Rennet Coagulation of Milk Retentates. 2. The Combined Effect of Heat Treatments and Protein Concentration

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ABSTRACT

The aim of this research was to study the clotting kinetics, curd hardening, and whey syneresis of milk concentrated by UF. Experiments were conducted on raw, pasteurized, and UHT-treated milks to verify whether the depression of coagulation characteristics caused by heat treatments of milk could be reversed by the increase in protein concentration.

Data demonstrate that rennin action on milk strongly depends on the severity of heat treatment and that curd consistency is influenced both by heat treatment and protein concentration. Ultrafiltration modifies the viscoelastic behavior of curd, resulting in a marked increase in the viscous component at increasing protein concentration. Syneresis decreases as protein concentration increases and is strongly affected by pasteurization and UHT treatment.

INTRODUCTION

The aim of this study was to analyze how the concentration of milk by UF modifies the kinetics of coagulation, curd hardening, and whey syneresis, which may be considered the fundamental phenomena occurring during the initial phase of cheese making. In addition, they are the most critical factors determining the chemical, physical, and structural characteristics of the cheese.

Another objective of this study was to compare the behavior of retentates obtained from raw, pasteurized, and UHT-treated milks to verify whether or not the depression of coagulation characteristics caused by heat treatment could be partially reversed by the increase of protein concentration resulting from ultrafiltration.

In recent years, various authors (5, 6, 8, 10, 11, 17, 21, 22) have analyzed the coagulation behavior of UF retentates. Because experimental approaches differed widely and the number of variables involved in retentates coagulation is high, it is difficult to compare data, and conflicting results may be obtained. A typical example of contrasting data is provided by various authors' findings (5, 6, 8, 10, 11, 15, 17 , 21, 22, 25) about the effects of protein concentration on coagulation time (CT), i.e., the time elapsed from rennet addition to the first macroscopic observation of floccules in the milk. Although most authors agree that an increase of protein concentration results in a decrease in CT (8, 10, 17, 22, 25), some have found that CT does not vary significantly in relation to the variation in protein content (6). Other authors have observed an inverse relationship between CT and protein concentration (5, 11, 21). In a previous work (15) we noted that CT was practically independent of protein concentration up to approximately 7% and slightly decreased at higher protein contents.

There are few data available on the rheology of curd, apart from the obvious observation that curd consistency increases as protein concentration increases. In particular, information is scarce on curd-hardening kinetics and quantitative relationships between consistency indices and protein concentration of retentates. According to Culioli and Sherman (5), the rigidity modulus of curd increases at a given rate up to a maximum consistency. Both curd hardening rate and maximum rigidity depend on protein concentration, whereas the time required to reach the maximum consistency value is the same regardless of the concentration ratio. Rheological behavior of curds with increasing pro-

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tein content has been studied by these authors (5) and related to the various bonding forces, which are active in the curd's tridimensional structure. Few papers (13, 16, 23) have been published on the kinetics of whey syneresis in curds from milk retentates, although this parameter has been proved the most indicative of variations in the functionality of milk constituents (3, 4, 19).

It is known that heat treatments of milk result in longer coagulation times as well as in a loss of adequate curd tension. These effects are mainly attributable to modifications in ionic equilibria and the formation of β-lactoglobulin-casein complexes (14, 24, 26). The interaction between heat treated κ-casein and β-lactoglobulin restricts the access of rennin enzymes to casein micelle surface and suppresses or retards the clotting reaction.

Furthermore, it may be interesting to consider the effects of heat treatment as a mean of reducing the consistency of curds obtained from UF retentates, thereby enlarging the technological possibility of mastering the sensory parameters of cheese.

MATERIALS AND METHODS

Samples

Experiments were carried out using raw, pasteurized, and UHT whole milk produced by different cow breeds and sampled from tanks containing approximately 250,000 L. Pasteurization and UHT treatments were conducted in industrial plants at 75°C for a holding time of 20 s and at 142°C for 5 s, respectively.

Raw and thermally treated milks were concentrated by UF with a lab unit type UF 35-2.25 (DDS, RO Division, Nakskov, Denmark). Operating conditions were: inlet pressure 3.8 atm, outlet pressure 3.2 atm, flow rate 6000 L/h, and temperature 48 to 52°C. Prevention of bacterial growth was assured by the addition of NaN_3 (.01% wt/vol) to milk before UF.

