Micellar Casein Gelation at High Sucrose Content

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ABSTRACT

This article investigates the effect of sucrose addition on the formation of casein gels by acidification and/or renneting of pure micellar casein. Gelation kinetics and gel properties were followed by rheological methods, and microscopy and syneresis measurements were used to obtain a more complete characterization of the structures formed. Sucrose content has been identified as a key parameter for controlling the kinetics of aggregation and the strength of the final gels. Results have shown that the effect of sucrose on gelation can vary such that effects can be completely reversed depending on the gelation route used. During acid gelation, addition of up to 30% (wt/wt) sucrose causes gels to form more rapidly and at higher pH values, and to have higher viscoelastic moduli and a more homogeneous microstructure than those without sucrose. By contrast, gels formed by renneting in the presence of sucrose are weaker and have longer gelation times. It is proposed that sucrose reduces solvent quality and causes the collapse of the “hairy” κ -casein brush on the surface of the casein micelles. This may explain why sucrose increases the possibility of gel formation during acidification and reduces the degree of κ -casein hydrolysis during renneting.

(Key words: casein micelle acid, gelation, rennet, sucrose)

Abbreviation key: CLSM = confocal laser scanning microscopy, CMP = casein macropeptide, GDL = glucono- δ -lactone, PCN = phosphocaseinate.

INTRODUCTION

Acidification and enzymatic treatment are the two procedures most commonly applied in the dairy industry to make milk gels. Casein is the main structure-building component of these gels, and in milk at pH 6.8, native caseins exist as large colloidal micelles in

association with calcium phosphate. The micelles are highly hydrated (average 3.7 g of water/g casein protein), have a large voluminosity (4.4 ml/g protein), and they range in size from 50 to 300 nm with a maximum distribution around a diameter of approximately 80 nm. They contain 93% (wt/wt) casein with the ratio of $\alpha_{s1}:\alpha_{s2}:\beta:\kappa$ -caseins present in a 3:1:3:1 proportion. Each micelle contains 20,000 to 150,000 casein molecules, giving an average molecular weight of 2.5×10^8 (Holt, 1975). The remaining 7% consists of inorganic calcium (2.87%), phosphate (2.89%), citrate (0.40%), and small amounts of magnesium, sodium, and potassium (Schmidt, 1982). Because of its amorphous character, the detailed structure of the casein micelle is not known exactly, but there is an accepted view that the particles are sterically stabilized by an external layer of κ -casein. One part of the κ -casein acts as an anchoring block (hydrophobic part), whereas the remaining charged hydrophilic part provides steric stabilization (Holt and Horne, 1996).

Gelation of casein micelles induced by acidification is completely different from that induced by renneting as far as modification of the casein micelles is concerned. Renneting affects mainly the surface of the micelles, whereas acidification leads to the collapse of the κ -casein layer and also the dissociation of the micelle core (dissolution of the colloidal calcium phosphate, and dissociation of casein molecules). During milk acidification, a number of physicochemical changes occur (Fox, 1990; Heertje et al., 1985; Roefs et al., 1985). The first step is the collapse of the hairy brush (charge neutralization of κ -casein) and the release of calcium phosphate. Then, molecules of casein—mostly β - and κ -casein—leave the micelle, and as their isoelectric point is passed, they become positive and try to reintegrate with the micelle (or casein particle), which still has an overall negative charge. The electrostatic interactions begin to override the dissociation forces, so that the altered micelles aggregate to form a three-dimensional gel network.

Rennet, like many other proteolytic enzymes, is able to clot milk. The active principle of calf rennet is chymosin. Rennet coagulation of milk has been described as a two-stage process (Horne and Davidson, 1990; de Kruif et al., 1992; McMahon and Brown, 1984). First, chymosin splits off a κ -casein macropeptide (CMP; Phe-

Received March 15, 2002.

Accepted June 19, 2002.

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Met bond); the casein micelles then become paracasein micelles, which are able to flocculate due to the reduced repulsion between the micelles resulting from loss of the κ -casein charged segment. The aggregates then form a network with pores a few micrometers in size and, as soon as a three-dimensional network forms throughout the milk, it becomes a gel. After formation of the gel network, many more bonds can, in principle, be formed between micelles due to more compact packing, and this leads to expulsion of liquid from the gel—a process known as syneresis. Depending on the renneting conditions used (pH, temperature, rennet concentration), milk gels can start to form at different levels of κ -casein hydrolysis. As an example, aggregation of micelles with lower levels of CMP hydrolysis will occur at higher temperatures.

