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Original article

**Rennet coagulation of skim milk and curd drainage:  
Effect of pH, casein concentration, ionic strength  
and heat treatment**

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**Abstract** — The effect of the pH of skim milk (6.4 to 6.0), the casein concentration (27 to 36 g·kg<sup>-1</sup>), a reduction in the ionic strength to 0.6 compared to that of unmodified milk and the effect of heat treatment on rennet coagulation, gel syneresis and drainage were tested. Coagulation was measured using a formagraph and by viscoelasticity, while syneresis was studied using a centrifugation test. Drainage was followed under conditions similar to those of cheesemaking, using either a mesophilic or thermophilic process. The effects of pH and casein concentration on rennet coagulation, gel syneresis and drainage were in accordance with those reported in the literature. Reduction in ionic strength led to shorter coagulation time and firming time. It also led to an increase in gel firmness, gel syneresis, drainage rate, final amount of expelled whey, solids content and hardness of drained curd. Ionic strength appeared to have an interactive effect with pH on coagulation and with the concentration of casein on syneresis and drainage. This effect of the reduction in ionic strength is discussed.

**rennet coagulation / pH / casein concentration / ionic strength / drainage**

**Résumé** — La coagulation présure du lait écrémé et l'égouttage : effet du pH et de la concentration en caséine, de la force ionique et du traitement thermique. L'effet du pH du lait écrémé (6,4–6,0), de la concentration en caséines (27–36 g·kg<sup>-1</sup>), de la réduction de la force ionique du lait à 0,6 fois celle du lait natif et du traitement thermique a été testé sur la coagulation présure, l'aptitude à la synérèse et l'aptitude à l'égouttage des gels. La coagulation était suivie à l'aide du formagraph et d'un viscoélastimètre, tandis que la synérèse était suivie par un test de centrifugation et l'égouttage était étudié par une méthode proche d'une fabrication fromagère. Les résultats confirment ceux de la littérature pour l'effet des facteurs pH et concentration en caséine sur la coagulation, la synérèse et l'égouttage. La réduction de la force ionique a entraîné une réduction du temps de prise et de

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raffermissement, une augmentation de la fermeté, de la synérèse, de la vitesse d'égouttage, du poids total de sérum égoutté, de l'extrait sec et de la dureté des caillés égouttés. L'effet de la force ionique est apparu en interaction avec le facteur pH lors de la coagulation et en interaction avec la concentration de caséines dans la synérèse et l'égouttage. L'effet de la réduction de la force ionique est discuté.

## coagulation présure / pH / concentration en caséine / force ionique / égouttage

### 1. INTRODUCTION

Many studies have been published on the effect of factors such as concentration of milk, pH and heat treatment of milk on the rennet coagulation of skim milk and on the drainage of the renneted milk gel [7, 29, 30, 33]. Very few of these take into account interactions between these factors by the use of an experimental design [18].

It is well known that reducing the pH of milk from 6.7 to 5.8 leads to faster rennet coagulation [7, 11] and faster syneresis [30]. The effect on milk clotting times of concentrating milk by ultrafiltration by a concentration factor between 1 to 4, and at adjusted pH values was reviewed by Garnot [10]: no effect or a slight increase of rennet clotting time was reported. Concentrating milk by ultrafiltration reduces the rate of syneresis [20, 30]. In highly concentrated milk, the degree of hydrolysis at the cutting time can

be lower than in the case of milk, and it is likely gel formation and syneresis probably will proceed differently [30].

Recent studies have reported the effect of reducing the ionic strength of milk on rennet coagulation by the use of native micelles prepared by microfiltration [8, 9, 21]. Rennet coagulation time (R), firming time (k20) and the firmness at 30 min studied using the formagraph are 14 min, 23 min and 2.8 mm, respectively, for milk and changed at almost 0 ionic strength to 6.5 min, 10 min and 4.4 mm, respectively, at the same pH and casein concentration. Addition of NaCl to milk in the range 17 to 117 mmol·L<sup>-1</sup> has a slight negative effect on syneresis [5], but the effect at higher concentrations is difficult to determine because rennet coagulation takes a very long time, as in the manufacture of Domiati cheese [14]. There has been no study on the effect of reducing ionic strength on drainage of rennet gel.

*List of abbreviations:* a1R, a2R: firmness calculated as the distance between the 2 arms of trace of Formagraph at 2 × and 3 × the rennet coagulation time (mm); cfu: colony forming unit; CN: concentration of casein (g·kg<sup>-1</sup>); D<sub>fract</sub>: displacement at fracture (mm); G: dynamic modulus (Pa); G', G'': storage and loss modulus (Pa); G'<sub>∞</sub>, G''<sub>∞</sub>: extrapolated value of the dynamic modulus at infinite time from Scott-Blair and Burnett's expression (Pa); HT: heat treatment; IS: ionic strength; k20: firming time calculated as the time to reach a distance of 20 mm between the 2 arms of the trace of a Formagraph (s); k<sub>G</sub>, k<sub>G'</sub>, k<sub>G''</sub>: firming time from the Scott-Blair and Burnett's expression for G, G' and G'' (s); M: micelle diameter (nm); NCN: non-casein nitrogen (g·kg<sup>-1</sup>); NPN: non-protein nitrogen (g·kg<sup>-1</sup>); pH: pH factor; PR: type of process; R: rennet coagulation time as the time of separation of the 2 arms of trace of Formagraph (s); r<sup>2</sup>: determination coefficient; SC<sub>curd</sub>: solids content of curd (g·kg<sup>-1</sup>); SC<sub>whey</sub>: solids content of whey (g·kg<sup>-1</sup>); S<sub>max</sub>: hardness as maximum strength at end of compression (N); Si: initial syneresis (%); Sf: final syneresis (%); S<sub>fract</sub>: firmness as strength at fracture (N); TA: loss tangent; t<sub>G</sub>, t<sub>G''</sub>: gel time of G' and G'' in Scott-Blair and Burnett's expression (s); TN: total nitrogen (g·kg<sup>-1</sup>); W: weight of whey collected during time t (g); W<sub>1</sub>: weight of whey during drainage at the end of phase 1 (g); W<sub>2</sub>: weight of whey during drainage at the end of phase 2 (g); W<sub>tot</sub>: W<sub>1</sub>+W<sub>2</sub> (g); τ<sub>1</sub>, τ<sub>2</sub>: characteristic times of drainage during phase 1 and phase 2 (s).

Milk heating hinders rennet clotting, but this effect is reversible provided that no denaturation of  $\beta$ -lactoglobulin occurs [7].

The effects of four factors on the construction of a gel network in renneted milk, on the drainage of the cut gel, and on the properties of the final drained curd were studied. These factors were: concentrations of casein from 27 to 36 g·kg<sup>-1</sup>, milk pH from 6.40 to 6.00, reduction in the ionic strength from 1 to 0.6 compared to that of unmodified milk and a heat treatment at 72 °C for 20 s. This paper is concerned firstly with rennet coagulation, with biochemical and rheological properties of renneted milk gels and secondly with drainage.

## 2. MATERIALS AND METHODS

### 2.1. Modified milk

Raw bulk skim milk was provided by a local dairy. Ultrafiltration was carried out at 52 °C using a pilot plant with ceramic membranes (Membralox, pore size 0.05  $\mu$ m, SCT, France). Milk was fractioned into retentate and permeate fractions. These fractions were mixed and a 50 g·L<sup>-1</sup> lactose solution was added to produce samples with the required casein concentration and ionic strength. The ionic strength of milk was modified by dilution of the aqueous phase. Ionic strength calculation was based on the concentration of potassium in milk, assuming an initial ionic strength of milk of 80 mmol·L<sup>-1</sup> [31]. Heat treatment was performed on an Actijoule apparatus (Actini, Evian les Bains, France) at 72 °C for 20 s. pH adjustment was performed at 30 °C by the gradual addition of 0.1 N lactic acid during rapid stirring. Milk samples were prepared at two levels of casein (about 27 and 36 g·kg<sup>-1</sup>), 2 levels of ionic strength (1 and 0.6 compared to that of unmodified milk, i.e. 80 and 48 mmol·L<sup>-1</sup>), 3 levels of pH (6.40, 6.20 and 6.00) and with or without heat treatment. Thus, there were 24 experiments for the study of rennet coagulation and syneresis.

The casein was concentrated by ultrafiltration. It is well known that ultrafiltration leads to a concentration of both caseins and whey proteins, but as caseins are the constitutive material for rennet gels, we used casein concentration as the main factor of the study, instead of the volume concentration factor.

