REVIEW ARTICLE

The mechanism and properties of acid-coagulated milk gels

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Abstract

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Acid-coagulated milk products such as fresh acid-coagulated cheese varieties and yogurt are important dairy food products. However, little is known regarding the mechanisms involved in gel formation, physical properties of acid gels, and the effects of processing variables such as heat treatment and gelation temperature on the important physical properties of acid milk gels. This paper reviews the modern concepts of possible mechanisms involved in the formation of particle milk gel aggregation, along with recent developments including the use of techniques such as dynamic low amplitude oscillatory rheology to observe the gel formation process, and confocal laser scanning microscopy to monitor gel microstructure.

Key words : acid-coagulated milk gels, gelation, adhesive sphere model, percolation, fractal structure model, rheology, microstructure, SEM, CLSM

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บทคัดย่อ

ชนกภัทร ผดุงอรรถ

กลไกและคุณสมบัติของเจลจากการตกตะกอนน้ำนมโดยกรด

ว. สงขลานครินทร์ วทท. 2548 27(2) : 433-448

ผลิตภัณฑ์นมหมัก เช่น ผลิตภัณฑ์เนยแข็งสดชนิดอ่อนชนิดต่าง ๆ และผลิตภัณฑ์โยเกิร์ต เป็นผลิตภัณฑ์อาหารนมที่มีความสำคัญ เนื่องจากมีการผลิตอย่างแพร่หลายทั่วโลก แต่ทว่าการศึกษาถึงข้อมูลสำคัญที่เกี่ยวข้องกับการเกิดโครงสร้างของเจล คุณสมบัติต่าง ๆ ของเจล รวมทั้งปัจจัยต่าง ๆ ที่อาจส่งผลกระทบต่อคุณสมบัติของเจล เช่น การให้ความร้อนแก่น้ำนม และอุณหภูมิที่ใช้ในการหมักเจลนั้น ยังไม่เป็นที่แพร่หลายมากนัก ดังนั้นบทความนี้จะกล่าวถึงแนวความคิดใหม่ของกลไกที่อาจเกิดขึ้นของการเกิดโครงสร้างของเจล รวมถึงเทคนิคใหม่ ๆ ที่ใช้ในการศึกษาคุณสมบัติต่าง ๆ ของเจล เช่น การใช้เทคนิคไดนามิค-โลว-แอมพลิทูด-ออสซิลาทอรี-รีโอโลจี (dynamic low amplitude oscillatory rheology) เพื่อศึกษากระบวนการเกิดเจล และคอนโฟคอล-เลเซอร์-แสกนนิ่ง-ไมโครสโคปี (confocal laser scanning microscopy) เพื่อศึกษาโครงสร้างของเจล

โปรแกรมวิชาวิทยาศาสตร์และเทคโนโลยีการอาหาร คณะวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยราชภัฏสุรินทร์ อำเภอเมือง จังหวัดสุรินทร์ 32000

Acidified milk gels are one of the oldest and the most popular foodstuffs produced throughout the entire world. The popularity of fermented milk products such as fresh acid-coagulated cheese varieties (cream cheese, Cottage cheese, Quarg, tvorog, and frais), and yogurt, is due to various health claims and curative benefits. Irrespective of the commercial importance of these acidified milk products, there is not much information regarding formation, structures and physico-chemical properties of acid-coagulated milk products (Horne, 1999; Kudryashov *et al*., 2001; Lucey and Singh, 2003).

A wide variety of acidified milk products are produced. Some of the main products are fresh acid-coagulated cheese as stated above and yogurt. The manufacture and technologies engaged in the production of fresh acid cheese varieties have been reviewed (Guinee *et al*., 1993; Puhan *et al*., 1994; Kosikowski and Mistry, 1997; Lucey, 2002). Additionally, there has been significant research on acid gels made with thermophilic cultures for the yogurt production (e.g., Tamime and Robinson, 1999). The objective of this article is mainly to give overall information of acidified milk gels and their physical, rheological and microstructural properties.

1. Protein gels

Several kinds of proteins are able to form gels, and the type of gel network contributes to the texture characteristics of many food products by acting as a matrix, holding water with fat and other components. In addition, proteins are able to form different types of network structures depending on several factors such as temperature, pH, and salts. Figure 1 shows different types of network structures, which can be divided roughly into fine-stranded gels (Figure 1(a) and (b)) and course aggregated gels (Figure 1 (c) and (d)), which commonly occurs in biopolymer gels. Fine-stranded gels are formed by an ordered association of molecules, and the networks are so small that these gel structures are transparent. The other type of networks, the aggregated gels, is formed into particulate gels that are not transparent, and this type of gels is common in milk and egg products (Hermansson, 1994).

