Effect of Different Membrane Separation Technologies (Ultrafiltration and Microfiltration) on the Texture and Microstructure of Semihard Low-Fat Cheeses

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Semihard low-fat cheeses made from ultrafiltered (UF) or microfiltered (MF) milk were compared. The use of MF membranes and milder pasteurization of the milk reduced the retention of whey proteins in the retentate to 35%, compared with ~100% retained in the UF process. Microbiological development, physicochemical composition, and cheese ripening were not altered by the concentration processes. The lower retention of whey protein in MF cheeses accounted for their higher hardness, which correlated with higher firmness values in the textural analysis. Microstructure showed a protein matrix with open spaces through the protein network, although micrographs of UF cheeses showed the presence of spongy structures linked to the casein, which did not appear in MF cheeses and which correspond to the denatured whey protein bound to the casein. Firmness was scored better in MF cheeses, although when MF membranes were used, the optimum yields achieved using UF membranes were not attained.

Keywords: *Low-fat cheese; ultrafiltration; microfiltration; texture; microstructure*

INTRODUCTION

Fat plays an important role in the characteristic cheese flavor, aroma, and acceptability (Emmons et al., 1980; Bryant et al., 1995). The chief obstacle to the manufacture of low-fat products is the difficulty of achieving acceptable sensory characteristics (Mann, 1994). A number of studies carried out for the purpose of producing low-fat cheeses have been reviewed recently (Mann, 1994; Drake and Swanson, 1995). To achieve products with acceptable characteristics, modifications to the conventional manufacturing technologies have been proposed, such as intensified pasteurization treatment of milk, lower temperature during drainage of whey, quicker curd handling (Jameson, 1994), and also the introduction of technological innovations. In this latter category, McGregor and White (1990) and Ardö (1994) proposed the use of milk concentrated by ultrafiltration (UF) to improve the characteristics of cheeses of this type. Also, the use of fat substitutes in these cheeses was reviewed (Huyghebaert et al., 1996).

Since the late 1960s when work began on the use of ultrafiltered milk for the manufacture of soft cheeses (Maubois et al., 1969), this technique has been used to make different kinds of fresh (Mahaut and Korolczuk, 1992; Schkoda and Kessler, 1996) and semihard cheeses, either full-fat (Goudédranche et al., 1980; Delbeke, 1987) or low-fat (De Boer and Nooy, 1980; De Koning et al., 1981). However, the results have not been altogether satisfactory, especially in the case of pressed-paste cheeses (Renner and Abd El-Salam, 1991).

Microfiltration (MF) has been used mainly in the dairy industry for bacterial removal from milk, but it has also been used for casein enrichment or to modify the α_s-/β-casein ratio of milk (Mistry and Maubois, 1993). However, as far as we know, MF concentration of milk for cheee-making has not been studied.

One characteristic that low-fat cheeses and cheeses made from UF milk have in common is poor aroma and flavor development (Bech, 1993; Ardö, 1994). In a previous paper we have described the manufacture of a semihard cheese made from a mixture of cow's, ewe's, and goat's milks concentrated by UF and with fat content reduced to 65% of the level in full-fat cheese (Rodríguez et al., 1996) and the development in these cheeses of aroma components (Rodríguez et al., 1997). The results showed that by using a starter culture especially selected for its high proteolytic activity (IFPL starter; Requena et al., 1992), it is possible to achieve ripening of such cheeses in half the time taken using a commercial starter. Although the aroma and flavor of these UF cheeses were acceptable, texture was judged "rather soft" in comparison with conventionally made semihard cheeses, due to the high incorporation of whey proteins (Rodríguez et al., 1996).

This paper describes the manufacture of the same type of semihard mixed-milk cheeses with 65% of the fat content of full-fat cheese, in which the concentration process of the original milk mixture was modified by using MF membranes instead of the UF ones. The use of MF membranes with a higher pore size than UF ones, in addition to milder pasteurization treatment of the milk mixture, should allow a lower retention of whey proteins in the MF retentate and enable their influence on cheese texture to be assessed. A comparative analysis

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of the effect of the two membrane separation technologies (UF and MF) on cheese yield, biochemical composition, textural characteristics, and microstructure of the resulting low-fat cheeses was also made.

MATERIALS AND METHODS

Concentration Processes and Cheese-Making. Milk concentration processes (UF and MF) and cheese-making were carried out in the pilot plant of the Laboratoire de Recherches de Technologie Laitière (INRA, Rennes, France), although not coincidentally. Approximately 190 kg of semiskimmed milk (2.1% fat content) made from skimmed cow's milk and whole ewe's and goat's milk in proportions of 55:15:30 was pasteurized in an Actijoule apparatus (Actini, Maxilly, Évian-Les-Bain, France) at 72 °C for 20 s (MF) and at 92 °C for 4 s (UF).

