Soft goat cheeses at different ripening stages: cheese structure, composition and non solvent water

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Abstract — Soft goat cheeses of the Sainte-Maure variety were studied at different ripening stages to characterise their microstructure in relation to their composition and water content. The internal liquid phase of the cheeses was extracted by hydraulic pressure and analysed. Non solvent water (NSW) in cheese was calculated according to the method of van Boekel and Walstra from the concentration of some completely dissolved components such as potassium (K), sodium (Na) or β -lactoglobulin (β -Lg) in the liquid fraction compared to that in the whole cheese. The values obtained for NSW in the 7-d-old cheeses were 1.1, 0.8, 2.1 g·g⁻¹ casein (CN) for K, Na and β -Lg, respectively. A reduction in NSW values was observed during ripening, down to 0.6 g·g⁻¹ CN for K and Na and 1.3 g·g⁻¹ CN for β -Lg in the 58-d-old cheese. Total water content of cheese decreased during ripening from 4.7 to 1.9 g·g⁻¹ CN, so that the relative proportion of NSW to total water increased during ripening. Very different cheese microstructures were observed at the beginning and end of the ripening period. In the early stage of ripening the structure of cheese was loose, characterised by accelerated enzymatic reactions which developed in the aqueous phase as a result of proteolysis. A rapid decrease in the water content occurred due to evaporation at the cheese surface, facilitated by the easy movement of water from the inner part of cheese to the surface through the open and porous structure. During these early stages of ripening, pressing of the cheese led to high flow rates of liquid. When cheese total solids reached 600–650 g·kg⁻¹, which occurred after ~43 d of ripening, the cheese microstructure became more compact. The residual water had a slower diffusion rate in the structure, as indicated by the lower variation in the cheese water content with time. Residual water was mainly non solvent. The proteolytic rate in the cheeses decreased, probably due to the lower content in solvent water. The kinetics of the pressing at this stage was characterised by a low flow rate. © Inra/Elsevier, Paris.

goat cheese / non solvent water / hydraulic pressing / desorption / structure

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Résumé — Caractérisation du fromage de chèvre à différents stades de maturation : structure du fromage, composition et détermination de l'eau non solvante. Des fromages de chèvre de type Sainte-Maure ayant des durées d'affinage de 7 à 58 j ont été caractérisés par leur microstructure, leur composition et leur teneur en eau afin de déterminer l'évolution se produisant au cours de l'affinage. La fraction liquide des fromages a été extraite par pressage hydraulique et analysée pour connaître la concentration des constituants se trouvant en solution dans les fromages. La comparaison de la concentration des solutés dans la fraction liquide et dans le fromage a permis de calculer la quantité d'eau non solvante du fromage relativement à deux types de constituants se trouvant entièrement à l'état soluble, des cations, le potassium (K) et le sodium (Na), et une protéine, la β -lactoglobuline (β -Lg). L'eau non solvante pour le K et le Na était respectivement de 1,1 et de 0,8 g·g⁻¹ de caséine (CN) dans le fromage à 7 j, et diminuait un peu au cours de la maturation pour atteindre 0,6 g·g⁻¹ CN pour les deux cations à 58 j d'affinage. Pour la β -Lg, ces valeurs étaient respectivement de 2,1 et 1,3 g·g⁻¹ CN. La valeur de l'eau non solvante pour la protéine était donc ~2,5 fois plus élevée. L'eau totale du fromage diminuait de 4,7 à 1,9 g·g⁻¹ CN, si bien que la proportion d'eau non solvante dans l'eau totale augmentait au cours de la maturation. L'ensemble des résultats obtenus par l'étude de la microstructure, de la cinétique de pressage et de la composition biochimique a permis d'interpréter les observations. Les fromages en début d'affinage ont une structure lâche et hydratée dont l'évolution est caractérisée par une dynamique élevée des phénomènes enzymatiques se déroulant en phase aqueuse, et par une diminution rapide de la teneur en eau, due à une évaporation de l'eau à la surface du fromage et à son renouvellement facile par un gradient d'eau de l'intérieur du fromage vers l'extérieur à travers une microstructure très ouverte et poreuse. À ce stade, l'eau est extractible par pressage avec des rendements élevés. Lorsque la teneur en extrait sec du fromage atteint 600–650 g·kg⁻¹, ce qui correspond à 43 j d'affinage, la microstructure du fromage apparaît comme compacte. L'eau résiduelle est majoritairement de l'eau non solvante. La dégradation protéique se ralentit du fait de la faible teneur du fromage en eau disponible comme solvant. La teneur en eau du fromage ne varie plus que lentement, sans doute du fait que l'eau résiduelle est fortement retenue par les brins de paracaseïne eux-mêmes et de ce fait la diffusion de l'eau résiduelle est plus lente dans ce milieu compact. © Inra/Elsevier, Paris.