2. Method of acidification of milk

The acidification method of milk can be done by using bacterial cultures, which ferment lactose to lactic acid, direct addition of acids, such as HCl, or the utilization of glucono-δ-lactone (GDL) (Lucey *et al*., 1997), which is hydrolyzed to

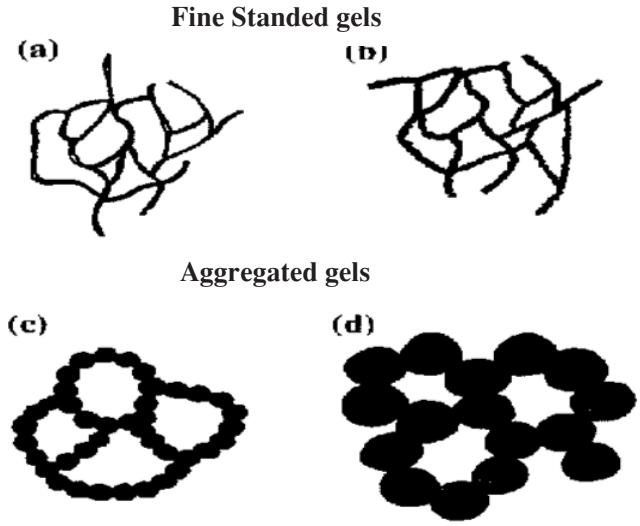

Figure 1. Diagrams of different types of gel networks: fine-stranded gels made up of (a) macromolecules and (b) globular proteins; (c) and (d) particulate gels. (Source: Hermansson, 1994)

gluconic acid, resulting in a reduction in pH (Lucey and Singh, 1998; Lucey and Singh, 2003). An extensive study of the formation and properties of milk gels made by cold acidification with direct addition of HCl and successive heating to the gelation temperature has been reported (Roefs *et al*., 1990; Roefs and van Vliet, 1990). The comparison of the formation of acidified milk gels made with a bacterial culture or GDL has also been reported (Lucey *et al*., 1998b). From their study, the rate of acidification of milk gels using different media is found to be different. GDL is rapidly hydrolyzed to gluconic acid; on the contrary, in milk added starter culture, the pH drops little initially, but then decreases steadily with time, as seen in Figure 2.

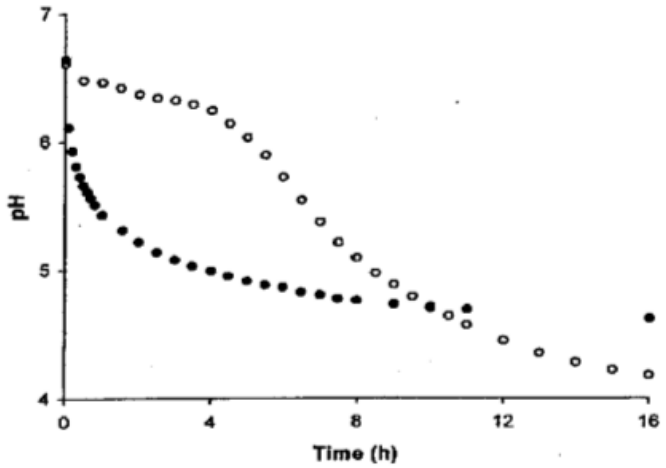

Figure 2. Changes in pH during the acidification of milk at 30ºC with (●) 1.3% glucono-δ-lactone (GDL) or (o) 2% (w/w) starter culture). Milk was heated at 85ºC for 30 min prior to being acidified. (Source: Lucey and Singh, 2003)

3. Effect of acidification on the properties of casein micelles

Caseins are phosphoproteins containing approximately 80% of the total content of milk proteins. Caseins are made up of many components. The main ones are α_{s1}-casein, α_{s2}-casein, β-casein, and κ-casein with considerable quantities of micellar or colloidal calcium phosphate (CCP) in the form of aggregates called casein micelles. Upon acidification, many of the physico-chemical properties of the casein micelles go through significant changes, especially in the pH range of 5.5 to 5.0, which includes voluminosity, salvation and dissociation of the caseins. In both heated and unheated milk, as the pH of milk is reduced to ~5.1 at a temperature above 22ºC, most of the CCP is dissolved, and caseins are liberated into the serum phase. Although the internal casein micelle structure is changed caused by the loss of CCP, the average hydrodynamic diameter of casein micelles remains the same. Consequently, aggregation of casein occurs as the isoelectric point (pH ~4.6) is reached (Brunner, 1977; Lucey and Singh, 1998; Lucey and Singh, 2003; Lucey, 2004).

