Effect of Controlled κ -Casein Hydrolysis on Rheological Properties of Acid Milk Gels

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ABSTRACT

An experimental method based on the controlled chymosin-induced κ -casein hydrolysis of milk was proposed to modify micellar reactivity. Milk samples with a degree of κ -casein hydrolysis of 19, 35, and 51% were obtained. The physicochemical properties of partially converted casein micelles were determined. The net negative charge of casein micelles was reduced with increasing degree of κ -casein hydrolysis and a small but significant decrease in hydrodynamic diameter and micellar hydration were noted. Dynamic low amplitude oscillatory rheology was used to monitor the rheological properties of acid milk gels (GDL) made with partially chymosin-hydrolyzed milks in comparison with those of strictly acid and rennet gels. An increase in the gelation pH value was observed with increasing the degree of κ -casein hydrolysis. The moduli values (G' and G'') reached 2 h after the point of gel were, for all degrees of hydrolysis tested, significantly higher than those of strictly rennet and acid gels. Comparison of changes in $\Delta G'/\Delta t$ with time indicated differences in gel formation that could be related to the increased values of G' obtained for acid gel made with chymosin-treated milk. At a given time after gelation (2 h), increasing the degree of κ -casein hydrolysis in milk led also to an increase in the loss tangent and the serum holding capacity of acid milk gels suggesting a correlation between these two parameters.

(Key words: κ -casein hydrolysis, acid milk gel, rheological property, serum-holding capacity)

Abbreviation key: CCP = colloidal calcium phosphate, CMP = caseinomacropéptide, DMSO = dimethylsulfoxide, E/I = enzyme/inhibitor, E/S = enzyme/substrate, GDL = glucono- δ -lactone, tg = gelation time.

INTRODUCTION

Chemical and enzymatic modifications of protein is generally performed with the intention of changing not only the functionality of food proteins (Vojdani and Whitaker, 1994), but also of improving the fundamental knowledge of the mechanisms involved in food processing (Vidal et al., 1998). Among enzymatic modifications of casein, the hydrolysis of protein can be considered as a way to modify the number of polar groups, the hydrophilicity/hydrophobicity balance, and the molecular configuration leading generally to changes in its functionality and more particularly in its gelation properties. The conversion of milk to gel by the action of chymosin is the result of the hydrolysis of κ -casein (Phe 105-Met 106). This highly selective modification of protein structure initiates a sequence of events resulting in a gelation phenomenon traditionally used in cheese making.

Chymosin action involves an enzymatic reaction, in which chymosin splits the casein macropéptide, followed by the aggregation of the newly formed paracasein micelles if temperature and calcium activity are high enough (Tarodo and Alais, 1975). In agreement with the concept of steric repulsion (Horne, 1986), measurable aggregation only occurs after most macropéptide forming hairs have been removed, about 85 to 90% according to Dalgleish (1979). The stability of casein micelles against aggregation is primarily due to steric repulsion caused by hydrophilic hairs of κ -casein but also, at low temperature of β -casein (Roefs et al., 1990a). The probability that casein molecules in different micelles comes into contact of each other for a sufficient time to create new intermolecular bonds appears to depend on electrostatic as well as steric repulsion, which thereby affect aggregation rate (Bringe and Kinsella, 1987). Because of the polar and charged nature of the caseinomacropéptide part of the κ -casein, repulsion is considered as the dominant factor controlling the stability of the casein micelle system (Horne and Parker, 1981).

Received February 16, 2002.

Accepted June 19, 2002.

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The aim of this study was to modify the micellar surface reactivity by using chymosin to realize a partial controlled hydrolysis of κ -casein. To control this reaction, a specific acid protease inhibitor was added to milk when a desired degree of κ -casein hydrolysis was reached. Such a method can be directly applied on casein micelles in their natural environment, unlike most reports dealing with enzymatic modification of isolated milk constituents. The extent of enzymatic modification was related to the degree of κ -casein hydrolysis that could be controlled with a low temperature of incubation (4°C). In such conditions, the two reactions implied in the chymosin hydrolysis, splitting of casein macropptide and aggregation of paracasein micelles were uncoupled. Acid milk gels (**GDL**) were made from partially chymosin-hydrolyzed milk, and the rheological properties of gels obtained were compared to those of strictly acid or rennet milk gels. The effects of the degree of κ -casein hydrolysis on acid milk gels formation were thus investigated, and a discussion relied on rheological properties and serum holding capacity of resulting acid milk gels was finally proposed.