For curd drainage experiments, only the effects of casein concentration and ionic strength were studied on heat treated milk at its initial pH value of 6.67, thus four experiments. Lactic bacteria were used to reduce the pH of the milk; rennet coagulation time was measured at pH 6.40.

### 2.2. Biochemical analysis

Total nitrogen (TN), non-casein nitrogen (NCN) and non-protein nitrogen (NPN) in milk were determined in duplicate using the Kjeldahl method, with 6.38 as the conversion factor from nitrogen to total protein [23]. Correction factors for differences in volumes between milk and filtrate for NCN and NPN were 0.998 and 0.987, respectively [1, 2]. Casein content was calculated as TN–NCN, and soluble protein as NCN–NPN. Minerals were measured in duplicate on milk and on ultrafiltrate obtained using a CF 25 Centriflo unit (Amicon, France) [15]. The concentrations of calcium and potassium were determined by atomic absorption spectrophotometry [4] and that of phosphorus by a colorimetric method [13].

Micelle diameter was determined in duplicate by a dynamic light scattering method, using the Coulter N4MD instrument (Coultronics, France) at 20 °C after a 1/300 dilution in Dalglish buffer [6]. Diluted milk was stabilised for 10 min in the cell holder at 20 °C and light scattered at 90° for 300 s was measured. The computed auto-correlation function of scattered light is related to the intensity-weighted average diffusion coefficient and to the diameter of the particle assuming a log-Gaussian distribution.

The solids content of whey and curd were determined by drying at 103 °C for 7 h.

The amount of water in drained curds was estimated from the weight of curd and the solid contents of the curd. The casein content of curd was calculated from the concentration of caseins in milk as follows: it was assumed that no casein was lost in whey; a correction was made to take into account the specific breakdown of the  $\kappa$ -casein by rennet (based on a  $\kappa$ -casein content of 12% in total casein, with molecular weights 19 000 g·mol<sup>-1</sup> of  $\kappa$ -casein and 6 500 g·mol<sup>-1</sup> of caseinomacropptide) and the general proteolysis of caseins that occurs during drainage. The latter has been calculated on the basis of the increase in NPN in whey during drainage as 2% of the casein. The increase in NPN was found in the last fractions representing 15% of the total weight of whey. Water content in curd was expressed as g water per g casein.

### 2.3. Rennet coagulation

Milk was held at 33 °C for at least 1 h before renneting. A 520 mg·L<sup>-1</sup> solution chymosin (SBI, Gand Gassiot) was freshly diluted with deionized water. Coagulation was studied at a concentration of 237 µg chymosin per litre of milk.

Rennet coagulation was studied in duplicate using the Formagraph at 33 °C. The values of R, k<sub>20</sub>, a<sub>1R</sub> and a<sub>2R</sub> were calculated, where R is the rennet coagulation time, k<sub>20</sub> is a measure of the firming rate expressed as the time to reach a distance of 20 mm between the 2 arms of the trace, and a<sub>1R</sub> and a<sub>2R</sub> are the firmness values after double and triple the clotting time, respectively [17].

Rennet coagulation was also studied using a Carri-med (CSL 50, Rhéo, France) at 33 °C in the oscillation mode at 1% shear strain and 1 Hz. This was performed with a 60 mm diameter, 3° 58' angle acrylic cone-plate geometry. Renneted milk was

placed at zero time in the gap and covered with liquid paraffin. Measurements began at the time of coagulation as measured by the Formagraph. Experiments were performed in triplicate. G', G'' and the loss tangent were measured. The moduli were fitted using the Scott-Blair and Burnett model [24]:

$$G = G_{\infty} e^{-k_G / (t - t_G)}$$

with G<sub>∞</sub> the value of the modulus extrapolated at infinite time; k<sub>G</sub> the firming time, as the time to reach G<sub>∞</sub>/e; t<sub>G</sub> the time for the beginning of modulus increase, either for G' or G''. The loss tangent at 3R from the renneting time was deduced.

### 2.4. Syneresis test

Two different syneresis tests were performed on milk samples. Firstly, 30 mL of milk were placed into a 50 mL tube, renneted (237 µg·L<sup>-1</sup> milk) and held undisturbed in a water bath at 33 °C. After 3 R from the time of rennet addition, the gel was centrifuged at 1 000 g for 15 min. The expelled whey was poured off, weighed and expressed as g whey per 100 g milk (Si). Secondly, the same procedure was performed, except that the samples were centrifuged at 6 R from the time of rennet addition (Sf). Tests were done in duplicate for each milk sample.

### 2.5. Curd drainage

Curd drainage was performed with heat-treated milk under experimental conditions similar to those used for cheesemaking and the addition of either a mesophilic or a thermophilic starter. Renneting and subsequent curd drainage were performed at a constant temperature, 33 °C for mesophilic starter MM100 and 42 °C for the thermophilic STB05 starter (Texel, Dangé-Saint-Romain, France). The mesophilic or thermophilic starters were added to 2 kg of milk at 7 × 10<sup>6</sup> cfu·mL<sup>-1</sup> or 3 × 10<sup>6</sup> cfu·mL<sup>-1</sup>,

respectively. When the milk reached pH 6.4, it was renneted with 0.912 mL of 520 mg·L<sup>-1</sup> chymosin solution (237 µg chymosin·L<sup>-1</sup> of milk). The gels were cut into 20 mm cubes after 70 or 20 min and then moulded in a soft cheese mould (11 cm internal diameter, with holes only at the bottom, initial height 13.5 cm) after 30 min or 48 min, for the mesophilic or thermophilic process, respectively. The mesophilic and thermophilic processes were performed on the same milk on 2 consecutive days. The mould was placed on a filter plate laid on a funnel. The amount of whey expelled was followed by weighing the whey drained off at 1 min intervals for about 10 min, then at 10 min intervals for 100 min and finally at about 30 min intervals for 18 h or 5 h for the mesophilic or the thermophilic process, respectively.

### 2.6. Rheological characterisation of the drained curd

The drained curds were covered with aluminium foil and stored before measurements at 13 °C for 2 h or overnight for the mesophilic or thermophilic process, respectively. The rheological properties were determined on the whole piece of drained curd without preliminary sampling. A 2.5 cm diameter plate and a 100 N load cell were used with the 4 501 Instron Universal testing machine with Séries IX software (Instron). The plate moved 15 mm or 7 mm into the curd at a rate of 30 mm·min<sup>-1</sup>, for the mesophilic or the thermophilic process, respectively, due to the different heights of the drained curds. Four compression tests were performed on each piece of curd. The maximum strength at the end of compression,  $S_{\max}$  (hardness), the strength at fracture,  $S_{\text{fract}}$  (firmness) and the displacement at fracture,  $D_{\text{fract}}$ , were measured. As thermophilic curd did not fracture, the maximum strength was used. This maximum strength was obtained at different compressions for mesophilic and thermophilic type curds.

### 2.7. Calculations

In the coagulation study, the levels for each factor were coded from -1 to +1 and the variables are listed in Table I. Micelle diameter, rennet coagulation properties and syneresis tests were studied using the linear regression model in Microsoft excel software. The factors were pH (pH), concentration of casein (CN), ionic strength (IS) and heat treatment (HT), the quadratic effect of pH centred on the mean pH squared calculated as  $[\text{pH}^2 - \text{mean value of pH}^2]$  and the two-factor interactions (pH × CN, H × IS, ...). The other effects were presumed to be insignificant and constituted the standard error. The *t*-test allowed cancellation of the insignificant factors at the 1% level. The final equation included significant factors, with the factor pH<sup>2</sup> as the quadratic effect of pH.

In the drainage study, drained curd and whey characterisation, only eight experiments (2 casein, 2 ionic strength levels and 2 types of processes, either mesophilic or thermophilic) were performed. The type of process was coded at PR = -1 for the mesophilic and PR = +1 for the thermophilic one. No extra data were available for the error estimation. Effects such as CN, IS, CN × IS and the type of process (PR) were calculated, but cannot be discussed in terms of significance.

## 3. RESULTS

### 3.1. Milk composition

The composition of the modified milk was as expected with respect to the known effect of the ultrafiltration of milk, heat treatment and dilution of milk with a lactose solution. Mean total nitrogen content was increased from 34.3 to 45.6 g·kg<sup>-1</sup> by ultrafiltration, and also the mean casein content (26.8 to 35.9 g·kg<sup>-1</sup>). NPN was reduced from 1.8 to 1.1 g·kg<sup>-1</sup> from the high to the low level of ionic strength, due to the

**Table I.** Effect of concentration of casein (CN), ionic strength (IS), heat treatment (HT) and pH on the rennet coagulation of milk and gel syneresis; linear regressions with significant factors at 1% level.