4. Mechanism of the acid coagulation

4.1 Theoretical approaches

Gels formed from milk protein are irreversible, unlike many other food gels, and are involved in the manufacture of many dairy and non-dairy foods. Acid milk gels are types of gel that are aggregated particle networks forming continuous structures expanding throughout the entire volume. There are at least three theoretical models, which are adhesive sphere model, percolation models and fractal models, which have been used to explain the formation of acid-induced milk gels (Green, 1980; Horne, 1999; Lucey and Singh, 2003).

1) Adhesive sphere model

It has been proposed, in this model, that casein micelles are sterically stabilized by the glycomacropeptide (GMP) part of κ-casein. The GMP acts as a polyelectrolyte brush, which collapses on the surface of the micelle as the pH of the system reaches the pK_a of the charged groups on the brush, or while the charge density is reduced. Hence, this model has been used to quantitatively describe the aggregation reactions of destabilized casein micelles while lowering pH. The steric stabilization of the casein micelles declines, resulting in attractive interactions among casein micelles, while pH is being reduced. Although the concept of gelation is well explained, the adhesive sphere model could solely apply to circumstances where weak attraction between particles is present. In addition, this model fails to describe the elastic properties of the gel and the kinetics of the development in the shear modulus (De Kruif *et al*., 1995; De Kruif, 1997; Horne, 1999; Lucey and Singh, 2003).

2) Percolation models

It is assumed, in this theory, that percolation clusters form random bonds in a lattice between adjacent micelles, resulting in an increase in the size of clusters as number of bonds increases, which can be calculated in computer simulation. When the aggregation of percolation clusters reaches a certain threshold, it can be detected and marked as a gelation point in a rheometer where the elasticity is measurable. Analogies can be drawn between this model and gelation, which is that as particles aggregate, they establish numbers of links and bonds until a certain threshold, a cluster is created, and it spans the container. At this stage, only a fraction of bonds have been incorporated into the system-spanning cluster and this mechanism could well explain the continued increase in storage modulus of the gel after gelation. The drawback of the percolation model is that, it is relevant only close to the gelation point and it is unable to predict the amplitude of the elastic moduli. Additionally, this model cannot easily explain the mechanical properties of acid gels (De Kruif *et al*., 1995; Horne, 1999; Lucey and Singh, 2003).

3) Fractal models

The fractal theory has been used to describe the formation of many casein gels, and has been recently reviewed by Lucey and Singh (1998, 2003), and Horne (1999). This model can be mathematically described with an assumption

that hard sphere particles of radius 'a' can move by the Brownian movement and that when they come across each other, they would aggregate, resulting in aggregation of aggregated forms. Once the particles are all incorporated, meaning no further changes happening among the particles in an aggregate, this cluster-cluster aggregation progression directs to aggregates which follow the equation (1);

$$\frac{N_p}{N_o} = \frac{(R)^{D-3}}{a_{eff}} \quad (1)$$

where N_p is the number of particles in an aggregate of radius R, N_O is the total number of primary particles that could form a floc, D is a constant value called the fractal dimension (D < 3), and a_{eff} is the radius of the effective building blocks, which form the fractal cluster (Horne, 1999; Lucey and Singh, 2003).

This model has effectively exemplified semi-quantitative aspects of casein gels, e.g. rheological properties. However, it fails to describe the aggregate rearrangement (before, during and after gelation) or interpenetration. There is also an assumption that all aggregates have the same size and are part of the gel network at the gelation point (Lucey and Singh, 1998, 2003; Horne, 1999).

4.2 Physico-chemical mechanisms

At normal milk pH, most proteins have a net negative charge value causing long-range electrostatic repulsion and short-range hydration repulsion between protein molecules, which stabilizes casein micelles (Bringe and Kinsella, 1987). Three pH regions from pH 6.7 to 4.6 of interest are discussed below.

1) pH 6.7 to ~ 6.0

The net negative charge on the casein micelles is lowered because of the decrease in pH, resulting in the reduction of electrostatic repulsion. The structural size of the casein micelles is mostly the same, because only a small amount of CCP is dissolved above pH 6.0 (Lucey, 2004).

2) pH 6.0 to ~ 5.0

The charged κ-casein hairs on the micelle surface provide a stabilizing layer including both sterically and electrostatically. Upon acidification, the net negative charge continues to get lowered, resulting in the decrease in both electrostatic repulsion and steric stabilization, which are both largely responsible for micelle stability. Therefore, the charged hairs may shrink, and the micelles are more sensitive to aggregation. In addition, the decrease in pH provokes a reduction in the ionization of the acidic functions of the caseins (aspartic - glutamic - phosphoserine residues). Thus, the surface potential is decreased. Accordingly, the sequestering power of the α_s - β caseins simultaneously lessens, causing increases of the solubility of the phosphocalcic salt in water. This consequence causes a progressive transfer of the calcium and the inorganic phosphate from micelle to the aqueous phase, that is, the CCP in milk is dissolved entirely by pH around 5.0. Moreover, the dissociation of casein from the micelle largely depends on both temperature and pH. The maximum pH of casein dissociation at temperature ≤ 20ºC is 5.2-5.4, owing to loosening of the molecular interactions between caseins as a result of the dissolved CCP, thereby causing increased electrostatic repulsion among the newly-exposed phosphoserine groups (Brule' *et al*., 2000; Lucey, 2004).

