Dynamic Rheology of Renneted Milk Gels Containing Fat Globules Stabilized with Different Surfactants

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ABSTRACT

Anhydrous milk fat was emulsified with α_{s1}-CN (casein), α_{s2}-CN, β-CN, κ–CN, α-lactalbumin, β-lactoglobulin, Tween 80, or phosphatidylcholine to produce a 30% fat cream in a 0.1 *M* imidazole pH 7 buffer. The creams were mixed with skim milk to yield a fat content of 3.4% and the viscoelastic properties of the recombined milks clotted with chymosin were measured. Recombined milk containing globules coated with the more amphipathic and phosphorylated α_{s2}-CN and β-CN clotted faster but gel firmness increased more slowly and weaker gels were formed. Gel firmness increased more rapidly for milks containing globules coated with of α_{s1}-CN and κ-CN that possess more uniformly distributed hydrophobic domains.
(**Key words:** emulsion, milk proteins, dynamic rheology, renneted milk gel)

Abbreviation key: CT = clot time, **G′** = storage shear modulus, **G″** = loss shear modulus, **G*** = complex shear modulus, **H** = homogenized cream, **PC** = phosphatidylcholine, $\mathbf{T_G}$ = time taken for renneted recombined milk to reach a complex shear modulus of 2.5 Pa, **UH** = unhomogenized cream, γ = shear strain, γ_c = critical strain at the point of nonlinearity of elastic moduli, δ = phase angle.

INTRODUCTION

The initial stage of manufacturing most varieties of cheese involves the rennet coagulation of milk; structural changes occurring during this stage have implications for the development of the physical properties of the finished cheese. There are two distinguishable phases in the milk clotting process. In the primary phase, 80 to 90% of the κ-CN molecules in the casein micelle are cleaved before casein aggregation is visible at the normal pH of milk (7). Further aggregation of micelles continues during the secondary phase to form the protein network or gel. The coagulum is usually cut 30 min after the addition of rennet during manufacture of Cheddar cheese, although the protein network continues to undergo new bond formation and rearrangement after this time (26).

The casein matrix of a renneted milk gel consists of strands of fused casein micelles that are about four micelles thick and 10 micelles long. The network becomes stronger and coarser over time as new links are formed between the protein strands (31). These changes are reflected in increases in the storage shear modulus (**G′**) and the loss shear modulus (**G″**) of milk gels, which most likely reflect the increase in the number and density of bonds between casein strands (28). The phase angle (δ), which is the inverse tangent of the ratio of G″ to G′, remains fairly constant after the first few minutes of renneting or may decrease slightly over time (28).

The effect of milk fat globules on the moduli of the casein gel varies depending on the nature of the coating on the globule surface (28). Globules that have been coated with casein micelles interact with the casein gel matrix and increase the number of junctions within the matrix and the rigidity of the gel.

A number of studies have shown or suggested that coating of the fat globules with casein micelles occurs during homogenization. The similarity of ζ-potentials of native casein micelles and homogenized fat globules supports the hypothesis that the micelles are adsorbed intact onto the fat-water interface (6). Under turbulent conditions, such as found during homogenization, larger species such as whole casein micelles will move to the interface first but under quiescent conditions, smaller surface-active species will migrate to an interface more quickly than larger species (27). The presence of a larger proportion of submicelles in homogenized cream (**H**) compared with that found in the serum phase of milk suggests that the adsorption process itself causes some disruption of micelles into submicelles (8). Despite the possibility of rupture during homogenization, micelles will quickly reform in minutes when not adsorbed onto an interface (27), indicating that the adsorption process, rather than homogenization, is re-

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sponsible for any micellar disruption on a long time scale.

Casein micelles have been observed on the surface of fat globules found in homogenized milk, and these globules have been shown by electron microscopy to participate as copolymers with casein micelles (12). Bridging of oil droplets by fat crystals was observed to occur in oil-in-water emulsions (12) and also between fat globules in homogenized milk by casein micelles as shown by thin-sectioning electron microscopy (15).

Previous research has examined the effect of stabilized fat globules on the rheological properties of whey protein gels (1, 2, 3, 11, 14, 19, 30) and acid milk gels (24, 25, 29), especially the interactions between the globule stabilizing layer and the surrounding bulk matrix. Particles with surface coatings that interact with the surrounding acid milk gel protein matrix have been shown to cause an increase in G′, compared with gels containing noninteracting particles (12).