In some dairy products, sucrose is commonly used, but surprisingly little systematic work has been reported on gel formation at low water activity. The aim of this study was, therefore, to look at the effect of sucrose on casein gel formation (acid and rennet) and to propose a hypothesis to explain the action of sucrose.

MATERIALS AND METHODS

Milk Solution

Native calcium phosphocaseinate. The micellar casein used in this study was a native calcium phosphocaseinate (PCN) purified by ultrafiltration and then freeze-dried. It was provided by INRA (Rennes, France) (Schuck et al., 1994; 1995), and was 90.7% total protein content, 5% noncasein proteins, 0.5% lactose, and 8.3% salts.

Solution preparation. PCN must be dissolved in a saline solution, which reproduces the salt content of milk, in order to maintain the integrity of the micelles. Thus, a milk buffer solution was prepared according to a method proposed earlier by Jenness and Koops (1962). Micellar casein dispersions were obtained by dispersing the PCN powder in the milk salt buffer while stirring with a paddle mixer (30 min at 60°C). Sucrose, when present, was added as a powder once the casein micelles were fully dispersed, followed by stirring at this temperature for a further 10 min.

Acidification and Sample Preparation

Two methods of acidification have been used to acidify 5% (w/w) PCN dispersions. Fast acidification was achieved by drop-wise addition (at a constant acidification rate) of 1M HCl to the milk salt solution at a temperature of 5°C, or below. Cold acidification was used in order to acidify systems to a given pH value without inducing any aggregation (de Kruif and Roefs, 1996).

The gelation process was followed by quiescently warming the system to 20°C in a controlled way. Slow acidification was achieved with the use of glucono- δ -lactone (GDL) from Sigma (St. Louis, MO), which slowly hydrolyzes into gluconic acid and which many authors have used to imitate the slow release of H_3O^+ ions by bacteria (Heertje et al., 1985). This ensures reproducibility of the acidification step and avoids the influence of bacteria on the ultimate network structure formed. The final pH chosen for this study was 4.7. To reach a final pH value of 4.7, 1.3 g of GDL was added per 100 g of 5% (wt/wt) micellar casein solution.

Renneting and Sample Preparation

Marshall rennet solution (chymosin activity 520 mg/L), provided by Texel (Rhodia, France), was diluted 1:100 (vol/vol) with deionized water. The concentration commonly used in this study was 0.25 ml/10 ml of casein dispersion. The diluted rennet was added at a temperature of 20°C.

Rheology Measurements

Small deformation measurements were made using a Physica US200 rheometer with Couette cylinder (Z3 DIN; Stuttgart, Germany) geometry to follow the gelation of casein micelles and to characterize the gels produced. The solution was transferred to the rheometer at 5°C, and the rheometer was then heated from 5 to 20°C, usually at a heating rate of 1.0°C/min. To prevent evaporation, samples were covered with silicone oil. Samples were oscillated at a frequency of 1 Hz, and the amplitude of deformation was chosen to be 1% strain in order to remain within the linear viscoelastic limits. A frequency sweep was generally performed after 60 h, covering a frequency range of 0.01 to 10 Hz.

Measurement of Enzymic Hydrolysis

Rennet was added to micellar casein dispersions (2.5 ml/10 ml) at 20°C, and tubes were then filled with a 10-ml aliquot of the sample at $t = 0$. TCA (12%) was added to each tube at appropriate times to stop the reaction. The samples were centrifuged for 10 min at $30,000 \times g$ to separate CMP from the other components. 2.5 ml of supernatant were filtered and introduced onto a HPLC column and the areas of CMP peaks were determined as a function of time.

Confocal Laser Scanning Microscopy (CLSM)

CLSM was used to visualize the microstructures of the gels. Rhodamine B (0.001% wt/wt) was added to the

dispersions in order to stain the proteins, and then GDL or rennet was added and mixed as usual. A small quantity of the sample was placed on a microscope slide with a cavity, and a cover slip was placed over the sample. The samples were stored for 24 h at 20°C, and then placed on a temperature-controlled stage. Visualization was carried out with laser excitation of 488 nm, provided by CLSM (Biorad MRC 600, Hemel Hempstead, England).

Syneresis

Syneresis was determined by measuring the amount of water formed on top of the samples stored in glass vials (2 × 6 cm) at a constant temperature of 20°C. The water was removed with absorbent paper and the samples were weighed in order to quantify water loss with time. The final water loss (%) was obtained from an average of five measurements with an error of ±3%.