**Tableau I.** Effet de la concentration en caséine (CN), de la force ionique (IS), du traitement thermique (HT) et du pH sur la coagulation présure du lait et la synérèse des gels. Régressions linéaires avec les facteurs significatifs au seuil 1 %.

| Equation                                                                                                                                                                  | $r^2$  |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------|
| $M = 190.68 - 2.13 \text{ CN} + 2.27 \text{ IS} - 2.39 \text{ pH} + 1.55 (\text{CN} \times \text{IS}) + 3.56 \text{ pH}^2$                                                | 0.7346 |
| $R = 260.01 + 14.44 \text{ CN} + 44.35 \text{ IS} + 135.04 \text{ pH} + 22.77 (\text{IS} \times \text{pH}) + 60.36 \text{ pH}^2$                                          | 0.9823 |
| $k_{20} = 106.69 - 38.99 \text{ CN} + 31.12 \text{ IS} + 53.84 \text{ pH} - 23.12 (\text{CN} \times \text{pH}) + 24.86 (\text{IS} \times \text{pH}) + 32.34 \text{ pH}^2$ | 0.9666 |
| $a_{1R} = 41.15 + 9.34 \text{ CN}$                                                                                                                                        | 0.8503 |
| $a_{2R} = 52.24 + 9.24 \text{ CN} - 2.10 \text{ IS} - 0.23 \text{ HT} - 1.47 (\text{CN} \times \text{HT})$                                                                | 0.9291 |
| $G'_{\infty} = 216.86 + 81.72 \text{ CN} - 28.79 \text{ IS}$                                                                                                              | 0.8109 |
| $k_{G'} = 490.26 - 27.81 \text{ CN} + 73.70 \text{ IS} + 257.22 \text{ pH}$                                                                                               | 0.9470 |
| $t_{G'} = 269.59 + 43.46 \text{ IS} + 131.88 \text{ pH} + 28.13 (\text{IS} \times \text{pH}) + 81.00 \text{ pH}^2$                                                        | 0.9687 |
| $G''_{\infty} = 62.00 + 23.76 \text{ CN} - 8.36 \text{ IS}$                                                                                                               | 0.8220 |
| $k_{G''} = 513.81 - 31.39 \text{ CN} + 78.98 \text{ IS} + 254.67 \text{ pH}$                                                                                              | 0.9513 |
| $t_{G''} = 273.56 + 41.70 \text{ IS} + 133.17 \text{ pH} + 31.95 (\text{IS} \times \text{pH}) + 71.93 \text{ pH}^2$                                                       | 0.9613 |
| $\text{TA} = 0.27 - 0.0044 \text{ pH} + 0.0071 \text{ pH}^2$                                                                                                              | 0.4691 |
| $\text{Si} = 61.99 - 11.26 \text{ CN} - 5.60 \text{ IS} - 12.10 \text{ pH} - 7.66 (\text{CN} \times \text{IS}) - 8.62 (\text{CN} \times \text{pH})$                       | 0.7525 |
| $\text{Sf} = 68.39 - 8.67 \text{ CN} - 3.40 \text{ IS} - 8.65 \text{ pH} - 7.10 (\text{CN} \times \text{IS})$                                                             | 0.5919 |

M: micelle diameter (nm); R: rennet coagulation time with Formagraph (s); k<sub>20</sub>: firming time (s); a<sub>1R</sub>, a<sub>2R</sub>: firmness at R and 2R after the coagulation time (mm); G'<sub>∞</sub>, G''<sub>∞</sub>: G' and G'' extrapolated to infinite time (Pa); k<sub>G'</sub>, k<sub>G''</sub>: firming times for G' and G'' (s); t<sub>G'</sub>, t<sub>G''</sub>: coagulation time for G' and G'' (s); TA: loss tangent at 2R after coagulation time measured with Formagraph; Si: initial syneresis (%); Sf: final syneresis (%); r<sup>2</sup>: determination coefficient.

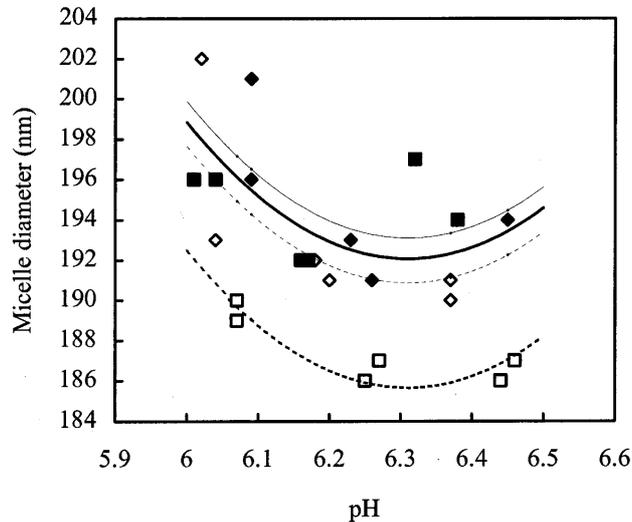
M: diamètre des micelles (nm); R: temps de coagulation présure mesuré avec le Formagraph (s); k<sub>20</sub>: temps de raffermissement (s); a<sub>1R</sub>, a<sub>2R</sub>: fermeté à R et 2R après le temps de coagulation (mm); G'<sub>∞</sub>, G''<sub>∞</sub>: G' et G'' extrapolés aux temps infinis (Pa); k<sub>G'</sub>, k<sub>G''</sub>: temps de raffermissement pour G' and G'' (s); t<sub>G'</sub>, t<sub>G''</sub>: temps de coagulation pour G' et G'' (s); TA: tangente de l'angle de perte à 2R après le temps de coagulation mesuré au Formagraph; Si: synérèse initiale (%); Sf: synérèse finale (%); r<sup>2</sup>: coefficient de détermination.

dilution of the aqueous phase of milk by the lactose solution. Whey proteins in heat-treated milk were reduced on average by 0.71 g·kg<sup>-1</sup> compared to raw milk. Whey proteins increased with the casein level from 6.35 to 8.40 g·kg<sup>-1</sup> for milk without ionic strength modification, and from 5.72 to 7.80 g·kg<sup>-1</sup> for milk with reduced ionic strength. Thus, the concentration of whey proteins in milk modified by dilution with a lactose solution was lower than without lactose dilution, indicating that a small part of whey proteins (less than 10%) was not rejected by the membrane. The reduction of pH from 6.4 to 6.0 led to an increase in the soluble minerals. Mean soluble calcium

represented 28, 33 and 40% of total calcium at pH 6.4, 6.2 and 6.0, whilst mean soluble phosphorus represented 39, 43 and 46% of total phosphorus at the same pH values, respectively. The proportions of soluble calcium and phosphorus were related to the concentration of casein and the ionic strength. At each pH value, the proportion of these soluble minerals decreased when CN was increased and when the IS was reduced (values not shown).

### 3.2. Micelle size

The micelle diameter decreased when CN was increased or when IS was reduced



**Figure 1.** Micelle diameter measured with photon correlation spectroscopy with respect to milk pH. Points are for experimental data at mean values given for concentration of casein and ionic strength. Curves were calculated from regression equations. CN 27.2 g·kg<sup>-1</sup>, IS 48 mmol·L<sup>-1</sup> (---◇---); CN 26.3 g·kg<sup>-1</sup>, IS 80 mmol·L<sup>-1</sup> (—◆—); CN 35.9 g·kg<sup>-1</sup>, IS 51 mmol·L<sup>-1</sup> (-□-); CN 35.9 g·kg<sup>-1</sup>, IS 80 mmol·L<sup>-1</sup> (—■—).