Both concentration processes (UF and MF) were carried out at 50 °C in a tubular Tech Sep apparatus (Tech Sep, Saint-Maurice de Beynost, Miribel, France) with two membrane subunits, the characteristics of which are described elsewhere (Goudédranche et al., 1980). The MF membranes used were Tech Sep M14 (cutoff = 0.14 μm), and the UF membranes were Tech Sep M1 (cutoff = 150 kDa). The total membrane area in both cases was 1.63 m^2. The starting conditions for the MF process were as follows: input pressure, 180 kPa; output pressure, 60 kPa. For the UF process these conditions were as follows: input pressure, 440 kPa; output pressure, 300 kPa. Initial permeate fluxes were 85.9 L h^{-1} m^{-2} for the MF process and 93.3 L h^{-1} m^{-2} for the UF one. Diafiltration was carried out when a 3-fold concentration was attained by adding a volume of water at 50 °C equal to 110% of the volume of the retentate at that time, to reduce lactose content to 1.7% (Goudédranche et al., 1980). The permeate flux in the MF process was 52.8 L h^{-1} m^{-2} on starting diafiltration and up to 77.9 L h^{-1} m^{-2} afterward. The same fluxes for the UF process were 35.6 and 56.4 L h^{-1} m^{-2}, respectively. Processes were continued to 6.5−7-fold concentration.

The cheese-making process was similar to that described by Goudédranche et al. (1980). The retentate (~30 kg after UF and ~23 kg after MF) was separated into three batches, and the different freeze-dried starter cultures were added to give counts of 10^{10} colony-forming units (cfu)/kg of retentate. Batch FD contained a commercial starter culture (Flora Danica MSP, Chr. Hansen, Denmark), consisting of a mixture of *Lactococcus lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis*, *L. lactis* subsp. *diacetylactis*, and *Leuconostoc mesenteroides* subsp. *cremoris*; batch IFPL contained strains from the culture collection of the Instituto del Frío (Department of Productos Lácteos), Madrid, Spain, consisting of a mixture of *L. lactis* subsp. *lactis* IFPL359 (80%), *Lactobacillus casei* subsp. *casei* IFPL731 (5%), *L. plantarum* IFPL935 (5%), *L. mesenteroides* subsp. *dextranicum* IFPL709 (5%), and *L. paramesenteroides* IFPL705 (5%); and batch IFPL+T1 contained the mixed starter of local strains (IFPL) supplemented with the Lac^- Prt^- variant of *L. lactis* subsp. *lactis* IFPL359 (*L. lactis* subsp. *lactis* T1) to give counts similar to the parental strain.

Starter cultures were added to the retentates at 30 °C, and the precheeses were left to acidify until pH 6.4. At that point salt (11 g/kg) and rennet (0.4 mL/kg of precheese; rennet contained 520 mg/L chymosin) were added. About 600−700 g of the mixture was placed in each mold and left to acidify overnight at 30 °C to reach pH ~5. Cheeses were treated with a solution of 3 g/L Delvocid (Gist-Brocades nv, Seclin, France) and ripened for 2 months at 13 °C and 90% relative humidity. Two whole cheeses in each batch were sampled in triplicate at each of 15, 30, 45, and 60 days of ripening from cheeses made in both concentration processes.

Microbiological and Physicochemical Analyses of Cheeses. Sample-taking and the necessary dilutions were carried out in accordance with the International Dairy Federation standards (IDF, 1985). Total viable microorganism counts were run on PCA incubated for 48 h at 30 °C. Lactobacilli and leuconostocs counts were performed following the procedures of Gómez et al. (1989).

Total solids (TS), fat, total protein, and lactose in milk, permeates, and whey were determined using a Multispec apparatus (Föss Electric, Nanterre, France). The pH was measured directly in milk and cheeses using a Schott CG-837 pH meter. TS and fat in cheeses were determined according to the International Dairy Federation standards (IDF, 1982 and 1986, respectively). Nitrogen and total protein were determined by using the Kjeldahl procedure (AOAC, 1975). Non-casein nitrogen (NCN) and non-protein nitrogen (NPN) were determined according to the procedure described by Kuchroo and Fox (1982). Free amino acids in cheeses were determined by cation exchange chromatography, using a Biochron 20 automatic amino acid analyzer (Pharmacia LKB, Uppsala, Sweden). Samples for free amino acid determination were prepared according to the method of Resmini et al. (1993). Chloride content was determined according to International Dairy Federation Standards (IDF, 1972).