fromage de chèvre / eau non solvante / pressage hydraulique / structure

1. INTRODUCTION

Cheese structure is heterogeneous, with some of its constituents present as a solid matrix (paracasein), some as a liquid phase (derived from residual whey) and others as fat, either solid or liquid according to cheese technology and temperature [4]. The properties of the cheese matrix and the composition of the cheese depend on the physico-chemical conditions existing during cheese making [7, 9]. Moreover, the specific conditions arising during the ripening period of each cheese type involve a particular scheme of breakdown of the cheese constituents through proteolysis, glycolysis and lipolysis [5]. These biochemical reactions predominantly proceed in the aqueous liquid frac-

tion of the cheese, and water is often involved in their reactions (e.g., hydrolysis). Thus, characterisation and quantification of the liquid fraction remaining in cheese is useful to determine the concentration of enzymes and substrates [3, 6, 19]. In short, cheese composition, matrix structure and evolution during ripening are highly specific for each type of cheese [2]. Characterisation of a cheese means not only determining its composition and the variation in composition during ripening, but also characterising the spatial organisation of the constituents in the matrix to determine the microstructure [8, 10].

Our objective in this study was to complete the characterisation of a French soft

goat cheese of the Sainte Maure variety. Preceding studies have determined its composition and the variation in composition during ripening, particularly as regards the evolution of the fat fraction, quantification and composition of total fatty acids, amounts of free fatty acids and of specific goat aroma compounds [12]. In addition, a comparison of the properties of goat cheeses made from milks containing different α_s -casein variants has been made through assessing the rheological properties of cheeses, which showed that their paracasein matrices were different [15]. The following work reports on additional observations made to characterise Sainte Maure goat cheese at different ripening stages. The microstructure of the cheese matrix was observed by scanning electron microscopy (SEM). Chemical analysis of the cheeses was performed to determine their biochemical ripening characteristics, gross composition, residual water and proteolysis. Extraction of the liquid aqueous phase of the cheeses by hydraulic pressing and its subsequent analysis was performed to determine the soluble solutes and their concentration, and to calculate the amount of non solvent water (NSW) for three different solutes naturally occurring in cheese, i.e. potassium (K), sodium (Na) and β -Lg, used as markers, according to the method of van Boekel and Walstra [19].

2. MATERIALS AND METHODS

2.1. Cheeses

Cheeses were provided by the Sèvre et Belle factory (Celles-sur-Belle, France). They were soft goat cheeses of the Sainte Maure type, prepared from raw milk according to the traditional process previously described [12]. The manufacture involved very low rennet amounts, 0.06 mL·L⁻¹ milk, and acidification of milk by mesophilic starters up to pH 4.3 before moulding (24 h). Salting was carried out by dusting the cheese surface with dry salt. Spores of *Penicillium candidum* were then sprayed onto the cheeses. Cheeses were transferred to the laboratory ripening room 2 d after their preparation

and ripening lasted for 58 d (13 °C; 90 % humidity). Cheese sampling for analysis took place at approximately weekly intervals.

2.2. Scanning electron microscopy

Observation of cheese microstructure was made on a Philips XL 20 microscope at 10 kV and equipped with a cold-stage Oxford CT 1500. Cheese samples were prepared according to the method described by Rousseau [16] as follows: small pieces of cheese 1 × 1 × 6 mm in size were cut and glued with carbon paste into the holes of a metal sample holder. After rapid freezing by immersion in liquid nitrogen (-190 °C), they were put into the cold-stage chamber and fractionated. They were then transferred to the microscope column at -150 °C. The temperature was increased to -85 °C and the samples were held a further 10 min to allow ice sublimation. Samples were then taken out from the column to the cold-stage chamber to be gold-coated. Finally, they were placed in the microscope column for observation.

