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Whey drainage during soft cheese manufacture and properties of drained curd as modified by casein concentration, whey protein to casein ratio, and pasteurisation of milk

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Abstract — Soft cheeses were prepared from milk modified by membrane technologies to modify the casein level (CN, 27–37 g·kg^{-1}) and the whey protein to casein ratio (WP/CN, 0.12–0.24). Characterisation of milk coagulation, of whey drainage kinetics and of the drained curd was performed to quantify the effect of the parameters on the process. Moreover, the effect of pasteurisation was tested at 2 casein levels. No effect of WP/CN was observed on coagulation nor drainage. Pasteurisation induced a decrease in the drainage rate at the beginning of the process finally resulting in the same amount of whey expelled. CN had an effect on coagulation, on drainage and on drained curd composition.

whey drainage / casein / whey protein / pasteurisation / soft cheese manufacture

Résumé — **Étude de l'égouttage et caractérisation du caillé égoutté en fromagerie à pâte molle, en relation avec la composition du lait (teneur en caséine et proportion protéines solubles/caséine) et la pasteurisation.** Un lait de fromagerie a été traité par des technologies à membranes afin de modifier la teneur en caséine (CN, 27–37 g·kg^{-1}) et le rapport de la teneur en protéines solubles à la caséine (WP/CN : 0,12–0,24). Par ailleurs, l'effet de la pasteurisation a été testé aux 2 teneurs en caséine. Des fromages de type pâte molle ont été préparés et les paramètres de coagulation et d'égouttage ainsi que les propriétés du caillé égoutté (composition et rhéologie) ont été déterminés. Aucun effet de WP/CN n'a été observé sur l'égouttage. Par contre la pasteurisation entraîne un retard de

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l'égouttage. Moins de sérum est recueilli au début de l'égouttage bien que la quantité de sérum total soit la même en fin d'égouttage. La teneur en caséine du lait a un effet sur la coagulation, sur la quantité de sérum obtenue, sur la cinétique de l'égouttage et sur la composition du caillé égoutté.

égouttage / caséine / protéine du lactosérum / pasteurisation / fromage pâte molle

1. INTRODUCTION

Cheesemaking is a separation and concentration process of milk components present as a colloidal dispersion (casein, calcium phosphate and fat), which are largely retained in the product, while the components in soluble form (whey proteins, lactose and some minerals) are separated to form the whey. A good cheesemaking practice implies that cheese is made with a constant composition that maximises financial return and hence, that retention of fat and protein is optimised. This implies several treatments such as protein and fat standardisation of cheese milk, control of the raw milk microflora, and addition of calcium chloride. While the manufacture of cheese from milk concentrated by ultrafiltration has been studied extensively in the last decade [1], there is comparatively little recent information concerning the effect of whey protein to casein ratio on cheese manufacture. Two methods are actually available to modify the whey protein to casein ratio of milk, the microfiltration and the heat treatment. The first is based on the physical separation of components of milk by a membrane process. The second approach is to denature whey proteins into an insoluble form so that they are entrapped in the renneted milk gel and hence in cheese. As a result of milk concentration, less moisture needs to be expelled from the curd and the retention of whey proteins into the curd increases the cheese yield. Pasteurisation of milk was usually applied in cheese manufacture. According to the literature, application of such heat treatment results in a slight increase in pH and in the rennet coagulation time (RCT) of milk [27]. An increase

in cheese yield can occur with pasteurised milk [44]. The presence of heat denatured whey proteins appears to have a relatively small effect on the structure of low pH, high moisture cheeses. Nevertheless, atypical flavours can be produced as a result of the hydrolysis of whey proteins, during the maturation of cheeses [38].

In the present study, the effects of the variation of whey protein to casein ratio and of heat treatment on the early stages of cheese manufacture were investigated, say, on the coagulation and the drainage process. The composition of total whey and the characteristics of curd were also estimated.

2. MATERIALS AND METHODS

2.1. Milk preparation

2.1.1. Experiment I: Study of WP/CN ratio

Raw bulk skim milk from a local dairy factory was concentrated either by microfiltration or by ultrafiltration. Ultrafiltration (UF) was carried out at 50 °C on a $2 \times 3P19$ multichannel ceramic membrane (Membralox, 1.8 m^2 area; 0.05 µm pore diameter, SCT, Tarbes, France) up to a 2.5 volumic concentration factor, to obtain UF-retentate and UF-permeate fractions. Microfiltration (MF) of milk to 2.16 fold concentration was carried out at 52 °C for about 1h30 using a multichannel ceramic membrane, type P19.40 (Membralox, 1.4 m^2 area; 0.1 µm pore diameter, SCT, Tarbes France). MF-Retentate fraction was combined with the UF-permeate in order to obtain milk with different casein

concentrations (about 27 and 37 g·kg⁻¹) and a constant whey protein to casein ratio (about 0.12). Milk with a WP/CN ratio equal to 0.24 was obtained by ultrafiltration, from the mix of UF retentate and permeate. Thermised cream containing about 450 g fat·kg⁻¹ was added to milk to obtain a fat/casein ratio equal to 1.0. The milk was then heat treated at 72 °C for 20 s, using an Actijoule apparatus (Actini, Evian-les-Bains, France).

