Cooling Effects on a Model Rennet Casein Gel System: Part II. Permeability and Microscopy

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Microscopy and permeability studies were performed to further illustrate the cooling effects on rennet casein gel structure and help interpret the rheological observations in the first part of this paper. Samples of gels cooled from 80 to 5 °C at four rates (0.5, 0.1, 0.05, and 0.025 °C/min) were studied with a confocal laser scanning microscope. A larger number of smaller flocs were generated at slower cooling rates, creating more cross-links within a network and corresponding to a stronger gel. Formation of a larger number of smaller flocs was hypothesized to result from a greater degree of doublet formation because the system spent more time within the temperature region where doublet formation is favored when cooled at slower rates. The doublets represent sites available for floc growth, similar to nucleation sites for crystal growth. Microscopy results further substantiated that the cooling effects were different from the aging effects because cooling affected floc size, and aging enabled the addition of idle flocs into the casein network. The conclusions for the cooling effects on floc size were further supported by permeability tests. A smaller permeability coefficient resulted from smaller flocs obtained with a slower cooling schedule. This study showed the importance of controlling floc numbers to modulate the strength of a gel, and cooling rates provide an approach of modulating functional properties when the chemical composition of a system is fixed.

Introduction

Casein and fat are major components of cheese. During the cooling stage of processed cheese production, fat crystallizes and casein forms a continuous network. Therefore, fat crystallization, protein−protein interactions, and the interactions between protein and fat are important for forming the microstructure of the processed cheese network that ultimately determines the texture and functionality.[1] Industrial researchers have observed that slower cooling yields a firmer processed cheese;[2] however, cooling mechanisms are poorly understood. A faster cooling rate creates more smaller-sized fat crystals.[3] More smaller-sized fat crystals embedded in a casein network dictate a firmer cheese,[4] opposite to cooling trends in processed cheese. Therefore, understanding casein gelation during cooling may help interpret cooling effects on cheese texture and functionality.

In the first part of this paper, a model system was formulated to include rennet casein and emulsifying salts used in processed cheese analogues.[5−7] Rheological data during cooling at different rates demonstrated the same trend as the industrial observations on processed cheese: a slower cooling rate generates a firmer gel. Fractal theory was applied to describe the casein network, and fractal dimension and floc size were proposed as two potential variables affecting microstructure and rheological properties. Estimation from rheological data gave similar fractal dimensions at different cooling rates, according to the scaling theory developed by Shih et al.[8] However, the rheological data did not enable the estimation of floc size, which may be obtained from microscopy techniques.

There are many microscopy techniques available, and the one selected must distinguish individual flocs and impose minimal structure damage during preparation and observation. Confocal laser scanning microscopy (CLSM) has been used to study whey protein aggregates at the micrometer scale.[9] Sample preparation for CLSM is straightforward, thereby avoiding complicating artifacts.[10] Furthermore, the scanning mode of CLSM enables the visualization of a relatively thick sample incapable of being observed by a conventional light microscope. The scanning mode of CLSM also enables the study of structures below a surface;[11] therefore, CLSM was selected for this study.

Another technique for studying protein gel microstructure is the permeability test developed by van Dijk and Walstra[6] to evaluate the syneresis of casein gels. This technique is based on the fact that a protein gel is a network of aggregates, and solvent can flow through the pores of this network if a driving pressure exists. The first step in a permeability test involves the formation of a gel column