Texture Analysis. Cheese texture was assessed using an Instron Universal Testing Machine model 4501 (Instron Corp., Canton, USA). Cheese samples were taken from near the center of the blocks in the form of cylinders (20 mm high and 18 mm diameter) and held at 20 °C for 24 h prior to analysis, which was also carried out at 20 °C. The cheese samples were compressed between parallel plates using a cell with a pressure capacity of 0.1−5.0 kN and a compression plate of 58 mm diameter.

A breaking compression test was carried out at 100 mm/min crosshead speed and deformation ratio of 75% to measure the maximum breaking compression force (*F*) in newtons and the percent deformation at the break point (%Def), respectively, according to the method of Creamer and Olson (1982), and firmness (Fi) in newtons per millimeter at this point.

A two-cycle compression analysis was also performed at 50 mm/min crosshead speed and 25% compression of the original sample height to determine elasticity (*E*), cohesiveness (*C*), and gumminess (*G*) of the cheese samples according to the method of Chen et al. (1979).

Structural Analysis by Scanning Electron Microscopy (SEM). The microstructure of UF and MF cheeses was examined by SEM at 15, 30, 45, and 60 days of ripening. Prisms, 1 mm × 1 mm × 15 mm, from five sample blocks from each cheese were cut at the same orientation (parallel to their bases) and fixed in 25 mL/L glutaraldehyde at 4 °C for 16 h. The samples were subsequently dehydrated in a graded ethanol series, defatted in chloroform, transferred into absolute ethanol, critical point-dried by carbon dioxide, mounted on aluminum SEM stubs, sputter-coated with gold, and examined in a JEOL (JSM-6400, Tokyo, Japan) scanning electron microscope operated at 20 kV (Gavaric et al., 1989).

Sensory Analysis. Sensory analysis was performed throughout cheese ripening by an untrained 15-member tasting panel who were selected from among our laboratory staff for being usual consumers of semihard full-fat mixed milk cheeses. All analyses were done in a climate-controlled sensory analysis room equipped with individual testing booths, and scores were of acceptability and like−dislike tests of appearance, aroma, flavor, texture, and general acceptability. Each of these attributes was marked on a scale of 0 (very poor) to 5 (very good). The panel also considered any defects in the sensory characteristics of cheeses (e.g., bitter tastes).

Statistical Analysis. The significance of differences between mean values of microbiological counts, physicochemical composition, proteolysis, and texture and sensory analyses was determined using a one-way analysis of variance with cheese batch or membrane process as the variable. This analysis was carried out using the BMDP software statistical package, programs 2D, 2V and 7D, on an Alpha 2100 under VMS computer (CTI, CSIC, Madrid, Spain).

RESULTS AND DISCUSSION

Concentration Processes. A high pasteurization treatment of the milk mixture for the UF process (92 °C, 4 s) was used to increase cheese yield by reducing

Table 1. Composition (Grams per Kilogram) and pH Values of the Milk Mixtures Used in the UF and MF Processes and of Permeates and Retentates Obtained

	pH		lactose		fat		total solids		NPN[a]		total protein		whey protein	
	UF	MF	UF	MF	UF	MF	UF	MF	UF	MF	UF	MF	UF	MF
mixture C/E/G[b]	6.7	6.7	48.6	50.7	22.7	20.5	116.6	114.8	ND[c]	ND	38.5	37.1	ND	ND
permeate	ND	ND	37.3	41.0	0.0	0.0	40.1	46.5	0.29	0.27	0.30[d]	0.88[d]	0.06	3.89
retentate	6.6	6.4	17.0	ND	132.0	ND	399.0	408.0	ND	ND	226.0	218.8	ND	ND

[a] NPN, non-protein nitrogen. [b] Skimmed cow milk (C) mixed with whole ewe (E) and goat (G) milk in proportions of 55:15:30. [c] Not determined. [d] Total nitrogen.

losses of fat and proteins in whey (Rao and Renner, 1988; Green, 1990) and to avoid the diffusion of water toward the surface of the cheeses during ripening. Furthermore, a high denaturation of whey proteins is an essential step for the manufacture of pressed-paste UF cheeses with adequate sensory quality (Lelièvre and Lawrence, 1988). However, a conventional pasteurization of the milk mixture was used for the preparation of the MF cheeses (72 °C, 20 s) to avoid excessive denaturation of whey proteins in the original milk, which would allow their incorporation in the retentate with the caseins (Dalgleish, 1993). Table 1 shows the composition of the original milk mixtures and the permeates and retentates obtained from the UF and MF concentration processes. About 190 kg of milk was concentrated by UF or MF, yielding 229 and 237 kg of total permeate (including the water added in diafiltration), respectively. Given the original amount of milk and the amount of retentate obtained from the two concentration processes, and considering that the volume of whey left after cheese-making was ∼2%, the cheese yield in relation to the original milk was calculated at 12.3% (MF process) and at 16.3% (UF process).