2.1.2. Experiment II: study of pasteurisation

Raw bulk milk was concentrated by ultrafiltration up to a 2.5 volumic concentration factor. UF-retentate and permeate were mixed to obtain milk at 27 or 37 g·kg⁻¹ CN concentration. Thermised cream was added as above to the milk, and milk samples were or were not heat-treated at 72 °C for 20 s (HT).

2.2. Curd preparation and drainage

Curd preparation was made with 2 kg of cheese milk. An addition of 1.54 g·100 L⁻¹ of a mesophilic starter was made (MM100 from Texel, Dangé-St-Romain, France), which corresponded to 7×10^6 CFU·mL⁻¹ in milk and a preincubation was performed during 17 h at 12 °C. The milk was then warmed up to 33 °C and poured into a polypropylene vat ($15 \times 15 \times 20$ cm) for incubation. When the pH of milk reached 6.4, renneting was performed by addition of a liquid rennet solution (520 mg·L⁻¹ chymosin, from SBI, Gand Gassiot, France), in order to obtain 7.4 mg·100 L⁻¹ of chymosin. At that time, a milk sample was picked up for coagulation studies. Forty minutes later, the coagulum was cut into 2 cm cubes with rectangular frames having metallic threads and then left to settle for 40 min. Moulding was then performed by pouring the coagulum with a ladle, into a perforated soft cheese mould placed in the drainage room of a pilot device, previously described [13].

At this time, automatic operations of data capture began and the weight of whey was registered over 18 h. The temperature in the chamber was set at the following values: 28 °C for 1 h; 25 °C for 4 h; 20 °C for 11 h; 18 °C for 2 h. Two turnarounds of the draining curd were done, after 1 h and 4 h of drainage respectively. Repeatability of the whey drainage kinetics was within 0.34 to 1.8%, as previously determined [14].

2.3. Characterisation of whey drainage kinetics

Each whey drainage kinetics curve was fit to a descriptive mathematical model, chosen of the exponential type, owing to the general shape of the curve. The equation used was:

$$W \, (g·kg^{-1}) = W1 \, (1 - \exp(-t/\tau1)) + W2 \, (1 - \exp(-t/\tau2)). \quad (1)$$

It involved 2 steps and 4 parameters, $W1$, $W2$, $\tau1$ and $\tau2$. W was the total weight of whey expelled at time (t), $W1$ and $W2$, the whey amounts obtained at the end of steps 1 and 2, and $\tau1$ and $\tau2$, the kinetics times of the step 1 and 2 of whey drainage. This equation was already used in a previous work [13, 14]. A 2 step exponential was chosen to describe W, as it gave a better adjustment to experimental data than a single step equation. Values for the parameters $W1$, $W2$, $\tau1$ and $\tau2$ were calculated for each experiment by fitting the W values of equation (1) to the experimental values of the whey drainage kinetics, using the least square method. Each experiment thus led to one value for each of the 4 parameters of the equation. The values obtained for the parameters were then analysed by a multiple regression analysis, keeping as variables the levels of the factors in the experimental design. This led to a quantification of the effects of factors on the parameters involved in equation (1) and to the determination of their average value in the design.

2.4. Characteristics of drained curd

2.4.1. Rheological properties of drained curd

Rheological properties of the drained curd were examined with an Instron Universal testing machine (model 4501) using the serie IX software. A compression test using a 100 N load cell and a plate of 60 mm was performed. Sampling was performed on the drained curds maintained at 12 °C and involved the cutting of 8 cylinders (20 mm in diameter and 20 mm in height), 5 near the ring and 3 at the centre of each piece of curd. They were equilibrated at this temperature for 1 h prior to compression. The crosshead speed was of 30 mm·min^{-1} from the surface of the sample towards 17 mm inside the curd. The rheological parameters were the fracture stress (Pa), the fracture (Hencky) strain and the Young's modulus (Pa). The fracture stress data were corrected for the increase of the cylinder surface during compression assuming a constant volume for the sample.

2.4.2. Scanning electron microscopy

Observation of curd microstructure was made on a Philips XL 20 microscope at 10 kV and equipped with a cold-storage Oxford CT1500. Curd samples were prepared according to the method described by Rousseau [39], as follows: small pieces of curd $1 \times 1 \times 6$ mm in size were cut at 10 mm of the ring and 5 mm of the surface and placed into the holes of a metal sample holder containing carbon paste. After rapid freezing by immersion in liquid nitrogen (−190 °C), they were put into the cold-storage chamber and fractured. 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-storage chamber to be gold coated. Finally, they were placed in the microscope column for observation. A cold storage technique was chosen as it maintained curd structure integrity, concerning fat globules as well as the protein network, thus providing more complete information on the native structure.

2.5. Analytic determinations

Rennet coagulation parameters were determined on an aliquot of cheese milk at pH 6.4, picked up from the cheese vat just before renneting, and coagulum firmness was determined at the cutting time of curd (40 min), using a Formagraph method at 33 ± 0.1 °C, on the aliquot of cheese milk at pH 6.4 picked up from the cheese vat before renneting. The rennet coagulation time (R), the firming time (k20), and the firmness at cutting time (a40) were measured.

