehaia and Cheryan [22] reported that while calcium had relatively little effect on the primary (enzymatic) phase of milk coagulation, it had a relatively large effect on secondary (non-enzymatic) phase interactions. It can be seen from Fig. 5 that the decreasing effect of calcium concentration on coagulation time is greatest in skim milk, and that as the protein content increases, the effect on coagulation time decreases. At the highest calcium concentration (0.045%) the coagulation time was nearly independent of the protein content. These observations were comparable with those reported, for cow milk, by Pahkala *et al.* [8].

Conclusions

From the foregoing results it could be concluded that rennet coagulation time of UF concentrated camel milk strongly affected by rennet and calcium concentrations and by the changes in the pH of the milk. Enzymatic coagulation of camel milk seems

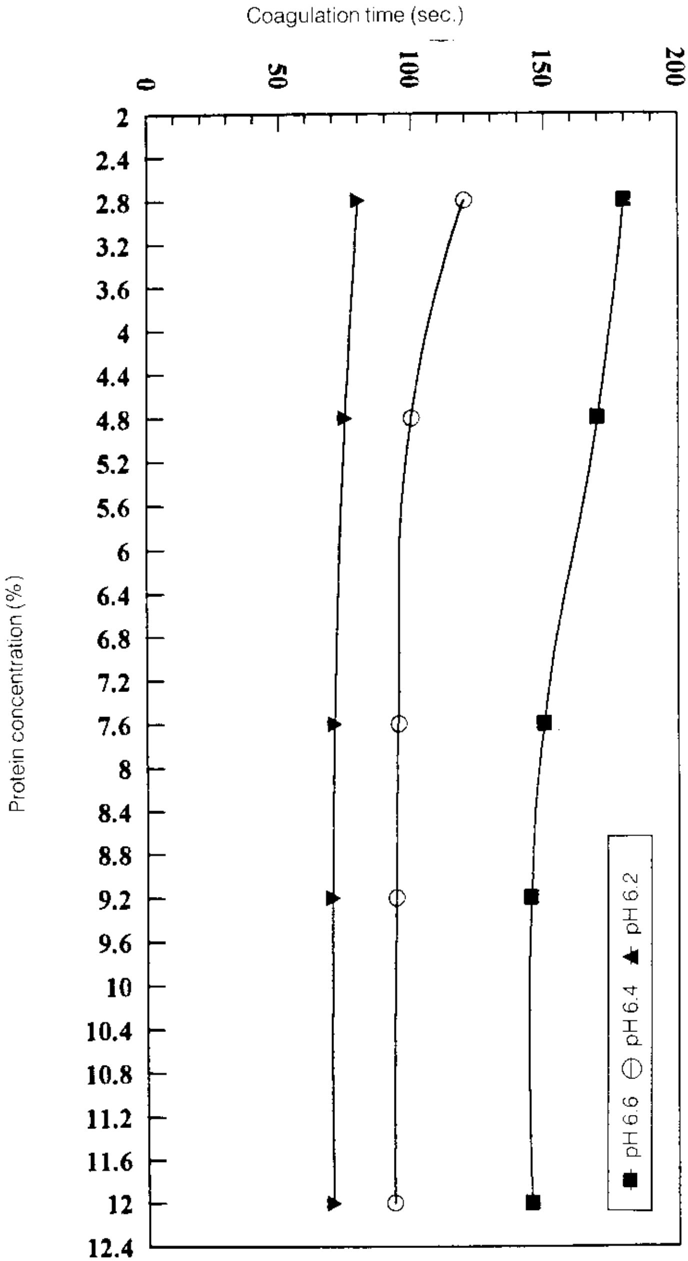

Fig. 4. The effect of protein concentration and pH on coagulation time of UF skim camel milk using 6 mg rennet/100 ml of milk at 35°C.