MATERIALS AND METHODS

Skim Milk Reconstitution

Reconstituted skim milk was made by dissolving a commercial low-heat powder (Lait Matines-SILI, Plouvien, France) at 12% (wt/vol) in deionized water. The powder used in this study contained 3% (p/p) moisture, 35.62% (p/p) protein (total nitrogen $\times$ 6.38) and had a whey protein nitrogen index of 6 mg of undenatured whey protein per gram of powder. Milk was stored at 4°C for 12 h after the addition of 250 mg/L of calcium as CaCl_2 solution. To prevent bacterial growth, 0.04% (wt/vol) sodium azide was added.

Preparation of Partially Chymosin Hydrolyzed Milk Samples

Chymosin hydrolysis of κ -casein was realized at 4°C by using defined amount of a liquid chymosin preparation at 600 mg/L (Maxiren 120, DSM, France). Adequate amounts of a 10-fold water diluted chymosin solution (10, 20, or 30 μl) were added to milk (100 ml) to give an enzyme/substrate (**E/S**) molar ratio of 8, 16, and 36×10^{-7} , respectively (substrate was considered as κ -casein). In such conditions, a final enzyme concentration of, respectively 2, 4, and $6 \cdot 10^{-10}$ M was obtained. After 15 h of incubation, the chymosin hydrolysis was stopped by the addition under vigorously stirring of an appropriate amount of Pepstatin A (Coger, Paris, France), an aspartic proteinase inhibitor, dissolved in dimethylsulfoxide (**DMSO**) to a concentration of 1 mM.

Pepstatin A was added to milk to give an enzyme/inhibitor (**E/I**) molar ratio of $2 \cdot 10^{-5}$. Totally κ -casein hydrolyzed milk samples were obtained by adding 40 μl of the chymosin preparation (Maxiren) to 100 ml of skim milk and incubating 1 h at 30°C.

Determination of the Degree of κ -Casein Hydrolysis

The quantitative determination of caseinomacropptide (**CMP**) was performed by cation-exchange chromatographic analysis according to Leonil and Mollé (1991) by relating the areas to the amounts of CMP injected (Sigma-Aldrich, France). The degree of κ -casein hydrolysis was determined by comparing the CMP content of different milk samples to a totally hydrolyzed milk considered as 100% κ -casein hydrolysis.

Micellar Hydration, Hydrodynamic Diameter, and Zeta Potential Measurements

The solvation water of casein micelles was determined by means of ultracentrifugation as previously described by Gastaldi et al. (1996) and was expressed as g of H_2O /g of dry pellet. Separation of the soluble and micellar phases was achieved by ultracentrifugation at $149,000 \times g$ for 35 min at 30°C with a Beckman ultracentrifuge, rotor Ti 30 (Beckman Instruments France S.A., Gagny, France).

A Malvern Zeta Sizer 3000 (Malvern Instruments, Worcestershire, England) was used to evaluate by dynamic light scattering with a 5 mW He-Ne laser ($\lambda = 633$ nm) both the average hydrodynamic diameter and the electrokinetic potential of casein micelles with different levels of κ -casein hydrolysis. Casein micelles were examined in their natural ionic environment by suspending them in milk permeate obtained by milk filtration through Amicon YM10 membranes (Epernon, France). The milk dilutions used were 1:50 and 1:200 for average hydrodynamic diameter and average electrophoretic mobility measurements respectively. Temperature was controlled at $30 \pm 1^\circ\text{C}$ by a Joule-Peltier device.

Gel Formation

Milks with different levels of κ -casein hydrolysis were acidified with 2% (wt/vol) Glucono- δ -lactone (**GDL**) (Merck, Darmstadt, Germany) at 30°C and the acidification was followed as a function of time. The pH of the samples was regularly measured at $30 \pm 1^\circ\text{C}$ using a Corning 125 pH meter and was about 4.5 after 3 h of incubation. To allow comparison between the different types of gels, a rennet milk gel was also prepared at 30°C by adding to milk 0.025% (vol/vol) of a commercial

calf rennet Naturen (Chr. Hansen's Laboratory, Copenhagen, Denmark) which contains 61% chymosin and 39% bovine pepsin and corresponds to a total milk clotting activity of about 145 IMCU (international milk-clotting units) ml^{-1} .