**Figure 1.** Le diamètre micellaire mesuré par spectroscopie de corrélation de photons en fonction du pH du lait. Les points représentent les données expérimentales obtenues aux niveaux moyens de CN et IS indiquées ci-dessous et les courbes sont calculées à partir des équations de régression. CN 27,2 g·kg<sup>-1</sup>, IS 48 mmol·L<sup>-1</sup> (---◇---); CN 26,3 g·kg<sup>-1</sup>, IS 80 mmol·L<sup>-1</sup> (—◆—); CN 35,9 g·kg<sup>-1</sup>, IS 51 mmol·L<sup>-1</sup> (-□-); CN 35,9 g·kg<sup>-1</sup>, IS 80 mmol·L<sup>-1</sup> (—■—).

and a minimum was observed at pH 6.3 (Fig. 1). The determination coefficient ( $r^2$ ) for micelle diameter was low (Tab. I) and only 73% of variations in diameter was explained by the model. This poor adjustment could result from a high standard error for the diameter measurement. The estimated differences between the lower and the higher levels of the factors are in the range of accuracy of micelle size measurement.

### 3.3. Rennet coagulation properties

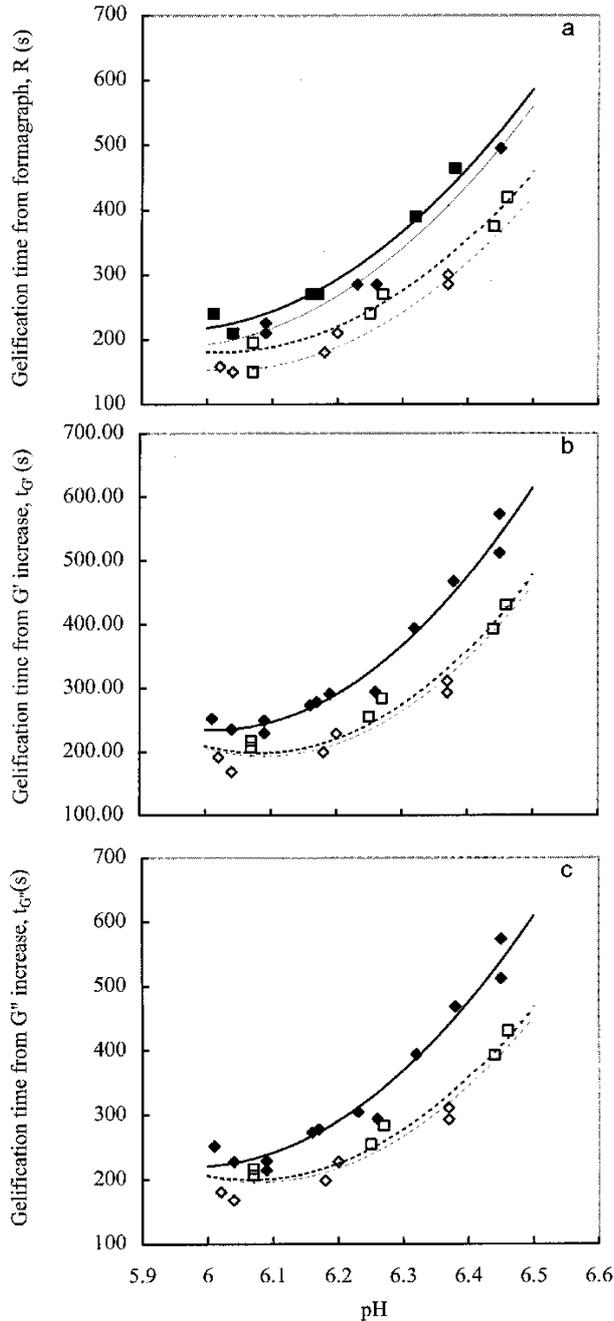
Equations of linear regression (Tab. I) showed that significant effects were obtained for CN, IS and pH factors. The effect of the studied heat treatment (HT) was insignificant, apart on a2R. The effect of heat treat-

ment appeared on a2R, in interaction with casein concentration.

Gel times ( $R$ ,  $t_G$ , and  $t_{G''}$ ) decreased with a reduction in pH (Fig. 2). Reducing CN and the IS led to a reduction in the coagulation time,  $R$  (Fig. 2a), while only an effect of reducing IS was noted for  $t_G$ , and  $t_{G''}$  (Figs. 2b and 2c). For example, at pH 6.5, for a reduction in ionic strength to 0.6 compared to that of unmodified milk, the calculated coagulation time was reduced by about 100 s (Fig. 2a).

Firming time parameters ( $k_{20}$ ,  $k_G$ , and  $k_{G''}$ ) decreased with the reduction of the pH (Fig. 3), apart from  $k_{20}$  at the higher concentration of casein and the lower ionic strength, where the quadratic effect was stronger than the linear effect of the pH, resulting in no relationship between pH and

k20. For these conditions, decreasing the pH led to a reduction of the R parameter, but the k20 parameter did not change. The parameter k20 which had lower values than the two other firming times,  $k_{G'}$  and  $k_{G''}$ , showed a quadratic effect of pH. The



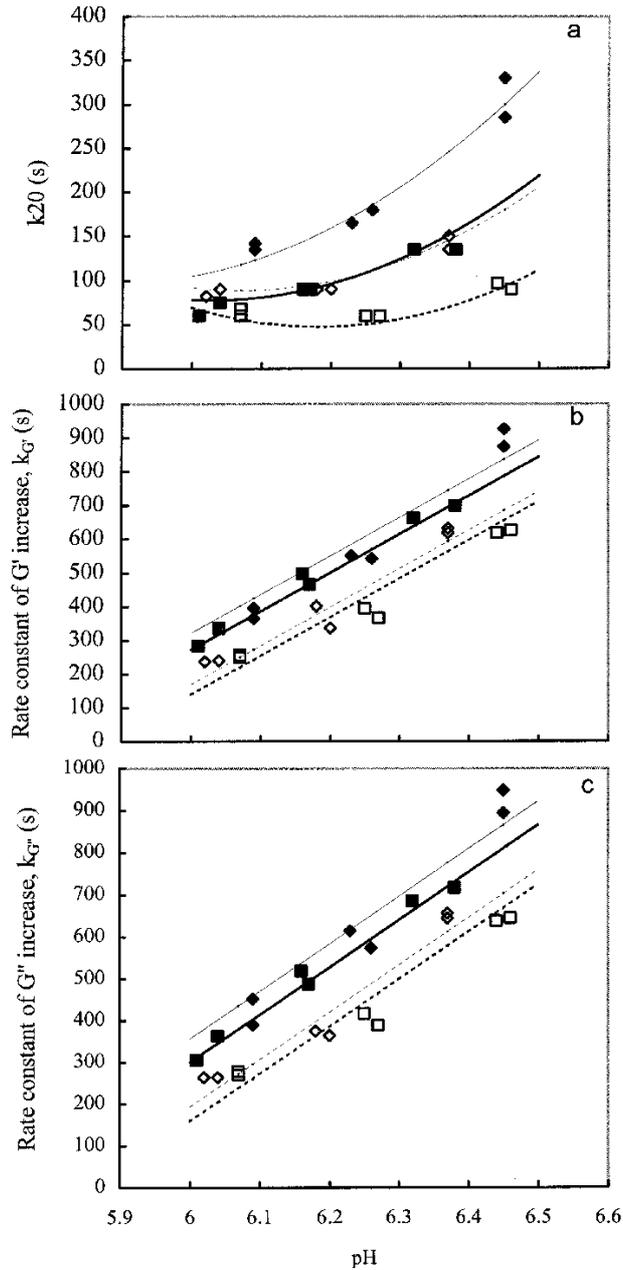
**Figure 2.** Gelification times in s with respect to milk pH. Points are for experimental data at mean values given for CN and IS. Curves were calculated from regression equations. (a) R from Formagraph; symbols as in Figure 1; (b)  $t_{G'}$  from  $G'$  increase; IS  $48 \text{ mmol}\cdot\text{L}^{-1}$  ( $\cdots\Diamond\cdots$ ); IS  $51 \text{ mmol}\cdot\text{L}^{-1}$  ( $--\square--$ ); IS  $80 \text{ mmol}\cdot\text{L}^{-1}$  ( $\cdots\blacklozenge\cdots$ ); (c)  $t_{G''}$  from  $G''$  increase; symbols as in b.