The durations of the MF and UF processes were very similar (∼3 h). The permeate fluxes were slightly higher during the MF process, and the pressures in the installation were lower when MF membranes were used [input pressure = 180−200 kPa (MF) and 430−470 kPa (UF) during the first 2 h of the process]. The final concentration factor (CF) obtained after the MF process was 6.7 and was 6.4 after UF, calculated in terms of the amount of original milk used and retentate obtained at the end of the processes.

The TN content of the permeate from the MF process was higher than in the permeate from the same milk mixture using UF membranes (Table 1) but was similar to the value reported by Fauquant et al. (1988) in concentration of skim milk with the same type of MF membrane. Given that a similar amount of NPN was lost in both permeates (∼0.3 g/kg), it was concluded that the difference in TN content between UF and MF permeates was due to the whey proteins (Table 1). The lower concentration of whey proteins in the MF retentate was due to larger pore size of the membrane and the milder pasteurization of the milk mixture (72 °C, 20 s, versus 92 °C, 4 s). This, in addition to the slightly lower fat and total protein contents of the original milk used to make the MF cheeses (Table 1), was considered to be the cause of the lower cheese yield obtained from the MF process. As a result of the higher loss of whey proteins found in the MF permeate, the protein content of the UF retentate was expected to be higher than that of the MF one, but, in fact, these contents were similar in both retentates (Table 1), probably due to the higher CF obtained in the MF process.

Microbiological Analysis and Physicochemical Composition of Cheeses throughout Ripening. The behavior of the different microorganisms during MF and UF cheese ripening was not altered by the change in technological process used to concentrate the milk, development being similar to that already detailed for UF cheeses (Rodríguez et al., 1996). Figure 1 shows, as an example, the development of lactococci, lactobacilli, and leuconostocs in the MF and UF cheeses made using the mixed starter of local strains (IFPL) supplemented with *L. lactis* T1 (Lac$^-$ Prt$^-$). Counts of lactococci and lactobacilli were comparable in IFPL cheeses, and leuconostocs were the least abundant flora in all cheeses.

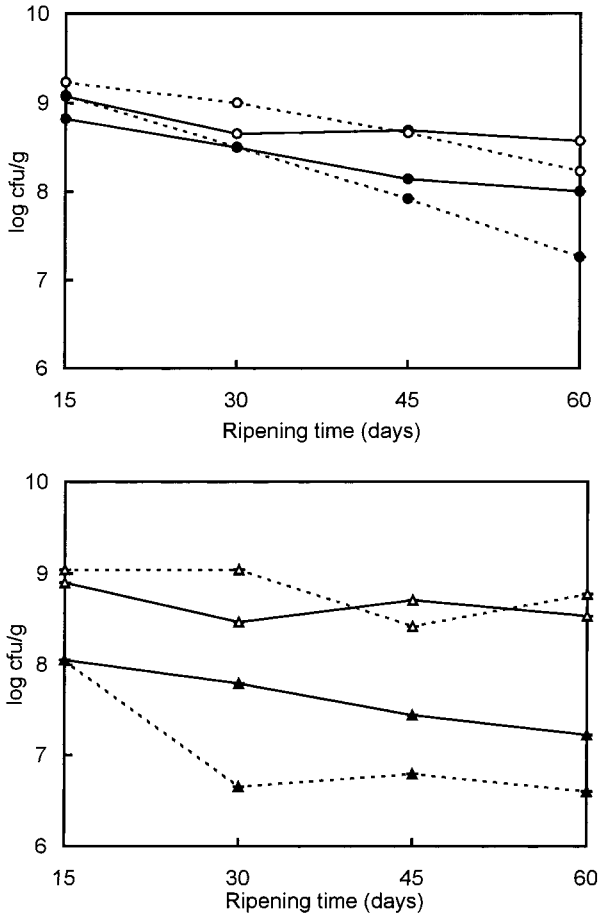

Figure 1. Change in *L. lactis* subsp. *lactis* IFPL359 (○) and T1 (●), lactobacilli (△), and leuconostocs (▲) in semihard low-fat cheeses manufactured from UF (—) or MF milk (- -) and with the mixed starter of local strains (IFPL) supplemented with *L. lactis* subsp. *lactis* T1 (Lac$^-$ Prt$^-$) during ripening (15, 30, 45, and 60 days).