Dynamic Rheological Measurement

Small deformation oscillatory measurements were performed on a Rheostress RS 100 (Haake, Karlsruhe, Germany) rheometer with a measuring system consisting in a cone-plate sensor system (60 mm diameter, 2° angle). Measurement of the storage modulus, (G'), the loss modulus, (G''), and the loss tangent, ($\tan \delta$), were performed continuously during milk gelation in oscillary mode. After the addition of GDL or rennet to milk, the mixture was stirred for 2 min and then an appropriate volume (2 ml) of the mixture was transferred to the measuring system. A glass cloche was placed on the system to prevent evaporation. Samples were submitted to oscillation at a frequency of 0.1 Hz and the measurement interval was set at 120 s with a stress of 0.02 Pa which was found to be within the linear region for all milk gels tested in this study. Gelation time (**tg**) was defined as the time when $G' = G''$ or $\tan \delta = 1$ (Tobitani et al., 1995).

Evaluation of Serum-Holding Capacity of Gel

A measure of serum-holding capacity was obtained by using a centrifugation test at $1000 \times g$ during 15 min according to Daviau et al. (2000). Aliquots of 15 ml of milk were placed into centrifugation tubes with appropriate coagulating agent (GDL or rennet) and held undisturbed in a water bath at 30°C . The centrifugation test was realized 2 h after gel point was reached. The supernatant was poured off, weighed, and expressed as a percentage of gram of serum per 100 g of gel.

Statistical Analysis

Experimental data were represented as the mean values of duplicate or more analysis depending on the type of analysis and were subjected to ANOVA (Stat-View, Abacus Concepts, Inc., Berkeley, CA). Fisher's protected least significant difference (PLSD) test was used to compare pairwise means and differences between means were considered to be significant at $P < 0.05$.

RESULTS AND DISCUSSION

Extent of κ -Casein Hydrolysis

Partially κ -casein hydrolyzed micelles were obtained by treating milk with low concentrations of chymosin

Table 1. Extent of κ -casein hydrolysis in milk as related to the concentration of chymosin added and the concentration of Pepstatin A used to inhibit the reaction after 15 h incubation at 4°C .

Chymosin concentration (M)	Pepstatin A concentration (M)	Degree of κ -casein hydrolysis (%) ¹
2.10^{-10}	1.10^{-5}	19 ± 0.8
4.10^{-10}	2.10^{-5}	35 ± 1.2
6.10^{-10}	3.10^{-5}	51 ± 1.5

¹Mean values of triplicate analysis.

(about 10-fold less than the amount usually used to make rennet milk gel) at low temperature (4°C) in order to slow the rate of κ -casein hydrolysis and to better control the enzymatic reaction inhibition. According to van Hooydonk et al. (1984), the temperature coefficient Q_{10} for κ -casein hydrolysis by chymosin varied between 1.3 and 2.0. On the other hand, the aggregation of para- κ -casein micelles did not occur at temperatures lower than 15°C . After incubation of milk overnight with different concentrations of chymosin, κ -casein conversion was stopped by adding to milk an appropriate amount of pepstatin A to finally obtain a degree of κ -casein hydrolysis comprised between 19 and 51% (Table 1). Pepstatin A, which contains the unusual amino acid statine ((3S, 4S)-4-amino-3-hydroxy-6-methylheptanoic acid), is recognized to be a universal and specific inhibitor of the members of the aspartic proteinase family as chymosin (Davies, 1990). The effectiveness of the hydrolysis inhibition was verified by CMP measurements realized on milk incubated at 30°C during 24 h after pepstatin A addition. Several E/I ratios have been tested and CMP measurements indicated that a molar ratio E/I of 2.10^{-5} was necessary to have a complete inhibition of the reaction.