3) pH < 5.0

The acidification from pH 5.0 to 4.6 induces the disassociation of the calcium complexes by the phosphoserines, whereas the previous pH region (pH 6.6 to 5.0) induces mainly the solubilization of the calcium phosphate, as shown in Figure 3. This association appears to induce a disorganization of the micelles and a reorganization of the micellar sub-units. While the pH decreases and approaches the isoelectric point, the net negative charge is neutralized; thereby causing a reduction in the amphiphilic character of the β and κ caseins. Owing to calcium phosphate solubilization, electrostatic interaction is strengthened, whereas the electrostatic repulsion is weakened, causing the depolymerizations of the α_s-caseins. Therefore, the hydrophobic interaction is increased. Consequently, this causes the

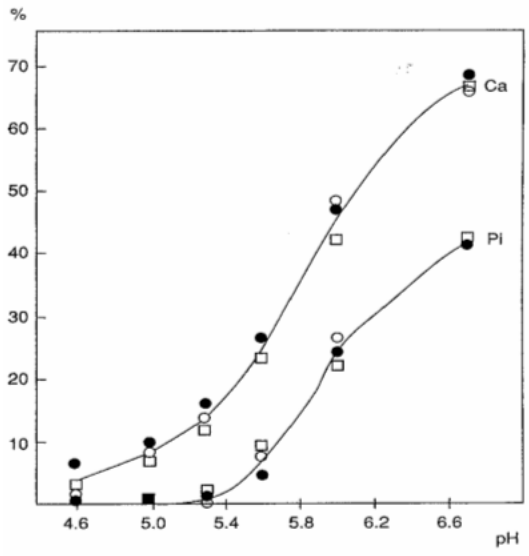

Figure 3. Sobilization of micellar calcium (Ca) and inorganic phosphorus (Pi) upon milk acidification. (Source: Brule′, 2000)

aggregation and formation of chains and cluster linked together as a three dimensional network (Brule′ *et al*., 2000; Lucey and Singh, 2003; Lucey, 2004).

Bringe and Kinsella (1987) stated that hydrophobic interactions are the main driving force for interactions between proteins for coagulation, gelation and gel syneresis. Therefore, the casein gels should be weaker with a decrease in temperature, because the hydrophobic interaction would be weaker. However, Lucey and Singh (2003) reported that hydrophobic interactions are not the major role for the strength of acidified milk gels, because the strength of the gels, as seen from viscoelastic moduli, are greater when the temperature is lowered. The example of this is shown in Figure 4, where milk gels were acidified with 1.3% (w/w) GDL at two different incubation temperatures (30 and 40ºC). Acidified milk gels incubated at lower temperature (30ºC) had higher storage modulus (G′) values than those incubated at higher temperature (40ºC).

Gelation in unheated milk gels, where acidification is the only coagulation method, occurs around pH 4.9; unless the acidification is performed at a very high temperature, where a higher gelation pH can be seen (Lucey, 2004). Additionally, the strength of gels made from unheated milk is low. Thus they have low values for the viscoelastic moduli because of the dense clusters of aggregated casein particles from the extensive particle rearrangements during the gel formation. As a result, many particles from those clusters would not be entirely cross linked throughout the network.

Acidified milk gels made from heated milk form a gel at higher pH values because of the denaturation of whey proteins by high heat treatment. Consequently, when heated milk is acidified and become susceptible to aggregation, the denatured whey proteins, associated with the casein micelles, especially κ-caseins, have a high isoelectric pH (e.g. the main whey protein, β-lactoglobulin has an isoelectric pH of ~ 5.3). This isoelectric pH is higher than that of the caseins, resulting in the high pH of gelation of heated milk. In addition, the strength of gels made from heated milk is higher than those made from unheated milk, because denatured whey proteins associated with casein micelles interact with each other and act as bridging materials, thereby increasing the strength and number of bonds between protein