Turbidity Experiments

Turbidity spectra were recorded on a UV-2101 spectrophotometer (Shimadzu, Scientific Instruments, Inc., Milton Keynes, England) between 400 and 800 nm, using 10-, 2-, or 1-mm optical pathlength cells depending on the concentration of the particles. The refractive index of the solvent and the refractive index increment were measured using a Bellingham+Stanley RFM34 refractometer (Kent, England). The refractive index increment of the casein was found to be 0.160 cm³/g.

The amount of light scattered by a particle in a given medium depends on several parameters and can be separated into different contributions (Doty and Steiner, 1949):

$$\tau = H \cdot Q \cdot S \cdot M \cdot c \quad (1)$$

where H , Q , and S are functions related to the optical constant, and the intra- and interparticle correction factors, respectively; M is molecular weight (g mol⁻¹); and c is concentration (g cm⁻³). H depends on the optical properties of both the solvent and particle and on the wavelength.

$$H = \frac{32 \cdot \pi^3 \cdot n_o^2 \cdot (dn/dc)^2}{3 \cdot N_a \cdot \lambda^4} \quad (2)$$

where n_o , dn/dc , N_a , and λ are the refractive index of the solvent, the refractive increment of the particle, Avogadro's number, and the wavelength of light in vacuum, respectively.

Using a simplistic expression for S , the turbidity (τ) per unit concentration (τ/c) is linked to the molecular

weight (M_w) of the casein particle and the second virial coefficient (A_2) via the relationship given by Doty and Steiner (1949):

$$H \cdot Q \cdot c / \tau = (1/M_w) + 2A_2 Q c \quad \text{for } \lambda \gg R_g \quad (3)$$

In the present work, turbidity at 800 nm and wavelength dependence (between 700 and 800 nm) of the turbidity ($d\log\tau/d\log\lambda$) are used. The derivative $d\log\tau/d\log\lambda$ can be written as follows:

$$d\log\tau/d\log\lambda = -4 + \alpha_1 + \alpha_2 + \beta + \beta' \quad (4)$$

where α_1 and α_2 come from the wavelength dependence of the refractive index of the solution and the refractive increment of the solute. These are small negative correction factors (Cancellieri et al., 1974) and are neglected here. β is the wavelength dependence of the dissipation factor ($d\log Q/d\log\lambda$), which is a function of the particle size and can be directly estimated from the relation between Q and D/λ , with D = the particle diameter. For small particles, $\beta = 0$, and the wavelength dependence of the turbidity is close to the -4 predicted by the Rayleigh theory (Cancellieri et al., 1974). β' accounts for the wavelength dependence of S , and is given by $d\log S/d\log\lambda$.

RESULTS AND DISCUSSION

Gelation via Acidification

Simultaneous acidification and gelation (with GDL). The acid gelation of micellar casein in the absence of sucrose was established at 20°C. A typical curve is illustrated in Figure 1, where GDL was added at $t = 0$. Initially, the system remains liquid and gelation occurs only after 21 h, at pH 4.7. At this point, G' increases and the gel firmness rises with increasing cure time, but maximal firmness is not achieved even after 60 h.

The effect of sucrose on the acid gelation of micellar casein is also shown in Figure 1. The results show that gelation time is significantly reduced as the sucrose content increases and that sucrose addition leads to much stronger gels (higher viscoelastic moduli), with an optimum at 30% (wt/wt) sucrose. The rate of pH reduction is independent of sucrose concentration, hence it is clear that gelation starts at a higher pH value in the presence of sucrose and consequently at a faster rate (Table 1). These results suggest that the differences in gelation rate can be attributed mainly to an effect of sucrose on solvent quality. At sucrose levels above 30% (wt/wt), it is assumed that a more grained and porous gel is formed. Also, at high sucrose levels, the micelle structure is more swollen (unpublished re-

Figure 1. Effect of sugars on the gelation curve of a micellar casein dispersion (5% wt/w) in the presence of glucano- δ -lactone (1.3%) and sucrose at $T = 20^{\circ}\text{C}$. (frequency = 1 Hz).

sults), and consequently the aggregates will be softer, which in turn will contribute to a lower gel modulus. Abbasi and Dickinson (2001) observed a similar effect of sucrose concentration on the modulus of skim milk gels formed by high-pressure treatment.