**Figure 2.** Le temps de gel en s en fonction du pH du lait. Les points représentent les données expérimentales obtenues aux niveaux moyens de CN et IS indiqués ci-dessous et les courbes sont calculées à partir des équations de régression. (a) R mesuré au Formagraph ; mêmes symboles que dans la figure 1 ; (b)  $t_{G'}$  obtenu par l'augmentation de  $G'$  ; IS  $48 \text{ mmol}\cdot\text{L}^{-1}$  ( $\cdots\Diamond\cdots$ ) ; IS  $51 \text{ mmol}\cdot\text{L}^{-1}$  ( $--\square--$ ) ; IS  $80 \text{ mmol}\cdot\text{L}^{-1}$  ( $\cdots\blacklozenge\cdots$ ) ; (c)  $t_{G''}$  obtenu par l'augmentation du  $G''$  ; mêmes symboles que dans b.

increase in CN and the reduction of IS led to a decrease in the firming time parameters. The effects of CN and IS on  $k_{20}$  were pH dependent, meaning that at pH 6.0, firming time  $k_{20}$  was shorter and no more depen-

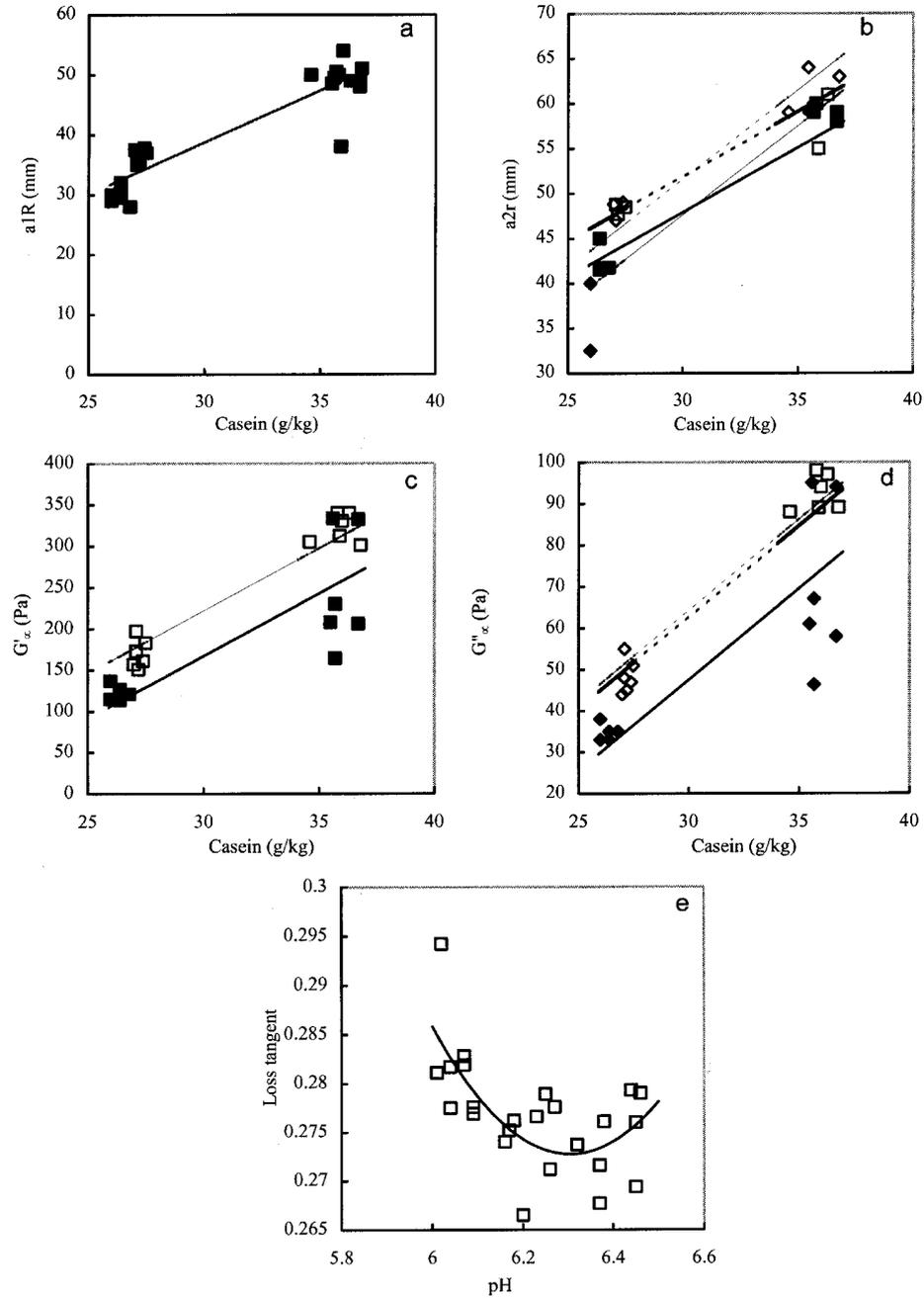
dent on the concentration of casein and the ionic strength value.

The firmness parameters ( $a_{1R}$ ,  $a_{2R}$ ,  $G'_{\infty}$  and  $G''_{\infty}$ ) were dependent on CN. The final firmness parameters,  $G'_{\infty}$  and  $G''_{\infty}$ , were



**Figure 3.** Firming time of rennet gelification in s with respect to milk pH. (a)  $k_{20}$  from Formagraph; (b)  $k_{G'}$  from  $G'$  increase; (c)  $k_{G''}$  from  $G''$  increase; symbols as in Figure 1.

**Figure 3.** Les temps de raffermissement du gel présure en fonction du pH du lait. (a)  $k_{20}$  mesuré au Formagraph ; (b)  $k_{G'}$  obtenu pour l'augmentation du  $G'$  ; (c)  $k_{G''}$  obtenu pour l'augmentation du  $G''$  ; mêmes symboles que dans la figure 1.



**Figure 4.** Firmness of rennet gel with respect to CN and loss tangent of the gel with respect to milk pH. (a) a1R, from Formagraph, in mm, for any condition of the design; (b) a2R, from Formagraph, in mm, IS 50 mmol·L<sup>-1</sup> without heat treatment (—◇—); IS 50 mmol·L<sup>-1</sup> with heat treatment (—□—); IS 80 mmol·L<sup>-1</sup> without heat treatment (—◆—); IS 80 mmol·L<sup>-1</sup> with heat treatment (—◆—).

(—■—); (c)  $G'_{\infty}$  in Pa, the infinite value of  $G'$  extrapolated with the Scott-Blair and Burnett model, IS 50 mmol·L<sup>-1</sup> (—□—); IS 80 mmol·L<sup>-1</sup> (—■—); (d)  $G''_{\infty}$  in Pa, the infinite value of  $G''$ , IS 48 mmol·L<sup>-1</sup> (-□-); IS 51 mmol·L<sup>-1</sup> (—◇—); IS 80 mmol·L<sup>-1</sup> (-◆-); (e) the loss tangent for any condition of the design.

**Figure 4.** La valeur de la fermeté du gel présure en fonction de CN et la tangente de l'angle de déphasage du gel en fonction du pH du lait. (a) a1R mesuré au Formagraph en mm pour toutes les conditions du plan ; (b) a2R, mesuré au Formagraph, en mm ; IS 50 mmol·L<sup>-1</sup> sans traitement thermique (—◇—); IS 50 mmol·L<sup>-1</sup> avec traitement thermique (-□-); IS 80 mmol·L<sup>-1</sup> sans traitement thermique (-◆-); IS 80 mmol·L<sup>-1</sup> avec traitement thermique (-■-); (c)  $G'_{\infty}$  en Pa, la valeur infinie de  $G'$  extrapolée à l'aide du modèle de Scott-Blair et Burnett ; IS 50 mmol·L<sup>-1</sup> (-□-); IS 80 mmol·L<sup>-1</sup> (-■-); (d)  $G''_{\infty}$  en Pa, la valeur infinie de  $G''$  en Pa, IS 48 mmol·L<sup>-1</sup> (-□-); IS 51 mmol·L<sup>-1</sup> (—◇—); IS 80 mmol·L<sup>-1</sup> (-◆-); (e) la tangente de l'angle de perte pour toutes les conditions du plan.

also dependent on IS (Fig. 4). An increase in casein concentration led to an increase in gel firmness. This positive effect of CN on a2R was less pronounced after the heat treatment, as shown by the a2R results. Decreasing IS led to an increase in firmness.

The loss tangent determined at 3R from the renneting time was minimal at pH 6.3 (Fig. 4), but the data were dispersed around the tendency curve. The determination coefficient for the loss tangent was low.

### 3.4. Syneresis of curd

Similar effects were observed for Si and Sf (Fig. 5). The difference between Si and Sf is that the value Sf was obtained after a longer time, and is likely related to the total syneresis and Si is likely to be related to the initial syneresis. Syneresis increased as pH was reduced. For the initial syneresis, this pH effect is greater at a high concentration of casein, while for the final syneresis, this effect of pH appeared to be independent of the concentration of casein. Reducing IS at a low CN led to a slight reduction in syneresis of 5%, while reducing IS at a higher CN led to an increase in syneresis of 20%. The determination coefficient for the syneresis was lower than for the other variables (Tab. I), indicating a poor adjustment of the model to experimental data.

