

# Production of brined soft cheese from frozen ultrafiltered sheep's milk. Part 1. Physicochemical, microbiological and physical stability properties of concentrates

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Some physicochemical, microbiological and physical stability changes in sheep's milk as a result of its concentration by ultrafiltration (UF) and long-term deep-frozen storage were studied. Skim milk was concentrated by UF to 19.37, 23.40 or 26.49% total solids (TS), mixed with cream to obtain recombined UF concentrates with 30.40, 33.95 or 36.90% TS, respectively, and then frozen and stored at  $-20^{\circ}\text{C}$  for up to 6 months. With the exception of highly concentrated milks frozen for 6 months, no significant differences in lipolysis (acid degree value) and fat oxidation (peroxide value) were observed between the control milk and the UF milk concentrates stored frozen for up to 6 months. The UF process resulted in significant increases in the bacterial and coliform counts in sheep's milk, which decreased during the frozen storage. The UF concentrates exhibited good protein stability throughout frozen storage, except for one obtained from milk to which 0.5% NaCl was added before UF, which destabilized after 2 months frozen storage.

## INTRODUCTION

The annual production of milk in Greece is 1.5 million tonnes, of which 35% is sheep's and 24% is goat's milk. The availability of sheep's milk is highly seasonal, since the lactation period of sheep lasts for only 5–6 months. In addition, much more milk is produced during the spring months than in early winter and summer. As a consequence, there is a peak of activity in dairies using sheep's and goat's milk in April and May and no activity from August to December. These dairies, therefore, encounter a fundamental problem in trying to achieve an even operation throughout the year (Alichanidis *et al.*, 1981). If the dairy industry solves this problem, i.e. is supplied with constant quantities of sheep's milk throughout the year, it will be able to produce sheep's milk products throughout the year, resulting in better utilization of personnel and equipment.

The first efforts to preserve milk by freezing were made in the early 1930s. However, it was only during World War II that research really began in this field. Several research studies have been carried out on the freezing of whole or concentrated cow's milk (Samuelsson *et al.*, 1957; Koschak *et al.*, 1981; Lonergan *et al.*, 1981). In a cheese factory at Lezay, France, goat's milk

is concentrated by ultrafiltration (UF) to 34–36% TS, at the rate of 20 000 l/d, for frozen storage (at  $-20^{\circ}\text{C}$ ) from summer to winter, when goat's milk supplies are very limited (Editorial, 1979a); the results obtained have been very satisfactory. Another dairy plant at Gencay, France, has been preserving goat's milk (capacity 20 000–60 000 l/day) by UF, evaporation under vacuum and freezing (Editorial, 1979b,c). When required, the product is thawed, diluted with water and made into cheese. The process is claimed to resolve the problem of storing goat's milk, increase output and improve the uniformity of cheese quality; the thawed product may be used without mixing with fresh milk. Various studies have been conducted on the preservation of unconcentrated ewe's milk by freezing for the preparation of yoghurt or cheese (Anifantakis *et al.*, 1980; Young, 1987; Decio, 1989). However, there is only one study on frozen concentrated (by UF) sheep's milk for the preparation of yoghurt (Kehagias *et al.*, 1980).

In order to solve the problem of the seasonal availability of sheep's milk and to balance the quantity of sheep's milk processed, the milk could be concentrated during peak production by UF, to reduce freezing and storage costs, and could be preserved by freezing.

The objective of this investigation, therefore, was to study the effects of concentration of sheep's milk by UF and frozen storage on some of its physicochemical, microbiological and stability properties. The compositional, physicochemical, microbiological and organoleptic properties of brined soft cheese produced from frozen ultrafiltered sheep's milk will be reported in Part 2 (Voutsinas *et al.*, 1995).