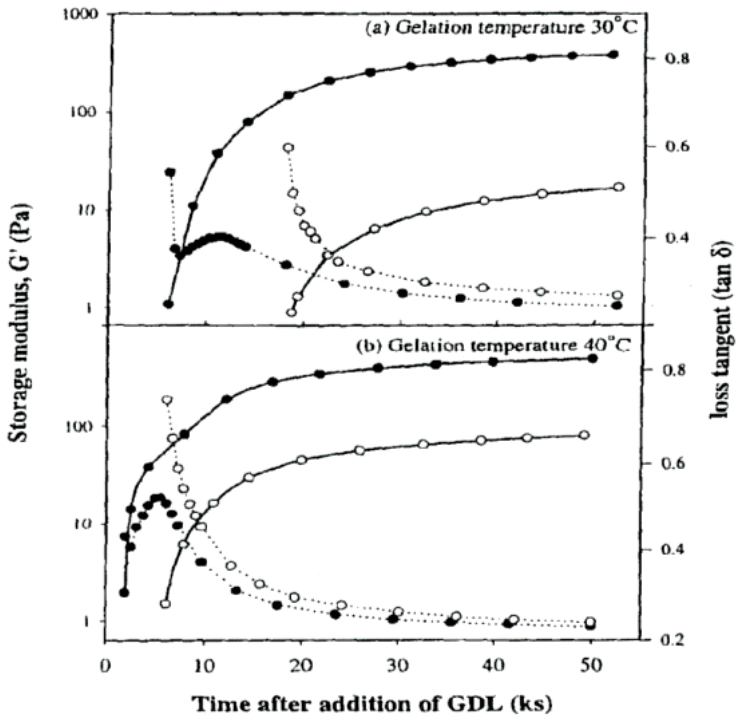

Figure 4. Effects of heat treatment and gelation temperature on the rheological properties of milk gels acidified with 1.3% (w/w) glucono-δ-lactone (GDL). Gelation temperatures: (a) 30ºC and (b) 40ºC. (o) represents unheated milk and (●) represents milk heated at 80ºC for 30 min prior to being acidified. (Source: Lucey and Singh, 1998)

particles. Figure 4 shows milk gels acidified with 1.3% (w/w) GDL. Gels made from milk heated at 80ºC for 30 min prior to GDL addition had higher G′ values than those made from unheated milk (Lucey and Singh, 2003).

5. Physical properties of acidified milk gels

5.1 Rheological properties

Acid gels are viscoelastic materials. The rheological and textural properties can be determined from several test methods, such as small amplitude oscillatory rheology (SAOR), large amplitude oscillatory shear, penetration, and texture profile analysis. The ideal technique should be able to observe the gel formation process, since gelation is the primary step in the formation of most acid-type cheese. Hence, in order to study the gelation phase of an acidified milk gel, dynamic non-destructive techniques, such as SAOR, are needed (Lucey and Singh, 2003; Lucey, 2004).

In fact, these dynamic rheological measurements have become popular in the last two decades because of their ability to perceive the formation of the gel network and differentiate the elasticity and viscosity of the gels. Many studies have been done using these techniques. For examples, the study of rheological changes during slow acid induced gelation of milk by D-glucono-δ-lactone by Kim and Kinsella in 1989, the study of structure of acid casein gels specified in formation and model of gel networks by Roefs and his colleagues in 1990, the study of permeability and rheological properties of microbially and chemically acidified skim-milk gels by Van Marle and Zoon in 1995, the study of effect of preheating of milk on the structure of acidified milk

gels by Van Vliet and Keetels in 1995, the study of rheological properties and microstructure of acid milk gels as affected by fat content and heat treatment by Lucey and his colleagues in 1998, and the study of micellar casein gelation at high sucrose content by Schorsch and his colleagues in 2002.

These SAOR tests give a strain response to an applied sinusoidal stress, or vice versa. The main parameters from these tests are the elastic or storage modulus (G′), a measure of the energy stored per oscillation cycle; the viscous or loss modulus (G″), a measure of the energy dissipated as heat percycle; and the loss tangent (tan δ), which is the ratio of the loss modulus to the storage modulus. These parameters are defined as follows:

$$G' = (\sigma_0 / \gamma_0)\cos\delta \quad (2)$$

$$G'' = (\sigma_0 / \gamma_0)\sin\delta \quad (3)$$

$$\tan\delta = G''/G' \quad (4)$$

where σ_0 is the amplitude of the shear stress, γ_0 is the amplitude of the strain and δ is the phase angle (Steffe, 1996; Rao, 1999; Lucey and Singh, 2003; Lucey, 2004). In addition, as already discussed, the acidified milk gels are basically casein particle gels, where the structure of the network is linked by weak interparticle interactions. These interactions can be effortlessly affected or destroyed by applied stress or strain used in SAOR tests, particularly in the vicinity of the gelation point. Therefore, the majority of preceding rheological measurements of milk gelation were performed using very small strains (≤ 1%) and oscillating strain rate (≤ 0.1 Hz) to avoid gel destruction (Van Marle and Zoon, 1995; Lucey *et al*., 1998a).