In the present study, the effect of different fat globule surface coatings on the renneting behavior and gel strength of coagulated milk was investigated. Fat globules were coated with individual caseins (α_{s1}-CN, α_{s2}-CN, β-CN, κ-CN) or whey proteins (α-LA, β-LG) isolated from milk, phosphatidylcholine (**PC**) or a nonionic surfactant polyoxyethylene sorbitan monooleate (Tween 80). Dynamic oscillatory measurements were employed to provide information regarding the conditions that exist in the milk coagulation stage of cheese making. We are not aware of previous publications on the rheology of chymosin-catalyzed clotting of milk containing milk fat globules coated with individual milk proteins.

METHODS

Preparation of Membrane Coating Material

Casein. Whole casein was prepared from fresh Holstein milk (13). Milk (6 L) was obtained from the University of Wisconsin Dairy Farms (Arlington, WI), defatted by centrifugation at the University of Wisconsin Dairy Plant and warmed to 30°C. The casein solution (pale brown viscous liquid) was freeze-dried and stored at room temperature until used. Kjeldahl protein analysis showed that the freeze-dried material was 91.9 ± 0.3% protein by weight. Approximately 30 g of freeze-dried material could be obtained in this way from 1 L of skim milk, assuming an 89% yield and that the skim milk comprised 2.8% casein.

Isolation and purification of casein fractions. A cation-exchange chromatographic method was used to isolate the four major caseins (18). The maximum amount of casein that can be loaded onto the column and still achieve good separation is 60 g per 1000 g of S-Sepharose Fast Flow cation-exchange medium (Sigma Chemical Company, St. Louis, MO). Casein concentration was kept below 12% to facilitate flow of the high viscosity solution through the column (J. Leaver, 1993, personal communication). All water used in this casein purification was deionized and filtered (Milli-Q Plus, Millipore Corporation, Bedford, MA). Flow rate was initially 600 ml/h per hour, but decreased to 300 ml/h when significant binding of caseins to the ion-exchange medium occurred.

The β-CN eluent was diluted with four volumes of deionized water and adjusted to pH 4.6 with 1 *M* HCl, and then left for several hours to allow the protein to sediment. The top clear aqueous layer was siphoned off and the remaining suspension centrifuged at $2800 \times g$ for 10 min followed by washing the precipitate in 17 volumes of deionized water. The centrifugation and washing was repeated three more times, and the concentrated washed β-CN suspension was then adjusted to pH 7.0 to dissolve the casein, then freeze-dried. The pooled eluents of α_{s1}-, α_{s2}-, and κ-CN were dialyzed against 15 volumes of deionized water at 4°C over a 24-h period with six changes of water using 12,000 to 14,000 kDa cut-off Spectra/Por dialysis tubing (Spectrum Medical Industries, Inc., Los Angeles, CA). The α_{s1}-CN and α_{s2}-CN precipitates were filtered through Whatman #1 filter paper, macerated in a blender, and freeze-dried in a small volume of water. The κ-CN was freeze-dried in solution.

Preparation of whey proteins. Milk (pH 6.7) was obtained as before, the fat was removed by centrifugation, and the temperature of the skim milk was adjusted 21 ± 2°C. Fractionation of α-LA and β-LG from the skim milk was achieved by pH adjustment and ammonium sulfate precipitation, by method Ic for β-lactoglobulin isolation and method IIb from the discarded portion of Ic to isolate α-LA (4). Flaky, pale yellow α-LA (5.5 g, approximately 30% yield) and flaky white β-LG (21.4 g, approximately 30% yield) were obtained and stored at room temperature in a desiccated chamber until used.

Phosphatidylcholine and Tween 80. Phosphatidylcholine was purified by alumina adsorption column chromatography (22). Alumina for chromatographic analysis (800 g, 80 to 200 mesh, Fisher Scientific) was activated at 120°C for 1 h and cooled. Crude lecithin (29.4 g) containing 60% PC (Sigma Chemical Company, St. Louis MO) was dissolved in 600 ml of chloroform with 0.01% 2,[6]-di-tert-butyl-*p*-cresol (butylated hydroxytoluene) antioxidant and loaded onto the column at room temperature (22 ± 2°C).