2.3. Hydraulic press

The hydraulic press and the general conditions for its utilization, previously described by Salvat-Brunaud et al. [18], were used in our experiments. Cheese samples for pressing consisted of 6 pieces of cheese (total weight ~1.2–1.6 kg) at each stage of ripening. Cheese with rind and mould intact was first frozen (-20 °C), then grated while in a frozen state, and rapidly mixed with sand (150–250 μ m). An adjustment of the proportion of sand added to cheese was necessary, as the 2:1 (w/w) sand/cheese ratio reported by other authors as suitable for cheeses with high total solids (TS) [1, 13, 17, 18] was not suitable for soft cheese. Preliminary experiments, which varied the sand/cheese ratio and total solids of cheeses showed that the amount of sand had to be adjusted as a function of TS in cheeses according to the following relation:

$$\text{Sand g}\cdot\text{kg}^{-1}\text{ cheese} = 0.7 \text{ TS} + 870$$

with TS in g·kg⁻¹ cheese.

For the goat cheeses with a 400–660 g·kg⁻¹ TS content, we used sand/cheese ratios 1.15–1.33.

The sand and cheese mixture was put in a hoop netted with cheese cloth. The temperature of the sand and cheese mixture was ~-5 °C. The hoop was then put on the press (room temperature

-20 °C), and pressing started immediately. Pressure was increased gradually (32 kPa·min⁻¹) up to 3.7 MPa, then a lower gradient was applied (10 kPa·min⁻¹) up to 4.5 MPa and the final pressure was maintained for 20 min. The whole pressing step lasted for 3 h. When pressure was applied, the outflow of liquid started within 15 min and the temperature of the expressed liquid was 15 °C, remaining constant for the duration of the pressing process. A rapid thawing of water in the samples occurred due to a rapid increase in the temperature of cheese during early pressing, meaning that part of the mechanical energy provided by pressing was converted into heat.

2.4. Analyses

The cheeses as well as the extracted aqueous liquid fractions were analysed. The TS contents were determined by weighing before and after drying in an oven (105 °C, 7 h). The fat content was determined by the acido-butyrometric method of van Gulik [21]. The Kjeldahl procedure was used to determine the nitrogen (N) content, which permitted calculation of the protein content ($N \times 6.38$). Total nitrogen matter (TNM) was determined by direct analysis of the samples, cheeses and liquid aqueous fractions. Soluble nitrogen matter (SNM) fractions in cheese were determined as the nitrogenous matter fraction soluble at pH 4.2 (isoionic point of goat casein [CN]), using a 0.98 correction factor to allow for the fat and paracasein volume adjustment during fractionation. CN in cheeses was calculated as $CN = TNM - SNM$. β -Lg was determined by RP-HPLC analysis (Varian 5000; C18 column Vydac, 15 cm length, 4.6 mm $\varnothing$). Eluents were as follows: A, 0.1 % trifluoroacetic acid (TFA) in water and B, 0.1 % TFA in 20:80 (v/v) water/acetonitrile. The gradient applied was 37–57 % of B eluent in 37 min. Detection was at 215 nm. For β -Lg quantification, calibration was made using pure goat β -Lg purified in the laboratory. Sample preparation for RP-HPLC analysis involved a dilution (d) of cheese in water, i.e. 0.2 g in 5 mL water ($d = 0.04$), dispersion by a Turrax blender (8 000 rev min⁻¹, 2 min) and centrifugation (1 000 g, 15 min). The supernatant was collected and filtered (0.45 μ m pore size). Analysis of β -Lg was on 50 μ L of the supernatant. Liquid aqueous fractions of the cheeses were diluted in water 25–100-fold ($d = 0.04$ –0.01), according to the total solids in the sample, with the dispersion mixed by blending with the Turrax

blender and then filtered (0.45 μ m). Analysis of β -Lg was performed on 50 μ L of the solution. K and Na were determined by atomic absorption spectrophotometry (Varian AA 300) on the solubilised ash fraction of the cheeses and on the liquid aqueous fractions [11].

Total water content was calculated as total cheese weight minus total solids (1 000 – TS). Water proportion in the non fat cheese fraction was calculated as $(1\ 000 - TS)/(1\ 000 - \text{fat})$. Total solid content of the cheese fraction remaining in the mould during pressing was calculated from the composition of the residual material in the mould at each time, taking into account the expelled amounts of the aqueous phase (water and TS) and that of the oil.