Biochemical analyses were performed on milk (m), whey (w) and drained curd (c), according to Daviau et al. [15]. They included the determination of total nitrogen matter (TN) from Kjeldahl determination of N (N $\times$ 6.38), and of the non-casein nitrogen matter (NCN) fractionated according to the Rowland procedure [40], dry matter (DM), fat, ash, total calcium (Ca) and pH. The casein content of milk (CN) was calculated as CN = TNm – NCNm. The water content in the non-fat moiety of drained curd was calculated as: W/nFc = [1 000 − DMc] / [1 000 − Fatc]. Denaturation of whey proteins was quantified as their decrease in solubility at pH 4.6, estimated from HPLC analyses.

2.6. Experimental design

The effects of the factors, CN, WP/CN and HT, were studied using two experimental designs (Tab. I) consisting of the combination of 2 factors at 2 levels. Fat content in milk was varied in the same extent as casein content to maintain a fat/CN ratio equal to 1.0. However, fat was not studied

Table I. Experimental designs performed to study the effect of: whey proteins to casein ratio (WP/CN) and casein concentration (CN, g·kg^{-1}), in Experiment I; heat treatment (HT) and casein concentration (CN, g·kg^{-1}), in Experiment II.

Tableau I. Plans d'expériences réalisés pour tester l'effet des facteurs suivants : rapport protéine du lactosérum sur caséine et teneur en caséine (CN, g·kg^{-1}) dans l'expérience I ; pasteurisation et teneur en caséine (CN, g·kg^{-1}) dans l'expérience II.

Combinations of factor levels			
Experiment I		Experiment II	
CN	WP/CN	CN	HT
27	0.12	27	0
27	0.24	27	72 °C 20 s
37	0.12	37	0
37	0.24	37	72 °C 20 s

separately as a factor so that its effect was included in the variation observed for the CN factor. The range of variation of the factor levels was 27 to 37 g·kg^{-1} for CN, 0.12 to 0.24 for WP/CN and without or with heat treatment. These factor levels were coded as −1 and +1 for the lower and higher level respectively. A correction was made if necessary to consider the real level achieved during the experimentation. A 4-time repetition of one of the combinations (CN−, WP/CN−; CN−, HT+) was performed to test the repeatability. The quantification of the effects of factors on drainage kinetics and rennet coagulation data was made using a linear regression model. Thus was obtained an average value for each of the dependant variables in the design corresponding to the constant b in an equation Y = aX + b and the value of the effects corresponding to each of the 3 factors studied. An effect quantified the variation from the average value induced by a variation of one coded unit of the factor level, meaning from 0 to +1. First order interactions between factors were tested. They measured the difference in a factor effect evenly existing according as the factor was tested at the high or low level of a second factor. Significant effects were retained at the level $P = 0.05$.

3. RESULTS AND DISCUSSION

3.1. Whey protein to casein ratio

3.1.1. Rennet coagulation and whey drainage kinetics

The compositions of milk samples are given in Table II. Increasing the casein concentration of milk led to an increase of rennet coagulation time and reduced the firming time of curd. In contrast, no effect of casein and fat content was reported on the firmness of coagulum at cutting time. The maximal firmness of coagulum was not reached at this time. The coagulation kinetic was probably different at low and high casein level, as it was indicated by the measure of R and k20. The WP/CN ratio had no significant effect on the rennet coagulation properties of milk nor on the curd firmness at cutting (Tab. III). No interaction was observed between these 2 factors. According to the literature, the clotting time is not modified or increases only slightly with milk protein concentration either by ultrafiltration [10, 12, 19, 22, 23] or by microfiltration [18] at pH 6.6. The aggregation phase of coagulum is generally accelerated in concentrated milk, according to the kinetics of Smoluchowski. Longer coagulation times are nevertheless obtained when the milk was enriched in casein and renneted at pH 6.2 [22]. Caron et al. [4] observe an increase in coagulation time and an increase in curd firmness when renneting was performed at pH 6.50 on native milk enriched with different milk retentate powders, up to 4.5 and 5.0 g total protein per kg. According to these authors, the increase in coagulation time is probably due to the higher concentration of casein micelles in enriched milk which, as expected by van Hooydonk et al. [43], must slow down the enzyme diffusion. According

Table II. Mean composition of milk samples in g·kg^{-1} except for WP/CN the concentration ratio.
Tableau II. Composition moyenne des laits modifiés en g·kg^{-1} excepté pour WP/CN le rapport de concentrations.

Experiment I	CN	WP	WP/CN	TN	NPN	NCN	Fat	DM
CN–WP/CN–	27.59	3.86	0.14	33.37	1.78	5.78	28.67	113.52
CN–WP/CN+	28.48	6.03	0.21	36.34	1.843	7.87	27.67	117.46
CN+WP/CN–	39.33	5.17	0.13	46.165	1.665	6.835	39.5	135.245
CN+WP/CN+	39.26	8.38	0.21	49.49	1.83	10.22	37.83	141.06

Experiment II	CN	TN	NPN	NCN	Fat	DM
CN–HT–	28.53	36.46	1.73	7.93	26.5	118.66
CN–HT+	28.45	36.51	1.92	8.06	27.25	118.54
CN+HT–	38.91	49.28	1.95	10.37	39.00	141.93
CN+HT+	39.07	49.34	1.92	10.26	39.82	139.64

Table III. Effect of casein level in milk (CN) and of the whey protein to casein ratio (WP/CN) on the rennet coagulation properties of milk and on the curd firmness at cutting time. ($P = 0.05$). ns: not significant.
Tableau III. Effet de la teneur en caséine du lait (CN) et du rapport protéine sérique sur caséine (WP/CN) sur les propriétés du lait à la coagulation présure et sur la fermeté des caillés au temps de tranchage. ($p = 0,05$). ns : effet non significatif.