Elutions shown by TLC to contain PC were pooled, and the methanol-chloroform solvent was extracted by rotary evaporation at 50°C under reduced pressure (Model 83 Thelco rotary evaporator, Precision Scientific Co., Chicago, IL), with 0.01% butylated hydroxytoluene

added as a preservative against lipid peroxidation. Deionized water (approximately 50 ml) was added to the syrupy orange liquid left after evaporation, and the resultant opaque white liquid was freeze-dried and stored at –18°C until required. Phosphatidylcholine (16.6 g, 57% yield) was slightly off-white, oily, and flaky in appearance.

Polyoxyethylene sorbitan monooleate nonionic surfactant (Tween 80, Sigma Chemical Company, St. Louis, MO) was used without further purification.

Anhydrous milk fat. Anhydrous milk fat was obtained frozen (Level Valley Dairy Company, West Bend, WI) and stored at 4°C until used. Several samples were taken from the surface and at different depths from the interior of the 20-kg cylinder of fat, and the melting profile was investigated by nuclear magnetic resonance (16). No significant differences were observed in the ratio of solid to liquid oil in the milk fat taken from different points of the cylinder, over the range 0 to 40°C. At 0°C, 45% of the oil was solid, and it was completely liquid at 40°C.

Preparation of Emulsions and Recombined Milk

Anhydrous milk fat (720 g) and a 0.1 *M* pH 7 imidazole buffer solution (1680 ml) were separately heated to 63°C and pasteurized by holding at this temperature for 30 min. Protein, Tween 80, or PC surfactant material (6 g) was mixed into the buffer, and the heated anhydrous milk fat added. The mixture was blended at 10,000 rpm with a 20-mm (150 mm long) saw tooth bottom probe (Pro 300D, Pro Scientific, Inc., Monroe, CT) for 2 min to disperse the milk fat into a coarse, 30% (wt/wt) fat emulsion, followed by homogenization in a one-stage homogenizer (type 8.30H, APV-Rannie Inc., St. Paul, MN) at 50°C and a pressure of 20.0 MPa. The outlet temperature of the homogenizer was in the range 40 to 48°C. Preheating the homogenizer by passing through several liters of hot water was necessary to ensure that the cream did not cool below 40°C during homogenization. The coarse emulsion was typically pale yellow and tended to separate into a cream layer and an aqueous layer within minutes, so continual stirring was necessary when adding to the homogenizer. The final H cream did not separate and was off-white.

Prepared 30% fat emulsions (113 ml) containing fat globules with different surface coatings were mixed with skim milk (887 ml, containing <0.10% fat) and mixed to yield a recombined milk with a fat content of 3.4%, as measured by the Babcock method. The recombined milk was warmed to 31°C immediately prior to renneting. Tenfold diluted double-strength, fermentation-derived recombinant chymosin (0.016% undiluted strength, Pfizer, Inc., Milwaukee, WI) was added to the recombined milk before measurement of the rheological properties of the milk gel.

Unhomogenized cream (**UH**) (30% fat, 113 ml) was separated from milk and a portion homogenized at 20.0 MPa and 50°C. The UH and H creams were mixed with skim milk (887 ml) as a control experiment, renneted and the dynamic rheological properties compared to that of the recombined milk gels containing fat globules with prepared membrane coatings.

Rheological Test Methods

A Bohlin VOR Rheometer (Bohlin AB, Lund, Sweden) was used to measure the dynamic rheological properties of milk gels containing coated fat globules (5). The measuring head was prewarmed to 31°C before measurements and comprised a 25-mm-diameter C25 cup and a 23-mm-diameter spindle, providing a 1-mm-wide annulus in which the sample resided. A lid was placed around the top of the measuring cup to minimize sample evaporation and heat loss. A small quantity of silicone oil (<1 ml) was injected into the top of the annulus containing the renneted milk to further prevent sample evaporation. Temperature was controlled by circulating water from a thermostat unit. A 5-min delay between renneting the milk and starting measurements allowed time for temperature equilibration and setting up of the rheometer hardware. The torque bar used was of rating 1.702 g.cm (0 to 0.6% dynamic range).

Torque on the inner spindle, shear strain (γ) during oscillation, and the δ between torque and γ were measured as a function of time after renneting. All rheological variables of interest, such as G″, G′, and the complex shear modulus (**G***) can be derived from torque, γ, and δ (21). Modeling of data was done using Bohlin software (BRS, version 4.05 and data processing module, version 3.01, Bohlin AB, Lund, Sweden). Measurements were taken at 31°C of triplicate batches of each milk except for a single batch of milk containing globules coated with κ-CN, and the means and standard deviations recorded.