The temperature history applied to the milk has a tremendous effect on the rheological properties of acidified milk gels. As can be seen from Figure 5, acid milk gels made from unheated milk usually forms a weak gel (G′ < 50 Pa), and the pH at gelation point is around 4.8-5.0. After the gelation point, G′ swiftly rises and starts to plateau during the aging of the gel around pH 4.6, while tan δ declines rapidly to < 0.4 and to about 0.2-0.3 during aging of the gel. Gels made from

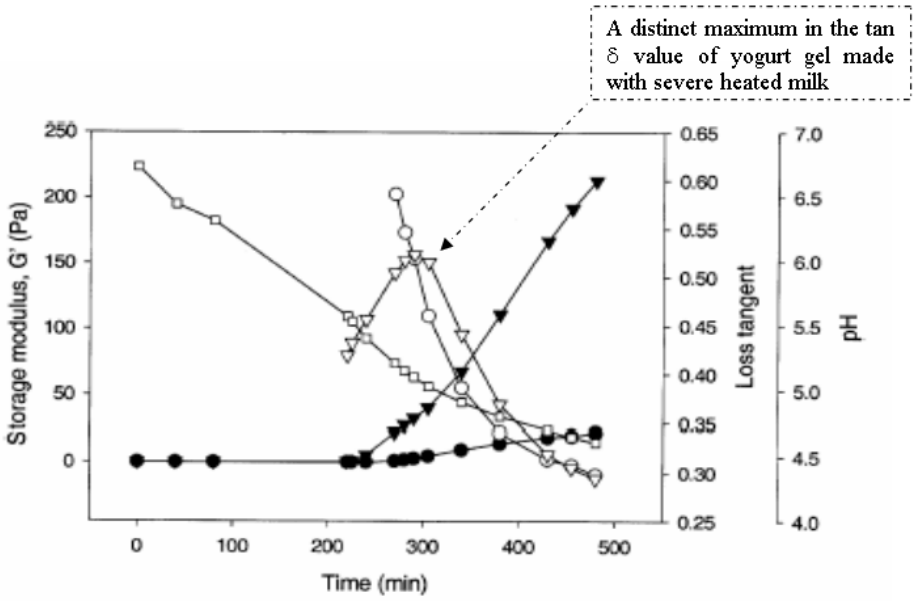

Figure 5. Effect of heat treatment on the rheological properties of a yogurt gel made at 40ºC from unheated milk (circles) or milk heated at 82ºC for 30 min (triangles). Solid and open symbols are the storage modulus and loss tangent, respectively. pH (□) was also measured as a function of time. (Source: Lucey, 2002)

heated milk show unusual trend, which is a distinct maximum in the tan δ value. The different trends of tan δ between acidified milk gels made from unheated and heated milk are shown in Figure 5. This maximum in the tan δ value of heated milk gels indicates that bonds and strands in the gel are easier to break or relax, resulting in more rearrangements of the gel. Furthermore, the maximum in tan δ is possibly because of a partial loosening of the weak initial gel network owing to the dissolved CCP, resulting in increased protein-protein attractions between casein particles as the net charge decreases while pH is lowered and reaches isoelectric point (Lucey, 2004).

5.2 Microstructure and textural properties

The textural properties of acidified milk gels are noticeably affected by the microstructure of these gels. The microstructural changes in casein micelles during the acidification of milk have been recently observed by Gastaldi *et al.* (1996). They studied the microstructure of acidified milk gels obtained from scanning electron microscopy