Physicochemical Properties of Partially Converted Casein Micelles

As expected, casein micelles size, zeta potential, and micellar hydration were both affected by partial hydrolysis of milk by chymosin (Table 2). Results indicated a reduction in the net negative charge of casein micelles as related to the degree of κ -casein hydrolysis that was accompanied by a small but significant decrease in hydrodynamic diameter and micellar hydration. Zeta (ζ)-potential obtained for untreated milk (-20 mV) was in accordance with values previously reported by Dagleish (1984). ζ -Potential obtained for casein micelles hydrolyzed by chymosin at 51% (-13.75 mV) appeared also consistent with result obtained by Dagleish (1984) for renneted micelles (-11 mV), indicating a good correlation between the reduction of ζ -potential values (Table 2) induced by the chymosin treatment and the

Table 2. Casein micelles hydrodynamic radius, zeta potential value, and micellar hydration as related to the degree of κ -casein conversion.

Degree of κ -casein conversion (%)	Hydrodynamic radius ¹ (nm)	Zeta potential value ¹ (mV)	Micellar hydration ² (g H ₂ O/g dry pellet)
0	227.5 ^a ± 0.5	-20.20 ^a ± 0.06	2.90 ^a ± 0.03
19	225.2 ^b ± 0.6	-17.15 ^b ± 0.08	2.66 ^b ± 0.06
35	223.8 ^c ± 0.4	-15.23 ^c ± 0.04	2.51 ^c ± 0.04
51	222.2 ^d ± 0.3	-13.75 ^d ± 0.07	2.38 ^d ± 0.03

^{a,b,c,d}Means with different superscripts differ ($P < 0.05$).

¹Mean values of triplicate analysis.

²Mean values of duplicate analysis.

amount of CMP liberated. Both electrostatic and steric repulsions seemed to be high enough to prevent aggregation of casein micelles hydrolyzed by chymosin at 51%.

The reduction of micellar negative charge induced by chymosin treatment was accompanied by the decrease in both hydrodynamic diameter and pellet hydration (Table 2). The gradual decrease in hydrodynamic diameter related to the increase in κ -casein hydrolysis could be attributed to the progressive cleavage of CMP by chymosin as previously shown by Walstra et al. (1981). Because casein micelle hairy layer is drained by solvent (Holt and Dalglish, 1986), its cleavage by chymosin came with a progressive reduction in micellar hydration in accordance to Creamer (1985) and Imafidon and Far-kye (1996). The total thickness of the hairy layer [between 5 and 12 nm according to Dalglish (1998)] appeared slightly greater than the hydrodynamic diameter decrease (5 nm) observed for the higher degree of κ -casein conversion (51%). Nevertheless, in these conditions, the amount of CMP remaining at the micellar surface seemed to be sufficient to induce both steric and electrostatic repulsions between micelles.

Rheological Properties of Acid Milk Gels Made with Partially Converted Casein Micelles Compared with Strictly Acid and Rennet Milk Gels

Dynamic low amplitude oscillatory rheology was used to monitor the acid milk gel formation process as related to the degree of κ -casein hydrolysis. As shown in Figure 1, the conditions of acidification were identical in the different types of gels formed. On the other hand, preliminary assays (results not shown) have permitted to assess that addition of DMSO and pepstatin A, from 1 to $3 \cdot 10^{-5}$ M, to milk had no effect on both gelation time and pH of resulting acid milk gels. Curves of the changes in G' and G'' as a function of time indicated a decrease of the gelation time (time at $G' = G''$) with increasing the degree of κ -casein hydrolysis (Figure 2). The comparison of the pH values at gelation time

showed an increase in the gelation pH as a function of the degree of κ -casein hydrolysis (Table 3). In accordance with the model developed by Horne (1998), the increase in the pH of gelation as increasing the degree of κ -casein hydrolysis could be interpreted as a shift of the forces balance in favor of the hydrophobic interactions allowing aggregation to proceed. The decrease in electrostatic repulsions due to the partial hydrolysis of κ -casein could be reinforced by the drop of pH that could alter the steric stabilizing capacity of the hairy layer (Horne, 1999). Previous report (van Hooydonk et al., 1986) that indicated a decrease in the percentage of CMP removed at the clotting time as lowering milk pH could also support this hypothesis.

Comparison of the storage and loss moduli of acid gels made with partially κ -casein hydrolyzed milk showed that chymosin hydrolysis before GDL addition had a marked effect on formation and aging of gels (Figure 2). As shown in Table 4, the moduli values (G' and G'') reached 1 and 2 h after the gelation time increased with the level of κ -casein hydrolysis. For comparison, moduli values of a gel made at 30°C by rennet addition were also shown (Table 4). Values obtained with partially hydrolyzed milk were in all cases (19, 35, and 51% hydrolysis) significantly higher than those of rennet milk gel. On the contrary, moduli values of rennet milk gel were as expected higher than those of strictly acid milk gel (0% hydrolysis).