Oscillation over time. Dynamic rheological variables G*, G′, G″, and δ were either measured or derived in an oscillatory experiment at a γ amplitude of 5% (γ = 0.0036 in dimensionless units) and a frequency of 1 Hz to investigate the curd coagulation profile over time. These conditions do not affect the coagulation process (9). Clot time (**CT**) was defined as the point where G′ deviated from the baseline value. It was determined by fitting the values of G′ over the 5-min period, after the visually estimated deviation, to a second-order polynomial and calculating the time at which the curve intersected the average baseline value. The time required for the renneted recombined milks to reach G* = 2.5 Pa

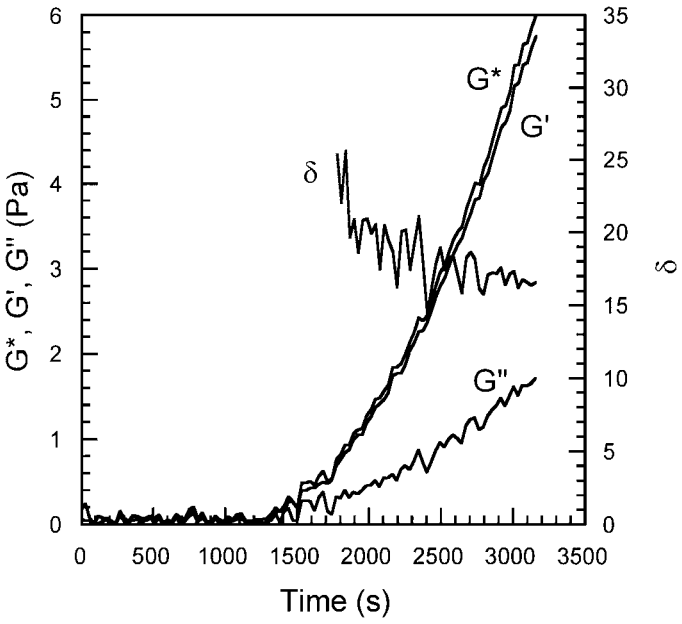

Figure 1. Dynamic rheological data over time for 3.4% fat recombined milk undergoing clotting by rennet and containing fat globules coated with α_{s1}-CN.

after renneting ($\mathbf{T_G}$) was also determined. Preliminary experiments on renneted milk (where the cream was not separated from the milk) showed that the point of cutting the curd during cheesemaking corresponded to a value of G* = 2.5 Pa. The value of T_G-CT was designated as the time of the secondary phase of rennet action and is an indication of the rate of increase of the firmness of the gels.

Shear strain sweep. The γ sweep experiments on the milk gels were started when G* = 2.5 Pa; values for G* were at 1 Hz and 5% γ amplitude. The γ was increased from 0.1 to 100% of the instrumental maximum value (0.00007 to 0.073 in absolute dimensionless units) in 30 steps with 5 s between each step at a frequency of 1 Hz; G*, G′, G″, and δ were measured or derived. Because the experiment took a finite amount of time to complete, and because the milk coagulum was continually increasing in firmness, all data were corrected (based on time) back to values corresponding to the point where G* = 2.5 Pa at a γ amplitude of 5% and a frequency of 1 Hz. The critical γ at the point of nonlinearity of elastic moduli (γ_c) was measured at the point where the elastic moduli decreased at increasing γ.

RESULTS

A clotting curve, showing dynamic rheological measurements over the period of milk renneting, is shown in Figure 1. During the early stages of the renneting reaction, the elastic moduli (G*, G′ and G″) remain constant, then begin to rise approximately 10 to 20 min after renneting. Values for CT and T_G-CT are recorded in Table 1.

The point at which G′ begins to rise is a direct measure of the point of clotting, as a clotted milk is by definition milk that has begun to become more viscous and solid-like. Values for CT ranged from 570 to 640 s for H cream milk, α_{s2}-CN and β-CN cream milks, to 1560 to 1780 s for PC, α-LA and α_{s1}-CN cream milks (Table 1), and agree with results obtained from Cheddar cheesemaking experiments (10).