	Effect of factors			
Coagulation parameters	CN	WP/CN	Average value	r^2
R (min)	2.2	ns	26.3	0.493
k20 (min)	–1.4	ns	6.3	0.862
a40 (mm)	ns	ns	37.6	0.407

to us, the changes in coagulation parameters could result from both the casein concentration and the changes in the mineral concentration of the milk aqueous phase. Specifically, milk was concentrated at pH 6.6 and renneting was performed at pH 6.4. Colloidal mineral solubilisation increased with the acidification of concentrated milk [2]. Otherwise, the ratio of the amount of acid solubilised minerals to the protein content of milk was lower in concentrated milk during acidification [2]. According to Lucey et al. [32], the removal of colloidal calcium phosphate (CCP) reduced the buffering capacity by 20.9%. In our experiments, pH in the soluble phase was measured by an electrode. So, it can be expected that the measured pH was certainly lower than that existing inside casein micelles, due to the presence of CCP. As a result of milk acidification, a gradient of protons between the soluble phase and the micellar phase can be formed. When milk is concentrated, the solubilisation of CCP during acidification is obtained for lower pH values than for non-concentrated milk [30]. So, in concentrated milk, at a given pH value, the micelle is certainly more mineralised than in

non-concentrated milk, and the buffering at the level of the micelle will be higher. So, the pH value at the surface of the micelle in concentrated milk is probably higher. As a consequence, the primary phase of rennet action could then require a longer time. The concomitant increase of ionic calcium in the aqueous phase of milks with acidification and concentration of milk could promote the aggregation phase of rennet coagulation. This latter will be more rapid in the concentrated milks due to the higher solubilisation of colloidal calcium in the retentate of milk [2, 30]. This resulted in an accelerated aggregation step and in an increased firming rate of the coagulum.

The WP/CN ratio had no significant effect on milk rennet coagulation parameters (Tab. III). So, we can expect that in the present experiment involving pasteurisation, whey proteins, native or partially denatured, did not affect the hydrolysis of κ casein by chymosin nor the subsequent formation of coagulum in the heat treatment applied here. A slight denaturation of whey proteins (5–7%) was reported to occur during pasteurisation of milk [19, 29]. Nevertheless, the reduction of the viscosity of the milk aqueous phase as WP/CN was decreased could have promoted aggregation by decreasing steric hindrance [43].

In Figure 1 the weights of expelled whey are reported for lower (CN 27 g·kg^{-1}) and higher casein levels (37 g·kg^{-1}) at two levels of WP/CN ratio, 0.12 and 0.24 respectively. Increasing CN concentration and also fat concentration in milk, led to a reduction of the weight of expelled whey all along the drainage process. This effect has been previously observed [5, 15, 33, 36], and can be mainly attributed to the reduction of the aqueous phase content of milk as a result of milk microfiltration. The changes in the WP/CN ratio seemed to have no significant effect on the amount of expelled whey. No difference in the course of pH decrease versus time was apparent (Fig. 2) during the drainage process. Nevertheless, the pH of whey was higher for milk at higher CN concentration whatever the WP/CN ratio, due to the retention of colloidal calcium into the curd. Calculation of whey drainage

Figure 1. Whey drainage kinetics for experiments made on milk at low and high casein levels, 27 g·kg^{-1} (—1—) or 37 g·kg^{-1} (—2—). Expanded scale in the inset where differences between the WP/CN = 0.12 (—*—) and WP/CN = 0.24 (——) are evidenced.

