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Structure development in a soft cheese curd model during manufacture in relation to its biochemical characteristics

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Summary. The structure development of a soft cheese curd model has been studied in relationship to its rheological properties and its biochemical characteristics (pH, amount and partition of minerals, casein proteolysis) at different technical steps including cutting, drawing, three turns and demoulding. Scanning electron microscopy was used to observe structural changes during the drainage of a fat-free soft cheese. The micrographs provided visual evidence of changes in the casein matrix from casein particles aggregated in clusters to uniform strands observed at the demoulding. The initial increase of loss tangent and of the exponent of the power law between G′ and G″ and frequency (that were maximal at the second turn) was related to the solubilization of micellar calcium phosphate, while intact caseins and large casein fragments accumulated in the curd. After the second turn, the strength, Youngs' and loss moduli of the curd increased greatly. The hydrolysis of α_{s1}-casein into α_{s1}-I-CN f(24–199) may facilitate the rearrangement of casein particles within the curd. The pH-induced solubilization of calcium phosphate continued throughout the manufacture process but was unexpectedly incomplete at the end of the drainage. Combination of electron microscopic observations with dynamic rheological measurements and chemical and biochemical assessments provided increased knowledge about the structure of soft cheese during drainage, an important but poorly understood cheese making stage.

Keywords: Structure, soft cheese, drainage, minerals, proteolysis.

During the manufacture of soft cheese, formation of curd results initially from the coagulation by rennet of a pre-acidified milk, and subsequently from drainage of the curd, leading to concentration of the casein. The basic chemistry and physics behind aggregation of casein, gel formation and syneresis have been reviewed (Mietton *et al.* 1994; Lomholt & Qvist, 1999). Curd formation is important for controlling the structure, moisture content and rheological properties of the cheese and thus its texture.

Curd consists of a casein matrix that retains fat and to a greater or lesser extent the whey, during the drainage. Curd is further brined and ripened to gain the desired organoleptic properties arising from the many diverse enzymatic reactions that lead

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to a mature cheese. As an example, the chemical composition of Camembert curd and the subsequent changes caused by the metabolism of surface flora such as *Penicillium camemberti*, together with the ripening conditions, determine the structure and consequently the texture of the mature cheese.

Rennet coagulation, acidification and drainage are the three complex phenomena which have been each extensively studied. But these phenomena have rarely been studied together despite their interdependence during curd formation. To date, extended studies on structure development in curd concerned few cheeses, among them Mozzarella cheese (Oberg *et al.* 1993). However, to understand and thereafter to control the manufacturing process of cheese, it is essential to find out how the structure of the curd developed and gained its particular properties. On one hand, the change in macro- and microstructure of the curd during manufacture can be observed; on the other hand, the chemical and physical parameters that are driving this change can be understood (McMahon & Oberg, 1999). Theses changes essentially deal with pH, content and partition of minerals, water content and proteolysis.

The aim of this work was to relate the biochemical changes during the formation of a soft cheese curd model to the structure of the curd observed using scanning electronic microscopic and rheological methods. This fat-free model includes filtered skim milk inoculated with a single lactococcal strain as starter used in order to better understand the structure development of the casein matrix.

Materials and methods

Manufacture of model soft cheese

Soft cheese curds were made in triplicate according to the process used to manufacture Camembert cheese with a pilot plant (Tecnal, F-79024 Niort, France). Raw skim milk (46 kg) was micro-filtered at 35 °C through a 1·4-μm Sterilox membrane (Société des Céramiques Techniques, F-65460 Bazet, France). This process allows milk filtration with bacterial retention rates of the contaminant flora in the discarded retentate above 99·9 % (Trouvé *et al.* 1991). The milk was inoculated with *Lactococcus lactis* subsp. *cremoris* AM2, a laboratory strain having high proteolytic activity and high ability to lyse (Boutrou *et al.* 1998). This lactococcal starter was cultured three times in sterile reconstituted skim milk at 30 °C and used to inoculate the micro-filtered milk at 34 °C at the rate of 15 ml inoculum/l milk. Renneting was carried out at pH 6·35 using 20 mg pure recombined chymosin (EC 3.4.23.4.; Maxiren 50, Gist Brocades, F-59472 Seclin, France)/l milk. At 31·5 min (3 × the average clotting time of 10·5 min), the coagulum was cut in 1·5 × 1·5 × 1·7 cm cubes and gently stirred 10 and 25 min after cutting to prevent cubes from sticking together. Ten kilograms whey were drawn off (i.e. 22 % of total cheese-milk weight) and the curd was poured out into 10·8-cm diameter moulds. The moulds were turned three times 1·5, 3 and 6 h after renneting and removed after 20 h. The room temperature was controlled at 34 °C from coagulation to drawing, 26 °C at moulding, 24 °C at the first turn, 22 °C at the second turn, and 18 °C from the third turn to demoulding. Some curds were collected at each processing step and analysed immediately (bacterial numeration, microscopic and rheological analyses) or quickly frozen at −20 °C until required for biochemical analysis. An additional manufacture without curd sampling was performed in order to estimate the actual quantity of whey expelled.