2.5. Non solvent water

Non solvent water content was calculated as the steric exclusion factor, h , according to the procedure of van Boekel and Walstra [19] from the levels of the completely dissolved constituents determined in cheese (y) and in the corresponding liquid aqueous fraction (x), taking into account the volume occupied by precipitated material and fat on the one hand in cheese, and on the other the respective dilutions made before the analysis:

$$\frac{x(1 - 1.01f - 1.0c - ch)}{y(1 - 1.01f' - 1.0c' - c'h)} =$$

where f and c : respectively represent the fat and CN mass fraction in cheese; f' , c' : respectively represent the fat and CN mass fraction in the cheese solution at dilution d , to be analysed for determination of the cheese composition, thus giving: $f' = fd$; $c' = cd$.

The coefficient relating to CN was maintained at 1.0, as no more colloidal calcium phosphate remained associated to the paracasein in the cheeses owing to the low pH (4.2–4.3).

If we set in Eq. (1), $i = 1 - 1.01f - 1.0c$ and $k = 1 - 1.01f' - 1.0c'$, h could be calculated as:

$$h \text{ g water} \cdot \text{g}^{-1} \text{ CN} = (yk - xi)/(yc' - xc).$$

3. RESULTS AND DISCUSSION

3.1. Composition and structure of cheeses

Cheese composition was determined at each of the 6 sampling periods from 7 to

Table I. Gross composition of goat cheeses (g·kg⁻¹) at different ripening times (7 to 58 d).**Tableau I.** Composition (g·kg⁻¹) des fromages de chèvre aux différents stades de maturation, de 7 j à 58 j.

Ripening time (d)	pH	TS	Fat	TNM	SNM	β-Lg	K	Na
7	4.5	398	208	153	23	2.5	1.59	4.70
14	4.6	414	211	162	31	3.0	1.74	4.40
23	5.0	491	265	194	44	2.8	1.97	6.22
35	5.3	594	313	232	60	2.2	2.33	6.89
46	5.8	653	349	252	67	2.6	2.69	6.54
58	6.0	664	359	254	78	3.4	2.70	7.69
58/7 d ratio		1.67	1.73	1.66	3.4	1.36	1.70	1.64

TS: total solids; TNM: total nitrogen matter; SNM: soluble nitrogen matter; β-Lg: β-lactoglobulin; K: potassium; Na: sodium.

TS : extrait sec total ; TNM : matière azotée totale ; SNM : matière azotée soluble ; β-Lg : β-lactoglobuline ; K : potassium ; Na : sodium.

58 d of ripening (*table I*). An increase in total solids (i.e. 1.67-fold) was observed during ripening, which resulted from the decrease in the water content of cheese due to the evaporation at the cheese surface: the

water proportion in the non fat cheese fraction decreased during ripening from 76 to 52 % (*figure 1a*). This resulted in an increase in all component levels by a quite similar ratio: fat, TNM, K, Na. However, β-Lg had

Figure 1. Soluble nitrogen matter (SN) in goat cheeses during ripening and water content: **a**: SN proportion in TNM (%), water proportion in non-fat cheese fraction (%), SN/water ratio in cheese; **b**: SN production rate during ripening (% TNM per d).

Figure 1. Teneur en matière azotée soluble (SN) des fromages de chèvre au cours de la maturation et teneur en eau ; **a** : proportion de SN dans la matière azotée totale (%), proportion d'eau dans la fraction non grasse du fromage (%), rapport SN/eau du fromage ; **b** : taux de production de SN pendant l'affinage (% de la matière azotée totale par j).

a lower ratio of increase (i.e. 1.36-fold), which could have resulted from an incomplete extraction of the protein before analysis. This may have been due to insolubilisation of the protein after denaturation induced by the low pH of cheese. Only the SNM fraction showed a higher increase (i.e. 3.4-fold), which resulted partly from the TS increase and partly from the proteolytic activity of surface moulds which produced high amounts of soluble peptides and amino acids from paracasein. The SNM/water content ratio in cheese increased linearly during ripening, meaning that proteolysis proceeded continuously in the aqueous phase of the cheese (*figure 1a*). The proportion of the SNM fraction in TNM also increased (*figure 1a*), but at a decreasing rate as the ripening process went on, as shown by the shape of the curve. Such a decrease in the water soluble nitrogen levels has already been observed in many cheeses [20], and could be related to the decrease in water content of cheese (*figure 1b*).