## MATERIALS AND METHODS

### UF concentration of milk

Bulk skim sheep's milk was obtained from the dairy plant of DODONI S. A., Ioannina, following clarification, separation, pasteurization (72°C for 15 s) and cooling to 4°C. The milk was concentrated in a batch-type UF unit (VERIND s.p.a., MI, Italy). The system contained two Abcor spiral-wound polysulfone membranes, type S4HFK 131 VYV (Abcor Inc., Paris, France) with a nominal molecular weight cut-off of 5000 Daltons and a total membrane area of 10 m<sup>2</sup>. Each membrane tube had a hold-up volume of 5.4 l. The milk was heated to 50°C before processing and held at that temperature during UF. During operation, the inlet pressure was maintained at 3.3 bar and the outlet pressure at 0.8 bar to give an average trans-membrane pressure differential of 2.5 bar. Recirculation of milk (350 kg) through the membrane system was continued until the TS content of skim milk was increased to 19.37, 23.40 or 26.49%, which required 75–90 min. The retentate (UFCS) was then recombined with (55% fat) cream (pasteurized at 80°C for 10 min and cooled to 50°C) in a pasteurizer equipped with a scraped-surface agitator to obtain a protein: fat ratio close to that of the control milk (0.93). Thus, recombined concentrates containing 1.75 times ( $\times$ ), 1.95 $\times$  or 2.12 $\times$ , respectively, the solids content of the control milk were prepared. These preparations are subsequently referred to as R-UFCS (30.40% TS), R-UFCS (33.95% TS), and R-UFCS (36.90% TS). In one trial, 0.50% NaCl was added to the skim milk just before UF in an attempt to reduce the buffering capacity of the concentrate. With the exception of the data on the physical stability of the milk, the data from this trial (37% TS) were combined with those of another UF trial (without NaCl added) having the same TS content (36.8%) and the means are reported. Since, in the first trials of this study, the total bacterial numbers in sheep's milk were increased greatly by the UF process, the concentrates obtained in the subsequent trials were heated at 70°C for 5 min. In the first trials, this heat treatment was applied to the concentrates just before cheesemaking.

Samples of the control milk, retentates, permeates and recombined UF concentrates were stored in glass-bottles at 3°C until analyzed (next day). Two trials were carried out for each milk treatment and the results obtained are the means of these trials.

### Packaging, freezing, storage and thawing

The recombined concentrates were packed in 1.5 l plastic bags (25x26cm, type PA/PE 5070 SKL, Dixie Union, Germany) which were heat-sealed with a special machine (type TISF-450, Tew Electric Heating Equipment Co. Ltd, Germany), leaving very little free space. Then, 10 bags were placed in a rectangular metal basket in which the bags were separated by wire screens to produce frozen milk blocks of standard thickness. The bags were promptly frozen quiescently in a moving air freezer overnight at -25°C and then stored in a still-air freezer at -20°C for up to 6 months. After freezing, each milk block weighed about 1.2 kg and was 20.5 cm long, 21.0 cm high and 2.7 cm thick. No free space was left in the bag.

After storage at -20°C for 2, 4 or 6 months, samples were fast-thawed quiescently in a water-bath at 40°C.

### Chemical analyses

Samples of milk, recombined UF concentrates and permeates were examined for pH (pH-meter Metrohm, AG, Switzerland), fat (Gerber method; British Standards Institution, 1955), titratable acidity (Dornic method), total solids (TS) (IDF, 1987), lactose (IDF, 1974), ash (AOAC, 1984), total N (IDF, 1986), non-casein N (NCN) (IDF, 1964), non-protein N (NPN) (Rowland, 1938), calcium (Pearce, 1977), and lipolysis by measuring the acid degree value (ADV; Deeth & Fitz-Gerald, 1976). The fat, total solids and lactose contents of concentrates were determined after dilution with an equal mass of distilled water. The Kjeldahl method was carried out by using the Kjeldatherm digestion system KT 20S and Vapodest-5 system equipped with a micro-processor for automatic distillation and titration (C. Gerhard, Bonn, Germany).