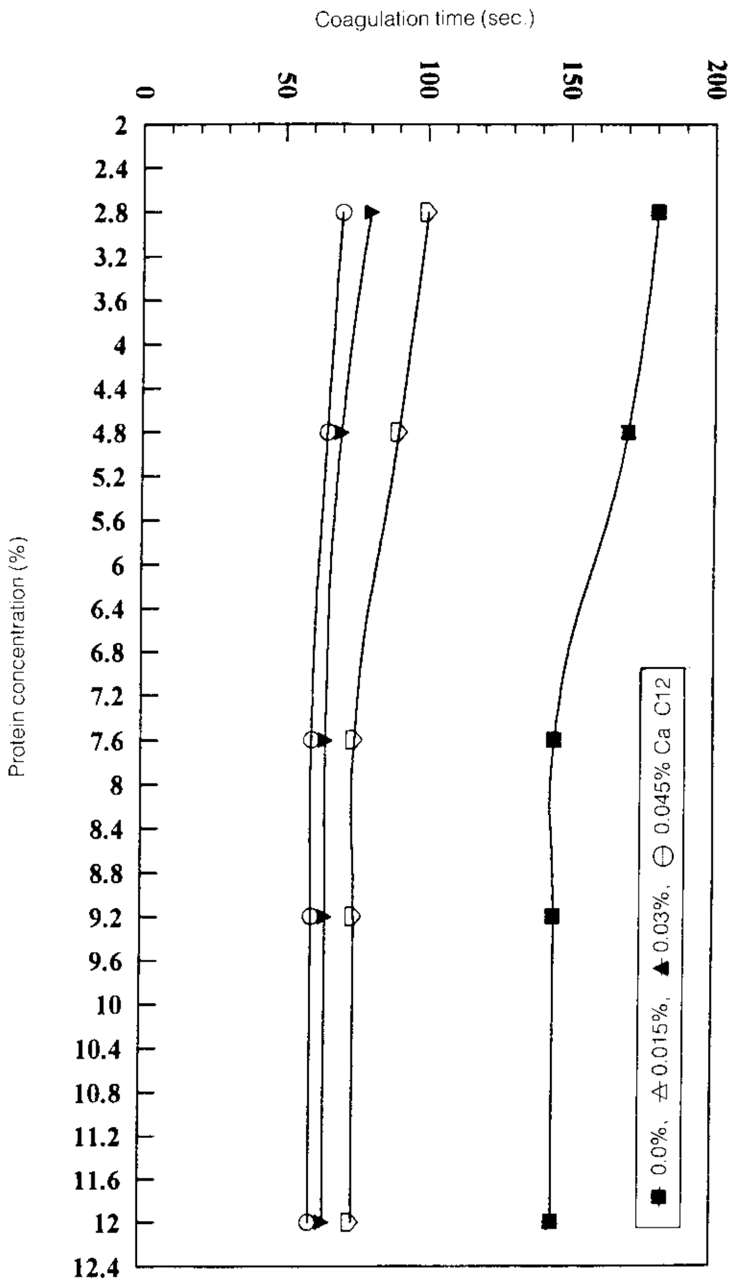

Fig. 5. The effect of protein and calcium concentration coagulation time of UF skim camel milk using 6 mg rennet/100 ml of milk at pH 6.6 and 35°C.

to be similar to that of cow milk. The rennet coagulation time decreased as the protein concentration increased but the precise influence of the later decreased as the rennet or calcium concentration increased or as the pH decreased. However, at the highest rennet (15 mg/100 ml), or calcium chloride (0.045%) concentration or at the lowest pH (6.2) the rennet coagulation time was nearly independent.

Rennet coagulation time decreased with increasing volume concentration ratio (protein content) possibly due to : (1) an increase in the number of effective collisions as a result of decrease in the volume of the aqueous phase, and (2) an increase in calcium concentration resulting in more secondary phase interactions. In general, the coagulum obtained from UF-concentrated camel milk appears to be more firmer than that of unconcentrated milk, this probably will improve cheesemaking from camel milk.

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دراسات على التجبن بالمنفحة لحليب الإبل الفرز المركز بواسطة الترشيح العال

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ملخص البحث. لقد تم تركيز حليب الإبل الفرز بواسطة أغشية الترشيح العال إلى نسب تركيزات حجمية مختلفة (١، ١,٧، ٢، ٢,٧، ٣، ٣,٣، ٤,٣) وتم تحليل مكونات عينات الحليب الفرز والحليب المركز. وتم دراسة زمن التجبن بالرنين للعينات باستعمال ثلاثة تركيزات من المنفحة، وكلوريد الكالسيوم ورقم حموضة pH وذلك عند درجة حرارة ٣٥°م.

النتائج أن بروتينات الحليب ركزت بواسطة الترشيح العال من ٢,٧٧٪ في الحليب الفرز إلى ٤,٦٩، ٥,٦٣، ٧,٦٦، ٩,٢، ١١,٢٪ في تركيزات ١,٧، ٢، ٢,٧، ٣، ٣,٣، ٤,٣ على التوالي. وكانت نسب الجوامد الصلبة الكلية المتطابقة هي ٨,٥٪ في الحليب الفرز و١٠,٨٧، ١١,٨١، ١٤,٣٥، ١٥,٨١، ١٨,٥٪ في التركيزات الناتجة من الترشيح العال، على التوالي. وقد انخفض زمن التجبن بالمنفحة بزيادة تركيز البروتين، وبزيادة تركيز كل من المنفحة أو الكالسيوم أو بانخفاض رقم الحموضة pH. ولكن عند التركيزات العالية من المنفحة (١٥مجم/١٠٠مل) أو من كلوريد الكالسيوم (٠,٠٤٥٪) أو عند أقل رقم حموضة ٦,٢ كان زمن التجبن بالمنفحة تقريبًا مستقلا عن تأثير البروتين (لم يتأثر زمن التجبن بزيادة تركيز البروتين في الحليب المركز).