The microstructure of cheeses was investigated by SEM (*figure 2*). The microstructure of a low TS cheese (cheese A, TS = 375 g·kg⁻¹) appeared to have a wide-mesh network of paracasein strands, enclosing the fat globules and surrounding large empty gaps in the structure. Cavities were large in size and appeared to form a continuous void space from the observation of the fracture surface. The paracasein strands and fat globules were unbroken, meaning that the fracture occurred along the void spaces of the microstructure, this having a lower resistance. It is likely that these empty cavities were initially filled with the aqueous liquid phase of the cheese. Such a microstructure would presumably confer a high porosity to the cheese, such that the liquid phase could easily move out, either forced by pressure or by a diffusion gradient in relation to the water evaporation on the cheese surface during ripening. The microstructure of a high TS cheese (cheese B, TS = 625 g·kg⁻¹) was more compact, i.e. showing the differ-

ent strands of paracasein to be quite compact, with cavities of a much smaller size and which were not very interconnected to one another. Paracasein strands and fat globules were cut by the fracture, which meant that the continuous void space present in cheese A had disappeared.

3.2. Cheese pressing

The liquid extracted from the cheeses consisted of two different phases: a supernatant lipid fraction (oil), with an average density of 0.88_{20 °C} (standard deviation, SD = 0.01) and an aqueous fraction, with an average density of 1.05_{20 °C} (SD = 0.03). The volumes collected increased with increasing pressure (*figure 3*). The total amounts of liquids extracted differed according to the stage of cheese ripening. The amount of expressible oil increased from 37 to 120 g·kg⁻¹ during ripening. The amount of the aqueous phase decreased from 450 to 113 g·kg⁻¹ (*table II*). For the 7-d-old cheese (*figure 3a*) a biphasic curve for the amount of the aqueous phase was observed as a function of pressure. The first step (step I), which occurred from the beginning to 1.6–2.0 MPa, was characterised by high flow rates (350–200 mL·kg⁻¹ cheese·MPa⁻¹). The amount (FI) collected during step I was related to the pressure (P) and to the water content of cheese (TW):

$$FI \text{ mL} \cdot \text{kg}^{-1} = (0.58 \times TW \times P) - 145$$

with TW in g·kg⁻¹ and P in MPa. This model explained 76–96 % of the variability according to the ripening stage. During step II, the amount of the liquid fraction collected (FII) remained quite constant, i.e. 64 (SD = 6) g·kg⁻¹ irrespective of the age of the cheese. It was independent of TW and related to the P increase only ($r^2 = 0.98$):

$$FII \text{ mL} \cdot \text{kg}^{-1} = 13.7 \text{ (SD = 3.6)} \times P + b$$

b being the relationship to the amount of liquid expelled during step I.

Figure 2. SEM of two goat cheeses, cheese **A** (TS = 375 g·kg⁻¹); cheese **B** (TS = 625 g·kg⁻¹). Three different magnifications. Scale bar = 5 μm.

Figure 2. Observation en microscopie électronique à balayage de deux fromages de chèvre, l'un (**A**) ayant un extrait sec de 375 g·kg⁻¹, l'autre (**B**) ayant un extrait sec de 625 g·kg⁻¹. Trois grossissements. Barre de référence = 5 μm.

The two aqueous liquid fractions FI and FII corresponding to step I and II were collected separately during pressing (*table II*).

The calculated TS contents of the residual cheese material in the press are reported in *figure 4*. At the end of step I, TS contents

were in the 550–653 g·kg⁻¹ range, corresponding to a mean value of 625 (SD = 58) g·kg⁻¹ for all the cheeses. At the end of step II, TS contents were ~670 (SD = 2) g·kg⁻¹ whatever the cheese, except for the 14-d-old cheese which showed an atypical value.

Table II. Total amounts of liquid aqueous fractions and oil fractions ($\text{g}\cdot\text{kg}^{-1}$ cheese) collected by hydraulic pressing from cheeses with different ripening times, i.e. 7 to 58 d.

Tableau II. Rendements en fraction aqueuse et en fraction huileuse ($\text{g}\cdot\text{kg}^{-1}$ fromage), obtenus lors du pressage des fromages aux différents stades de maturation entre 7 et 58 j.