In both types of cheeses (UF and MF), the three cheese batches did not differ significantly ($P < 0.05$) in total protein and fat contents during ripening. Mean ± SD values were 533.2 ± 1.9 and 388.0 ± 2.0 g/kg of TS, respectively, for the MF cheeses. The same values for the UF cheeses were 533.3 ± 1.6 and 360.4 ± 1.6 g/kg of TS, respectively.

Table 2. Change in pH, Moisture Content, and Proteolysis of UF and MF Low-Fat Cheeses Manufactured with the Mixed Starter of Local Strains (IFPL) Supplemented with *L. lactis* T1 (Lac⁻ Prt⁻), at 15, 30, and 60 Days of Ripening

membrane process	ripening time (days)	pH	moisture content (%)	NCN (%TN)[a]	FAA[b] (μmol/g)
UF	15	5.07	57.6	19.4	53.3
	30	5.27	56.3	22.0	60.0
	60	5.23	51.6	27.5	97.4
MF	15	5.22[c]	56.9[c]	15.8[c]	37.2[c]
	30	5.53[c]	53.7[c]	17.6[c]	59.5
	60	5.37[c]	49.3[c]	23.4[c]	90.1[c]

[a] NCN (%TN), non-casein nitrogen as a proportion of total nitrogen. [b] FAA, total free amino acid content. [c] Significantly different ($P < 0.05$) from values of UF cheeses at the same ripening time. Values are means of six cheese samples.

Table 2 shows the change in pH and moisture content of UF and MF cheeses made with the IFPL starter supplemented with *L. lactis* T1. During all of the ripening period, the pH in the MF cheeses was significantly ($P < 0.05$) higher than that obtained in UF cheeses. Furthermore, moisture content was significantly ($P < 0.05$) lower in MF cheeses than in those made from ultrafiltered milk throughout ripening, for the reasons cited above: that is, milder pasteurization of the milk mixture and lower retention of whey proteins in MF cheeses. The moisture levels in nonfat solids ratios (MNFS) at the outset of ripening of MF and UF cheeses were 67.4 and 68.0%, respectively, which are slightly higher than that reported by Martín-Hernández et al. (1992) and Fontecha et al. (1994) in the manufacture of semihard cheeses by conventional procedures using whole goat's and ewe's milk (63.8 and 63.4%, respectively).

The mean pH values of MF and UF cheeses at the outset of ripening were 5.16 and 5.07, respectively. NaCl contents were similar in MF and UF cheeses and equal to the amount of salt added to the precheese (mean value at the outset of ripening = 12.1 g/kg. At the end of ripening, the mean value ± SD of NaCl content in both types of cheeses was 21.5 ± 0.4 g/kg.

Table 2 also shows the proteolysis during cheese ripening, estimated on the basis of NCN as a proportion of TN, and total free amino acid content in the cheeses made with the IFPL starter supplemented with *L. lactis* T1, where proteolysis was most intense. Detailed effect of starter in proteolysis and volatile components has already been described for UF cheeses (Rodríguez et al., 1996, 1997). The main difference between the types of cheeses was a significantly ($P < 0.05$) lower content of NCN in MF than in UF cheeses, due to the lower retention of whey protein in these cheeses (Table 1), although the increases in NCN content during ripening time were similar (Table 2). As Table 2 shows, secondary proteolysis, estimated on the basis of total free amino acids content in the cheeses, was little affected by modification of the milk concentration process. Although the compositions of the UF and MF retentates were different, there was no change in the viability of the microorganisms or the intensity of proteolysis in the cheeses. Moreover, as found for UF cheeses (Rodríguez et al., 1996), ripening of MF cheeses made using the IFPL starter supplemented with *L. lactis* T1 was achieved in half the time taken when a commercial starter was used (results not shown). However, comparison of secondary proteolysis in the two types of cheeses (UF and MF) with a full-fat semihard cheese made with goat's milk and the local starter IFPL (Requena et al., 1992) showed that the use of either UF or MF milk concentrate caused a slowing of cheese ripening due to the higher retention of whey proteins, which have been described to inhibit proteinase activity (Harper et al., 1989; Bech, 1993). Besides, the decreased cheese proteolysis may have been due at least in part to the reduced fat content of the cheeses (Ardö, 1994; Katsiari and Voutsinas, 1994). Asensio et al. (1996) reported comparable results for secondary proteolysis when using the same IFPL starter culture to manufacture low-fat goat's milk cheese by conventional methods.