Figure 1. Cinétique de l'écoulement du lactosérum pour des laits ayant des taux de caséine égaux à 27 g·kg^{-1} (—1—) ou 37 g·kg^{-1} (—2—). L'expansion d'échelle dans l'insert permet de voir les différences entre WP/CN = 0,12 (—*—) et WP/CN = 0,24 (——).
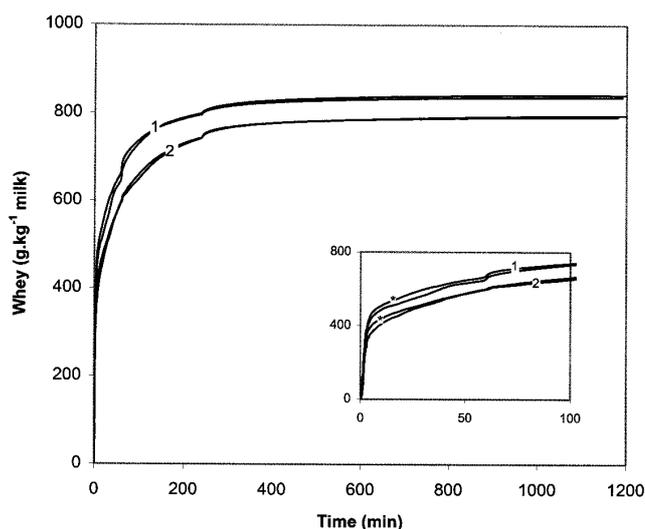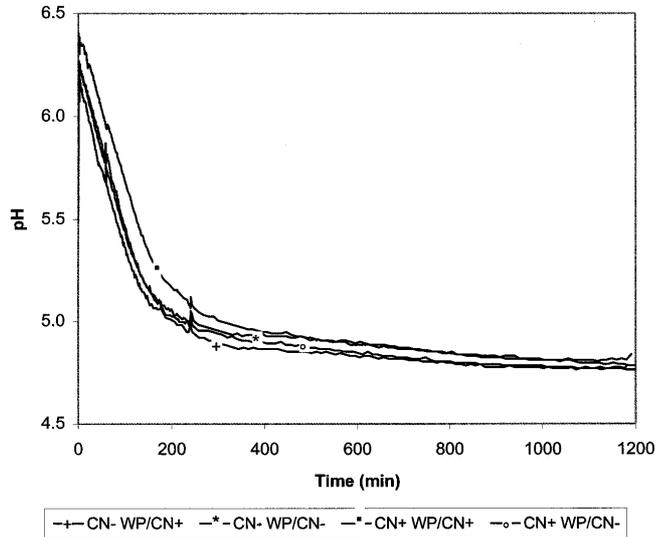
Figure 2. Changes in the pH of whey during the whey drainage kinetics. Experiments reported were made on milk at casein levels 27 g·kg^{-1} (CN−) or 37 g·kg^{-1} (CN+), and at two levels of WP/CN ratio: 0.12 and 0.24.

Figure 2. Évolution du pH du lactosérum pendant la cinétique d'égouttage. Les expériences étaient réalisées sur des laits à des taux de caséine égaux à 27 g·kg^{-1} (CN−) ou 37 g·kg^{-1} (CN+) et à deux niveaux du rapport WP/CN : 0,12 et 0,24.

kinetics parameters from the mathematical equation described in Section 2.3, confirmed these results (Tab. IV). The WP/CN ratio had no significant effect on these kinetics parameters. W1 decreased with increasing casein concentration. A positive effect of CN was also observed on τ2, meaning that the drainage rate was reduced during the second step of the drainage process. The same effect of CN concentration was already reported in previous works [14, 15], nevertheless the values obtained were different, perhaps due to the different technology applied, as ultrafiltration was used to

Table IV. Effect of casein content in milk (CN) and of the whey protein to casein ratio (WP/CN) on the yield of whey obtained after 1 100 min of drainage (g·kg^{-1} milk), and on the parameters of the whey drainage kinetics calculated according to the relation:
W = W1 × (1 − exp (−t/τ1)) + W2 × (1 − exp (−t/τ2)).
W, W1, W2 in g·kg^{-1}; τ1, τ2 in min. *(P = 0.05).* ns: not significant.

Tableau IV. Effet de la teneur en caséine du lait (CN) et du rapport protéine sérique sur caséine (WP/CN) sur les rendements en lactosérum (g·kg^{-1} lait) obtenus après 1 100 min d'égouttage et sur les paramètres de l'équation permettant de décrire la cinétique d'égouttage :
W = W1 × (1 − exp (−t/τ1)) + W2 × (1 − exp (−t/τ2)).
W, W1, W2 en g·kg^{-1} ; τ1, τ2 en min. (*p* = 0,05). ns : non significatif.

| | Effect of factors | | | |
	CN	WP/CN	Average value	r^2
Yield (g·kg^{-1})	−32.6	ns	820.5	0.985
W1	−52.3	ns	456.4	0.794
τ1	ns	ns	3.0	0.190
W2	ns	ns	357.9	0.454
τ2	6.1	ns	90.6	0.519
W1 + W2	−34.5	ns	814.4	0.984

Table V. Effect of casein content in milk (CN) and of the whey protein to casein ratio (WP/CN) on the average composition of the whey collected during the whole drainage (g·kg^{-1}). ($P = 0.05$). ns: not significant.

Tableau V. Effet de la teneur en caséine du lait (CN) et du rapport protéine sérique sur caséine (WP/CN) sur la composition moyenne du lactosérum collecté (g·kg^{-1}). ($p = 0,05$). ns : non significatif.

| | Effect of factors | | | |
	CN	WP/CN	Average value	r^2
DM	2.51	2.12	66.6	0.949
TNM	1.7	1.2[a]	10.0	0.997
NPN	ns	ns	3.0	0.471
TN-NPN	1.6	1.1	7.1	0.985
pH	ns	ns	5.86	0.176

DM, dry matter ; TNM, total nitrogen matter; NPN, non protein nitrogen.
[a] Significant interaction WP/CN × CN = 0.28.

DM, extrait sec ; TNM, matières azotées totales ; NPN, azote non protéique.
[a] Interaction significative WP/CN × CN = 0,28.

increase milk CN concentration in previous work, instead of microfiltration in the current work. The reduction in viscosity with the decrease of the WP/CN ratio was certainly not important enough to observe an increase in whey drainage due to an increase in permeability.