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Bacterial numeration

Curd sample (10 g) was homogenized with a Warring Blender (Labo-Standa, F-14000 Caen, France) for 1 min at 13500 rpm in 90 ml 20 g/l sodium citrate. The lactococci were numerated on lactose M17 agar plates after 48 h incubation at 30 °C.

Scanning electron microscopy

From the curd centre, $2 \times 2 \times 3$ mm pieces of curd were taken in the vertical direction, and immediately fixed in 0·1 M-sodium cacodylate, pH 7·2 containing 25 ml glutaraldehyde/l at 4 °C for 24 h. After rinsing with cacodylate and water, pieces were dehydrated in alcohol series and critical point dried. Fractured samples were glued on the sample holder, gold coated and observed at 10 kV on a Philips XL20 scanning electron microscope (FEI Philips Electronique, F-93000 Bobigny, France). At least five micrographs were taken at each technological step on the three cheese manufactures. This kind of preparation has been widely used either on acid gels (Famelart *et al.* 1996) or on cheeses (Emmons *et al.* 1980; Rousseau & Le Gallo, 1990; Haque *et al.* 1997) except that we did not post-fix in osmium tetraoxide. This latter step is only required when fat is present. According to Rousseau & Le Gallo (1990), fixation in glutaraldehyde maintains the network structure and the observed aspect of micelles agrees with previous descriptions. Emmons *et al.* (1980) reported a good agreement of the Cheddar cheese structure observed by this preparation and thin-section electron microscopy.

Rheological properties

Uniaxial compression. Curds under drainage at the first, second and third turn and at demoulding were rapidly cut at 30 mm height in such a manner that the lower part of the curd before each turn was discarded. At demoulding, the curd height was 30 mm. Uniaxial compression was performed at room temperature at 120 mm/min with an Instron Universal testing machine 4501 (Instron S.A., F-78284 Guyancourt, France), using a 35-mm diameter plate and a 100-N (1000 N for the sample at the end of drainage) load cell. Many authors used a compression speed of 30 mm/min, but a higher speed allowed a faster compression of the sample of soft cheese as used by Kfoury *et al.* (1989) and Mpagana & Hardy (1986) on Camembert cheese. Indeed, the sample was clearly draining during storage. As the size of the measuring probe was smaller than the curd sample, the measurements were not absolute rigidity measurements, but they could be compared with each other. Although the results were not expressed in stress and strain data for simpler calculations, they could be compared with trends in other cheeses. A mean temperature of the sample between 22 and 27 °C was assumed, from its own initial temperature and the temperature losses during preparation in the 22-°C Instron room. Rheological measurements were not corrected for the effect of temperature. The strength at 5 mm, the displacement and the force at rupture and the Young's modulus were recorded. Strengths at 5, 10 and 15 mm were initially collected and showed the same trend.

Harmonic measurements. From the same piece of curd as the one used for uniaxial compression, a 40-mm diameter sample was cut using a borer, in the vertical direction. With an apparatus equipped with two wires, 4 mm-high slices were cut. Just after cutting, the slice was placed between two cross-hatched parallel plates at 26 °C. The temperature was chosen as the average temperature of the curd during the whole draining process and was probably the least disturbing testing condition. An aluminium solvent trap cover was placed above the upper plate to prevent

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evaporation. A logarithmic frequency sweep (0·5–10 Hz) at 2 % strain was performed with 10 points per frequency-decade. Conditions were adapted from previous studies (Horne *et al.* 1993; Solorza & Bell, 1995). The frequency range was reduced to reach a shorter time for the whole sweep and strain at 2 % was in the linear region. The slope of the loss modulus and the storage modulus versus frequency in a log–log representation (respectively expG' and expG'') was collected, together with the mean loss and storage moduli and the mean loss angle (delta).

Biochemical analysis

The pH of the curd was measured using a penetrating electrode (Ingold) linked to a Hi 9025 pH-meter. Total solids (TS) of grated cheese was determined after desiccation at 102–105 °C for 7 h (International Dairy Federation, 1982). The lactose concentration was determined by ion chromatography as described by Morgan *et al.* (1999).

For the determination of mineral content in the curd, 3 g curd was homogenized in 100 g 0·02 M-nitric acid solution. The homogenate obtained had a pH ≈ 3 leading to total mineral solubilization. After standing 1 h at room temperature, the solution was filtered through a 0·42-μm filter (Minisart, Sartorius, D-37070 Göttingen, Germany). To estimate the proportion of mineral associated with the casein matrix, the mineral content in the aqueous phase of the curd was also determined. About 30 g curd was centrifuged at 3000 ***g*** for 30 min, and the supernatant was filtered through a 0·42-μm filter. The aqueous phase of the milk was obtained by centrifugation of milk at 1800 ***g*** for 30 min, ultrafiltration of the supernatant on an Ultrafree 15 membrane (molecular mass cut-off 10000 Da) (Millipore, F-78280 Saint Quentin en Yvelines, France) and filtration through a 0·42-μm filter.