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(1) Fox, P. F.; Guinee, T. P.; Cogan, T. M.; McSweeney, P. H. *Fundamentals of Cheese Science*; ASPEN Publishers: Gaithersburg, MD, 2000.
(2) Carić, M.; Kaláb, M. In *Cheese: Chemistry, Physics and Microbiology*; Fox, P. F., Ed.; Chapman and Hall: New York, 1993; Vol. 2, p 467.
(3) Lopez, C.; Bourgaux, C.; Lesieur, P.; Bernadou, S.; Keller, G.; Ollivon, M. *J. Colloid Interface Sci.* **2002**, *254*, 66−78.
(4) Tunick, M. H.; Nolan, E. J.; Shieh, J. J.; Basch, J. J.; Thompson, M. P.; Maleeff, B. E.; Holsinger, V. H. *J. Dairy Sci.* **1990**, *73*, 1671−1675.
(5) Bowland, E. L.; Foegeding, E. A. *J. Dairy Sci.* **1999**, *82*, 1851−1859.
(6) Bowland, E. L.; Foegeding, E. A. *J. Dairy Sci.* **2001**, *84*, 2372−2380.
(7) Dees, A. L. Effect of Various Ingredients on a Model Process Cheese System. M.S. Thesis, North Carolina State University, 2002.
(8) Shih, W.; Shih, W. Y.; Kim, S.; Liu, J.; Aksay, I. A. *Phys. Rev. A* **1990**, *42* (8), 4772−4779.
(9) Verheul, M.; Roefs, S. P. F. M.; Mellema, J.; de Kruif, K. G. *Langmuir* **1998**, *14*, 2263−2268.
(10) Blonk, J. C. G.; Vanaalst, H. *Food Res. Int.* **1993**, *26* (4), 297−311.
(11) Dürrenberger, M. B.; Handschin, S.; Conde-Petit, B.; Escher, F. *Lebensm.-Wiss. Technol.* **2001**, *34*, 11−17.

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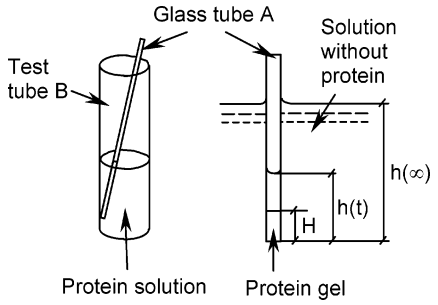

Figure 1. Conventional setup for measuring the permeability coefficient of a protein gel.

inside a glass tube (inside diameter, 2.0 or 3.7 mm) positioned in a bulk protein solution (Figure 1). As soon as the gelation is complete, the glass tube with a gel column is placed in a solution reservoir similar in composition to the protein gel but without protein. As the gel column is positioned at a level lower than the solution reservoir, a pressure gradient drives solution through the protein gel. By measuring the volume of solution penetrating through the gel column, one can estimate the permeability coefficient of a protein gel. The method has been used for whey protein gels[9,13,14] as well as casein gels.[15−20]

The objective of this part of the work was to study the cooling rate effects on the microstructure of rennet casein gels by microscopy and permeability. Microscopy tests enabled the measurements on floc size and provided visual support to the proposed scheme for rennet casein gelation, discussed in part I of this paper. Cooling effects on the model rennet casein gel system were thus fully interpreted by the fractal theory. As a supplementary technique, the permeability test discussed above was modified to enable the estimation of permeability coefficients of rennet casein gels used in the present experimental conditions.

Materials and Methods

Materials and Sample Preparation. The samples investigated contained 18% (w/w) rennet casein powder from New Zealand Milk Products (USA), Inc. (Lemoyne, PA), 0.3% food grade monosodium phosphate and 2.5% disodium phosphate donated by Rhodia, Inc. (Cranbury, NJ), 2.0% salt (sodium chloride), and 77.2% deionized water. During sample preparation, deionized water was heated to 50 °C, followed by the dissolution of salts. Rennet casein powder was then dissolved into solution with a stirring bar rotating at 350 rpm. The sample was stored overnight in a refrigerator at 5 °C.

Microstructure Observation. The samples for microscopy tests were cooled in the rheometer cup from 80 to 5 °C at four different rates (0.5, 0.1, 0.05, and 0.025 °C/min). During cooling, rheological data were obtained as previously detailed. Immediately following a rheological test, a small segment of the sample was taken from the rheometer cup bottom and applied as a thin layer to a glass slide. The slide was then observed with an Olympus Fluoview FV 300 confocal laser scanning biological microscope (Olympus, Tokyo, Japan) using a 100× immersion oil objective. The laser was operated at 488 nm and a scanning aperture of 3.0. The samples were evaluated at different horizontal and vertical positions.