In order to test for the effect of sugar specificity, some experiments were carried out using glucose or glycerol in place of sucrose (wt/wt). The results were similar to those obtained for sucrose, showing that the effect is not sugar specific.

Successive acidification and gelation. Acidification with GDL is slow, which means that the gelation time is long and that acidification and gelation are coupled. Acidification to pH 4.7 with HCl at a temperature below 5°C allows the pH to be reduced from 6.8 to 4.7 within a few minutes so that acidification and gelation can be decoupled. If the system is then reheated to 20°C at a constant rate, gelation can be followed at this temperature. Figure 2 illustrates the gelation curves obtained in this way for sucrose concentrations between

Figure 2. Effect of sucrose on the gelation curve of a micellar casein dispersion (5% wt/wt) acidified at $T = 2^{\circ}\text{C}$ with HCl to pH 4.7 in 30 min. Heated at a rate of $1^{\circ}\text{C}/\text{min}$. to $T = 20^{\circ}\text{C}$ (frequency = 1 Hz).

0 and 60% (wt/wt). These results show that stronger gels are formed in the presence of sucrose with an optimum occurring at 30% (wt/wt). Above this concentration, the gels are weaker. There is no effect on gelation time as in all cases the gel is formed as soon as the system reaches a temperature close to 20°C . Using this method of acidification, it was observed that sucrose addition resulted in an increase of the pH at which gelation started, with values similar to those observed when GDL was used.

Gelation via Renneting

Results for the effect of sucrose on the formation of rennet gels are in opposition to those obtained for acid gels. Figure 3 illustrates the effect of sucrose on the

Figure 3. Effect of sucrose on the gelation curve of a micellar casein dispersion (5% wt/wt) after rennet addition (1/100; 0.25 ml/10ml) at $T = 20^{\circ}\text{C}$. (rennet added at $t = 0$). (frequency = 1 Hz).

Table 1. Gel time and pH of gelation for glucano- δ -lactone-induced milk protein gels as a function of sucrose concentration.

Sucrose concentration, % wt/wt	Gel time, h	pH of gelation
0	21	4.7
10	16	4.8
20	6	5.2
30	2	5.4
40	1.05	5.5
60	0.25	5.7

Figure 4. Effect of sucrose on the kinetics of caseinomacropeptide (CMP) release after rennet addition to a micellar casein dispersion (5% wt/wt). Sucrose concentration: 0 (▼), 10 (◇), 20 (●), and 30 (□)% wt/wt.

gelation of casein micelle dispersions at 20°C after rennet addition. The gels containing sucrose are weaker than those without sucrose, and the lag times before gelation are longer. These results are in good agreement with the previously published data of Pearce (1976) and Famelart (1994). In order to clarify these results, rennet activity measurements were carried out by measuring the rate of CMP release from casein micelle dispersions at different sucrose concentrations (see Figure 4). To check whether there is an effect of sucrose on rennet activity, experiments were first performed on pure κ -casein molecules. The results showed only slightly slower kinetics of CMP release above 20% (wt/wt) sucrose, whereas a similar experiment performed on ca-

Figure 5. Effect of pH value on the viscoelastic moduli [G' (■) and G'' (○)] of renneted casein gels obtained after 4 h at $T = 20^\circ\text{C}$. (frequency = 1 Hz).

sein micelles gave very different results, as shown in Figure 4. Firstly, the rate of hydrolysis is significantly reduced by sucrose addition, and secondly, the final concentration of hydrolyzed CMP is also reduced.

Gelation via Combined Acidification and Rennet

The combination of acidification and renneting on casein micelle gelation was also explored. Rennet action is known to promote hydrolysis of κ -casein when casein dissociation and micelle solvation decrease over the pH range 6.6 to 4.6. Both of these changes enhance micelle aggregation and, therefore, shift the balance of aggregating to disaggregating forces during the early stages of acidification such that gelation begins at a higher pH.

In these studies, micellar casein dispersions were acidified at low temperature using HCl, and rennet was added immediately afterward. The gelation process was then followed at 20°C. Results showed that combined acidification and renneting leads to instantaneous gelation and a stronger gel with an optimum in gel modulus occurring around pH 6.0 (Figure 5). Similar results were found in the presence of sucrose, except that the G' values are much greater and the maximum gel strength is obtained at a slightly lower pH value (5.8, instead of 6.0). This study demonstrates that the addition of rennet at a reduced pH gives two advantages: firstly, casein gels are formed instantaneously, and secondly, the gels produced are stronger than those at pH 6.8. Both effects occur whether sucrose is present or not.