The time to reach the point of cutting the curd in cheesemaking after renneting is represented by T_G, and ranged from 1990 to 2180 s for H cream milk, α_{s2}-CN, β-CN and κ-CN cream milks, to 2680 to 2980 s for UH cream milk, α-LA, β-LG and PC cream milks. These times were generally longer than required for unmodified renneted milk to clot prior to cutting in Cheddar cheese manufacturing trials (10).

The time taken for milk to clot subtracted from the time taken to reach G* = 2.5 Pa gives an indication of the secondary phase of rennet action and is expressed as T_G-CT in Table 1. Values ranged from 1040 to 1530 s for most of the renneted milks, with the exception of milk containing fat globules coated with α_{s1}-CN (870 s) and κ-CN (840 s).

Values for G*, G′, G″, and δ were measured or calculated from the γ sweep experiments; examples of two typical trends of elastic moduli as a function of γ are shown in Figures 2 and 3. At γ less than γ_c the elastic moduli were constant, where γ_c is a measure of the extent of linear viscoelasticity.

All renneted recombined milks exhibited linear viscoelasticity to the maximum γ possible on the rheometer ($\gamma = 0.07$), with the exception of milks containing globules coated with either α_{s2}-CN or β-CN (Table 1). These two recombined milks showed a decrease in G′ at γ greater than $(1.3 \pm 0.3) \times 10^{-3}$, indicating that at the frequency of oscillation chosen (1 Hz), bonds between structural elements in the renneted milk were being broken. Gels from renneted milk containing α_{s2}-CN coated globules were observed to be very weak; the gel broke apart easily when removed from the measuring device after the conclusion of the experiment.

DISCUSSION

The γ sweep experiment was conducted to determine the region of linear viscoelasticity of milk curd, that is, the amplitude of oscillation below which the viscous and elastic moduli are independent of γ. Above the limit of linear viscoelasticity, bonds within the curd structure will break and re-form during oscillation in the rheome-

Table 1. Dynamic rheological properties of coagulated recombined milk containing coated globules (n = 3).

Coating	CT[1]		T_G[2]		T_G-CT		$1000 \times \gamma_c$[3]	
	(s)							
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
UH Cream	1404	30	2780	30	1376	60	>72.95	. . .
H Cream	616	40	2000	110	1384	150	>72.67	. . .
α-LA	1560	30	2710	130	1150	160	>73.08	. . .
β-LG	1190	140	2680	280	1490	420	>73.20	. . .
α_{s1}-CN	1580	40	2450	80	870	120	>58.43	. . .
α_{s2}-CN	640	80	1990	140	1350	220	1.3	0.3
β-CN	570	190	2100	300	1530	490	1.3	0.3
κ-CN[4]	1340	. . .	2180	. . .	840	. . .	>58.45	. . .
Tween 80	1370	100	2410	160	1040	260	>73.02	. . .
Phosphatidylcholine	1780	80	2980	30	1200	110	>58.59	. . .

[1]Clot time based on storage shear modulus (G′) measurement.

[2]Time taken to reach a complex shear modulus of G* = 2.5 Pa after rennet addition.

[3]Dimensionless critical strain, above which the moduli cease to be linear. Values expressed as ">" are linear up to the maximum shear strain possible for particular measuring heads on the rheometer.

[4]n = 1 for κ-CN.

ter, and the elastic moduli will decrease with increasing γ.

The data for oscillatory rheometry shown in Table 1 were reasonably consistent between gels containing the various coated globules, with some exceptions. Clot times were lower for milk containing H cream or globules coated with either α_{s2}-CN or β-CN. Values for T_G, were shorter for these recombined milks, as was milk containing globules coated with κ-CN. The interval between clotting of milk and the point where curd would be cut in cheesemaking, T_G-CT, was lowest for milks containing globules coated with either α_{s1}-CN or κ-CN. The γ at which the elastic moduli became nonlinear, γ_c, was lowest for milks in which globules were coated with either α_{s2}-CN or β-CN. The onset of nonlinearity (Figure 3) occurs when the oscillatory γ is large enough to break bonds within the gel or to stretch bonds beyond the simple harmonic limit. As the strength of the bonds between casein strands in the protein matrix does not depend upon the nature of the fat globule membrane present, any rheological differences must either be the