Analytical Methods

Total solids, protein, and ash were determined according to AOAC (1) methods (16.032, 16.036, and 16.020, respectively). The NPN was determined by the AOAC method 16.036 after protein precipitation by 12% solution of trichloracetic acid. Total calcium was evaluated by atomic absorption (wavelength 422.7) with a Pye Unicam SP9 spectrometer (Pye Unicam Ltd., Cambridge, England) according to the method reported by Brule et al. (2).

Evaluation of the Initial Velocity of the Primary Phase

The initial velocity of the primary phase of clotting was evaluated by the increase of NPN released by rennin action on κ-casein. Renneting was performed at 38°C by adding 2 ml of .025% rennet solution to 100 ml of milk and retentates. The NPN was determined at various times after rennet addition according to a turbidimetric method proposed by Qvist (20). By reference to a calibration curve, the turbidity readings were transformed into the absolute values of NPN. The initial NPN content of each sample before renneting was subtracted from the values obtained after various times of rennet action.

Rheological Evaluation of Curds

Curd samples were prepared as reported in a previous paper (4). The rheological properties of curd were evaluated by compression-relaxation tests carried out with an Instron Universal Testing Machine model 3140 (Instron Ltd., High Wycombe, England). After 10% compression performed at a crossbar speed of 200 mm/min, curd samples were allowed to relax for 5 min. The elastic modulus E (N/m^2) was evaluated from the compression part of the curve, and its value at infinite time (E_∞) was computed from the relaxation curve, according to Peleg (18).

Evaluation of Syneresis Curves

Curd syneresis was evaluated according to Marshall (6) on curd samples prepared as reported previously (4).

RESULTS AND DISCUSSION

In a previous work, we demonstrated that the thermal and mechanical effects associated with UF treatment performed without milk concentration do not significantly modify the coagulation ability of milk (4).

TABLE 1. Proximate analysis of raw milk and retentates.

Sample code	Total solids	Protein[1]	Concentration ratio[2]	NPN	Ash	Calcium	Total N in whey[1]
	(g/100 g)			(g/100 g)		(mg/L)	(g/100 g)
1A	11.67	2.90	1.0	.16	.70	129	1.11
1B	12.53	3.37	1.2	.16	.71	146	1.12
1C	12.78	3.84	1.3	.17	.72	163	ND[3]
1D	14.80	4.86	1.7	.17	.83	214	ND
1E	19.15	8.32	2.9	.17	.98	299	1.19

[1]N × 6.38.

[2]Calculated on the basis of protein content.

[3]Not determined.

Tables 1, 2, and 3 report the composition of retentates obtained from raw, pasteurized, and UHT milk, respectively. As noted in Table 2, UF retentates from pasteurized milk were obtained from two UF batches; the first included samples 2B, 2C, and 2D, and the second included samples 2E, 2F, and 2G. Tables 1 and 2 also report the total N content of whey obtained

TABLE 2. Proximate analysis of pasteurized milk and retentates.

Sample code	Total solids	Protein[1]	Concentration ratio[2]	NPN[1]	Ash	Calcium	Total N in whey[1]
	(g/100 g)			(g/100 g)		(mg/L)	(g/100 g)
First batch							
2A	10.77	2.92	1.0	.16	.73	110	.80
2B	13.30	3.87	1.3	.16	.76	150	.97
2C	16.19	5.69	1.9	.17	.89	150	1.36
2D	18.85	6.30	2.2	.16	.95	200	1.65
Second batch							
2A	11.52	3.07	1.0	.18	.70	150	.98
2E	28.97	10.77	3.5	.16	1.27	360	3.32
2F	30.77	12.92	4.2	.16	1.44	460	3.87
2G	36.14	16.28	5.3	.15	1.62	660	4.92

[1]N × 6.38.

[2]Calculated on the basis of protein content.

TABLE 3. Proximate analysis of UHT milk and retentates.

Sample code	Total solids	Protein[1]	Concentration ratio[2]	NPN[1]
	(g/100 g)			(g/100 g)
3A	11.11	2.86	1.0	.16
3B	20.06	8.18	2.9	.16
3C	30.22	10.96	3.8	.16
3D	32.50	14.67	5.1	.17

[1]N × 6.38.