3.1.2. Composition of whey and characteristics of drained curd

The average composition of the whey obtained at the end of the drainage process depended on the composition of milk (Tab. V). The protein content of whey, as judged by the DM and TNM measurements, increased at higher CN concentration and at higher WP/CN ratio, the latter being explained by the heat treatment applied. Drained curds from higher CN milk were drier and more mineralised but their rheological properties were not modified. Different rheological properties of cheese are reported in the literature to be dependent on an increase in DM content and mineralisation of curd due to ultrafiltration of milk [9, 15, 34, 35, 37]. It has been suggested that undenaturated whey proteins act as inert filler in the protein network of curd [16], resulting in a cheese with a smoother texture [31]. This is probably the same explanation for the difference in curd texture according to whether it is prepared from microfiltrated milk or ultrafiltrated milk. Variation of WP/CN ratio did not modify the composition, rheological properties and microstructure of curd (Tab. VI, Fig. 3). The decrease of WP/CN ratio was probably too small to allow the measurement of a change in the structure and also in the texture of curd as expected from the literature, as a result of the incorporation of undenaturated whey protein in the protein network [25, 29, 31].

3.2. Heat treatment

3.2.1. Whey drainage kinetics

The compositions of milk samples are given in Table I. The effect of CN concentration and of heat treatment on whey drainage kinetics was reported in Figure 4 and Table VII. The weight and pH of whey were recorded as a function of time during the curd drainage. Drainage decreased as

Table VI. Effect of casein content in milk (CN) and of the whey protein to casein ratio (WP/CN) on the composition of drained curd (g·kg^{-1} curd) and the rheological properties. P = 0.05. DM, dry matter; TNM, total nitrogen matter; W/nFc: water in non-fat cheese.

Tableau VI. Effet de la teneur en caséine du lait (CN) et du rapport protéine sérique sur caséine (WP/CN) sur la composition du caillé égoutté (g·kg^{-1} caillé). p = 0,05. DM, matière sèche ; TNM, matière azotée totale ; W/nFc : eau dans le fromage dégraissé.

| | Effect of factors | | | |
	CN	WP/CN	Average value	r^2
DM	10.1	ns	417.7	0.683
TNM	7.34	ns[a]	196.1	0.842
Fat	ns	ns	182.5	0.085
Ash	1.36	ns	17.3	0.748
pH	ns[b]	ns	4.82	0.391
W/nFc	−0.013	ns	0.712	0.802
Young modulus (10^3 Pa)	ns	ns	99.6	0.412
Fracture stress (10^3 Pa)	ns	ns	28.3	0.033
Fracture strain	ns[c]	ns	0.421	0.660

[a] Significant at P = 0.10, WP/CN = −1.7; [b] significant at P = 0.08, CN = 0.04; [c] significant at P = 0.08, CN = 0.024.

[a] Significatif à p = 0,10, WP/CN = −1,7 ; [b] significatif à p = 0,08, CN = 0,04 ; [c] significatif à p = 0,08, CN = 0,024.

the protein concentration increased, as may be expected, and was strongly affected by heat treatment. Application of pasteurisation led to a reduction of 43.6 g of the whey amount per kg of milk in the early drainage and, inversely, to an increase in the whey drainage process in the second part of the whey drainage kinetics. The weight of expelled whey of the second part of the drainage process presumably corresponded to the flow of whey not expelled during the first part (Tab. VII). A negative effect of heat treatment on drainage has already been reported [3, 6, 11]. The late effect we observed was never reported in the literature, presumably because the studies of draining times generally did not exceed 200 min. No interaction between heat treatment and casein concentration was observed in the present study. As a consequence, the yield of whey at the end of curd drainage was not modified by the pasteurisation of milk. In contrast, Casiraghi et al. [7] and Smith and McMahon [42] observed that the drainage rate was markedly reduced when heat treatment was applied to milk with a higher protein level; the intensity of heat treatment was different from our own and it was applied before the milk concentration.