Micellar Casein Gel Properties

The microstructures of casein gels produced by acidification, renneting, or by a combination of acidification

(a)

(b)

Figure 6. Confocal scanning laser microscope photo of a micellar casein dispersion (5% wt/wt) at pH 6.0 after rennet addition (1/100; 0.25 ml/10 ml). No sucrose (a); 30% wt/wt sucrose (b). Scale bar represents 25 μ m.

and renneting, in the presence or absence of sucrose, were compared using CSLM. Figure 6 illustrates casein gels formed with rennet at pH 6.0 in the absence or presence of 30% (wt/wt) sucrose, respectively. In the absence of sucrose, large water-containing pores are present between the casein aggregates, whereas in the presence of sucrose, the gel is more homogeneous with smaller aggregates linked together to form a fine-meshed network. The network remains intact on storage, suggesting that sucrose can prevent the local phase separation normally present in acid- or rennet-induced casein gels. The observed microstructures are in good agreement with the measured syneresis behavior of the gels (Figure 7). Without sucrose, all of the casein gels show some syneresis, and the extent is largely dependent on the method of preparation. The level of syneresis

(a)

(b)

Figure 7. Syneresis measurements at 20°C on micellar casein gels (5% wt/wt) formed after acidification (using glucono- δ -lactone or HCl) and/or renneting. No sucrose (a); in 30% wt/wt sucrose (b).

increases with decreasing pH for renneted gels, which is probably due to an effect of the gelation process on the pore size of the gels. However, in the presence of 30% (wt/wt) sucrose, all gels show almost no syneresis regardless of the gelation route used.

Effect of Sucrose on the Structure of Micellar Casein

Turbidimetry was used to investigate the effect of sucrose on the structure of casein micelles. Figure 8 illustrates the concentration dependence of the function $H \times Q \times c/\tau$ (at a single wavelength of 800 nm) for a dispersion of casein micelles at 5°C and 20°C. These curves can be divided into three concentration regimes: a dilute regime up to 2% (wt/wt), a semidilute regime between 2 and 6% (wt/wt), and a concentrated regime above 6% (wt/wt). In the dilute regime ($S = 1$), $H \times Q$

Figure 8. Concentration dependence of the function $H \times Q \times c/\tau$ (at 800 nm) for a micellar casein dispersion at $T = 20^\circ\text{C}$ (▼) or 5°C (▽).

$\times c/\tau$ (which is proportional to $1/M$) increases (negative slope) as the concentration decreases, indicating a dissociation process. In the semidilute regime (where the equation $H \times Q \times c/\tau = 1/M_w + 2A_2Qc$ applies), the positive slope indicates the dominance of excluded volume effects, whereas at higher concentrations, it is due to particle-particle interactions.

Figure 8 also shows that in the dilute regime, when the temperature is lowered (e.g., to 5°C), the dissociation process is more pronounced due to a reduction in hydrophobic interactions between the casein fractions. This allows some of the casein components, particularly β -casein, to leave the micelle and migrate into solution. It is reported in the literature that at 4, 20, and 30°C , the maximal amount of dissociated casein is found to be 60, 30, and $<10\%$, respectively, of the total casein content (Dalglish and Law, 1988; Rose, 1968).

Results for the effect of sucrose on casein micelles at 20°C are illustrated in Figure 9. It can be seen that in the presence of 30% (wt/wt) sucrose, the shape of the curve changes, suggesting dissociation is less pronounced or even absent. Similar behavior was also observed at 5°C .

Extrapolation of the wavelength exponent (between 700 and 800 nm) to an infinite dilution ($\beta' = 0$) of casein micelle dispersions, gives a value around -2.8 . Based on equation (4),

$$d\log Q/d\log \lambda = \beta = -2.8 + 4.0 = 1.2$$

which corresponds to $D/\lambda \sim 0.4$ and $Q \sim 0.5$ (based on the dependence of Q on D/λ for a sphere) (Cancellieri et al., 1974) and an average particle radius (for a sphere)

of around 150 nm, in agreement with the data of Holt (1975). The wavelength exponent is almost constant with protein concentration, suggesting that the particle size is constant and that the concentration dependence of the function $H \times Q \times c$ is due to a change in molecular weight and/or second virial coefficient.