Ripening time (d)	Total water in cheese	Aqueous fraction			Residual water in cheese	Total fat in cheese	Oil fraction	Residual fat in cheese
		FI	FII	Total				
7	602	391	59	450	152	208	37	171
14	586	335	60	394	196	211	57	154
23	509	281	57	338	169	265	61	204
35	406	185	70	263	146	313	98	215
46	347	92	71	163	187	349	102	247
58	336	46	67	113	226	359	120	239
Average value		64			179			
SD		6			30

Figure 3. Amounts of liquid fractions ($\text{mL}\cdot\text{kg}^{-1}$ cheese) collected from goat cheeses as a function of the applied pressure in cheeses after different ripening times from 7 to 58 d; (a): aqueous fraction; (b): oil fraction.

Figure 3. Quantités ($\text{mL}\cdot\text{kg}^{-1}$ de fromage) de fraction aqueuse (a) et de fraction grasse (b) collectées par pressage hydraulique à partir des fromages de chèvre à différents stades de maturation, de 7 à 58 j.

3.3. Composition of the aqueous fractions

The concentration of TS, K and Na in the aqueous fraction FI of cheeses showed a 2.6-fold increase during the course of ripening (table III), which was higher than that of the original cheese (~ 1.6 -fold; table I). A higher increase was observed for TNM (6.9-fold), which was due to the TS increase and also to the increase in soluble nitrogen resulting from CN proteolysis. In fraction FII, concentrations of most solutes were lower than in FI for the same cheese. The amounts of TS were lower by $20\text{ g}\cdot\text{kg}^{-1}$ in FII. Higher differences were observed for β -Lg, with β -Lg levels in FII being 50 % lower than in FI. For example, β -Lg levels in 7-d-old cheese were respectively $6.5\text{ g}\cdot\text{kg}^{-1}$ in FI and $3.0\text{ g}\cdot\text{kg}^{-1}$ in FII. For K and Na a difference was also observed, but this was not as great or as consistent.

One may question whether the freezing of cheeses could have induced such differences in the composition of FI and FII. Freezing of the aqueous fractions in cheese certainly induced concentration gradients of the solutes. However, this was overcome in our experiments during cheese preparation as the frozen cheeses were grated and mixed with sand into a homogeneous mix, lead-

Figure 4. Evolution of total solids in the cheese remaining in the mould during pressing (g.kg^{-1} cheese).

Figure 4. Évolution de la teneur en extrait sec du fromage dans le moule au cours du pressage (g.kg^{-1} fromage).

ing to a uniform average composition of cheese in all the parts of the mould. Moreover, thawing was achieved in one step at the beginning of pressing by the heat due to the process of compression, which was evenly distributed over the entire volume, so that we could expect a constant composition for the thawed and expelled liquids.

Concerning the soluble minerals in cheese, it could be possible that the mineral equilibria were modified by concentration gradients during freezing and that these might not be strictly reversible at thawing and that some ions, precipitated during the concentration steps, were not resolubilised. However, this was unlikely as the pH remained at quite low values. Concerning the oil fraction, the measured density, D (0.88) was low compared with that of cow milk fat ($D = 0.92$). Some of the longer-chain triglycerides could remain crystallised, thus decreasing the D of oil. However, it is also possible that it was a specific characteristic of the fat in goat milk, as no value for its D has previously been reported.

3.4. Non solvent water (NSW) in cheese

The concentration of entirely soluble constituents such as K or Na was higher in the aqueous fraction (*table III*) than in the cheese (*table I*). This could mean that the volume of the solvent phase in cheeses was different for the two kinds of solutes. The amount of non solvent water (h) was calculated according to the method of van Boekel

Table III. Gross composition of liquid aqueous cheese fractions collected by hydraulic pressing (g.kg^{-1} liquid aqueous fraction). Abbreviations as in *table I*.

Tableau III. Composition de la fraction aqueuse des fromages recueillie lors du pressage à six stades de maturation, de 7 à 58 j (g.kg^{-1} de fraction aqueuse). Abréviations, voir *tableau I*.