Table 3. Mean Values[a] for the Textural Characteristics of the Low-Fat Cheeses Made from Semiskimmed Milk Concentrated by UF or MF at 30, 45, and 60 Days of Ripening

	30 days of ripening		45 days of ripening		60 days of ripening	
	UF[a]	MF[a]	UF	MF	UF	MF
breaking compression test						
force (N)	41.2	76.2[b]	55.8	83.3[b]	76.4	85.6
deformation (%)	73.7	62.1[b]	62.9	58.9	56.0	53.7
firmness (N/mm)	2.8	6.2[b]	4.5	7.1	6.8	8.0
two-cycle compression test						
force (N)	5.4	28.3[b]	7.6	20.7[b]	25.0	29.4
firmness (N/mm)	1.1	5.7[b]	1.5	4.1[b]	5.0	5.9
elasticity (%)	97.3	98.0	97.6	98.0	96.3	97.9
cohesiveness	0.86	0.84	0.88	0.84	0.80	0.84
gumminess (N)	4.6	23.8[b]	6.6	17.6[b]	19.6	24.5
chewiness (N)	448	2335[b]	649	1730[b]	1892	2394

[a] Values are means of the three cheese batches (nine cheese samples each value). [b] Significantly different ($P < 0.05$) from values of UF cheeses at the same ripening time.

Cheese Texture. The study of UF and MF cheese texture is outlined in Table 3. The different starter cultures employed did not modify the cheese rheological properties studied; therefore, results are means of the three cheese batches. It is recognized that the rheological properties of cheese depend to a large extent on the structure and composition of the curd. At 20 °C the textural properties of cheese are largely governed by the moisture and total content of fat (Emmons et al., 1980), which did not vary in this study.

Despite protein breakdown, the breaking compression test showed increasing values of maximum breaking compression force (F) and firmness (Fi) and decreasing values of %Def throughout ripening for UF and MF cheeses, due to the loss of moisture from the cheeses. These results agree with the findings reported by other authors for Camembert (Kfoury et al., 1989) and ewe's milk cheese (Fontecha et al., 1996), in which the F and Fi values rose at the end of the ripening period. However, decreased values for the same characteristics were found for Saint-Paulin cheese (Kfoury et al., 1989).

Due to the higher protein retention during UF as compared to MF, MF cheese was expected to be softer than UF, but, in fact, breaking compression and two-cycle compression tests show that both the maximum breaking compression force (F) and firmness (Fi) values were lower in UF cheeses during the ripening period (Table 3), most likely due to the lower moisture content in the MF cheeses (Table 2). Whey protein aggregates may also interfere with the degree of curd fusion and, consequently, with the network arrangement during ripening. The rheological properties of cheese with similar pH, salt and fat contents, and casein degrada-