These differences were mainly related to the thermal denaturation of whey proteins, which was strongly influenced by factors such as pH, temperature and ionic strength [8, 21]. According to de Wit [17], denaturation of whey proteins at a heat treatment above 60 °C involves an initial loss of their compact globular conformation. On a mild heating, the conformational changes may be reversible, but on a more severe heating, the whey proteins tend to become associated with one another, through hydrophobic interaction or disulfide linkage with the casein micelles [41]. Whey protein denaturation in milk at pH close to the isoelectric point has often been reported in the literature [26, 44]. The rate of denaturation of the milk whey proteins has been estimated at
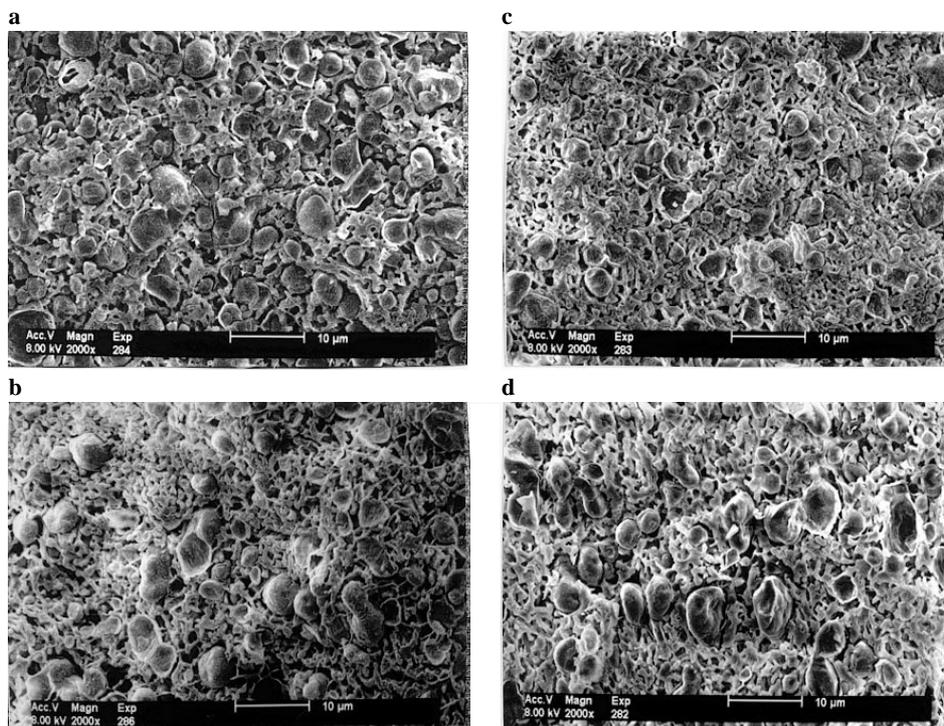
Figure 3. Microstructure of drained curds prepared from milk at casein 27 g·kg^{-1} (CN–) or 37 g·kg^{-1} (CN+), and at two levels of WP/CN ratio: 0.12 (WP/CN–) and 0.24 (WP/CN+); **a**, CN(–) WP/CN (–); **b**, CN(–) WP/CN(+) ; **c**, CN(+) WP/CN(–); **d**, CN(+) WP/CN(+). Bar = 10 μm.

Figure 3· Microstructure de caillés égouttés obtenus à partir de laits à des taux de caséines égaux à 27 g·kg^{-1} (CN–) ou 37 g·kg^{-1} (CN+) et à deux niveaux du rapport WP/CN : 0,12 (WP/CN–) et 0,24 (WP/CN+) ; **a**, CN(–) WP/CN(–) ; **b**, CN(–) WP/CN(+) ; **c**, CN(+) WP/CN(–) ; **d**, CN(+) WP/CN(+). Barre = 10 μm.

Figure 4. Whey drainage kinetics for experiments made on milk at casein levels 27 g·kg^{-1} (—1—) and 37 g·kg^{-1} (—2—), with (—*—) and without (——) heat treatment. The kinetics of pH decrease during drainage are shown and cannot be distinguished from an experiment to another.

Figure 4. Cinétique d'égouttage du lactosérum sur des laits à des concentrations en caséine de 27 g·kg^{-1} (—1—) ou 37 g·kg^{-1} (—2—) avec (—*—) ou sans (——) traitement thermique. Les cinétiques de pH sont présentées et ne montrent aucune différence d'une expérience à l'autre.
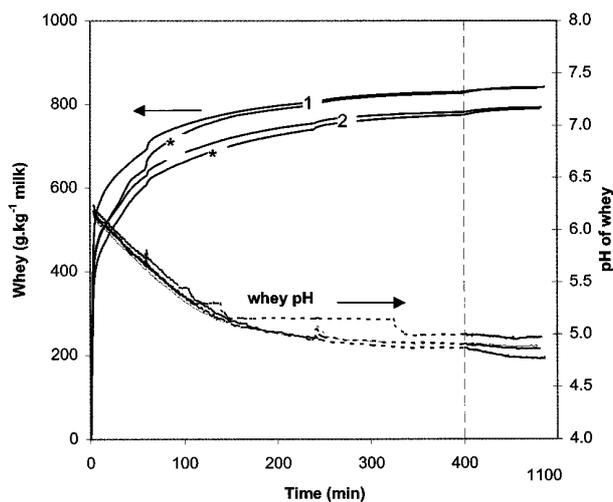
Table VII. Effect of casein content in milk (CN) and heat treatment (HT) on the yield of whey and on the parameters of the whey drainage kinetics calculated according to the relation:
$W = W1 \times (1 - \exp(-t/\tau1)) + W2 \times (1 - \exp(-t/\tau2))$.
W, W1, W2 in $g \cdot kg^{-1}$; $\tau1$, $\tau2$ in min. ($P = 0.05$). ns: not significant.