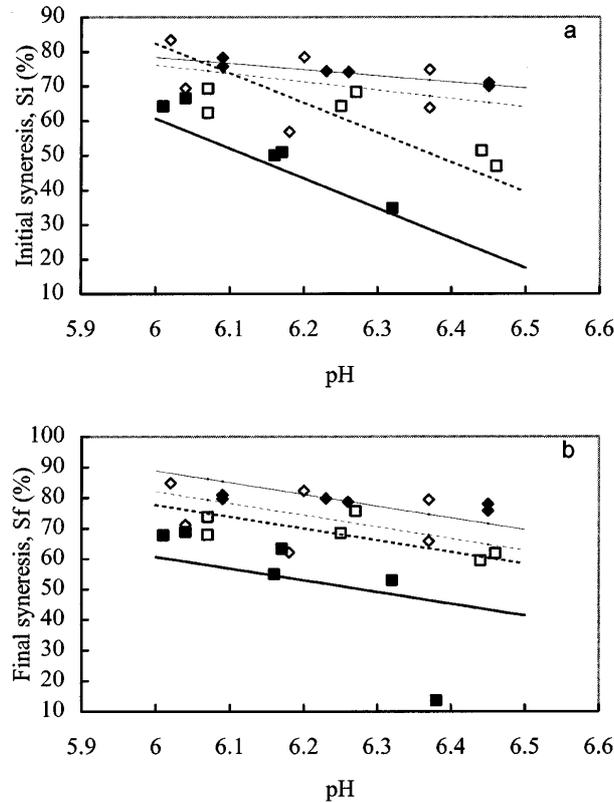
### 3.5. Curd drainage

Drainage curves for milk at 2 concentrations of casein and 2 values of ionic strength with the mesophilic and thermophilic processes are shown in Figure 6. The weights of whey were 1 200–1 400 g within a few minutes for the thermophilic process, while in the mesophilic process, the increase in weight was more progressive. The curves were fitted to a 2 phase exponential equation describing the drainage in 2 successive phases:

$$W = W_1 \left( 1 - \exp\left(-\frac{t}{\tau_1}\right) \right) + W_2 \left( 1 - \exp\left(-\frac{t}{\tau_2}\right) \right)$$

where  $W$  is the whey weight collected during the time  $t$ ;  $W_1$  and  $W_2$  are the final whey weights at the end of the successive phases 1 and 2;  $\tau_1$  and  $\tau_2$  are the characteristic times of drainage during phases 1 and 2, respectively. The characteristic times,  $\tau_1$  and  $\tau_2$ , represented the time to obtain 63% of  $W_1$  and  $W_2$ , respectively. Table II shows values of  $W_1$ ,  $W_2$  and  $W_{\text{tot}}$  ( $W_1 + W_2$ ), and  $3\tau_1$  and  $3\tau_2$ , which are the times needed to reach 95% of  $W_1$  and  $W_2$ .

The effects of the factors on the parameters  $W_1$ ,  $W_2$ ,  $3\tau_1$  and  $3\tau_2$  and their mean values (constant of the linear model) were calculated (calculations are not shown). The results indicated a faster initial rate of drainage in the thermophilic process compared to the mesophilic, presumably due to



**Figure 5.** Syneresis of rennet gels as measured with laboratory test based on gel centrifugation. (a) initial syneresis (at 3 times R); (b) final syneresis (at 6 times R); symbols as in Figure 1.

**Figure 5.** Synèrèse des gels présure mesurée par le test de laboratoire basé sur la centrifugation du gel. (a) synèrèse initiale (à 3 fois R); (b) synèrèse finale (à 6 fois R), mêmes symboles que dans la figure 1.

the higher temperature [20, 30], with a small increase in the final weight of whey. In fact,  $W_1$  (mean value 1 123 g) greatly increased in the thermophilic process (effect = +243 g), while  $W_2$  (mean value 607 g) greatly decreased (effect = -196 g). When IS was reduced,  $W_1$  increased (effect = -51 g),  $W_2$  decreased (effect = +36 g) and  $W_{tot}$  increased (effect = -15 g). These results revealed faster drainage at a lower ionic strength. The increase in the rate of drainage with the reduction of ionic strength was more pronounced at a higher concentration of casein. The increase in concentration of casein led to a slight decrease in the total drainage of -25 g (mean difference for  $W_{tot}$ ). If the ratios between the whey amount ( $W_{tot}$ ) and the water content of milk (1 000-dry matter of milk) are calculated, these ratios that took into account the lesser available

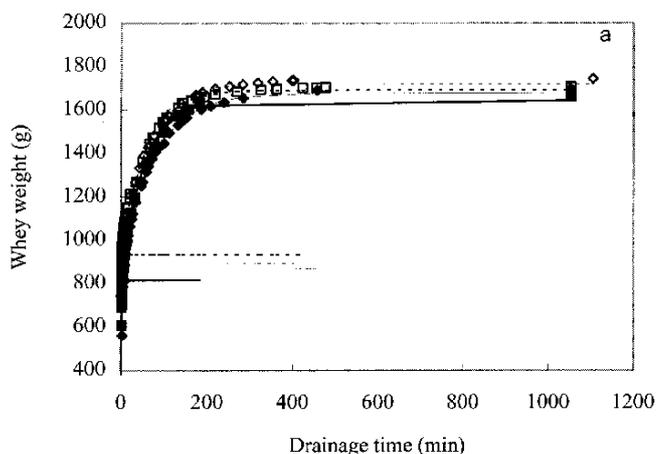
content of water in ultrafiltered milk were not greatly different for the two concentrations of caseins.

The effects were the same on the  $3\tau$ : a reduction of  $3\tau_1$  and  $3\tau_2$  in the thermophilic process compared to the mesophilic one and with the ionic strength reduction, meaning a faster drainage.

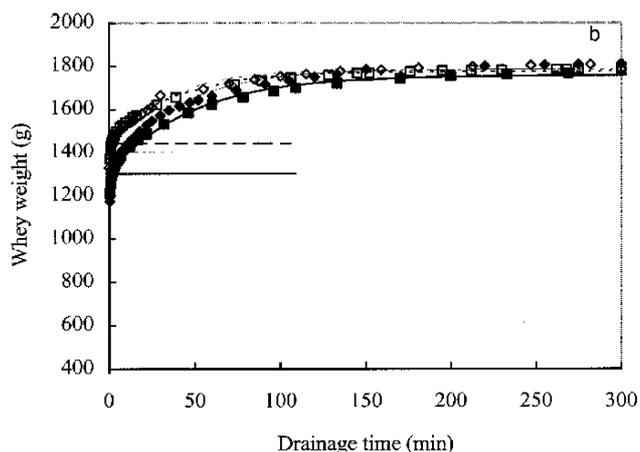
### 3.6. Curd and whey characterisation

The hardness of the curd,  $S_{max}$  (mean value 74 N), was much greater in the thermophilic process compared to the mesophilic one (effect = +9 N) (Fig. 7). Increasing the ionic strength led to a reduction in curd hardness (effect = -14 N) and firmness (effect = -5 N for a mean value of 41 N) for the mesophilic curd (Fig. 7). Increasing the

**Figure 6.** Drainage of rennet gels during time. Curves were calculated using the 2 exponential equation for drainage. Straight lines are for final amount of whey at the end of first step of model. **(a)** in mesophilic process; **(b)** in thermophilic process; CN 26.4 g·kg<sup>-1</sup>, IS 80 mmol·L<sup>-1</sup> (—◆—); CN 36.7 g·kg<sup>-1</sup>, IS 80 mmol·L<sup>-1</sup> (—■—); CN 27.5 g·kg<sup>-1</sup>, IS 48 mmol·L<sup>-1</sup> (—◇—); CN 36.3 g·kg<sup>-1</sup>, IS 51 mmol·L<sup>-1</sup> (—□—).