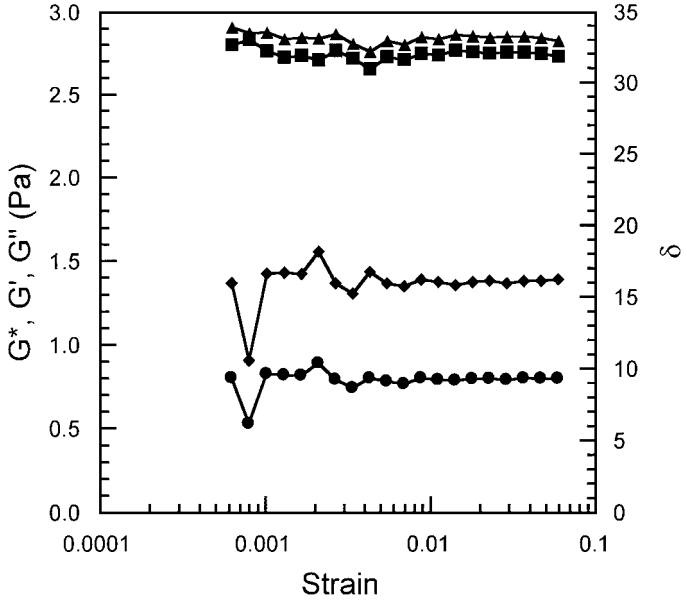

Figure 2. Dynamic rheological data as a function of shear strain for 3.4% fat recombined milk undergoing clotting by rennet and containing fat globules coated with phosphatidylcholine. G* (▲),G′ (■),G″ (●), δ (♦).

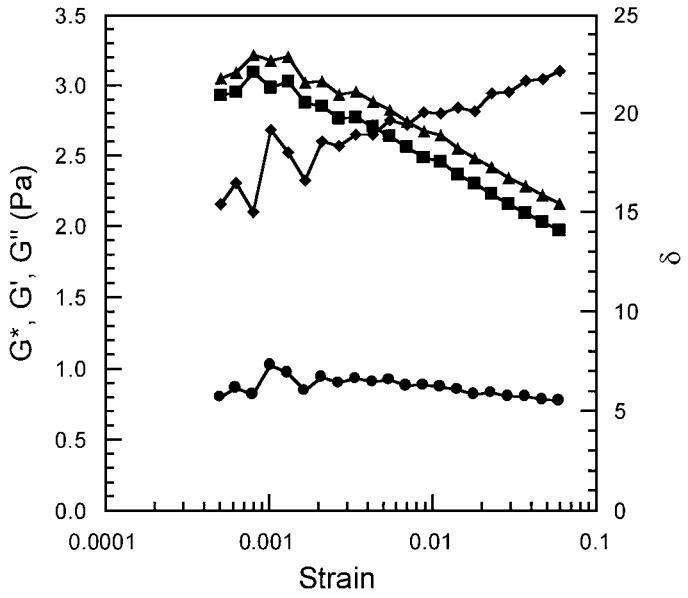

Figure 3. Dynamic rheological data as a function of shear strain for 3.4% fat recombined milk undergoing clotting by rennet and containing fat globules coated with α_{s2}-CN. G* (▲),G′ (■),G″ (●), δ (♦).

result of casein-fat globule interactions or interactions between fat globules.

These deviations appear to be consistent with the inherent properties of the proteins coating the fat globules, or the impact of homogenization of milk fat into skim milk. The shorter CT for α_{s2}-CN and β-CN milk may be explained by the highly phosphorylated and amphipathic structure of these molecules in which the hydrophobic C-terminal domain would be associated with the fat and the phosphorylated, hydrophilic N-terminal domain extends out into the aqueous phase. Evidence for this hydrophilic layer on an oil-water interface has been given for β-CN (17), and may also represent the interfacial conformation of α_{s2}-CN based on the relatively hydrophilic 1-89 and hydrophobic 90-207 regions (23). The amphipathicity of these two proteins may result in the formation of a "hairy emulsion droplet."

At pH 6.6, ionic calcium would be associated with the phosphate groups of β-CN and α_{s1}-CN, which would induce the coated fat globules to participate in the initial aggregation of renneted casein micelles in the skim milk. None of the other coating components possesses this characteristic. However, the hydrophilicity and degree of phosphorylation of the exposed domains of these two proteins on the globule surface may have hampered further integration of the coated globules into the casein matrix or the renneted milk, as indicated by the longer time periods from CT to T_G, and the weaker gel structure as indicated by γ_c. Cheese containing fat globules coated with α_{s2}-CN had a lower resistant force at 80% compression and lower stress at fracture than cheese containing globules coated with other milk proteins (10), providing further evidence that globules coated with α_{s2}-CN weaken the surrounding milk protein network.