Tableau VII. Effet de la teneur en caséine du lait (CN) et du traitement thermique du lait sur le rendement de lactosérum et sur les paramètres de l'équation permettant de décrire la cinétique d'égouttage :
$W = W1 \times (1 - \exp(-t/\tau1)) + W2 \times (1 - \exp(-t/\tau2))$.
W, W1, W2 en $g \cdot kg^{-1}$; $\tau1$, $\tau2$ en min. ($p = 0.05$). ns : non significatif.

| | Effect of factors | | | |
	CN	HT	Average value	r^2
Yield ($g \cdot kg^{-1}$)	−30.2	ns	814	0.956
W1	−50.5	−43.6	485	0.922
$\tau1$	ns	ns	3.2	0.186
W2	ns	43.7	325	0.824
$\tau2$	ns	ns	96.9	0.608
W1 + W2	−34.45	ns	814.4	0.984

4.3%, in our experiments, on the basis of the decrease of protein nitrogen solubility at pH 4.6. This value, although lower than the 7% predicted by Lawrence [29] for a mild pasteurisation (72 °C for 20 s or 73 °C for 15 s), cannot explain the differences in whey drainage kinetics observed in our experiments. The conformational change of whey proteins during heating, particularly the unfolding of the protein structure, probably hinders the whey outflow to a higher extent as the pH decreased towards the isoelectric point of whey proteins. As a consequence, the expulsion of whey slowed down during the early drainage. The increase of whey drainage in the second part of the curve could be attributed to the whey content that was not expelled at the beginning of drainage. The loss of affinity of denatured whey proteins for water could be responsible for the improvement of the whey drainage kinetics during the second step of the curd drainage process. The evolution of the pH versus time during curd drainage showed that the pH of whey obtained from pasteurised milk was higher than the one obtained from raw milk in the early drainage. Conversely, it was lower in the second step of the whey drainage kinetics.

These results are essentially due to the change in mineral equilibria between micelles and the soluble phase [20, 24], to lactose hydrolysis by bacteria, and to formic acid production during milk pasteurisation. The increase in pH of whey at the beginning of the process with pasteurisation can be attributed to the incorporation of soluble minerals towards the micelles. Inversely, the reduction of pH of pasteurised milk at the end of the drainage process was in relation to the complete solubilisation of colloidal minerals, the production of lactic and formic acid. The changes in the salt balance and also in the curd mineralisation with heat treatment could affect the structure of curd and consequently the rate of syneresis.

3.2.2. Composition of whey and characteristics of drained curd

Contrary to the CN concentration, heat treatment had no significant effect on the composition of total whey nor on the composition and rheological properties of drained curd (Tabs. VIII and IX). These results are contrary to those of the literature, since higher yield and higher nitrogen recovery were obtained from cheese made

Table VIII. Effect of casein content in milk (CN) and heat treatment of milk (HT) on the average composition of the whey collected during the whole drainage (g·kg⁻¹). ($P = 0.05$). ns: not significant.

Tableau VIII. Effet de la teneur en caséine du lait (CN) et du traitement thermique du lait (HT) sur la composition moyenne du lactosérum collecté (g·kg⁻¹). ($p = 0,05$). ns : non significatif.

	Effect of factors			
	CN	HT	Average value	r^2
DM	ns[a]	ns	70.7	0.933
TNM	2	ns	11.7	0.974
NPN	ns	ns	3.0	0.710
TN-NPN	1.7	ns	8.7	0.952
pH	ns	ns	5.77	0.529

DM, dry matter ; TNM, total nitrogen matter; NPN, non protein nitrogen.
[a] Significant factor ($P = 0.10$) ; CN = 2.9.

DM, extrait sec ; TNM, matières azotées totales ; NPN, azote non protéique.
[a] Facteur significatif ($p = 0,10$) ; CN = 2,9.

Table IX. Effect of casein content in milk (CN) and of heat treatment (HT) on the composition and rheological properties of drained curd (g·kg⁻¹ curd). $P = 0.05$. DM, dry matter; TNM, total nitrogen matter; W/nFc: water in non-fat cheese.

Tableau IX. Effet de la teneur en caséine du lait (CN) et du traitement thermique (HT) sur la composition et les propriétés rhéologiques du caillé égoutté (g·kg⁻¹ caillé). $p = 0,05$. DM, matière sèche ; TNM, matière azotée totale ; W/nFc : eau dans le fromage dégraissé.

	Effect of factors			
	CN	HT	Average value	r^2
DM	11.3	ns	419	0.820
TNM	4.9	ns	195.6	0.895
Fat	ns	ns	179.4	–
Ash	1.12	ns	17.35	0.964
pH	ns	ns	4.79	0.469
W/nFc	−0.013	ns	0.708	0.949
Young modulus (10^3 Pa)	ns	ns	113	0.581
Fracture stress (10^3 Pa)	ns	ns	30	0.172
Fracture strain	ns	ns	0.405	0.513

from pasteurised milk [28, 38, 44]. According to Lau et al. [28], approximately 5% of the whey proteins are associated with casein micelles after pasteurisation. This could result in an increase in cheese yield of about 1–4%. Pasteurisation (63 °C for 30 min) has conversely, little effect on fat recovery in cheese [7, 28] and it has been observed that the elastic modulus of curd obtained from pasteurised milk was lower than those obtained from raw milk. Moreover, the whey obtained from pasteurised milk has a protein content lower than that obtained from raw milk [7].

These results suggest that the effect of the WP/CN ratio and of pasteurisation on the curd drainage process and on curd characteristics depends mainly on the level and the composition of the proteins in cheese, whether whey proteins are undenatured or denatured and whatever the extent of their complexation with casein.

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