The oxidation of milk and concentrates was determined as follows: 0.5–1 kg of sample was heated to 40°C and centrifuged at 1540 g for 30 min (VARIFUGE K, Heraeus Crist, Germany). The cream was transferred to a FUNKE-GERBER (Berlin, Germany) whipped cream tester, cooled to 12–14°C and churned for 5–10 min at 50–60 rpm, intermittently (5 s on, 10 s off). The buttermilk was drained off (5–10 min), the butter granules washed repeatedly with cold water (2–4°C) and then transferred to 100 ml centrifuge tubes. Clear butterfat was obtained from butter by melting slowly in a water-bath at 40°C, centrifuging at 1900 g for 5 min and filtering through N° 1 Whatman paper. When this procedure failed to give a clear fat, it was followed by filtration through N° 4 Whatman paper in an oven at 40°C. The peroxide value (PV) of extracted fat was determined (AOAC, 1984) and expressed as meq peroxide/kg fat.

### Microbiological analyses

Counts (cfu/ml) of total bacteria (TVC) and coliforms in milk and concentrates were determined using the

pour-plate method (APHA, 1967), the former on plate count agar (Merck) at 32°C for 3 days, the latter on desoxycholate lactose agar (Merck) at 32°C for 1 day.

### Physical stability of concentrates

#### Apparent viscosity

The apparent viscosity of fresh control and concentrated samples, and of freshly-thawed concentrates was measured at 20°C using a Brookfield Synchro-Lectric Viscometer, Model RVT (Brookfield Engineering Laboratories, Inc., Stoughton, MA, USA). Viscosities below 100 cps were determined by using the UL adaptor while spindles #2 and #4 were used for samples with viscosities above 100 cps. The spindle speed was set at 50 rpm. The viscosity reading was recorded after 1 min of shearing. Duplicate readings were obtained for each sample.

#### Sedimentation

Samples of concentrates were reconstituted with distilled water to the TS content of the control milk (17.4%). Samples (40 ml) of control or diluted concentrates were centrifuged at 1540 g for 10 min; the sediment was dried at 105°C for 3 h and weighed (Anifantakis *et al.*, 1980). Protein stability was expressed as g of sediment per 40 ml of sample, a value of dry weight > 1.0 being regarded as indicative of instability (Koschak *et al.*, 1981).

### Statistical analysis

The data were analysed by Analysis of Variance using Statgraphics (Statistical Graphics Corp., Rockville, MD, USA). At each storage time studied, the experimental means were compared with the control mean and between themselves. When significant ( $P < 0.05$ )

differences were found among treatments, means were separated by Tukey's test (Steel and Torrie, 1960).

## RESULTS AND DISCUSSION

### Composition of UF concentrates and permeates

The composition of the control milk and various UF concentrates is shown in Table 1. The TS content of the recombined concentrates (R-UFCS) ranged from 30.40 to 36.90%. Thus, concentration factors (CF) of 2.06×, 2.49× and 2.77×, based on protein content, were achieved. The composition of the control milk was typical of standardized milk normally used for Feta cheese in the factory. With the exception of NPN, which remained constant, and lactose which decreased, all other milk constituents increased with increasing CF. These results agree with those of other investigators (Glover, 1986; Bastian *et al.*, 1991; Premaratne & Cousin, 1991). Green *et al.* (1984) also found that the low molecular weight components comprising the NPN, which appeared to be entirely accounted for by urea, amino acids and NH<sub>3</sub>, were not concentrated by UF. Table 2 shows the composition of various permeates obtained from UF concentration of sheep's skim milk to different degrees. The permeates contained no measurable fat (100% retention), as expected considering the size of the fat globules (Yan *et al.*, 1979). Similar levels of NPN were detected in all permeates, whereas the protein, lactose, ash and Ca contents of permeates increased with increasing CF. However, only the differences in the protein content of the permeates were found to be significant ( $P < 0.05$ ). The results of Table 2 agree with those of other authors (Glover, 1985, 1986). Sutherland (private communication, 1987), cited by Bastian *et al.* (1991), using Abcor spiral-wound membranes, found