Cation (calcium, magnesium, sodium and potassium) and anion (lactate, chloride, inorganic phosphate, citrate) concentrations were determined on 0·42-μm filtrates using atomic absorption spectrometry (Varian AA300 spectrometer, F-91941 Les Ulis, France) (Brulé *et al.* 1974) and ion chromatography (Dionex, F-78354 Jouy-en-Josas, France) (Gaucheron *et al.* 1996), respectively. Concentrations were expressed in g/kg. From the calcium and inorganic phosphate concentrations in the curd and in its corresponding aqueous phase, the percentage of ions still associated to the casein matrix was calculated as follows:

$$\% \text{ associated} = 100 \times \left(1 - \left(\frac{\text{mineral content in the aqueous phase}/(1000 - \text{TS}_{\text{aqueous phase}})}{\text{mineral content in the curd}/(1000 - \text{TS}_{\text{curd}})}\right)\right).$$

From the mineral content (calcium, magnesium, sodium, potassium, lactate, chloride, inorganic phosphate and citrate) determined in the aqueous phase of the curd, a computer program calculated the mineral partitions and the activities of each ion and the ionic strength (Holt *et al.* 1981). From activities, the solubility product for brushite was calculated. Brushite was chosen because several authors found, in milk, an invariant ion activity product to be approximately that of a dicalcium phosphate (Holt, 1982; Chaplin, 1984). The calculations were carried out in the pH range where calcium phosphate salts were still at least partially in the micellar phase (pH between 6·7 and $\approx 4{\cdot}8$). The results of these theoretical calculations were of semi-quantitative significance as assumed by Morris *et al.* (1988).

For the determination of nitrogen content of the curd, an aliquot of grated curd was suspended in a sodium citrate solution as described by Gripon (1975). The

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content of total nitrogen (TN), pH 4·6 soluble nitrogen (SN) and non-protein nitrogen (NPN) soluble in 12 % trichloroacetic acid were determined in duplicate by the Kjeldahl method.

Urea-PAGE containing 180 g acrylamide/l in 4·33 M-urea–0·0375 M-Tris-HCl buffer, pH 8·8 was used in a Mini Protean II gel system (BioRad, F-94203 Ivry-sur-Seine, France) according to the method of Andrews (1983). Insoluble fraction of the curd was prepared. Sodium citrate solution of cheese obtained as previously described, was centrifuged at 10000 ***g*** at 20 °C for 10 min and the pellet was resuspended to the initial volume with 8·75 M-urea. Samples were denatured with 0·0625 M-Tris-HCl buffer, pH 6·8, containing, 3·3 M-urea, 50 ml β-mercaptoethanol/l, 100 ml glycerol/l and 0·5 g bromophenol blue/l. A volume of the insoluble fraction equivalent to 10 μg TN measured in the curd was loaded by lane. Migration was for 2·5 h at 20 °C and 200 V constant voltage. The protein bands were revealed with R_{250} Coomassie blue staining.

RESULTS

Drainage, acidification, lactose fermentation and bacterial growth

After renneting, the pH of the curd continued to decrease until pH 4·8 at demoulding (Table 1). The major part of whey (76 kg/100 kg milk) was drained before the first turn. Thereafter, whey continued to be expelled, but the quantity decreased drastically, especially from the second turn to demoulding (Table 1). As the times of the successive turns were 1·5, 3 and 6 h after renneting, the changes with time of expelled whey were very tiny.

Bacterial growth was concomitant with the decrease of lactose concentration and the proportional increase in lactate concentration (Table 2). The *Lc. lactis* AM2 grew slowly within the curd during the drainage (Table 1). The bacteria were visible by electron microscopy from the first turn to the end of the drainage, either alone or dividing (Fig. 1). No evidence was found of bacteria connected to the casein matrix. They appeared more in void spaces, surrounded by whey, as in Cheddar cheese (Haque *et al.* 1997), in Cottage cheese (Kalab, 1978) or in yogurt (Kalab, 1979; Kalab *et al.* 1983; Tamime *et al.* 1984).

Scanning electron microscopic observations

Electron microscopy was used to visualize the structure of the curd at different steps in the manufacturing process. The micrograph presented at each step is representative of the three cheeses manufactured (Fig. 1). Water was removed during sample drying, leaving only the casein matrix and bacteria to be visualized. Open spaces, that contained the aqueous phase before sample drying, were always uniformly dispersed and of the same size throughout the drainage. Observed particles supposed to be micelle aggregates (about 0·25–0·5 μm) agglomerated to form clusters (0·5–4·0 μm) that were visible at the first turn. There were still strands connected to the rest of the network at one end only (Fig. 1*a*). Holes between 2 and 6 μm showed the pores of the matrix. Particles within the clusters rearranged into chains but the micelle-based structure was still visible at the second turn (Fig. 1*b*). At the third turn, initial particles became difficult to visualize and the fusion between particles progressed, thus thickening and smoothing the strands (Fig. 1*c*) until the curd had lost the cluster structure leading to the completely fused amorphous network at demoulding (Fig. 1*d*). The chains observed at demoulding had a diameter $\approx$ 0·6–1·5 μm and were larger than the average diameter of individual particles at the first turn. This is particularly evident while comparing with lactococci cells.