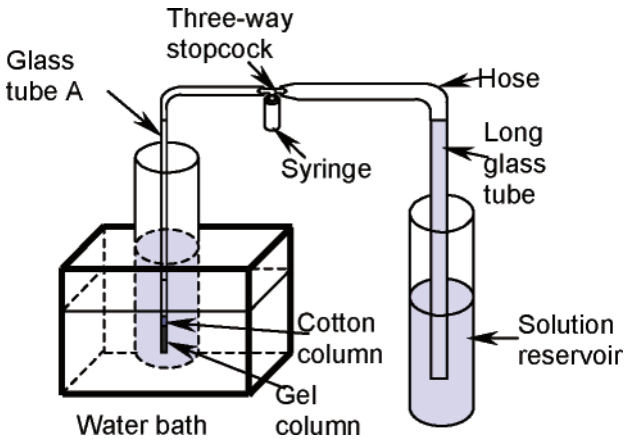

Figure 2. Modified permeability test setup by generating an underpressure in the system.

Image Analysis. The microscopy images were first converted to gray scale using Adobe Photoshop 5.5 software (Adobe, San Jose, CA), and the aggregate size was measured with Scion imaging software (version Beta 4.0.2, Scion Corporation, Frederick, MA). Average aggregate sizes and standard deviations were obtained by measuring more than 200 randomly selected flocs.

Permeability Tests. Preliminary tests confirmed that slip of the protein gel at the capillary walls was a problem, and a cotton plug was successful in maintaining the gel column in a secure position. Initially, solution permeation was slow during exposure to low, literature-based[14] pressure gradients. To accelerate the test, the setup in Figure 1 was modified to generate a steady underpressure above the gel column, thereby increasing the driving force across the gel; see Figure 2. The glass tube (A) containing the gel column was connected to a long glass tube (2 cm i.d.), and the opposite end was immersed into another solution reservoir. When the glass tube was connected to the long tube, the vapor in tube A was in equilibrium with the solution, avoiding mass transfer between tube A and the atmosphere. A three-way stopcock was placed between glass tube A and the long glass tube. Next, a syringe was used to suction air from the system, generating an underpressure measured by the solution column height inside the long glass tube. Preliminary tests identified an underpressure equaling a solution column height of ∼2.50 m was required to observe solution movement across the gel.

Gel Column Preparation. To make a gel column, samples were prepared by the method previously described. A salt solution was made to have the same formulation as an 18% rennet casein gel without the rennet casein powder. Next, a short cotton plug was positioned in glass tube A (i.d. = 5.0 mm) at a distance of 2 cm from the tube end. The cotton was soaked in salt solution and agitated until no free solution was visible. Approximately 20 g of the sample was then loaded into test tube B (i.d. = 3 cm, as illustrated in Figure 1), and the opening was covered with Parafilm to minimize moisture loss. The sample was melted in a programmable water bath at 80 °C for 6 min. Using a siphon, glass tube A was positioned and filled with protein solution to the cotton plug. A layer of mineral oil was applied at the top of the protein solution in test tube B, and the opening of glass tube A was sealed with Parafilm to minimize moisture loss. Next, test tube B was positioned in the water bath and cooled to 5 °C at the same temperature ramps used for rheological testing.

Solution Permeation Observations. After cooling, glass tube A was removed, and the outside surface was cleaned. A solution column was injected above the cotton plug to wet the surface, as described by Verheul and Roefs.[14] Before connecting glass tube A to the hose, a layer of vacuum grease was applied to prevent leakage. Then, glass tube A was positioned in the solution reservoir maintained at 5 °C by a water bath, and the gel column