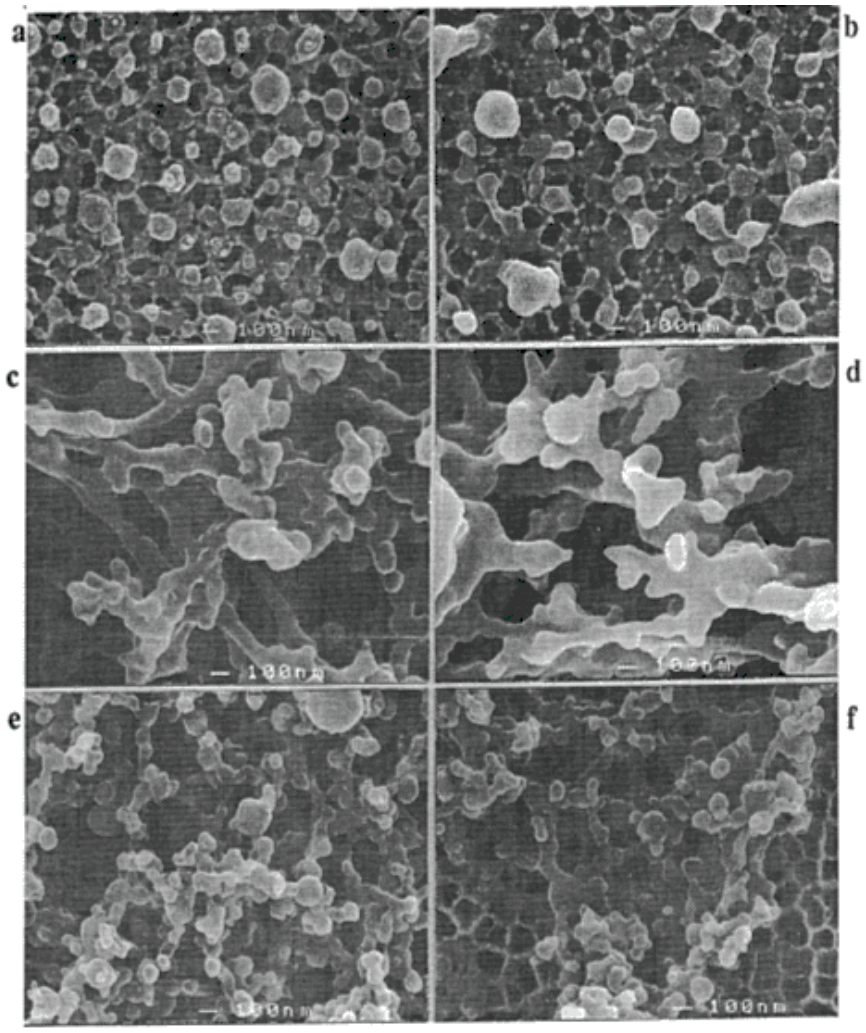

Figure 6. SEM micrographs of acidified milk samples with a comparison of the two different techniques of sample preparation for SEM. Critical point dried samples: a) pH 6.7, c) pH 5.3, e) pH 4.6; Freeze dried samples: b) pH 6.7, d) pH 5.3, f) pH 4.6. Scale bar represents 100 nm. (Source: Gastaldi *et al.*, 1996)

(SEM). pH-induced changes in casein micelles during direct acidification and bacterial fermentation of reconstituted skim milk at 20ºC were monitored. Acidified milk samples were taken by dipping an ANODISC® alumina inorganic membrane with an average pore diameter of 0.2 µm. The membranes with attached casein micelles were either directly dehydrated using an alcohol gradient and dried to CO_2 critical point, or dipped in pH-adjusted simulated milk ultrafiltrate (SMUF). The latter samples were then rapidly frozen in Freon 22 (cooled to -155ºC with liquid nitrogen) and then freeze-dried at -80ºC. Dried samples were gold-coated and examined with a JEOL JSM-6400F field emission scanning electron microscope operated at 15 kV. Five main pH ranges of microstructural changes in casein micelles during milk acidification were proposed, which were: 1) The first stage of aggregation (pH 6.7 to 5.8), micelles started to loose their individuality, came closer and formed clusters, but the initial shape was still seen (Figure 6a,b; Figure 7a); 2) pH 5.5 to 5.3, most casein particles were significantly deformed, stretched, and extensively coalesced; thus form-