**Figure 6.** L'égouttage des gels présure. Les courbes sont calculées à l'aide du modèle d'égouttage à 2 exponentielles. Les droites représentent la quantité finale de sérum à la fin de la première phase du modèle **(a)** dans le procédé de fabrication mésophile ; **(b)** dans le procédé de fabrication thermophile ; CN 26,4 g·kg<sup>-1</sup>, IS 80 mmol·L<sup>-1</sup> (—◆—) ; CN 36,7 g·kg<sup>-1</sup>, IS 80 mmol·L<sup>-1</sup> (—■—) ; CN 27,5 g·kg<sup>-1</sup>, IS 48 mmol·L<sup>-1</sup> (—◇—) ; CN 36,3 g·kg<sup>-1</sup>, IS 51 mmol·L<sup>-1</sup> (—□—).



casein concentration led to an increase in firmness (effect = +11 N). Effects on the displacement at fracture were very slight.

The regression equation for the solids content of curd as a function of the levels of factors was:

$$SC_{\text{curd}} = 375.4 + 16.9 \text{ CN} - 20.6 \text{ IS} - 6.7 (\text{CN} \times \text{IS}) + 56.6 \text{ PR}$$

while that of whey was:

$$SC_{\text{whey}} = 67.0 + 2.6 \text{ CN} + 1.6 \text{ IS} - 1.5 (\text{IS} \times \text{CN}) - 1.1 \text{ PR}$$

with PR at -1 and +1 for the mesophilic and the thermophilic processes, respectively.

This means that the solid content of thermophilic curd was higher than for the mesophilic curd and decreased as CN was decreased and with the increase in IS (Fig. 8). Solids content of whey decreased as CN was decreased, as IS was decreased and when the thermophilic process was applied compared to the mesophilic one (Fig. 8). The effect of the concentration of casein appeared to be lower as ionic strength increases (Fig. 8).

The water content of the curd, expressed in g per g casein (Tab. II), decreased with the reduction of IS, with the increase in CN and

**Table II.** Parameters of drainage equation and water retention of rennet curd. Whey was expelled from the gel obtained by rennet coagulation of 2 kg of milk in mesophilic or thermophilic processes. Whey weights were adjusted with the following equation:

$$W = W_1 \left( 1 - \exp\left(-\frac{t}{\tau_1}\right) \right) + W_2 \left( 1 - \exp\left(-\frac{t}{\tau_2}\right) \right)$$

with  $W$  the whey weight collected during time  $t$ ;  $W_1$  and  $W_2$  the final amounts of expelled whey at the end of successive phase 1 and 2;  $\tau_1$  and  $\tau_2$  the characteristic times of drainage during phase 1 and 2.  $W_1$ ,  $W_2$  and  $W_{\text{tot}}$  ( $W_1 + W_2$ ) are shown in the table, together with  $3\tau_1$  and  $3\tau_2$ , which are the times taken to reach 95% of final amounts of expelled whey of each phase. Water content of drained curd is calculated in g per g casein.

**Tableau II.** Paramètres de l'équation d'égouttage. Les poids de sérum sont expulsés du coagulum obtenu par coagulation présure de 2 kg de lait avec les procédés mésophile ou thermophile. Ces poids ont été ajustés par l'équation :

$$W = W_1 \left( 1 - \exp\left(-\frac{t}{\tau_1}\right) \right) + W_2 \left( 1 - \exp\left(-\frac{t}{\tau_2}\right) \right)$$

avec  $W$ , le poids de sérum recueilli au cours du temps  $t$ ;  $W_1$  and  $W_2$ , les poids finaux des phases successives 1 et 2;  $\tau_1$  and  $\tau_2$ , les temps caractéristiques des phases 1 et 2. Dans le tableau sont présentés  $W_1$ ,  $W_2$  et  $W_{\text{tot}}$  ( $W_1 + W_2$ ), et  $3\tau_1$  and  $3\tau_2$ , les temps pour obtenir 95 % du poids final de chaque phase. Les teneurs en eau des caillés égouttés sont calculées en g par g de caséine.

| CN                    | -1    |       | +1    |       |
|-----------------------|-------|-------|-------|-------|
|                       | -1    | +1    | -1    | +1    |
| Mesophilic            |       |       |       |       |
| $W_1$ (g)             | 893   | 868   | 934   | 816   |
| $W_2$ (g)             | 827   | 810   | 757   | 827   |
| $W_{\text{tot}}$ (g)  | 1 720 | 1 678 | 1 691 | 1 643 |
| $3\tau_1$ (s)         | 53    | 518   | 93    | 182   |
| $3\tau_2$ ( $10^3$ s) | 10.25 | 13.58 | 10.11 | 9.40  |
| g water/g casein      | 2.67  | 3.02  | 2.35  | 2.96  |
| Thermophilic          |       |       |       |       |
| $W_1$ (g)             | 1 403 | 1 305 | 1 443 | 1 304 |
| $W_2$ (g)             | 381   | 482   | 333   | 453   |
| $W_{\text{tot}}$ (g)  | 1 784 | 1 787 | 1 776 | 1 757 |
| $3\tau_1$ (s)         | 16    | 56    | 31    | 36    |
| $3\tau_2$ ( $10^3$ s) | 5.58  | 6.90  | 6.32  | 8.41  |
| g water/g casein      | 1.55  | 1.88  | 1.47  | 1.70  |

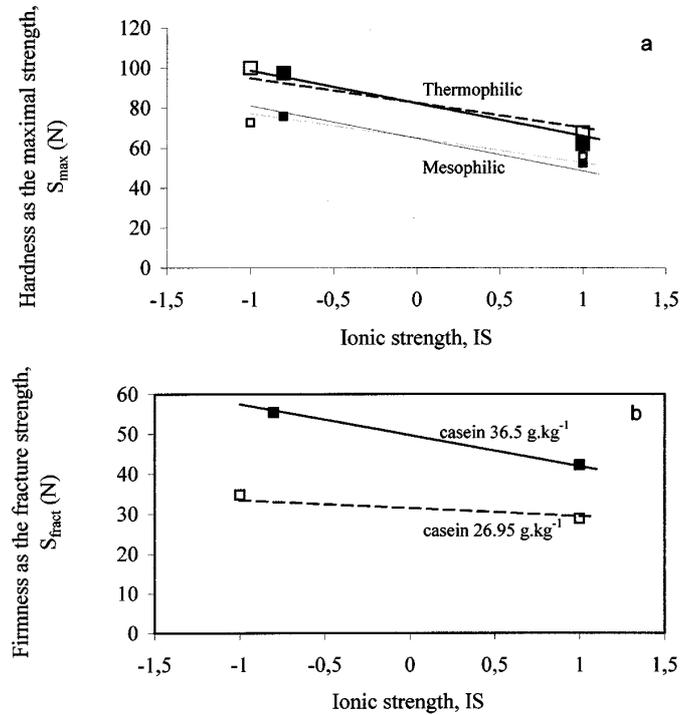
with the thermophilic drainage compared to the mesophilic one.

#### 4. DISCUSSION

Many studies have already reported the effect of factors such as concentration of casein or pH of milk on rennet coagulation

or on curd drainage. In the present study, interactions between factors were quantified, in addition to the effects of the individual factors.

For example, the effect of pH on the gel time, on the gelification rate and on the syneresis of rennet gels was pointed out and agreed very well with previous results. Decreasing pH in the range studied resulted



**Figure 7.** Rheology of drained curd with respect to milk IS. (a) hardness of drained curd as maximum strength,  $S_{max}$  (N) in a compression test; mesophilic curd, CN 26.9 g.kg<sup>-1</sup> (—■—); CN 36.5 g.kg<sup>-1</sup> (-□-); thermophilic curd, CN 26.9 g.kg<sup>-1</sup> (-□-); CN 36.5 g.kg<sup>-1</sup> (-■-); (b) firmness of mesophilic drained curd as fracture strength,  $S_{fract}$  (N) in a compression test; CN 26.9 g.kg<sup>-1</sup> (-□-), CN 36.5 g.kg<sup>-1</sup> (-■-).