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Table 1. *Characterization and rheological properties of the milk and the curd throughout drainage. Cheese was prepared on three separate occasions*

(Values are mean ± SD for $n = 3$ or various §)

	Milk	Cutting	1st turn	2nd turn	3rd turn	Demoulding
Whey expelled† kg/100 kg milk	—	0·0	76·0	86·8	89·5	90·5
Whey expelled‡ kg/100 kg milk	—	0·0 ± 0·0	73·8 ± 0·92	82·2 ± 0·67	84·2 ± 0·72	85·3 ± 1·12
PH	6·56 ± 0·01	6·22 ± 0·03	5·91 ± 0·06	5·23 ± 0·09	4·95 ± 0·06	4·83 ± 0·06
Temperature, °C	34·4 ± 0·17	34·0 ± 0·15	31·8 ± 0·25	27·7 ± 0·85	20·9 ± 0·93	16·5 ± 0·47
Strength at 5 mm, N	—	—	3·67 ± 0·74$^{(9)}$	6·92 ± 0·74$^{(9)}$	16·91 ± 2·05$^{(9)}$	53·07 ± 11·19$^{(19)}$
Young's modulus, Pa	—	—	32 566 ± 5629	59 039 ± 7178	152 611 ± 11 609	576 403 ± 91 101
Loss modulus, Pa	—	—	9545 ± 2985$^{(8)}$	22 864 ± 4000$^{(8)}$	32 503 ± 2798$^{(8)}$	79 241 ± 14 127$^{(18)}$
Storage modulus, Pa	—	—	3292 ± 1020	8808 ± 1410	11 883 ± 982	25 310 ± 4544
exp G′	—	—	0·21 ± 0·013	0·23 ± 0·01	0·22 ± 0·0032	0·19 ± 0·0064
exp G″	—	—	0·23 ± 0·0057	0·23 ± 0·003	0·22 ± 0·0041	0·21 ± 0·0047
Loss angle, degree	—	—	18·95 ± 0·30	21·11 ± 0·32	20·07 ± 0·16	17·86 ± 0·28
Cell count, log cfu/ml	6·59 ± 0·45	6·93 ± 0·37	8·54 ± 0·08	8·72 ± 0·46	9·00 ± 0·3	9·11 ± 0·46

† Without curd sampling (one manufacture).
‡ With curd sampling (three manufactures).
§ Superscript numbers in parentheses indicate the total number of measurements for either compression or oscillation tests.

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Table 2. *Biochemical properties of the milk, the curd and its aqueous phase throughout drainage. The results determined in the aqueous phase either of the milk or of the curd (supernatant of centrifugation as described in M&M) are denoted by*^{aqu}

(Values are means ± SD for replicates form three cheese-making trials)

	Milk	Cutting	1st turn	2nd turn	3rd turn	Demoulding
Calcium	1·32 ± 0·03	1·46 ± 0·09	4·68 ± 0·18	6·20 ± 0·77	6·20 ± 0·7	6·44 ± 0·23
Calcium^{aqu}†	0·37 ± 0·02	0·48 ± 0·01	1·06 ± 0·2	3·35 ± 0·17	5·08 ± 0·11	7·15 ± 0·15
Magnesium	0·12 ± 0·01	0·12 ± 0·01	0·22 ± 0·01	0·26 ± 0·02	0·25 ± 0·01	0·33 ± 0·01
Magnesium^{aqu}	0·07 ± 0·01	0·09 ± 0·01	0·12 ± 0·01	0·21 ± 0·01	0·26 ± 0·01	0·31 ± 0·01
Sodium	0·42 ± 0·01	0·49 ± 0·02	0·48 ± 0·06	0·47 ± 0·03	0·48 ± 0·07	0·41 ± 0·03
Sodium^{aqu}	0·41 ± 0·01	0·44 ± 0·01	0·47 ± 0·02	0·52 ± 0·02	0·51 ± 0·02	0·52 ± 0·02
Potassium	1·72 ± 0·05	1·77 ± 0·08	1·74 ± 0·18	1·61 ± 0·24	1·66 ± 0·37	1·57 ± 0·30
Potasium^{aqu}	1·55 ± 0·03	1·66 ± 0·02	1·76 ± 0·03	1·80 ± 0·07	1·87 ± 0·07	1·93 ± 0·09
Inorganic phosphate	nd	2·41 ± 0·21	5·57 ± 0·23	7·01 ± 0·68	7·09 ± 0·71	8·35 ± 2·48
Inorganic phosphate^{aqu}	0·99 ± 0·03	1·13 ± 0·02	1·75 ± 0·24	4·22 ± 0·16	6·11 ± 0·20	8·48 ± 0·48
Citrate^{aqu}	1·66 ± 0·09	1·72 ± 0·08	1·71 ± 0·09	2·15 ± 0·12	2·55 ± 0·13	3·06 ± 0·16
Chloride^{aqu}	1·11 ± 0·04	1·11 ± 0·08	1·04 ± 0·05	0·99 ± 0·04	0·99 ± 0·04	1·01 ± 0·02
Lactate^{aqu}	nd	0·66 ± 0·02	3·50 ± 0·77	11·34 ± 0·74	16·41 ± 1·43	23·44 ± 2·60
Log solubility product brushite^{aqu} (no unit)	—	−6·24	−5·85	−5·87	−5·75	−5·68
Ionic strength^{aqu} (mM)	64	71	108	217	284	363
Lactose^{aqu}	nd	49·61 ± 1·82	47·29 ± 0·43	35·8 ± 1·23	28·41 ± 1·63	19·38 ± 2·51
TS	89·86 ± 0·45	nd	234·22 ± 11·07	279·69 ± 4·01	308·25 ± 4·80	330·25 ± 55·03
TN	33·58 ± 0·31	60·49 ± 13·93	165·21 ± 11·45	215·80 ± 4·04	243·53 ± 2·72	268·44 ± 30·90
SN	8·00 ± 0·36	9·85 ± 0·79	11·58 ± 0·79	13·3 ± 0·74	13·78 ± 0·79	18·59 ± 3·61
NPN	1·67 ± 0·17	1·78 ± 0·82	2·79 ± 0·41	4·36 ± 0·45	5·45 ± 0·38	7·68 ± 2·05
TN − SN	25·6	50·64	153·63	202·49	229·75	249·85