The rate of change in G' with time ($\Delta G'/\Delta t$) as a function of time of acid milk gels made with partially converted casein micelles were compared to those of

Table 3. Gelation time and pH obtained for acid milk gel made by glucono- δ -lactone addition as a function of the degree of κ -casein hydrolysis.

Degree of κ -casein hydrolysis (%)	Gelation time ¹ (min)	Gelation pH ¹
0	72.2 ± 1.3	5.15 ± 0.03
19	40.1 ± 0.9	5.38 ± 0.02
35	26.5 ± 0.4	5.48 ± 0.01
51	18.3 ± 0.7	5.65 ± 0.02

¹Mean values of duplicate analysis.

Figure 1. Effect of the degree of κ -casein hydrolysis on pH values as a function of time at 30°C after glucono- δ -lactone addition (2%, wt/vol): 0%, $\diamond$; 19%, $\circ$; 35%, $\triangle$; 51%, $\square$. Mean values of duplicate experiment.

strictly acid and rennet gel (Figure 3). The initial rate of gel firmness ($\Delta G'/\Delta t$) appeared steeper for acid gels made with chymosin hydrolyzed milk than those of strictly acid gel. On the contrary, no significant difference was observed in regard to the initial rate of gel firmness for acid gels made at the three levels of chymosin hydrolysis (19, 35, and 51%). For these three acid gels, the rate of change in G' with time appeared quite complex with two stages separated by a shoulder (shallow local minimum in the curve) which corresponded to pH values of 5.3, 5.2, and 5.15 for acid gel made with milk hydrolyzed at 51, 35, and 19%, respectively. During this second stage, the curves of $\Delta G'/\Delta t$ continued to rise as a function of time, without important changes in the shape of the curves. Although the slope of the three curves appeared identical during the first stage, a small increase in the slope of the curves could be noted during the second stage as increasing the level of κ -casein hydrolysis (Figure 3). The effect of the extent of κ -casein hydrolysis on gel formation appeared to be more significant in the second stage of the change in G' with time than in the first one, i.e., as soon as the pH value of about 5.2 has been reached. The higher values of gel firmness obtained at $t_g + 1$ h and $t_g + 2$ h for acid gels made with hydrolyzed milk (Table 4) seemed to be a consequence of the important increase in the rate of gel firming during the second stage of the time derivative G' curves. Rennet milk gel exhibited steeper initial rate of gel formation than acid gels made with chymosin-treated milks but the time derivative G' curve slowed down rapidly to reach a plateau and finally decreased as previously observed for strictly acid gel (Figure 3).

Figure 2. Effect of the degree of κ -casein hydrolysis on the storage (G' : plain symbol) and the loss (G'' : open symbol) moduli of acid gel made by glucono- δ -lactone addition as function of time at 30°C: 0%, $\diamond$; 19%, $\bullet$; 35%, $\circ$; 51%, $\triangle$; 51%, $\square$. Mean values of duplicate experiment.

The particular shape of the time derivative G' curves obtained for acid gels made with partially chymosin-hydrolyzed milk could be related to results recently reported by Tranchant et al. (2001). According to these authors, the curves of $\Delta G'/\Delta t$ obtained for milk acidified in minimal renneting conditions presented also irregularities ("maximum-minimum") near pH 5.2 (approximately 40% hydrolysis of κ -casein) that were more pronounced than those shown in Figure 3. However, in the present study, the κ -casein hydrolysis of milk was controlled before GDL addition contrarily to the study presented by Tranchant et al. (2001) in which milk acidification and renneting evolved simultaneously.