Turbidity measurements were also carried out in order to follow the changes in casein particle size due to acidification or renneting. Measurements were made at a protein concentration of 1% (wt/wt) where an infinitely sized network is not formed (the critical concentration for gelation is about 2% wt/wt). Thus only the first stages of aggregation can be followed by this method. The effect of acidification on casein micelles and casein micelles with 30% (wt/wt) sucrose are illustrated in Figure 10. After an initial lag time, the wavelength exponent (and the turbidity) increased quite significantly, suggesting the formation of heterogeneities due to aggregation. However the time-dependence of the wavelength exponent after the first few minutes shows a clear difference between systems with and without sucrose. Without sucrose, there is an initial decrease of the wavelength exponent (by 0.4 units, which is significant), indicating a reduction in the particle size. It is known that during acidification, temperature-dependent solubilization of bound calcium occurs and this in turn leads to the release of individual caseins from the micelle (Davies and White, 1960; Singh et al., 1996; van Hooydonk et al. 1986). As acidification proceeds, the free caseins will aggregate near their isoelectric point to form part of the final gel network (Bannon and Hardy, 1992; Fox and Mulvihill, 1990).

By contrast, for the sample containing sucrose, the wavelength exponent remains constant before the step

Figure 9. Concentration dependence of the function $H \times Q \times c/\tau$ (at 800 nm) for a micellar casein dispersion at $T = 20^\circ\text{C}$ without sucrose (▼) or in the presence of 30% wt/wt sucrose (□).

Figure 10. Time dependence of the wavelength exponent for a micellar casein dispersion (1% wt/wt). Acidification with glucono- δ -lactone (1% wt/wt) at 20°C. No sucrose (▼); in 30% wt/wt sucrose (□).

2 transition, which tends to suggest that sucrose prevents the dissociation of the micelles. This protective role of sucrose is consistent with the results obtained at neutral pH. It is proposed that the presence of sucrose leads to a reduction in solvent quality, which prevents dissociation of soluble casein from the micelles, and when combined with charge neutralization during acidification, leads to the collapse of the κ -casein hairy brush. This reduction in steric stabilization of the micelles leads to gel formation at a faster rate and at a higher than normal pH. It should also be pointed out that because the gels are stronger and more homogeneous (even though sucrose is a poor solvent for casein), this suggests that sucrose accelerates the kinetics of gelation by making the particles stickier. The system is then trapped so that the coarsening of the gel network due to phase separation cannot take place. This in turn leads to reduced syneresis.

Similar results were also obtained for rennet gels. The time dependence of the wavelength exponent again showed two steps, but in the first step, the wavelength exponent remained constant both in the absence and in the presence of sucrose. This suggests that the removal of CMP from the casein micelles does not change the average size of the casein micelles prior to aggregation and gel formation. However, in the presence of sucrose, the aggregation process is clearly less pronounced. It is suggested, consistent with the mechanism already proposed, that sucrose induces the collapse of the κ -casein molecules onto the casein micelle surface by decreasing solvent quality. The κ -casein will then be inaccessible to chymosin, resulting in the observed reduction in CMP hydrolysis (Figure 4b) and

reduced aggregation of the paracasein micelles. These effects may be directly responsible for the observed changes in the kinetics of gel formation and final gel firmness.

CONCLUSIONS

This study has shown that sucrose concentration is a very important parameter for controlling the kinetics of gel formation as well as the strength and stability properties of casein gels, although the effect of sucrose varies depending on the gelation route used. It was also found that during acid gelation, the addition of up to 30% (wt/wt) sucrose allows gels to be formed more rapidly and at a higher pH. At sucrose levels above 30% (wt/wt), it is assumed that a more grained and porous gel is formed. At high sucrose levels the micelle structure is more swollen (due to greater hydration), and consequently, the aggregates are softer. Also, during renneting, the addition of sucrose leads to weaker gels and longer gelation times. The degree of CMP hydrolysis is reduced by the presence of sucrose, suggesting a specific effect on the micelle structure. A key parameter to control during renneting in order to manipulate gel time and gel strength is pH. In addition, good agreement between microstructure and syneresis behavior has been found, suggesting that aggregate size and subsequent pore size determine the extent of syneresis. Greater pore size means more syneresis, and in this respect, sucrose appears to prevent syneresis by producing a finer and more homogeneous gel structure.

It is proposed that the presence of sucrose reduces solvent quality and causes the collapse of the κ -casein "hairy" brush on the surface of the micelles. This would explain why sucrose reduces dissociation of the casein micelle on cooling at both neutral and acid pH, and why it increases the possibility of gel formation during acidification. It may also explain the reduced ability for κ -casein hydrolysis during renneting.

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