nd, not determined.

† Concentrations (g/kg) measured in the aqueous phase of either milk or curd (supernatant from centrifugation as described in M&M) are denoted by^{aqu}.

TN, total nitrogen; TS, total solids; SN, soluble nitrogen; NPN, non-protein nitrogen; TN–SN, estimated casein concentration.

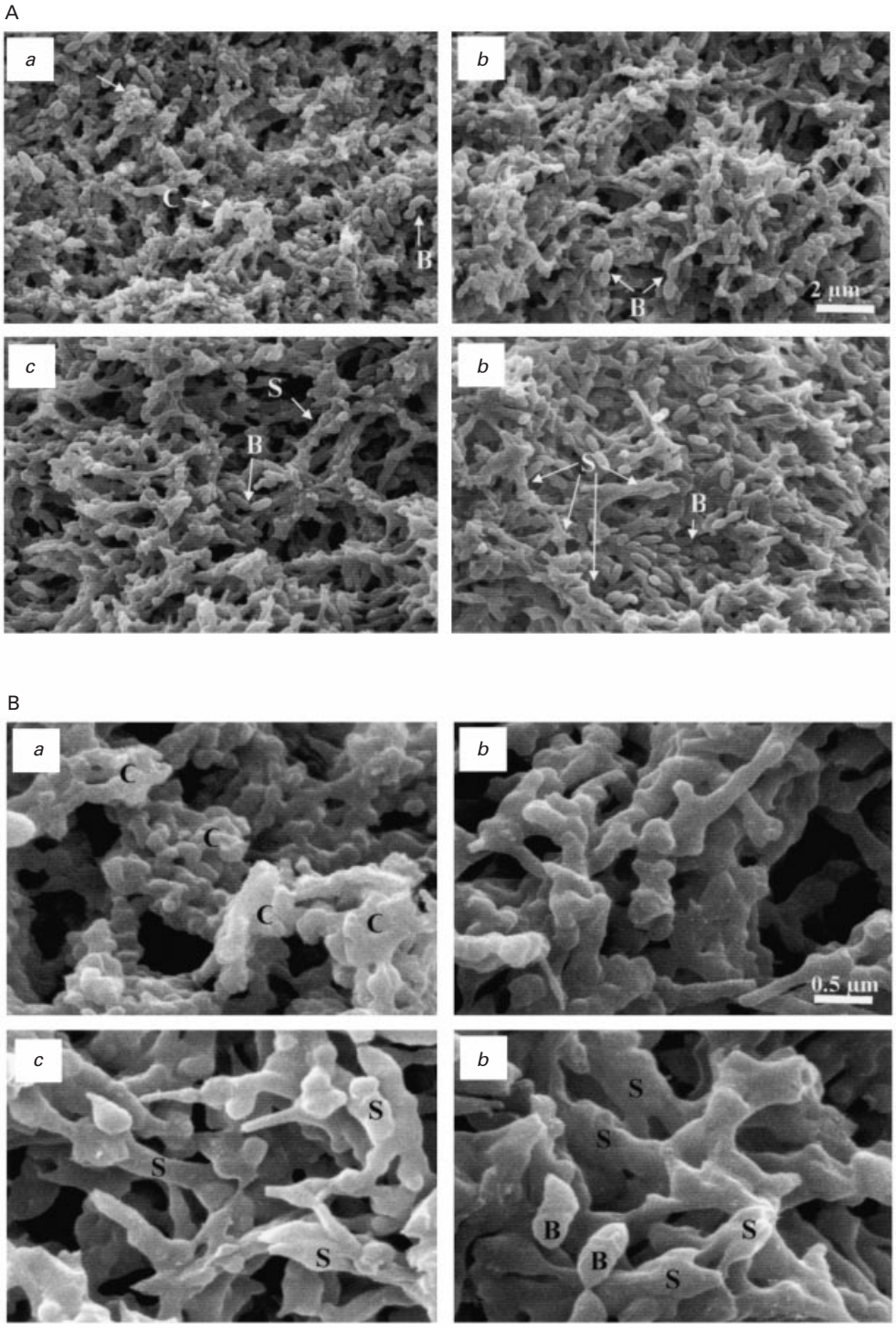


Fig. 1. Scanning electron micrographs of curd at (*a*), the first turn (*b*), second turn (*c*), third turn and (*d*), at demoulding with magnification ×5000 (A) and ×20000 (B). B = bacteria, S = strands, C = cluster of particles.

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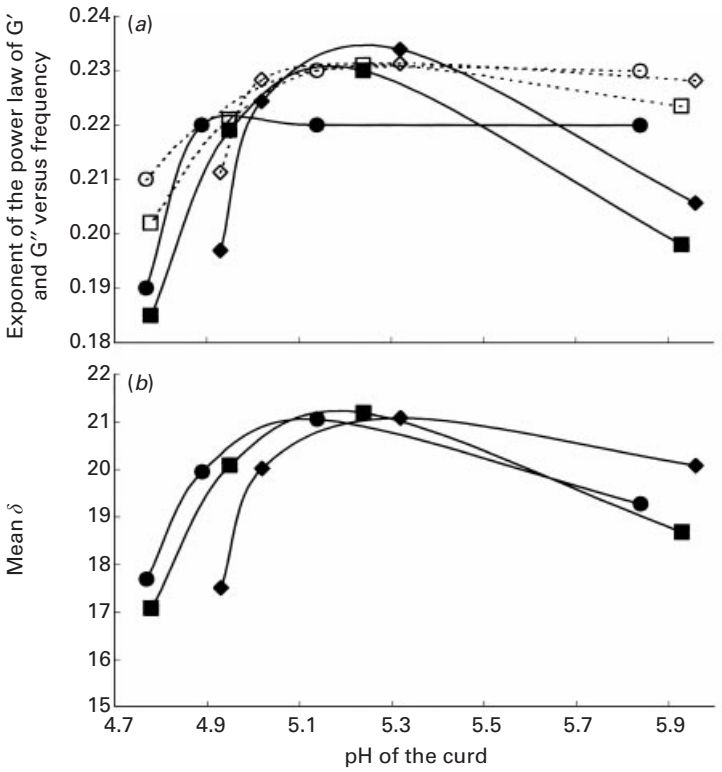


Fig. 2. (*a*) Relation between the pH of the curd and the exponent of the power law of G′ (●, ◆, ■), G″ (○, ◇, □) versus the frequency; (*b*) mean value of loss angle (●, ◆, ■) versus the pH of the curd. The three different symbols are for the triplicate manufactures.

Rheological properties

The compression curves of curd under drainage were obtained with relatively constant slopes between the force and the displacement. A rupture was observed on the compression curves only at the third turn and at the demoulding. The displacement and the force at rupture were 14–16 mm and 56–74 N for the third turn and 10–12 mm and 120–165 N for demoulding (results not shown). This means a higher brittleness of the curd while draining.

The Young's modulus and the force at 5 mm compression increased 2, 5 and 16 times relative to values at the first turn, for the second turn, third turn and demoulding, respectively. At the same times, G′ and G″ moduli increased 2, 3 and 8 times on average (Table 1). The increase of G″ was greater at the second and third turn and lower at demoulding, compared with G′. The slopes of the log modulus − log frequency curves (exp G′, exp G″) and the loss angle values were calculated. exp G′ and exp G″ increased at the second and third turn and decreased at demoulding. This indicates a more liquid-like material at the second and third turns and a more solid-like material at demoulding; firmness of the curd increased weakly before the third turn and strongly after.

A power law dependence of the rheological characteristics such as the strength measured at 5 mm on the casein concentration (estimated as TN − SN) was found with exponent values of 1·30 [strength = constant × $(TN-SN)^{1\cdot30}$]. Similarly the Young's modulus, G′ and G″ depended on the casein concentration with a power law