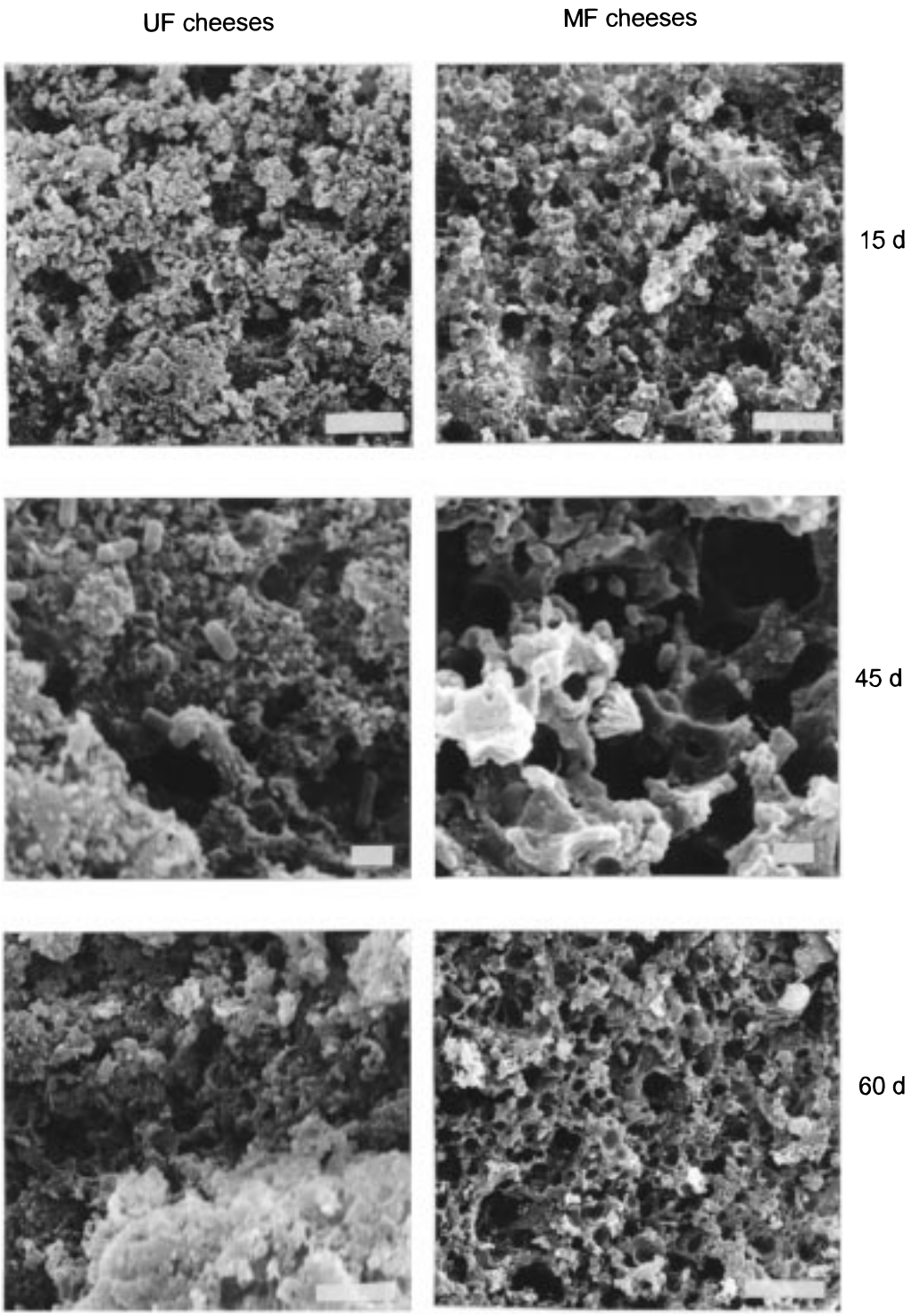

Figure 2. Scanning electron micrographs of semihard low-fat cheeses manufactured from milk concentrated by UF or MF during ripening (15, 45, and 60 days). Scale bars: 10 μm (15 and 60 days) and 1 μm (45 days).

tion were therefore regulated by their moisture content. The firmness of different types of cheeses is reported to be influenced markedly by relatively small variations in moisture (Lawrence et al., 1987). Several authors have reported an inverse relation between hardness and moisture content of cheeses (Chen et al., 1979; Creamer and Olson, 1982; Amantea et al., 1986). After 45 days of ripening, the values tended to converge as a result of moisture losses and no significant differences were found between UF and MF cheeses (Table 3).

The whey proteins retained in UF cheeses influenced their texture, although the effect of their presence is still unclear and depends on the concentration and the way in which they are incorporated in the UF cheeses, either in a soluble (native) or denatured form (Lelièvre and Lawrence, 1988). It seems that the whey proteins incorporated in the UF cheese in a native form are fully resistant to the proteolytic activity of rennet and play the role of an inert filling (De Koning et al., 1981). Nevertheless, the denatured whey proteins form complexes with the caseins and reduce the casein interaction interfering with the consistency of the UF cheese (Lelièvre and Lawrence, 1988; Raphaelides et al., 1995). Because part of the casein is replaced by whey proteins

in these types of cheeses, several authors have reported that the UF process accounted to a great extent for the softening of cheeses (Lelièvre and Lawrence, 1988; Spangler et al., 1990; Raphaelides et al., 1995).

In the two-cycle compression test in which the %Def applied to the cheese sample was constant (25%), the *F* and Fi values did not vary with ripening time. The higher *F* and Fi values in MF cheeses as compared to UF cheeses were related to the increased TS content in MF retentate rather than to the rate of casein aggregation. The elasticity (*E*) and cohesiveness (*C*) of the cheese samples were barely affected by the milk concentration process and showed high values during the ripening period, presumably due to the reduced fat content of the cheeses (Emmons et al., 1980; Bryant et al., 1995). The MF cheeses showed higher values for the parameters of gumminess and chewiness as compared to UF cheeses; however, after 45 days of ripening, no differences were found (Table 3).

Microstructure. Figure 2 shows the scanning electron micrographs of the semihard low-fat cheeses made from a retentate obtained by UF or MF during ripening (15, 45, and 60 days). Micrographs of both types of cheeses showed a protein matrix with open spaces through the protein network. These void spaces indicate the localization of fat globules before they were extracted with chloroform during the cheese sample preparation. The average hole size in both types of cheeses was ~2−3 μm (Figure 2, 45 days of ripening). This involves a reduction in the normal milk fat globule size, which has an average diameter of 3−5 μm (Alais, 1984). Sometimes, a reduction in fat globule size occurs early in the UF process, and disruption of the fat globule membranes takes place (Green et al., 1984), although the extent of this homogenization of the milk fat during the concentration process depends on plant design (Green et al., 1983).