Table 1. Composition of whole sheep's milk and various recombined ultrafiltration concentrates

| Treatment of milk   | Concentration factor <sup>b</sup> | Total solids (%) | Fat (%) | SNF (%) | Protein (%) | NPN (%) | NCN (%) | Lactose (%) | Ash (%) | Ca (mg/100 ml) |
|---------------------|-----------------------------------|------------------|---------|---------|-------------|---------|---------|-------------|---------|----------------|
| Control milk        | 1.00×                             | 17.40            | 6.00    | 11.40   | 5.6         | 0.05    | 0.20    | 4.87        | 0.86    | 211            |
| R-UFCS <sup>a</sup> | 2.06×                             | 30.40            | 13.10   | 17.30   | 11.5        | 0.05    | 0.29    | 4.37        | 1.39    | 392            |
| R-UFCS              | 2.49×                             | 33.95            | 14.70   | 19.25   | 13.9        | 0.05    | 0.36    | 4.02        | 1.50    | 414            |
| R-UFCS              | 2.77×                             | 36.90            | 15.90   | 21.00   | 15.5        | 0.06    | 0.41    | 3.73        | 1.74    | 442            |

<sup>a</sup>R-UFCS = recombined ultrafiltration concentrate of skim milk.

<sup>b</sup>Concentration factor based on protein.

Table 2. Composition and some physicochemical properties of various permeates obtained from ultrafiltration of sheep's skim milk to different concentrations

| Treatment of milk             | Total solids (%) | Fat (%) | Protein (%)       | NPN (%) | Lactose (%) | Ash (%) | Ca (mg/100 g) | pH   | Acidity (°D) |
|-------------------------------|------------------|---------|-------------------|---------|-------------|---------|---------------|------|--------------|
| UFCS <sup>a</sup> (19.37% TS) | 5.35             | 0.00    | 0.26 <sup>b</sup> | 0.05    | 4.67        | 0.44    | 32.2          | 6.45 | 7.50         |
| UFCS (23.40% TS)              | 5.68             | 0.00    | 0.31 <sup>c</sup> | 0.05    | 4.83        | 0.46    | 33.9          | 6.46 | 7.00         |
| UFCS (26.49% TS)              | 6.13             | 0.00    | 0.34 <sup>c</sup> | 0.05    | 5.36        | 0.52    | 34.7          | 6.42 | 7.00         |

<sup>a</sup>UFCS = ultrafiltration concentrate of skim milk.

<sup>b,c</sup>Experimental means in each column without a superscript or bearing a common superscript did not differ significantly ( $P > 0.05$ ).

that permeate N levels normally increased during UF, and the levels were higher with membrane age, cleaning frequency, and cleaning intensity. Bastian *et al.* (1991) also reported that, during some UF trials, permeate N levels increased. Table 2 also shows that the permeates did not differ ( $P > 0.05$ ) in pH and acidity.

#### Physicochemical properties of UF concentrates

The effects of the UF process and frozen storage on some physicochemical properties of sheep's milk are shown in Table 3. UF alone or in combination with frozen storage did not affect ( $P > 0.05$ ) the pH of sheep's milk, which remained nearly constant during the frozen storage. The UF concentrates (fresh or frozen) had significantly ( $P < 0.05$ ) higher titratable acidity than the control milk, and their acidities fluctuated slightly during the frozen storage. The increased buffering capacity in UF concentrates, mainly because of their high protein content, is probably the reason for their higher acidities.

Some UF plants damage the fat globules in milk, causing some homogenization, and this effect is particularly marked in batch processing (Glover, 1985). In addition, Green *et al.* (1984) reported that reduction of fat globule size occurred early in the UF process, damage to the fat globule membrane was indicated and the milk became more susceptible to lipolysis. They suggested that lipolysis would be affected by plant design, but it seemed unlikely that disruption of the globules could be avoided completely. For this reason, and since the concentrates in this study were to be stored frozen for a long time, skim milk was used as the starting material.