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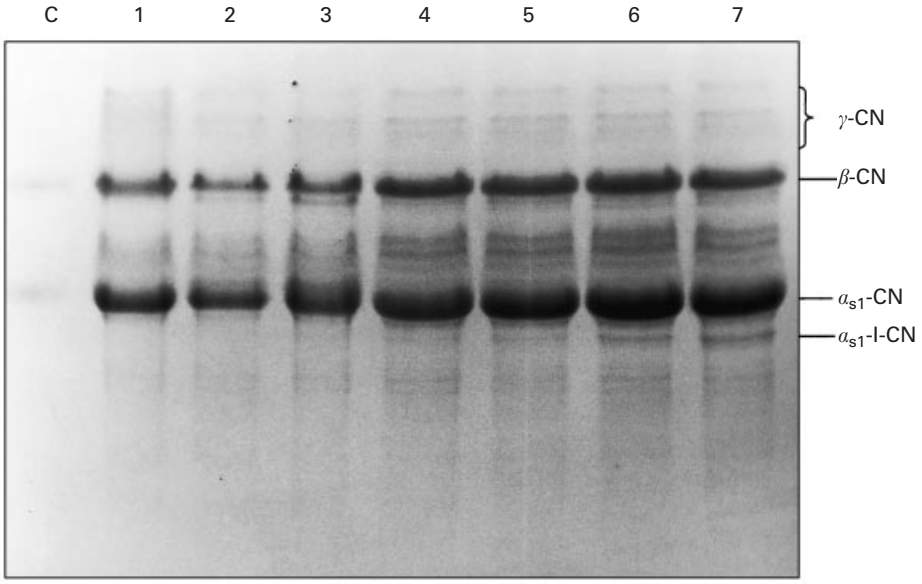


Fig. 3. Urea-PAGE of caseinate (C), the micro-filtered skim milk (lane 1) and the insoluble fraction of the curd at different steps of the cheese-making: cutting (lane 2), drawing (lane 3), first turn (lane 4), second turn (lane 5), third turn (lane 6) and demoulding (lane 7). The location of casein is given.

dependence with exponent values of 1·38, 1·12 and 1·11, respectively ($P < 0{\cdot}002$ for linear regression of log–log). Values for $\exp G'$, $\exp G''$ and the loss angles were plotted versus pH at each turn (Fig. 2); a convex graph was obtained, with a maximum around pH 5·2.

Biochemical analysis

As a consequence of the high quantity of whey expelled during the drainage, the TS of the curd increased during cheese manufacture (Table 2). In parallel, the mineral content of the curd (expressed in mg/kg curd, i.e. in matrix and aqueous phase) and its corresponding aqueous phases (expressed in mg/kg of aqueous phase) increased (Table 2). Firstly, the increase observed in the curd was related to the expulsion of whey that induced a concentration of the minerals in the casein matrix. Secondly, the decrease of pH induced a solubilization of micellar calcium phosphate, contributing to the increased mineral contents in the aqueous phase of the curd. Due to the low proportion of aqueous phase within the curd at the end of the drainage, the concentration of minerals in the aqueous phase was higher than the concentration in the curd. The rate of inversion of these concentration values between the curd and corresponding aqueous phase was slower for minerals that were strongly bound to the casein matrix (calcium, magnesium and inorganic phosphate), than for those free in solution (sodium and potassium).

Extensive whey drainage led to concentration of TN in the curd. SN and NPN amounts also increased due to the proteolysis of casein (Table 2). Moreover the amount of TN and TN−SN (corresponding primarily to intact casein, α_{s1}-I-casein and γ-caseins) also increased because the amount of SN was low compared with TN. As observed by urea- PAGE, the intensity of α_{s1}- and β-casein bands increased in the insoluble fraction of the curd (Fig. 3). This result showed that the proportion of casein to TN increased in the curd throughout drainage. The concentration of α_{s1}- and β-casein was visible although their concomitant degradation was evidenced by

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the appearance, from the second turn, of bands with electrophoretic mobility corresponding to that of α_{s1}-I- and γ-casein, respectively.

DISCUSSION

Structure development of a soft cheese curd begins with the formation of aggregated micellar particles resulting from the rearrangement of casein micelles subsequent to the action of chymosin on κ-casein, the acidification that solubilized minerals, and the drainage of the curd that concentrates solids. Using scanning electronic microscopy, we visualized casein particles agglomerated in clusters within the curd at the first turn, i.e. 1·5 h after renneting. At this stage, 76% of the whey had already been expelled. The subsequent acidification and mineral solubilization therefore proceeded within an almost drained curd, because only about 15% of whey was subsequently expelled before demoulding.

At the first turn, a slight pH-induced solubilization of micellar calcium phosphate, magnesium and citrate ions from the casein matrix to the aqueous phase has begun (Pyne & McGann, 1960). About 80% of the calcium and 75% of the inorganic phosphate were still associated with the casein matrix. Consecutive to acidification, ionic strength of the aqueous phase increased (Brulé *et al*. 1974; van Hooydonk *et al*. 1986; Visser *et al*. 1986; Dalgleish & Law, 1989; Le Graët & Brulé, 1993; Famelart *et al*. 1996; Gastaldi *et al*. 1996; Gaucheron *et al*. 1996). In the soft cheese curd model, casein concentration throughout drainage slowed the pH-induced solubilization of minerals compared with that observed in milk (Le Graët & Brulé, 1993). The high retention of minerals in the curd has probable implications for the structure of the casein matrix. The retention of calcium ions in the curd occurred probably by binding to the casein. The two consequences of this binding were a reduced negative charge on the casein and a more compact particle structure due to connections within the casein structure by this divalent cation.