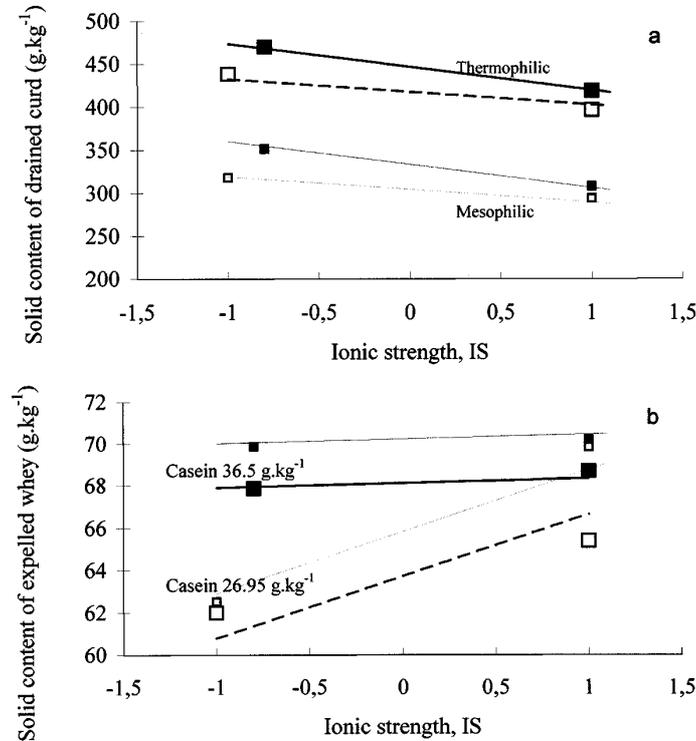
**Figure 7.** Rhéologie du caillé égoutté en fonction de la force ionique du lait. (a) dureté du caillé ou force maximale,  $S_{max}$  (N) dans le test de compression ; caillé mésophile, CN 26,9 g.kg<sup>-1</sup> (-□-); CN 36,5 g.kg<sup>-1</sup> (-■-); caillé thermophile, CN 26,9 g.kg<sup>-1</sup> (-□-); CN 36,5 g.kg<sup>-1</sup> (-■-); (b) fermeté du caillé mésophile ou force à la rupture,  $S_{fract}$  (N) dans le test de compression ; CN 26,9 g.kg<sup>-1</sup> (-□-); CN 36,5 g.kg<sup>-1</sup> (-■-).

in a reduction of the coagulation time and of the firming time, in accordance with previous results [22] and in an increase in syneresis [16, 19, 25, 30, 32].

Although it has been reported that the concentration of casein had no effect on the coagulation time [33], increasing the concentration of casein in the current study led to a small reduction in  $R$ , of about 29 s, while no effect on  $t_{G'}$  and  $t_{G''}$  was observed. The  $k_{20}$ ,  $k_{G'}$  and  $k_{G''}$  were reduced and the rennet gel firmness increased with the increase in the concentration of casein, as widely reported. Increasing the concentra-

tion of casein led also to a decrease in syneresis, according to previous results [30].

Concerning drainage, increasing the concentration of casein from 27 to 36 g.kg<sup>-1</sup> led to a reduction in the final weight of whey (mean reduction of 25 g), as generally reported. But, this can result from the reduction in water content of concentrated milk. The increase in the concentration of casein was also reported to increase the rate of syneresis [32], but we observed no effect of the concentration in casein on the rate of drainage (Fig. 6). The low ultrafiltration



**Figure 8.** Solid content of drained curd (a) and of drained whey (b) with respect to milk ionic strength; symbols as Figure 7.

**Figure 8.** Extrait sec du caillé égoutté (a) et du sérum d'égouttage (b) en fonction de la force ionique du lait ; même symboles que dans la figure 7.

volume concentration factor ( $\times 1.33$ ) in the current study could explain why no effect of the concentration of casein appeared on the rate of drainage. According to van Hooydonk et al. [27], the hydration of para-casein is  $1.2 \text{ g of water} \cdot \text{g}^{-1}$  at pH 4.6. On this basis, the theoretical weight of substances not retained in the curd would be  $943 \text{ g}$  for  $27 \text{ g} \cdot \text{kg}^{-1}$  casein (calculated as  $1\ 000 - 27 \times 0.96 (1 + 1.2)$ ) and  $924 \text{ g}$  for  $36 \text{ g} \cdot \text{kg}^{-1}$  casein (calculated as  $1\ 000 - 36 \times 0.96 (1 + 1.2)$ ), with the factor 0.96 taking into account the removal of CMP. This leads to a difference of  $38 \text{ g}$  for  $2 \text{ kg}$  of milk. This value compares with the  $25 \text{ g}$  difference obtained between the amounts of whey at the low and high concentration of casein.

The differences in the composition of milk can explain a part of the differences in the final weight of whey obtained by drainage. It seems that other factors intervened, such as different structures of gel obtained at different concentrations of caseins.

The increase in concentration of casein led to an increase in the solids content of the drained curd (mean increase of  $25.5 \text{ g} \cdot \text{kg}^{-1}$ ), which can explain the increase in the firmness of the mesophilic curd with the higher concentration of casein. Calculation of the water content per g of casein enabled a comparison of curds at different concentrations of casein. It revealed that para-casein retained less water in the curd when milk

was concentrated slightly. The gel with a higher concentration of casein was firmer and the curd grains presumably have less deformability. Thus, the whey channels in the curd could stay more open. In a softer curd, the channels became more tortuous and closer, thus reducing the flow. A hard gel could lead to increased loss of water per g casein. Such an hypothesis needs further investigations to be confirmed.

The effect of reducing the ionic strength on rennet coagulation has not been studied extensively and its effect on drainage is not known. A reduction in the rennet coagulation time of milk and in the firming time was observed when the ionic strength was reduced. This result confirmed previous studies [9, 21]. The increase in ionic strength of milk by addition of NaCl has been previously reported to increase the rennet coagulation time [8, 12, 26]. The effect of adding NaCl to a concentration between 10 and 110 mmol·L<sup>-1</sup> has been confirmed on model substrates by Visser et al. [28]. The increase in ionic strength leads to a screening of the negative charge on the chymosin and of the positive charge on the  $\kappa$ -casein cluster, which hampers enzyme-substrate attraction [28]. Addition of anions inhibited both the breakdown of  $\kappa$ -casein by chymosin and the aggregation of para-casein micelles [3]. Anions bind to cationic sites on  $\kappa$ -casein and this confirmed the role of these sites in rennet coagulation. Rennet coagulation has been studied on casein micelles dispersed in water or NaCl solutions [8, 9]. The rates of enzymatic and aggregation steps were both reduced by the increase in the concentration of NaCl in micelle dispersions [8]. Differences between voluminosity of micelles with different soluble phases were suggested to explain differences in the rate of the aggregation phase, while the rate of the enzymatic phase could be related to the screening of charges on the enzyme and its substrate. Anyway, in the current study, the effect of IS appeared alone and also in strong interaction with the pH factor. This interaction resulted in a reduction of gel time by

2 or 3 min when IS was reduced at pH 6.5, while this reduction is less than 30 s at pH 6.0. This indicated that electrostatic interactions together with steric repulsions, which are changing during the coagulation process, played complementary roles in the destabilisation of the system.

The reduction in ionic strength led to an increase in firmness parameters  $a_2R$ ,  $G'_\infty$  and  $G''_\infty$  (Fig. 4). This was a rather surprising result, as micelles in suspensions of reduced ionic strength appeared more compact and less hydrated than in higher ionic strength in the range of pH between 6.6 and 6.0 [9]. These characteristics of micelles in acidified suspensions do not promote an increase in rennet gel firmness although the characteristics of paracasein micelles in this pH range are not known.

The main effect of reducing the ionic strength was observed on syneresis. Again, the study showed that ionic strength acted in interaction with the concentration of casein. Reducing ionic strength at a high concentration of casein greatly increased the gel syneresis as reflected by Si and Sf.

The effect of ionic strength was confirmed by the drainage behaviour. Reducing the ionic strength gave higher final whey weight and higher initial rate of drainage, as can be seen on the drainage curves (Fig. 6). As for the syneresis results, an interaction between ionic strength and concentration of casein was observed. Reducing the ionic strength led to an increase in W1, and this increase is much higher when it was performed at a high CN. The effects were opposite for W2, which decreased as reducing IS. The higher the concentration of casein, the greater the decrease of W2 with the reduction of ionic strength. This interaction probably results from the fact that the effect of changing ionic strength depends strongly on the absolute value of ionic strength. Indeed, milk at the higher concentration of casein had a higher ionic strength than un-concentrated milk as the pH decreased. This suggested that diafiltration

could be a potential solution for modified milk showing low rates of syneresis or drainage.

The effect of reducing the ionic strength was obvious on  $W_1$  and  $W_{tot}$  either with the mesophilic or thermophilic process. This can be explained by the fact that the reduction in ionic strength leads to an increase in the isoelectric pH value of casein, to a reduction in the negative charge of caseins from pH 6.6 to 5.0 and to a reduction in the affinity of casein for water. This increase in syneresis led to an increase in the solids content of drained curds, and in curd hardness and firmness.

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