Ripening time (d)	FI						FII		
	pH	TS	TNM	β -Lg	K	Na	TS	K	β -Lg
7	4.7	92	19.3	6.5	3.04	8.4	70	2.61	3.0
14	5.0	87	31.8	7.4	3.41	9.8	73	3.07	3.8
23	5.2	114	56.7	8.0	4.18	11.7	120	3.88	3.8
35	5.4	163	83.9	11.3	5.82	17.3	148	5.96	6.8
46	5.6	203	108.9	12.8	6.74	19.7	183	7.06	8.7
58	5.7	242	133.4	14.4	7.69	21.2	214	7.86	10.4
58/7 d ratio		2.6	6.9	3.1	2.5	2.5	3.1	3.0	5.1

and Walstra [19] for three different cheese constituents, i.e. K, Na and β -Lg, comparing the analytical determinations made on the cheeses and on the aqueous fraction FI. NSW values obtained at different ripening stages of the cheeses, expressed as gram of water per gram of total CN ($\text{g}\cdot\text{g}^{-1}$ CN) as a function of total solids in the cheeses, are presented in figure 5.

3.4.1. NSW in fresh cheeses

NSW values in 7-d-old cheeses with a TS value of $\sim 400 \text{ g}\cdot\text{kg}^{-1}$ were $2.1 \text{ g}\cdot\text{g}^{-1}$ CN for β -Lg, and 1.1 and $0.8 \text{ g}\cdot\text{g}^{-1}$ CN respectively for K and Na. The accuracy of the

Figure 5. Amounts of different kinds of water obtained in goat cheeses at different ripening stages, as a function of cheese total solid content (TS, $\text{g}\cdot\text{kg}^{-1}$). Water in $\text{g}\cdot\text{g}^{-1}$ CN. Total water (total), NSW for K (non sol. K), Na (non sol. Na) and β -lactoglobulin (non sol. β -Lg). Residual water after step I pressing (Pr step I) and step II pressing (Pr step II).

Figure 5. Quantification des différents types d'eau ($\text{g}\cdot\text{g}^{-1}$ CN) observés dans les fromages de chèvre à différents stades de maturation, en fonction de l'extrait sec du fromage ($\text{g}\cdot\text{kg}^{-1}$). Eau totale (total), eau non solvante pour K (non sol. K), Na (non sol. Na) et β -lactoglobuline (non sol. β -Lg). Eau résiduelle après l'étape I du pressage (Pr step I) et l'étape II du pressage (Pr step II).

determination, calculated from the precision of the analytical methods and from the repeatability obtained, was $\sim 0.15 \text{ g}\cdot\text{g}^{-1}$ CN for K and Na, and $\sim 0.63 \text{ g}\cdot\text{g}^{-1}$ CN for β -Lg expressed as SDs. The NSW value obtained for β -Lg was higher than for K and Na presumably due to its larger molecular weight, leading to a different steric exclusion volume [3]. In contrast, it was lower than that reported for cow milk β -Lg, i.e. $3.4 \text{ g}\cdot\text{g}^{-1}$ CN [19] and closer to the NSW found for the whole whey protein fraction, i.e. $2.4 \text{ g}\cdot\text{g}^{-1}$ CN. β -Lg values for cow milk were determined on liquid milk, which is obviously a substance that is very different from goat cheese, and could explain the observed differences. Moreover, goat β -Lg does not have the same ability to polymerise [14] as bovine β -Lg, so that its apparent molecular weight in solution remains unchanged whatever the pH or concentration. This could contribute to the differences between goat and cow milk, as the amount of NSW is related to the molecular weight of the reference component [3]. On the other hand, the high NaCl content in goat cheeses, $19.7 \text{ g NaCl}\cdot\text{g}^{-1}$ water in the 7-d-old cheese, could contribute to lowering the NSW value for β -Lg in the same manner as that reported for lactose [3].

The NSW values for the minerals K and Na in goat cheeses were far higher than those usually reported for cow milk or cheese slurries from cow milk, which were in the 0.1 – $0.2 \text{ g}\cdot\text{g}^{-1}$ CN range [3]. One study has mentioned a higher value, $0.55 \text{ g}\cdot\text{g}^{-1}$ CN, obtained from measurements of chloride (Cl) concentration [3]. If freezing were connected with the increase in NSW values, we would also have found a higher value for β -Lg, which was not the case. The high values of NSW obtained could mean that strong ionic effects were involved.