The changes in $\tan \delta$ values during the formation of the different types of gels studied were presented in Figure 4. A maximum in $\tan \delta$ was observed at about pH 5.2 for acid gels made with chymosin-hydrolyzed milk, whatever the degree of hydrolysis, whereas a shoulder was noted around pH 5.1 for strictly acid gel (0% hydrolysis). After this maximum (or shoulder in the case of acid gel) $\tan \delta$ decreased progressively, and the rate of decrease tended to fall with time. In contrast, no maximum was observed for rennet gel and $\tan \delta$ continued to decrease until reaching a plateau (Figure 4). Such behavior in $\tan \delta$ was recently reported by Lucey et al. (2000) for both GDL and combined gels made with heated milk. In all cases the maximum in $\tan \delta$ was observed around pH 5.15. A maximum in $\tan \delta$ in the same range of pH was also noted by Tranchant et al. (2001) for acid and combined gels. Likewise higher

Table 4. Storage and loss moduli values¹ of acid milk gel with different degree of κ -casein hydrolysis and rennet milk gel determined at 1 and 2 h after their gelation time (tg).

Type of gel	G' (Pa) at tg + 1h	G'' (Pa) at tg + 1h	G' (Pa) at tg + 2h	G'' (Pa) at tg + 2h
Acid milk gel				
0% κ -casein hydrolyzed	19.7 $\pm$ 1.5	6.7 $\pm$ 0.3	37.8 $\pm$ 0.5	10.8 $\pm$ 0.3
19% κ -casein hydrolyzed	38.4 $\pm$ 1.4	15.7 $\pm$ 0.2	116.2 $\pm$ 0.6	36.1 $\pm$ 0.9
35% κ -casein hydrolyzed	47.2 $\pm$ 1.8	21.2 $\pm$ 0.4	177.2 $\pm$ 1.1	61.6 $\pm$ 0.8
51% κ -casein hydrolyzed	52.7 $\pm$ 1.7	25.0 $\pm$ 0.3	192.0 $\pm$ 1.5	76.6 $\pm$ 0.7
Rennet milk gel	40.0 $\pm$ 1.5	11.2 $\pm$ 0.2	80.0 $\pm$ 0.9	24.2 $\pm$ 0.4

¹Mean values of duplicate experiment.

values of $\tan \delta$ were found by Roefs et al. (1990b) at pH 5.2 for gels made by acidification with HCl in the cold and renneting. All of these observations permitted us to assess that the maximum in $\tan \delta$ appeared as a pH-induced effect only and was independent of the extent of κ -casein hydrolysis or the aging time of the gel. As reported by Lucey et al. (2000) for combined gels, the solubilization of CCP at low pH values led to a partial loosening of bonds within the casein particles in gel network. Solubilization of CCP may alter the balance between viscous and elastic components in the network modifying protein-protein interactions, and hence may be responsible for the increased loss tangent of gel observed.

Results obtained indicated that partial chymosin-hydrolysis of milk prior to GDL addition caused much stiffer gels to be formed. As previously reported by Roefs et al. (1990b) higher values of the storage moduli were found for gels obtained by combining acidification at pH 5.1 (or below) and renneting compared with those observed for strictly acid gels. In agreement with Tran-

chant et al. (2000), the effects of partial κ -casein hydrolysis and milk acidification appeared as synergistic, with acidification enabling the aggregating tendency of partially hydrolyzed casein particles. The unmasking of supplementary reactive sites on casein micelles due to the release of CMP might counterbalance the destabilizing effect of casein demineralization on gel cohesiveness and contribute to reinforce the structure of the gel. According to Roefs et al. (1990b) an important and gradual increase in G' with time might be largely attributed to increased fusion of casein particles due to rearrangement of both inter- and intramolecular forces. Such behavior was observed for acid gels made with partially chymosin-hydrolyzed milk after the gel has formed and especially from pH 5.2, pH range where an extensive fusion of casein particles coming with important protein conformational rearrangements was previously reported by Gastaldi et al. (1996).

Relation Between Rheological Properties and Water-Holding Capacity of Acid Milk Gels Made with Partially Converted Casein Micelles

Table 5 summarizes loss tangent values and serum-holding capacity of gels made by GDL addition in milk

Figure 3. Changes in $\Delta G'/\Delta t$ as a function of time for acid glucono- δ -lactone gels made with increasing degree of κ -casein hydrolysis: 0%, $\blacktriangle$; 19%, $\diamond$; 35%, $\triangle$; 51%, $\square$, and for rennet milk gel $\bullet$ (at 30°C). Mean values of duplicate experiment. **Figure 4.** Changes in loss tangent ($\tan \delta$) as a function of time for acid glucono- δ -lactone gels made with increasing degree of κ -casein hydrolysis and for rennet milk gel (at 30°C): 0%, $\blacktriangle$; 19%, $\blacklozenge$; 35%, $\triangle$; 51%, $\blacksquare$, and for rennet milk gel, $\bullet$ (at 30°C). Mean values of duplicate experiment.