[2]Calculated on the basis of protein content.

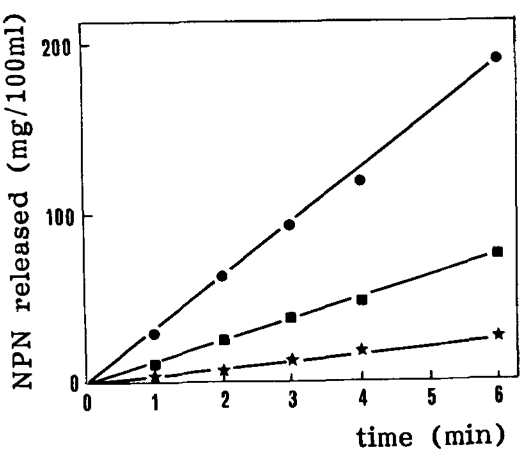

Figure 1. Initial velocity of the enzymatic reaction for different thermally treated milks: raw (●); pasteurized (■); UHT (★).

from the cut curds. These values do not appear in Table 3 since curds could not be obtained from UHT retentates with low protein concentration and since curds obtained from high protein retentates do not release whey.

Figure 1 shows the kinetics of NPN release by rennet action in raw, pasteurized, and UHT milk. These data clearly demonstrate that the enzymatic action is significantly reduced in pasteurized milk and almost completely inhibited in UHT milk.

Figure 2 represents the kinetics of curd hardening in terms of variation of elastic modulus as a function of time elapsed since rennet addition. Contrary to the findings of Culioli and Sherman (5), the curds obtained from retentates with different protein content do not appear to reach their maximum consistency values at the same time. In fact, the elastic modulus tends to

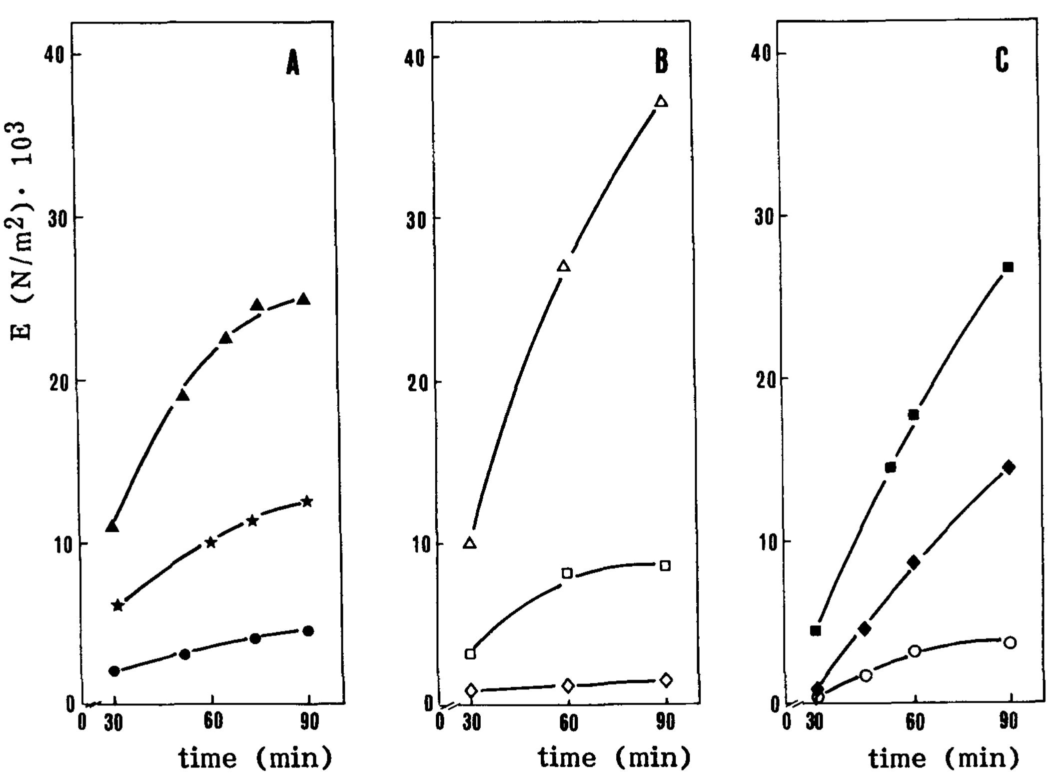

Figure 2. Elastic modulus of curds as a function of time since rennet addition. A) Raw milk and retentates: sample 1A (●), sample 1D (★), sample 1E (▲). B) Pasteurized milk and retentates: sample 2A (◊), sample 2D (□), sample 2E (Δ). C) UHT retentates: sample 3B (O), sample 3C (♦), sample 3D (■).