Table 3 shows that the fresh concentrates had slightly higher, but not significantly ( $P > 0.05$ ), ADVs than the control milk, and that the ADV of the concentrates remained nearly constant during the first 4 months of frozen storage and then increased appreciably. Thus, a significant ( $P < 0.05$ ) difference in ADV

was observed only between the control milk and the R-UFCS (36.90% TS) stored frozen for 6 months. The low ADV of the fresh UF concentrates as compared to the control milk may be attributed to the use of pasteurized milk in the UF process as well as to the high temperature (50°C) maintained during UF. Pierre and Real del Sol (1978) reported that the ADV of the untreated raw milk was 0.7 and that of the concentrate obtained after UF for 250 min at 40°C was 4.1.

The degree of fat oxidation, expressed as peroxide value (PV), of sheep's milk was not affected ( $P > 0.05$ ) by the UF process (Table 3). In addition, a gradual increase in the PV of the UF concentrates was observed during frozen storage. However, significant differences ( $P < 0.05$ ) in PV were observed only between the control milk and the R-UFCS with 33.95 and 36.90% TS stored frozen for 6 months. Pierre (1978) determined the level of oxidation, expressed as thiobarbituric acid (TBA) value, in ultrafiltered goat's milk before storage and after 9 months storage at -20°C, and found that no lipid oxidation occurred. Kehagias *et al.* (1980) reported that the PV of ultrafiltered whole sheep's milk increased with the storage time at -25°C. With regard to frozen milk, it is essential to exclude air (oxygen) which causes oxidation (rancidity) or changes in colour and flavour, to close effectively and to use gas impermeable packaging material (Samuelsson *et al.*, 1957). Table 3 indicates that the packaging (material and procedure) of the UF concentrates in this study was very effective in maintaining the quality of the fat during the frozen storage.

#### Microbiological quality of concentrates

The effects of the UF process, post-concentration heat treatment and frozen storage on the total bacterial and coliform counts of sheep's milk are shown in Table 4. The UF process resulted in significant ( $P < 0.05$ ) increases in both bacterial and coliform counts. Theoretically, about a two-fold increase in TVC was expected

Table 3. Effects of ultrafiltration concentration and frozen storage on some physicochemical properties of sheep's milk

| Duration of frozen storage (months) | Treatment of milk               | pH   | Acidity (°D)        | ADV (meqKOH/100 g fat) | Peroxide value (meq/kg fat) |
|-------------------------------------|---------------------------------|------|---------------------|------------------------|-----------------------------|
| 0                                   | Control milk                    | 6.53 | 26.5 <sup>b</sup>   | 0.46 <sup>b</sup>      | 0.07 <sup>b</sup>           |
|                                     | R-UFCS <sup>a</sup> (30.40% TS) | 6.52 | 36.5 <sup>c</sup>   | 0.54 <sup>b</sup>      | 0.08 <sup>b</sup>           |
|                                     | R-UFCS (33.95% TS)              | 6.52 | 39.0 <sup>c,d</sup> | 0.50 <sup>b</sup>      | 0.09 <sup>b</sup>           |
|                                     | R-UFCS (36.90% TS)              | 6.51 | 42.5 <sup>d</sup>   | 0.58 <sup>b</sup>      | 0.11 <sup>b</sup>           |
| 2                                   | R-UFCS (30.40% TS)              | 6.47 | 32.5 <sup>c</sup>   | 0.52 <sup>b</sup>      | 0.09 <sup>b</sup>           |
|                                     | R-UFCS (33.95% TS)              | 6.44 | 36.5 <sup>d</sup>   | 0.53 <sup>b</sup>      | 0.14 <sup>b</sup>           |
|                                     | R-UFCS (36.90% TS)              | 6.41 | 38.0 <sup>d</sup>   | 0.52 <sup>b</sup>      | 0.17 <sup>b</sup>           |
| 4                                   | R-UFCS (30.40% TS)              | 6.44 | 35.0 <sup>c</sup>   | 0.52 <sup>b</sup>      | 0.10 <sup>b</sup>           |
|                                     | R-UFCS (33.95% TS)              | 6.42 | 38.5 <sup>c</sup>   | 0.49 <sup>b</sup>      | 0.15 <sup>b</sup>           |
|                                     | R-UFCS (36.90% TS)              | 6.35 | 38.0 <sup>c</sup>   | 0.77 <sup>b</sup>      | 0.19 <sup>b</sup>           |
| 6                                   | R-UFCS (30.40% TS)              | 6.44 | 35.5 <sup>c</sup>   | 0.65 <sup>b,c</sup>    | 0.16 <sup>b,c</sup>         |
|                                     | R-UFCS (33.95% TS)              | 6.38 | 35.0 <sup>c</sup>   | 0.73 <sup>b,c</sup>    | 0.21 <sup>c</sup>           |
|                                     | R-UFCS (36.90% TS)              | 6.48 | 40.5 <sup>c</sup>   | 0.89 <sup>c</sup>      | 0.23 <sup>c</sup>           |