Table 5. Loss tangent values ($\tan \delta$), serum-holding capacity [g of serum drained/100 g of gel (%)] and pH of acid milk gels with different degree of κ -casein hydrolysis and rennet milk gel determined at 2 h after their point of gel (tg).

Type of gel	$\tan \delta^1$	Serum-holding capacity (%) ²	pH ¹
Acid milk gel			
0% κ -casein hydrolyzed	0.28 ^a $\pm$ 0.02	82.12 ^a $\pm$ 0.12	4.67 $\pm$ 0.02
19% κ -casein hydrolyzed	0.31 ^b $\pm$ 0.01	85.91 ^b $\pm$ 0.25	4.76 $\pm$ 0.03
35% κ -casein hydrolyzed	0.35 ^c $\pm$ 0.03	87.64 ^c $\pm$ 0.12	4.82 $\pm$ 0.04
51% κ -casein hydrolyzed	0.39 ^d $\pm$ 0.02	90.21 ^d $\pm$ 0.32	4.90 $\pm$ 0.02
Rennet milk gel	0.30 ^b $\pm$ 0.01	85.64 ^b $\pm$ 0.23	6.67 $\pm$ 0.03

^{a,b,c,d}Means with different superscripts differ ($P < 0.05$).

¹Mean values of duplicate experiment.

²Mean values of triplicate determination.

having different levels of κ -casein hydrolysis. A strictly rennet gel was also proposed for comparison. At a given time after gelation (2 h), increasing the degree of κ -casein conversion in milk led to an increase in loss tangent and serum-holding capacity of acid milk gels. As a comparison, strictly rennet gel exhibited $\tan \delta$ values and serum-holding capacity higher than strictly acid gel, but lower than acid milk gel made with chymosin treated milk at the two highest levels (35 and 51%). The small changes in pH observed between different gels (Table 5) were probably insufficient to explain these results. So, acid gels made from partially converted milk appeared to be more prone to serum separation than strictly acid gels. Such behavior might be explained by taking into consideration the effects of κ -casein hydrolysis which is thought to alter mainly the type of interactions involved in gel network.

In general, most rheological parameters characterizing milk gels depend on the number and strength of bonds between the casein particles, on the structure of the latter, and the spatial distribution of the strands making up these particles (Roefs et al., 1990a). According to Lankes et al. (1998), G' values resulted from stronger proteins bonds contributing to the elastics characteristics such as electrostatic interactions and hydrogen bonds, whereas G'' values were due to weaker bonds such as hydrophobic and Van der Waals bonds. On the other hand, $\tan \delta$ was independent of the number of bonds but was related to the types of bonds (van Vliet and Walstra 1985). As a consequence, the increase in $\tan \delta$ of acid gels made with partially converted micelles could be attributed to change in the balance between viscous and elastic components in the network in favor of weaker interactions characterized by the viscous component. As reported by Roefs et al. (1990a), a higher value of $\tan \delta$ indicated an increased relaxation behavior of the protein-protein bonds that could be related to a transition from an "acid" to a "rennet" gel character (Lucey et al., 2000). On the other hand, a higher value of

the loss tangent was often accompanied by an increased susceptibility of the gel to rearrangements and syneresis (Van Vliet et al., 1991). These trends appeared consistent with the increase in both $\tan \delta$ of the gel and its serum-holding capacity as increasing the level of κ -casein conversion in acid gels.

Results obtained in this study demonstrate that it is possible to modify rheological and serum-holding behavior of acid gels by changing micellar surface reactivity with controlled κ -casein chymosin hydrolysis. Although the use of Pepstatin A and DMSO appeared difficult to be applied in cheese making, acid gel made with partially chymosin hydrolyzed milk constituted an useful model system for studying the effect of changes in chemical interactions during acid milk gel formation. However, for industrial applications, complementary studies should be performed to find another way to inhibit chymosin, notably with an appropriate heating treatment.

ACKNOWLEDGMENTS

The financial support of the scientific council of the University of Montpellier II is gratefully acknowledged. Authors wish to thank Pr Blas Tarodo de la Fuente for helpful scientific advices at the initiation of this work.

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