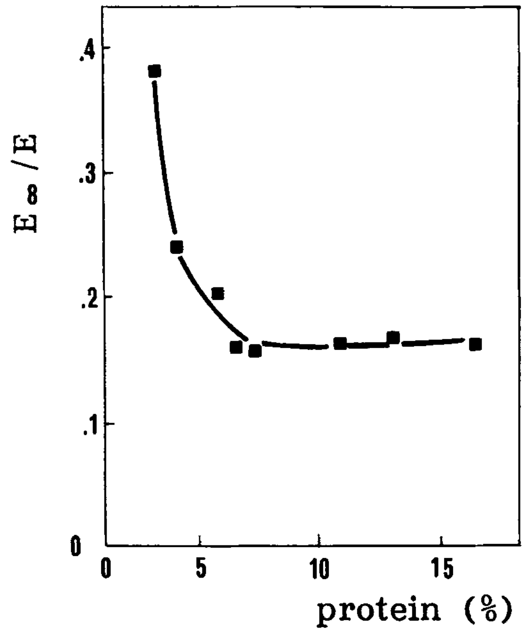

Figure 3. Viscoelastic characteristics of curd (E_∞:E) obtained from pasteurized milk as a function of protein concentration.

level off in less time at lower protein concentrations. A comparison of the curves in Figure 2 clearly shows that the consistency of curds is highly dependent on both heat treatment and protein concentration.

Representation of the experimental values by a mathematical model that allows interpolation between data shows that, at given times, the elastic moduli (E) are linked to protein concentrations by a simple power equation: $E = kP^n$ in which constant k may be considered as the reference consistency parameter; in fact, it represents the elastic modulus value at unit protein concentration. The exponent n weights the dependance of E on protein concentration. As an example, Table 4 presents the equations valid for 60 min after renneting. Parameter k decreases almost exactly by one order of magnitude going from raw to pasteurized milk and then, again, from pasteurized to UHT-treated milk. Furthermore, the elastic modulus dependance on protein concentration increases by increasing the severity of heat treatment. This means that the difference in consistency of curds obtained from different heat-treated milks tends to decrease at high protein concentrations.

TABLE 4. Correlations between elastic moduli (E) and protein contents (P) for raw, pasteurized, and UHT milk retentates.

Samples	Equation	Correlation coefficient
Curds from raw milk retentates	$E = .61P^{1.71}$	.987*
Curds from pasteurized milk retentates	$E = .06P^{2.57}$	.995*
Curds from UHT milk retentates	$E = .005P^{3.05}$	.999*

*$P<.01$.

An obvious caution is to limit the application of these expressions to the protein concentration range that has been experimentally investigated. In other words, the equation in Table 4 may be used for interpolation, not extrapolation, of data. It should also be noted that E is a consistency parameter, which does not provide any information on the viscoelastic characteristics of curd.

Relaxation tests were carried out to determine differences in viscoelastic behavior of samples, and the data were treated according to Peleg (18) and Inokuchi (12). The results of these evaluations, which are reported in detail elsewhere (3, 9), are not always consistent or sufficiently explanatory. The only parameter that appears to have significant meaning is the ratio E_∞:E between the elastic modulus at infinite time and at zero time: the higher is this value, the less viscous is the curd.