Some of the micrographs show residues of the fat globule membranes attached to the protein matrix in the form of lacelike structures (Figure 2, 45 days of ripening). However, the protein matrices of UF and MF cheeses were different as a consequence of the differences in the processes used in each case. Probably, the presence of the whey proteins bound to the caseins in the UF milk, as a consequence of the high pasteurization treatment, induces differences in the coagulation of milk and probably this also modified the microstructure of the resulting cheeses. Micrographs of UF cheeses showed a more closed microstructure with less open spaces as compared to MF ones, due to the presence of spongy structures linked to the casein micelles, which did not appear in MF cheeses. The higher heat treatment to which the milk mixture was subjected for UF cheeses, compared to MF ones, enabled whey protein to be incorporated quantitatively due to the denaturation of β-lactoglobulin and its interaction with κ-casein (Dalgleish, 1993). McMahon et al. (1993) found that casein micelle size increased on heating skim milk, due to a large accumulation of protein material (denatured β-lactoglobulin) adhering to the casein micelle surfaces. Therefore, the spongy structures over the protein network that appear in the microstructure of the UF cheeses corresponded to the denatured whey protein bound to the casein.

Micrographs shown in the present work are very similar to those reported by Park et al. (1996), who studied the effects of the incorporation of β-lactoglobulin on the rheological properties and microstructure of casein gels. Casein gels with β-lactoglobulin showed the presence of many fine grains on their surfaces, which were thought to be the β-lactoglobulin and κ-casein complexes produced by the heat treatment. Also, the microstructure of the MF cheeses agrees with images reported by other authors for low-fat Cheddar cheese (Emmons et al., 1980; Bryant et al., 1995; Drake et al., 1996a), although when they are compared to the microstructure reported for different types of full-fat cheeses (Kaláb, 1977; Drake et al., 1996a; Fontecha et al., 1996), they appear more compact with fewer open spaces and more uninterrupted protein matrix, due to decreased fat content (Bryant et al., 1995; Drake et al., 1996b).

In both types of cheeses micrographs show no orientation of protein. This finding is in agreement with the images reported for Kaláb (1977) in Edam and Gouda cheeses. Furthermore, the surface appearance of the protein matrix of the UF and MF cheeses became smoother as the cheese ripened (Figure 2), which typically occurs in reduced-fat cheeses (Anderson and Mistry, 1994).

Some of the more magnified electron micrographs (Figure 2, 45 days of ripening) show the presence of starter bacteria (lactococci and lactobacilli), usually in contact with the fat globule membrane or at the casein−fat interface. In general, bacteria seem to have affinity to the fat phase (Laloy et al., 1996).

Sensory Analysis. The general acceptability of MF cheeses was good, with an average score of 4 at the end of ripening. Like the same type of cheeses made with UF milk, the greatest differences were found in aroma and flavor development, which were significantly ($P < 0.01$) greater in the cheeses made with the IFPL starter supplemented with *L. lactis* T1. The improved aroma and flavor development in the IFPL cheeses could be due to higher levels of proteolysis and volatile components (Rodríguez et al., 1997).

No significant differences in texture were found among the different cheese batches. The MF cheeses were judged to be harder than UF cheeses, which the tasting panel considered "rather soft" (Rodríguez et al., 1996), and similar in point of firmness to the remembrance of conventionally made semihard full-fat cheese. The reason for the harder texture detected in the MF cheeses was that retention of whey proteins, and hence also moisture content, was lower than in the UF cheeses, as a result of which breaking compression force and firmness values in texture analysis were higher throughout ripening (Table 3).

Conclusions. In conclusion, semihard low-fat cheeses with acceptable sensory characteristics were successfully made from liquid precheeses obtained by MF or UF. The use of MF membranes improved cheese texture to the extent that it was more appreciated than UF cheeses and reminiscent of the texture of conventionally made semihard full-fat cheeses. The lower retention of whey protein in MF cheeses accounted to a great extent for their higher hardness and correlated with the higher texture panel scores. However, when MF membranes were used, the optimum yields achieved using UF membranes were not attained, although the cheese yield was nonetheless greater than normally found in conventional processes (35% whey protein retention in MF cheeses). Which of the two processes is chosen in any

given case will depend basically on economic considerations and market strategies.

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