(12) Van Dijk, H. J. M.; Walstra, P. *Neth. Milk Dairy J.* **1986**, *40*, 3−30.
(13) McGuffey, M. K.; Foegeding, A. *J. Texture Stud.* **2001**, *32*, 285−305.
(14) Verheul, M.; Roefs, S. P. F. M. *Food Hydrocolloids* **1998**, *12*, 17−24.
(15) Roefs, S. P. F. M.; van Vliet, T.; van den Bijgaart, H. J. C. M.; de Groot-Mostert, A. E. A.; Walstra, P. *Neth. Milk Dairy J.* **1990**, *44*, 159−188.
(16) Van Marle, M. E.; Zoon, P. *Neth. Milk Dairy J.* **1995**, *49*, 47−65.
(17) Lucey, J. A.; van Vliet, T.; Grolle, K.; Geurts, T.; Walstra, P. *Int. Dairy J.* **1997**, *7*, 381−388.
(18) Lucey, J. A.; Tamehana, M.; Singh, H.; Munro, P. A. *Food Res. Int.* **1998**, *31* (2), 147−155.
(19) Lucey, J. A.; Teo, C. T.; Munro, P. A.; Singh, H. *Food Hydrocolloids* **1998**, *12*, 159−165.
(20) Mellema, M.; Heesakkers, J. W. M.; van Opheusden, J. H. J.; van Vliet, T. *Langmuir* **2000**, *16*, 6847−6854.

was kept below the water bath level. An underpressure was generated as previously discussed. The solution level in the long glass tube was checked periodically and adjusted when necessary to maintain a constant underpressure. The solution level inside glass tube A (above the cotton column) was measured with a cathetometer (Eberbach Inc., Ann Arbor, MI).

Calculation of the Permeability Coefficient. As discussed by van Dijk and Walstra[12] and Verheul and Roefs,[14] flow through a gel column can be treated as flow through a homogeneous porous matrix, and macroscopic fluid motion (Darcy's equation) may be expressed as[21]

$$v_0 = -\frac{B}{\mu}(\nabla p - \rho g) \tag{1}$$

where v_0 is the average velocity of fluid through the matrix, B is the permeability coefficient of the protein gel, μ is the Newtonian (fluid) viscosity, ∇p is the pressure gradient across the gel column, ρ is the fluid density, and g is the acceleration due to gravity.

The underpressure magnitude corresponds to the solution column height (V) in the long test tube. Knowing the underpressure, the pressure gradient and average velocity may be expressed by eqs 2 and 3, respectively:

$$\nabla p = \frac{\mathrm{d}p}{\mathrm{d}x} = -\rho g\frac{[V + h_\infty - h(t)]}{H} \tag{2}$$

$$v_0 = \frac{\mathrm{d}h(t)}{\mathrm{d}t} \tag{3}$$

where h_∞ is the solution level for the solution reservoir, $h(t)$ and h_0 are the solution levels in glass tube A at a specific time and at the beginning of the test, and H is the gel column height.

Substituting eqs 2 and 3 into eq 1 and integrating with respect to time, the permeability coefficient (B) may be expressed as a function of the solution level:

$$Bt = -\frac{\mu H}{\rho g}\ln\left[1 + \frac{h_0 - h(t)}{V + h_\infty - H - h_0}\right] \tag{4}$$

Equation 4 can be further simplified by using only the first term in the Taylor series expansion of a logarithm function. Neglecting the resistance from the cotton plug, the gel permeability coefficient was approximated by a linear regression of $f(t)$ with respect to time:

$$Bt = \frac{\mu H}{\rho g}\frac{h(t) - h_0}{V + h(\infty) - H - h_0} = f(t) \tag{5}$$

The permeability coefficient is a function of floc size,[9] and microscopy and permeability tests are thus complementary to each other. Microscopy enabled the determination of floc size, another important parameter in the fractal theory besides the fractal dimension. The results from microscopy studies were compared with those of the permeability tests, and the fractal concepts illustrated cooling effects on rennet casein gel rheology and microstructure.

Results and Discussion

Casein Floc Size. The microscopy images of casein gels did not permit the visualization of the primary protein particles due to the limit of microscope resolution. However, the casein flocs and their arrangement were clearly observed. As a typical example, Figure 3 shows the structure of 18% casein gels cooled at 0.5 and 0.025 °C/min. In each image, the flocs appear approximately spherical, and the arrangement of the flocs was similar to the schemes presented in part I of this paper.

After taking more than 200 random measurements, the average aggregate sizes for 18% rennet casein gels cooled at different rates are presented in Table 1. The aggregate size as well as the standard deviation was smaller at a slower cooling rate which may have resulted from the slow cooling process.