<sup>a</sup>R-UFCS, as in Table 1.

<sup>b,c,d</sup>Experimental means in each column, regardless of the storage time, bearing a common superscript with the control mean did not differ significantly ( $P > 0.05$ ) from it; experimental means in each column and at the same storage time without a superscript or bearing a common superscript did not differ significantly ( $P > 0.05$ ).

on the basis of the CF achieved. However, the results in Table 4 show that the TVC for the R-UFCS (33.95% TS) was more than five-fold higher than that of the control milk. This finding is in contrast to the results of others (Lonergan *et al.*, 1981; Glover, 1985, 1986) who reported that microbial growth during UF is not a serious problem in a continuous operation at about 50°C, and that by far the more important factor is the hygienic quality of the milk before processing. Glover (1985) reported that the TVC for whole milk batch-processed for 1 h at 50°C were less than expected on the basis of the counts in the ingoing milk and the CF. The increase observed in this study in the bacterial counts in sheep's milk caused by the UF process may be attributed to the high bacterial counts of the starting material, and to the non-continuous operation of the UF (batch operation). In addition, a certain degree of post-concentration contamination was unavoidable, since the experiment was carried out under factory, and not laboratory, conditions.

Table 4 shows that the post-concentration heat treatment applied to some UF concentrates (R-UFCS with 30.40 or 36.90% TS) greatly reduced their TVC and coliform counts. The TVC and coliform counts of the UF concentrates decreased during the frozen storage (Table 4). A similar lethal effect of frozen storage (-20.5°C) on the TVC has been observed by Samuelsson *et al.* (1957) for pasteurized milk and by Alekseeva *et al.* (1974) for concentrated skim milk. However, Kehagias *et al.* (1980) reported that the TVC for ultrafiltered whole

sheep's milk remained nearly constant during storage at -25°C for 9 months.

#### Physical stability of concentrates

Changes in the stability of protein in sheep's milk due to UF alone or in combination with the frozen storage are shown in Table 5. The fresh UF concentrates had higher values for protein sediment than the control milk, which increased with CF. However, significant ( $P < 0.05$ ) differences in protein sediment were observed only between the control milk and the R-UFCS (37% TS) obtained from milk to which 0.5% NaCl was added before UF. It is also evident from Table 5 that the protein sediment in the UF concentrates increased slightly during the frozen storage, except for the concentrate containing added NaCl in which the protein precipitate increased markedly. This concentrate exhibited good protein stability only during the first 2 months of storage, whereas the other concentrates demonstrated good protein stability throughout the frozen storage. The decreased protein stability in the former concentrate compared to the others was probably due to the addition of NaCl to the sheep's milk before UF. Sodium replaces bound calcium and lowers the stability of the casein (Glover, 1985; Lawrence, 1989). It must also be noted that, on freezing, the effec-