Figure 3 shows how the E_∞:E ratio varies in pasteurized milk curd as a function of the protein content of the retentate. Even a small increase in the protein content dramatically reduces E_∞:E ratio, which then becomes relatively constant at protein contents higher than 6%. In other words, an increase in protein concentration increases the viscous component more than the elastic one. In the practical language of cheese makers, this phenomenon is called "loss of nerve" of curd, and it obviously represents an unavoidable change in the curd's usual rheological behavior. These findings are in agreement with the hypothesis suggested by

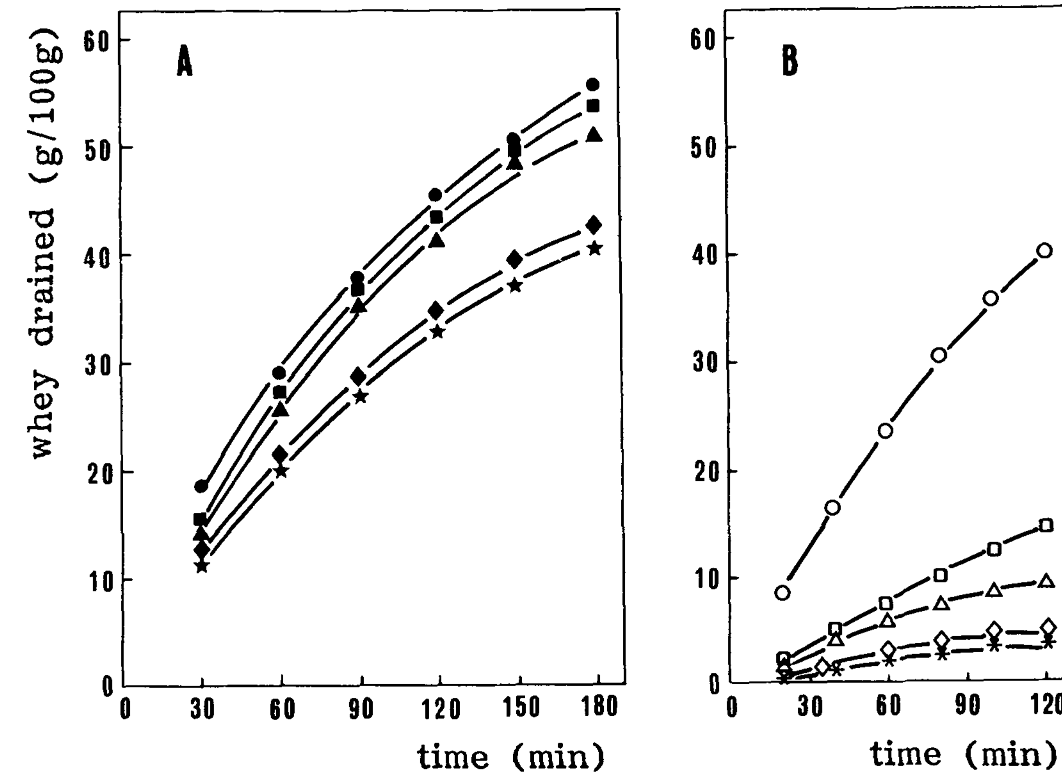

Figure 4. Amount of whey released as a function of time since rennet addition. A) Curds obtained from raw milk and retentates: sample 1A (●), sample 1B (■), sample 1C (▲), sample 1D (♦), sample 1E (★). B) Curds obtained from pasteurized milk and retentates: sample 2A (O), sample 2C (□), sample 2E (Δ), sample 2F (◊), sample 2G (*).

Dalgleish (7) that curd obtained from UF retentates contains a higher proportion of free, unbound casein micelles, which contribute to the increased viscosity of the dispersed phase.

Figure 4 shows the kinetics of whey syneresis for raw and pasteurized milk curds. Data for UHT milk curd is not included since, as mentioned, syneresis does not occur at any protein concentration. Analysis of Figure 4 demonstrates that syneresis, as may be expected, decreases as protein concentration increases. However, a mass balance (3), which is not included here for brevity, shows increasing retention of whey protein in the curd obtained from retentates with increasing protein content. This may be due to retention of a more concentrated solution in the curd or to a proportionally weaker contraction of the curd and a lower tendency toward whey expulsion. The most significant conclusion based on Figure 4 concerns the marked difference in the behavior of curd obtained from raw and pasteurized milk retentates. Pasteurization reduces whey syneresis considerably; for instance, the rate and the extent of whey separation observed in pasteurized milk curd is similar to that of a twofold raw milk retentate.

This result is further proof that pasteurization, although considered to be a mild heat treatment, dramatically changes the functionality of milk proteins as well as the cheese making ability of milk.

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