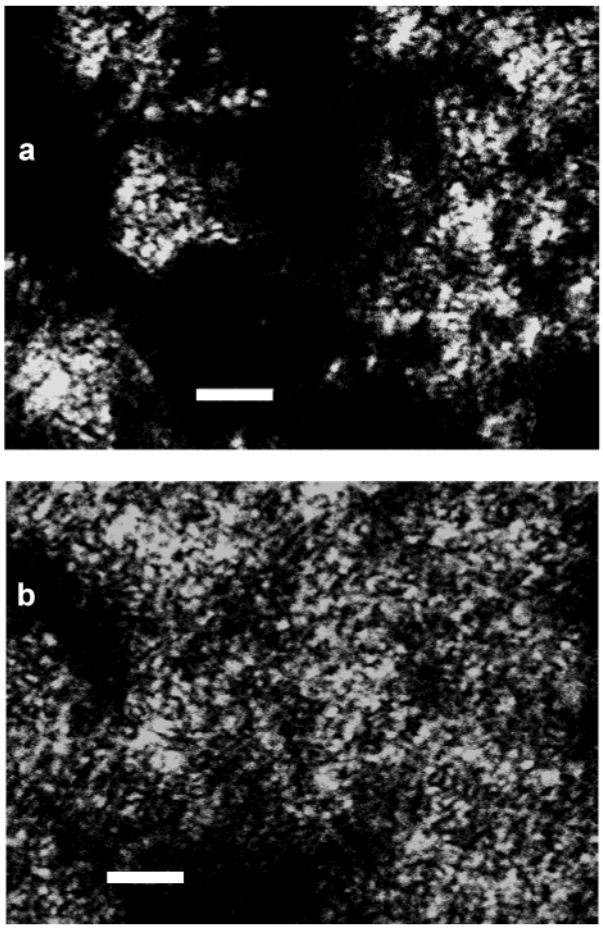

Figure 3. Confocal microscopy image of the microstructure of 18% rennet casein gel cooled at 0.5 (a) and 0.025 (b) °C/min. Bars = 2.0 μm.

Table 1. Floc Sizes and Permeability Coefficients of 18% Rennet Casein Gels as a Function of Cooling Rate

	floc size (μm)		
cooling rate (°C/min)	average	standard deviation	permeability coefficient ($\times 10^{-18}$ m^2)
0.025	0.26	0.06	5.38
0.05	0.31	0.06	8.02
0.1	0.35	0.08	9.00
0.5	0.43	0.10	20.60

Equation 6 represents a scaling relationship between the size (R_f) of a fractal object (an individual casein floc) and the number (N_p) of constructing units (protein particles).[22,23] Since the total amount of protein particles ($N_{p,tot}$) was the same at different cooling rates (same protein concentration), eq 7 must be conserved because all protein particles are included in flocs. At a slower cooling rate, the floc size was smaller; thus, the number of flocs ($N_{f,tot}$) was greater because the flocs were of a similar fractal dimension as previously determined.

$$N_p \sim (R_f/a)^{D_f} \tag{6}$$

$$N_{p,tot} \sim N_{f,tot}(R_f/a)^{D_f} \tag{7}$$

where a is the particle size and D_f is the fractal dimension.

(21) Bird, R. B.; Stewart, W. E.; Lightfoot, E. N. *Transport Phenomena*, 2nd ed.; John Wiley & Sons: New York, 2002.

Table 2. Permeability Coefficients (*B*) for Whey* and Casein Gels**

concentration	gelation method	B (m^2)	refs
10.5% (w/w) skim milk powder**	renneting (pH ~ 6.6)	10^{-13}	12
1.2−6.0% (w/w) casein**	acidification to pH ~ 4.6	10^{-12}−10^{-14}	15
11.8% (w/w) skim milk powder**	acidification to pH ~ 4.3	10^{-13}−10^{-14}	16
12% (w/w) skim milk powder**	acidification to pH ~ 4.6	10^{-13}	19
4% (w/w) casein**	renneting (pH 5.3−6.6)	10^{-11} (pH 5.3)−10^{-13} (pH 6.6)	20
2.4−7.9% (w/v) protein*	heating	10^{-12}−10^{-16}	9, 14
9% (w/v) protein*	heating	10^{-13}−10^{-14}	13