The clotting characteristics of milks with α_{s2}-CN-coated fat globules suggest that the Phe_{88}-Tyr_{89} peptide bond in this casein was not cleaved even though it is the primary site of rennet action on this protein when it is in solution (20). Removal of this hydrophilic and highly phosphorylated peptide (23) should produce a membrane surface that would be more hydrophobic and possibly flatter. The modified α_{s2}-CN-stabilized emulsion would then not have the necessary concentration of phosphate groups to affect a fast clot and would exhibit a faster T_G-CT. As the rate of clot formation was relatively fast and gel firming was slow, the rate of rennet action on Phe_{88}-Tyr_{89} was probably low when this protein was adsorbed onto an oil-water interface, as was the case in the present study.

In contrast, milk gels containing globules coated with α_{s1}-CN had relatively fast secondary coagulation times, as represented by T_G-CT in Table 1. The hydrophobic domains of this protein are fairly uniform throughout its primary structure, accounting for 68% of the amino acid residues (23), suggesting that this protein provided a more hydrophobic fat globule surface which could participate in the milk gel structure development.

Cleavage of the Phe_{23}-Phe_{24} peptide bond of α_{s1}-CN by rennet would probably not allow the hydrophilic 44 to 90 portion from the interfacial membrane protein to extend freely out into the aqueous phase, similar to what has been postulated to occur with β-CN (17), because it would still be anchored to the globule surface by the hydrophobic 24 to 44 portion. The lack of cleavage of this peptide bond is suggested by the apparent minimal effect of removing this hydrophilic peptide on the secondary phase of rennet action (development of the gel structure).

The participation of hydrophobic associations is suggested also by the data for κ-CN. Globules coated with this protein had little effect on the initial stages of clotting, as indicated by CT approximating that of milk containing UH cream. However, the increase in gel strength from CT to the point where G* = 2.5 Pa was relatively rapid. These trends may be explained by κ-CN on the globule surface being acted upon by rennet to remove the glycomacropeptides and expose the hydrophobic, positively-charged para-κ-CN. The rate and extent of enzymatic cleavage of interfacial κ-CN may not have been sufficient to impact on the initial clotting of the recombined milk, but the hydrophobic associations became important in the later integration of the coated fat globules into the casein network. It is also possible that hydrophobic associations are not the dominant forces during the earlier stage of clotting as suggested by Walstra and van Vliet (28).

Fat globules coated with the other components, and the natural globules in UH cream, appeared to have less of an effect on the initial clotting process and the subsequent formation of the casein network in the milk. Clot time for milk containing H cream was short, which may have resulted from the fat globules being clustered, thereby accelerating the initial clot formation. There was greater interaction between H fat globules compared with that between globules coated with the surfactants used in this study, as shown by the higher viscosity, longer time to reach an equilibrium viscosity during constant shearing, and the presence of a Bingham yield stress for 30% fat H cream (10). The fat globules in the H cream would have been partially coated with casein micelles and thereby been a participant in the gel formation after rennet action. It is not clear why the casein micelle-coated fat globules in the H cream sample did not affect a faster formation of the milk gel to G* = 2.5 Pa after clotting. There may have been a limited number of micelles adsorbed on the glob-

ule surface during homogenization because of the lower amounts of caseins in the serum phase of the 30% fat cream.

CONCLUSIONS

Renneted milk clotted fastest when containing globules coated with the more amphipathic and phosphorylated proteins α_{s2}- and β-CN, as well as for milk containing H cream. However, for the case of globules coated with either of these two caseins, the short clotting time was accompanied by a long secondary phase of coagulation and a relatively weak gel structure that fractured easily during oscillation. Milk containing globules coated with α_{s1}- and κ-CN exhibited the fastest rate of gel firming during the secondary phase. These two proteins are distinguished by having more uniformly distributed hydrophobic domains in the primary protein structure, particularly so after rennet action on κ-CN to remove the hydrophilic glycomacropeptide. An exposed hydrophilic and phosphorylated peptide on the fat globule membrane seemed to provide for a fast rate of clotting of renneted recombined milk, whereas a hydrophobic fat globule membrane surface will hasten the formation of a strong gel by acting as a copolymer with the surrounding casein matrix.

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