**Table 4. Microbiological changes in sheep's milk as a result of ultrafiltration concentration, post-concentration heat-treatment and frozen storage**

| Duration of frozen storage (months) | Treatment of milk                            | TVC (cfu/ml) × 10 <sup>3</sup> | Coliforms (cfu/ml)  |
|-------------------------------------|----------------------------------------------|--------------------------------|---------------------|
| 0                                   | Control milk                                 | 55.0 <sup>d</sup>              | 3.0 <sup>d</sup>    |
|                                     | R-UFCS <sup>a</sup> (30.40% TS) <sup>b</sup> | 43.0 <sup>d</sup>              | 0.0 <sup>d</sup>    |
|                                     | R-UFCS (33.95% TS) <sup>c</sup>              | 304.0 <sup>e</sup>             | 225.0 <sup>e</sup>  |
|                                     | R-UFCS (36.90% TS) <sup>b</sup>              | 77.5 <sup>d</sup>              | 58.0 <sup>d,e</sup> |
| 2                                   | R-UFCS (30.40% TS) <sup>b</sup>              | 44.5 <sup>d</sup>              | 0.0 <sup>d</sup>    |
|                                     | R-UFCS (33.95% TS) <sup>c</sup>              | 249.5 <sup>e</sup>             | 137.0 <sup>e</sup>  |
|                                     | R-UFCS (36.90% TS) <sup>b</sup>              | 68.5 <sup>d</sup>              | 27.5 <sup>d</sup>   |
| 4                                   | R-UFCS (30.40% TS) <sup>b</sup>              | 42.0 <sup>d</sup>              | 0.0 <sup>d</sup>    |
|                                     | R-UFCS (33.95% TS) <sup>c</sup>              | 288.5 <sup>e</sup>             | 55.0 <sup>e</sup>   |
|                                     | R-UFCS (36.90% TS) <sup>b</sup>              | 53.0 <sup>d</sup>              | 12.0 <sup>d</sup>   |
| 6                                   | R-UFCS (30.40% TS) <sup>b</sup>              | 38.0 <sup>d</sup>              | 0.0 <sup>d</sup>    |
|                                     | R-UFCS (33.95% TS) <sup>c</sup>              | 275.5 <sup>e</sup>             | 85.5 <sup>e</sup>   |
|                                     | R-UFCS (36.90% TS) <sup>b</sup>              | 56.0 <sup>d</sup>              | 3.0 <sup>d</sup>    |

<sup>a</sup>R-UFCS, as in Table 1.

<sup>b</sup>These UF concentrates were heated at 70°C for 5 min after recombination, whereas the others (33.95% TS) before cheese-making.

<sup>c</sup>Microbial counts were determined before heat treatment of these concentrates.

<sup>d,e</sup>Experimental means in each column, regardless of the storage time, bearing a common superscript with the control mean did not differ significantly ( $P > 0.05$ ) from it; experimental means in each column and at the same storage time without a superscript or bearing a common superscript did not differ significantly ( $P > 0.05$ ).