The gel network structure dictates its strength and rheological properties. For polymer gels, the complex shear modulus (G^*) is proportional to the number of cross-links between "blobs".[24] Carlson et al.[25] used this concept to describe the properties of a casein micelle gel by using the analogy between flocs in a particle gel and the blobs in a polymer gel.[8] The number of cross-links among flocs is thus proportional to G^*. In part I of this paper, rheological data suggested a stronger gel at a slower cooling schedule. The observation resulted from more cross-links among flocs as discussed above, and the cooling effects observed were thus caused by different number of flocs created during cooling.

Permeability Coefficients. The permeability coefficients of 18% rennet casein gels at 5 °C subjected to different cooling schedules are reported in Table 1. The coefficients were lower than those reported for casein gels and whey protein gels in Table 2. A permeability coefficient is strongly dependent on the protein concentration, pH, and salt concentration,[9,14] and the higher the protein concentration, the smaller the permeability coefficient. The concentration of rennet casein gel in the present work was higher than all the protein concentrations used in the literature, resulting in a smaller permeability coefficient.

The permeability coefficient for a packed column is a function of component size, as described by the Kozeny−Carman equation (eq 8).[26] For casein gels, solution penetrates through the space between flocs, and floc sizes are thus applicable in eq 8:

$$B = \frac{\epsilon R_f^{\,2}}{f_1} \tag{8}$$

where ϵ is the porosity and f_1 is the Kozeny constant.

At a slower cooling rate, more smaller-sized flocs resulted in a smaller permeability coefficient (Table 1). The results from microscopy and permeability tests thus supported each other.

Physical Origin of Cooling Effects. Rennet casein structure evolution follows subsequent steps from molecules to particles to flocs and finally to a network. Before forming into flocs, protein particles diffuse by Brownian motion. Under the conditions at which flocculation is possible, doublets are first formed by two particles "sticking", followed by the growth of flocs. Depending on the sticking possibility, flocculation can be classified as a diffusion-limited or reaction-limited process, creating flocs of different fractal dimensions. For a fractal dimension in a reaction-limited regime ($D_f > 2.0$), flocculation is slow because particles stick together before attempting additional configurations.[27]

The doublet formation rate can be described as the rate of disappearance of individual protein particles by

$$\frac{dN_{p,tot}}{dt} = -J \tag{9}$$

where t is the time and J is the rate of flocculation as related to the total interaction energy (W_{tot}) by

$$J = \frac{4k_BT}{3\mu a}\frac{N_{p,tot}^2}{\int_{2a}^{\infty}\frac{\exp(W_{tot}/k_BT)}{r^2G(r)}\,dr} \tag{10}$$

where $G(r)$ is the density distribution of protein particles, r is the center-to-center distance between two particles, k_B is the Boltzman constant, and T is the absolute temperature.

Upon the transformation from time to temperature for a cooling process with a constant rate, the doublet formation rate at a specific temperature can be expressed as

$$T = T_0 - \alpha t \tag{11}$$

$$dT = -\alpha\, dt \tag{12}$$

$$\frac{dN_{p,tot}}{dT} = \frac{1}{\alpha}J \tag{13}$$

where T_0 is the initial temperature and α is the cooling rate in °C/min.

Casein particles are the constructing units of a casein network. A quantitative description of the interactions between casein particles is challenging. The interactions are temperature dependent, further complicated by the strong (and difficult to quantify) hydration interactions.[27] Despite these complexities, the following qualitative analysis still applies for the same casein sample subjected to different cooling schedules.