3.4.2. NSW in ripened cheeses

During ripening, a decrease in NSW occurred. For both K and Na, the final values were $0.6 \text{ g}\cdot\text{g}^{-1}$ CN, and the average val-

ues over the whole ripening period were 0.7 ($SD = 0.3$) $\text{g}\cdot\text{g}^{-1}$ CN. For β -Lg, the NSW decreased to 1.3 $\text{g}\cdot\text{g}^{-1}$ CN, with a mean value of 1.7 ($SD = 0.3$) $\text{g}\cdot\text{g}^{-1}$ CN. Ripening processes influenced in different ways (pH variation, proteolysis, total water content) and modified the parameters included in the calculation of NSW (amount of soluble K and Na, β -Lg, CN level). The increase in pH due to the growth of the surface flora may increase the protein charge and result in some increased ion binding to casein [2], which may then decrease the proportion of soluble K and Na, and could lead to the decrease observed in the corresponding NSW value. The β -Lg mass balance in cheeses at the end of ripening could not account for $\sim 18\%$ of β -Lg; this loss was either due to proteolysis or to an insolubilisation of the protein. In either case, this could interfere with NSW calculations as the amounts of β -Lg in the cheeses and in the aqueous fractions were both affected. Proteolysis altered the CN value which was kept as a reference for NSW determination, as CN was calculated as $\text{TNM}-\text{SNM}$. The activity of surface mould proteases gave rise to high amounts of pH 4.2 soluble peptides, which were quantified in SNM and thus considered as non CN material in the CN calculation ($\text{TNM}-\text{SNM}$). However, it seems likely that many of them, particularly those with a higher molecular mass, could have participated in the exclusion phenomena. This would result in a lowering of the CN value and thus to an increase in the values of NSW as ripening proceeded. As a result of these changes, it is likely that the surface area of the protein matrix was different. Moreover, the lowering of the total water content in the cheeses during ripening might be related to a decrease in all the NSW values, as has been observed by Geurts et al. [3].

3.4.3. Total water in cheeses

Total water content in cheeses has also been reported in *figure 5*. It amounted to 4.7 $\text{g}\cdot\text{g}^{-1}$ CN in fresh cheese and decreased

during ripening to 1.9 $\text{g}\cdot\text{g}^{-1}$ CN in cheeses with a TS level of 664 $\text{g}\cdot\text{kg}^{-1}$. Thus, during the course of ripening, total water content decreased greatly, while NSW for either β -Lg, K or Na, remained reasonably constant. Consequently, at the end of ripening the water content was mainly NSW.

3.4.4. Residual water after pressing

Residual water content remaining in cheeses after steps I and II of hydraulic pressing has also been reported in *figure 5*. Water collected during step I was mainly solvent water for both minerals and β -Lg except for the cheeses with the highest TS, in which some NSW for β -Lg was also expelled in this fraction. During step II, the liquid fraction obtained was solvent water for minerals but not for β -Lg, which was in agreement with the analytical results obtained for FII composition and which is shown in *table III*.

4. CONCLUSION

Some conclusions regarding cheese structure were reached from the results. The microstructure of fresh goat cheese consisted of a smooth network of paracasein strands originating from the renneted and acidified micelles (pH 4.2), with the fat globules evenly distributed and large pores between the strands enclosing large amounts of water. This open structure seemed to include the liquid aqueous fraction as a continuous phase. Water was present mainly as solvent water (55% of the total) which favoured the development of proteolytic reactions and the diffusion of water in response to the gradient produced by water evaporation (from the centre to the surface of the cheese). Thus, in the early stages of ripening, the changes in cheese composition were rapid, involving an increase in soluble nitrogen as well as a decrease in water content, down to a 'water in non fat cheese' fraction in the range 52 – 53% . This

value corresponded to a TS level in cheese of 600–650 g·kg⁻¹. At this stage, the residual water was mainly non solvent water (70 % of the total). The same TS limit was observed as ripening proceeded and during hydraulic pressing of the cheeses at the end of step I, with the pressure causing a high flow rate of water, which stopped at approximately the same cheese TS, i.e. ~600–650 g·kg⁻¹.

As cheese water content decreased, cheese microstructure changed to become more compact, with very few cavities remaining between the strands. This also highlighted the initial mobility of the network and the ability of the strands to reorganise and form a more compact network. This resulted in a microstructure in which the paracasein strands and fat globules were more closely packed. The residual water was present as a dispersed phase, contained partly in the residual cavities and partly in the paracasein strands themselves. Such a dispersion could explain the properties observed: a higher resistance to compression, a lower accessibility of water for solutes in relation to the high amount of non solvent water, a lower porosity that decreased the diffusion rate of water and led to a reduced variation in the water content of cheese at the end of ripening.

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