At the second turn, particles within clusters rearranged into chains but the micelle-based structure was still visible. As a consequence of the decrease in pH, the negative charge of caseins was reduced (Lomholt & Qvist, 1999), and micellar calcium phosphate moved out of the casein strands to the aqueous phase. Both phenomena might strengthen hydrophobic interactions at the expense of calcium bridge interactions and lead to the observed thickening of the strands during subsequent drainage. It is noteworthy that at this step, about 60 and 55% of the total calcium and inorganic phosphate were still associated with the casein matrix. These values are higher than those obtained during milk acidification (without drainage), as it is generally admitted that at pH 5·2 all inorganic phosphate is solubilized (van Hooydonk *et al*. 1986; Visser *et al*. 1986; Dalgleish & Law, 1989; Le Graët & Brulé, 1993; Gaucheron *et al*. 1996; Le Graët & Gaucheron, 1999). Indeed, as the acidification occurred during drainage (resulting in casein concentration), a shift of mineral solubilization towards lower pH has been observed (Gastaldi *et al*. 1997; Le Graët & Gaucheron, 1999), that corresponds probably to a large and rapid increase in the ion activity product of calcium phosphate salts in the aqueous phase of the curd.

Another interesting way to obtain structural insights in the casein matrix is to determine its composition. Intact caseins as well as α_{s1}-I-casein and γ-caseins accumulated in the curd during drainage. The appearance, from the second turn, of α_{s1}-I-casein in the insoluble fraction of curd demonstrated the continuous action of chymosin and indicated that α_{s1}-I-casein is a constitutive element of the casein

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matrix. Concomitantly the α_{s1}-CN f(1–23) has been identified in the corresponding whey (Boutrou *et al.* 2001). The appearance of αs_1-I-casein may facilitate rearrangement of the remaining casein particles possibly through hydrophobic or electrostatic interactions, and thus prepare the change of texture that happens during the ripening of soft cheese. De Jong (1976) showed that the change in consistency during ripening of soft cheese was caused by the breakdown of α_{s1}-casein, while the β-casein remained almost unchanged. According to Noomen (1977) the weakening of the soft cheese body was closely related to the protein breakdown under the existing conditions in the cheese, especially the pH. This was confirmed by Vassal *et al.* (1986). Changes in matrix composition can explain the initial increase of loss tangent at the second turn. Indeed, the loss tangent and the exponents of the modulus – frequency relation were maximal at pH 5·2. Similar trends were observed in many studies on rennet gels (Roefs, 1986) and on young Gouda cheese (Luyten, 1988) and this has been related to the maximum of casein voluminosity. Tan δ can increase with the micellar calcium phosphate solubilization. Zoon *et al.* (1988*b*) reported that reducing the concentration of micellar calcium phosphate below half the amount present in milk (i.e. pH 5·7 according to van Hooydonk *et al.* (1986), and pH 5·2 in our study) leads to an increase in tan δ.

From the third turn to the demoulding, the rearrangement of casein particles continued towards a continuous matrix. The acidification rate slowed and the rheological properties of the curd, especially strength, Young's modulus and loss modulus greatly increased. Studying the relationship between the firmness or moduli and protein concentration is relevant because this relationship characterizes the organisation of the network and its changes with time. Many authors reported an increase in firmness exponentially related to casein or nitrogen or solid content, with an exponent value (exp) greater than 1 for acid gels (Harwalkar & Kalab, 1980; exp = 2·6), for rennet gels (Zoon *et al.* 1988*a*; exp = 2·4 for gels aged for 7 h) and for cheeses (De Jong, 1978; exp = 5·7 for Meshanger experimental ripened cheese; Luyten, 1988; exp = 10 for fat Gouda cheese from 1 week to 13 months ripening). According to Luyten (1988), this effect is due, first to the increase in concentration of the stress carrying component of cheese and second, to the lower freedom of the less swollen particles with a lower moisture. The exponent values of the power law were low compared to cited values (Roefs, 1986; Zoon *et al.* 1988*a*), but it means that in all these gels, the number of stress carrying strands was not strictly proportional to the number of particles. This was probably due to the rearrangement of casein particles during the drainage.

At the end of drainage, 20% of calcium and inorganic phosphate was still associated with the casein matrix. Such an incomplete solubilization showed that in the structure development of soft cheese curd, the role of minerals is probably underestimated and even at pH 4·8, they can still participate in the structure of the curd by formation of bridges between two negative sites on casein molecules (organic phosphate of phosphoserine, carboxyl groups of acid amino acid) and/or inorganic phosphate. From this point of view, to determine the nature of the calcium phosphate still associated with the casein matrix was of major interest. The relatively constant ion activity product of the calcium phosphate salt suggested that it was probably close to dicalcium phosphate (Table 2).

This work has investigated the structure development of the casein matrix of a fat-free soft cheese curd model during drainage. The cheese curd thus forms the basis of the cheese, which is later modified by salting and ripening. Further studies are required to include the role of fat in the structure of soft cheese curd. The effect of

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salting and the presence of other micro-organisms during ripening should also be considered.

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