During cooling to a "critical" temperature, doublets are formed, followed by the addition of other particles, that is, floc growth. The formation of doublets results from a decrease in short-range repulsive forces that decrease upon cooling. For the casein system during cooling at different rates, the overall interaction energy at which doublets are formed should be the same under different cooling conditions, meaning J should be the same. At a slower cooling rate, eq 13 illustrates that the doublet formation rate ($dN_{p,tot}/dT$) at a certain temperature is larger because α is smaller. Physically, since the formation of doublets occurs at a characteristic temperature, protein particles have more time at this temperature when cooled at a slower rate, generating more doublets. The addition of individual protein particles onto doublets stops when all

(22) Bremer, L. G. B.; Bijsterbosch, B. H.; Schrijvers, R.; van Vliet, T.; Walstra, P. *Colloids Surf.* **1990**, *51*, 159−170.
(23) Bremer, L. G. B.; Bijsterbosch, B. H.; Walstra, P.; van Vliet, T. *Adv. Colloid Interface Sci.* **1993**, *46*, 117−128.
(24) Doi, M. *Introduction to Polymer Physics*; Oxford University Press: Oxford, U.K., 1996.
(25) Carlson, A.; Hill, C. G., Jr.; Olson, N. F. *Biotechnol. Bioeng.* **1987**, *29*, 612−624.
(26) Carman, P. C. *Flow of Gases Through Porous Media*; Academic Press: New York, 1956.
(27) Russel, W. B.; Saville, D. A.; Schowalter, W. R. *Colloidal Dispersions*; Cambridge University Press: New York, 1989.

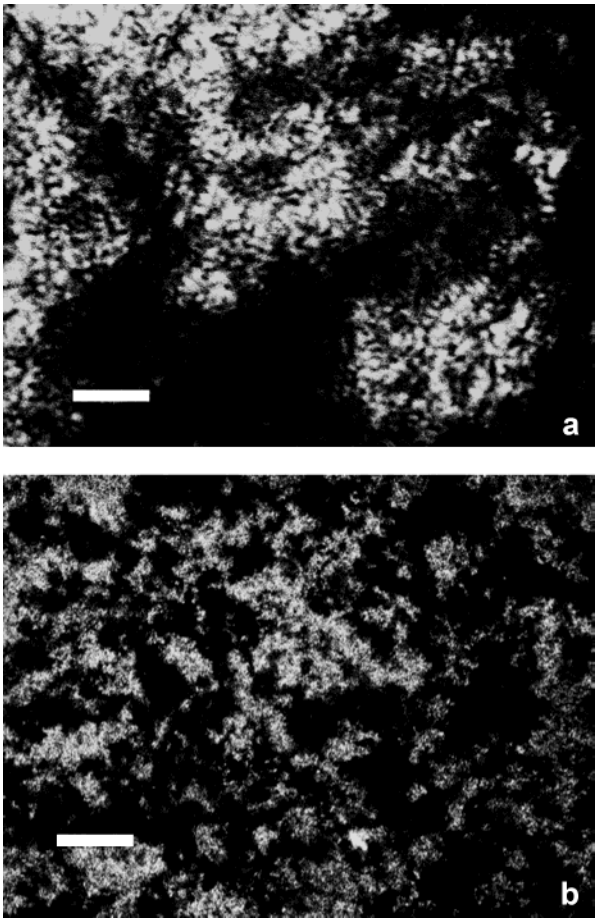

Figure 4. Confocal microscopy image of the microstructure of 18% rennet casein gel cooled at 0.5 °C/min followed by incubation at 5 °C. Bar = 2.0 (a) or 20.0 (b) μm.