**Table 5. Physicochemical changes in sheep's milk as a result of ultrafiltration concentration and frozen storage**

| Duration of frozen storage (months) | Treatment of milk                                   | Sediment (g dry wt/40 ml) | Apparent viscosity (cp) |
|-------------------------------------|-----------------------------------------------------|---------------------------|-------------------------|
| 0                                   | Control milk                                        | 0.15 <sup>e</sup>         | 2.98 <sup>e</sup>       |
|                                     | R-UFCS <sup>a</sup> (30.40% TS) <sup>b</sup>        | 0.060 <sup>e,f</sup>      | 29.58 <sup>f</sup>      |
|                                     | R-UFCS (33.95% TS)                                  | 0.090 <sup>e,f</sup>      | 33.80 <sup>f</sup>      |
|                                     | R-UFCS (36.80% TS) <sup>b,c</sup>                   | 0.150 <sup>e,f</sup>      | 81.92 <sup>g</sup>      |
| 2                                   | R-UFCS-NaCl <sup>d</sup> (37.00% TS) <sup>b,c</sup> | 0.225 <sup>f</sup>        | 483.00 <sup>h</sup>     |
|                                     | R-UFCS (30.40% TS) <sup>b</sup>                     | 0.185 <sup>e,f</sup>      | 39.88 <sup>f</sup>      |
|                                     | R-UFCS (33.95% TS)                                  | 0.190 <sup>e,f</sup>      | 36.43 <sup>f</sup>      |
|                                     | R-UFCS (36.80% TS) <sup>b,c</sup>                   | 0.320 <sup>f</sup>        | 90.40 <sup>g</sup>      |
| 4                                   | R-UFCS-NaCl <sup>d</sup> (37.00% TS) <sup>b,c</sup> | 0.590 <sup>g</sup>        | 860.00 <sup>h</sup>     |
|                                     | R-UFCS (30.40% TS) <sup>b</sup>                     | 0.265 <sup>e,f</sup>      | 42.90 <sup>f</sup>      |
|                                     | R-UFCS (33.95% TS)                                  | 0.260 <sup>e,f</sup>      | 39.69 <sup>f</sup>      |
|                                     | R-UFCS (36.80% TS) <sup>b,c</sup>                   | 0.390 <sup>f</sup>        | 104.20 <sup>g</sup>     |
| 6                                   | R-UFCS-NaCl <sup>d</sup> (37.00% TS) <sup>b,c</sup> | 2.790 <sup>g</sup>        | 2,492.00 <sup>h</sup>   |
|                                     | R-UFCS (30.40% TS) <sup>b</sup>                     | 0.300 <sup>f</sup>        | 55.60 <sup>f</sup>      |
|                                     | R-UFCS (33.95% TS)                                  | 0.210 <sup>f</sup>        | 39.12 <sup>f</sup>      |
|                                     | R-UFCS (36.80% TS) <sup>b,c</sup>                   | 0.475 <sup>g</sup>        | 153.50 <sup>g</sup>     |
|                                     | R-UFCS-NaCl <sup>d</sup> (37.00% TS) <sup>b,c</sup> | —                         | 3,840.00 <sup>h</sup>   |

<sup>a</sup>R-UFCS, as in Table 1.

<sup>b</sup>These UF concentrates were heated at 70°C for 5 min after recombination, whereas the others (33.95% TS) before cheese-making.

<sup>c</sup>One trial.

<sup>d</sup>NaCl = 0.5% NaCl was added to the skim milk before ultrafiltration.

<sup>e,f,g,h</sup>Experimental means in each column, regardless of the storage time, bearing a common superscript with the control mean did not differ significantly ( $P > 0.05$ ) from it; experimental means in each column and at the same storage time without a superscript or bearing a common superscript did not differ significantly ( $P > 0.05$ ).

tive added NaCl concentration may increase from 0.5% to a much higher level as available water decreases. Perhaps this may contribute to salting-out and destabilization of the protein on freezing the milk. Various workers (Koschack *et al.*, 1981; Lonergan *et al.*, 1981; Muir, 1984) have reported protein precipitation to be one of the two most important forms of instability observed in frozen concentrated cow's milk. However, Kehagias *et al.* (1980) reported that the weight of the protein sediment in ultrafiltered whole sheep's milk remained nearly constant throughout a 9 month storage at  $-25^{\circ}\text{C}$  and assumed that the proteins in sheep's milk are probably more stable than those in the cow's milk.

Table 5 also shows the apparent viscosity of the control milk and various UF concentrates. The UF process, as expected, significantly ( $P < 0.05$ ) increased the viscosity of sheep's milk. Several investigators have reported that when the protein content of UF retentate exceeds 12–14%, there is a dramatic increase in viscosity (Maubois & Mocquot, 1975; Glover, 1985). With the exception of the R-UFCS (33.95% TS), the viscosity of which remained almost constant during frozen storage, the viscosity of the UF concentrates, especially that containing NaCl, increased with increasing storage time. Although the R-UFCS (30.40% TS) had a lower viscosity than the R-UFCS (33.95% TS) before freezing, the former exhibited a higher viscosity than the latter during the frozen storage. This indicates that the post-concentration heat treatment applied to the UF concentrates adversely affected their viscosity during the frozen storage, probably because it caused protein denaturation which promoted protein-protein interactions and formation of protein complexes. It can also be seen from Table 5 that the addition of NaCl to sheep's milk before UF resulted in a concentrate with much higher viscosity than the R-UFCS having similar TS content (36.80%). The viscosity of the former concentrate increased markedly during frozen storage, another clear indication of its poor protein stability.

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