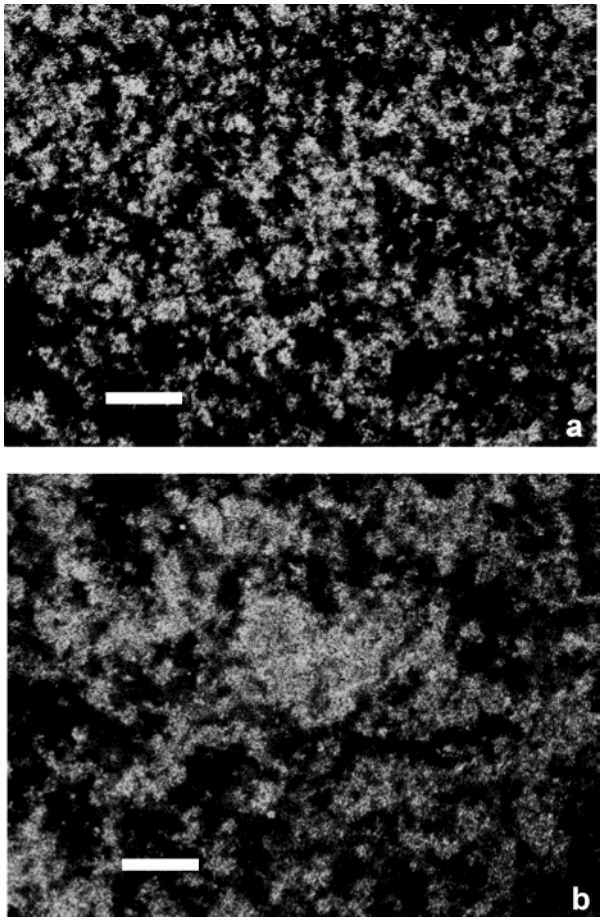

Figure 5. Confocal microscopy image of the microstructure of 18% rennet casein gel when cooled at 0.5 (a) and 0.025 (b) °C/min. Bars = 20.0 μm.

particles are consumed. Therefore, at a slower cooling rate, more smaller-sized flocs are generated, developing a stronger gel.

Cooling Effects versus Aging Effects. Rheological tests were performed and reported in the first part of this paper to distinguish the cooling effects from the aging effects (a strengthening of a network after gelation at a constant temperature). The sample of 18% rennet casein gel was first cooled at 0.5 °C/min from 80 to 5 °C and was subsequently incubated at 5 °C for a total test time equaling that used during a cooling rate of 0.025 °C/min. During the incubation at 5 °C, the storage modulus increased slightly until some period of time and remained at a final value lower than that cooled at 0.025 °C/min.

To explain the aging effect, Verheul et al.[9] speculated that the increase of the storage modulus with time was attributed to the "decoration" of individual molecules or particles into the network, strengthening the structure. The authors reported a protein network composed of aggregates that were approximately spherical and smaller than 1 μm, similar to our observations. When initially cooled to 5 °C at 0.5 °C/min, the images (Figure 3a) showed some "idle" flocs that were not connected with other flocs. On the contray, there were fewer idle flocs when the system was cooled to 5 °C at 0.025 °C/min (Figure 3b) or incubated at 5 °C after cooling at 0.5 °C/min (Figure 4a). When observed on a larger scale (Figures 4b and 5a), gels did not show a noticeable difference with or without incubation and had fewer flocs than gels cooled at 0.025 °C/min (Figure 5b). The floc size after incubation was 0.42 μm with a standard deviation of 0.10 μm, very similar to the floc size average and standard deviation for gels without incubation. The strengthening of the network during incubation most likely resulted from the addition of idle flocs into the network, creating more cross-links between the flocs. When the idle flocs became a part of the network, no more cross-links were created, and the gel showed a plateau of the storage modulus. On the other hand, because there were fewer flocs when cooled faster, fewer cross-links produced a weaker gel when compared with gels prepared from a slower cooling rate even though each received identical time treatments.

Conclusions

During cooling, casein molecules formed a network consisting of fractal flocs, and the fractal dimension and size of these flocs are two important parameters that determine the rheology and microstructure of rennet casein gels. Microscopy studies revealed that smaller flocs were generated during slower cooling schedules, supported by the permeability tests. Physically, protein particles spent more time at a temperature range favoring doublet formation when cooled at slower rates, creating more doublets at the initial stage of aggregation and more sites for floc growth. More smaller-sized flocs and more cross-links among flocs were created, resulting in a stronger gel. Additional microscopy studies showed no differences in the floc size and distribution for gels with or without incubation after cooling, and the development of the storage modulus during incubation thus resulted from bonding idle flocs into the protein network. Fractal theory successfully described the structure of rennet casein gels. The amount of flocs determined the strength of the rennet

casein gels and was affected by differences in floc fractal dimension and size. Fractal dimension is a function of chemical composition, while floc size can be manipulated by rates of thermal treatments.

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