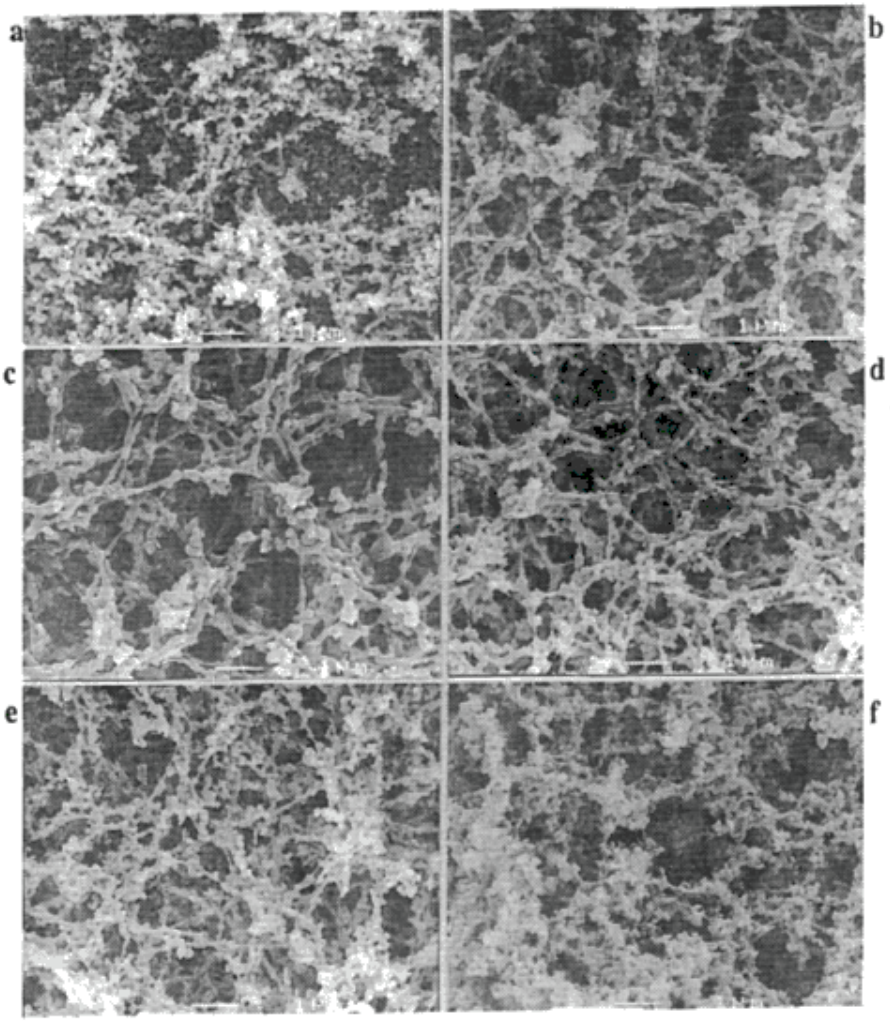

Figure 7. SEM micrographs of acidified milk critical-point dried samples; a) pH 5.8, b) pH 5.5, c) pH 5.3, d) pH 5.0, e) pH 4.8, f) pH 4.7. Scale bar represents 1 µm. (Source: Gastaldi *et al.*, 1996)

ing a pseudo network with a very open structure (Figure 6c,d; Figure 7b,c); 3) pH 5.3 to 4.8, the network became denser and fragmented into small units with new casein particles acting like individual characteristics (Figure 7c,d,e); 4) pH 4.8 and 4.7, casein particles with the previous stage of fusion were followed by a stage of contraction and rearrangement, resulting in new casein particles with spherical shapes (Figure 7e,f); 5) pH 4.6, the formation of acidified milk gels was completed with the casein particles aggregated into a true three-dimensional network of chains and clusters (Figure 6e,f).

Confocal laser scanning microscopy (CLSM) has also been used to study on acid milk gels. CLSM is one of the most useful microscopy techniques to observe the microstructure of a wide variety of foods, especially dairy foods. A most important quality of the CLSM is its ability to "optically section" through samples, giving a 3-dimensional view of a food product with minimal sample disturbance, where other microscopy techniques would require physical disruption of the sample (Auty, 2003; http://anka.livstek.lth.se:2080/microscopy/f-visit2.htm). Some researchers have found by using this technique that acid gels consist of a course particulate network of casein particles linked together in clusters, chains and strands (Lucey, 2004), as shown in Figures 8 and 9.

An example of confocal micrograph of acidified milk gels made from heated milk is shown in Figure 10. It seems that this structure is more interconnected than milk gel made from unheated milk gels, especially if a small amount of rennet is added (Figure 11) (Lucey, 2004).

A study performed at Moorepark examined the effect of whey protein and ionic environment on microstructure of acid-induced milk protein gels. Phosphocasein (obtained by micro-filtration) and heat denatured whey protein (DWP) were dispersed (5% protein) in water, simulated milk ultra-filtrate (SMUF) or two-fold concentrated SMUF. Individual protein dispersions and 80:20 phosphocasein:whey protein mixtures were acidified at 40ºC with glucono-δ-lactone until reaching pH 4.6 after two hours. CSLM allowed direct visualization of protein aggregation and subsequent network formation. The phosphocasein gels consisted of a coarse, open network of

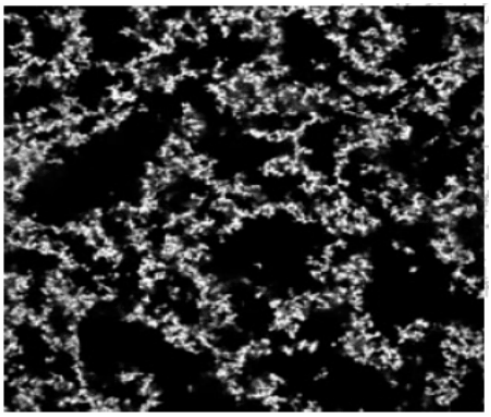

Figure 8. Confocal laser scanning microscopy image of acid casein gel. Protein network appears white/gray on dark background.
(Source: Merete Færgemand, 2003; http://www.mli.kvl.dk/dairy/special/microstructure/microstr.htm)

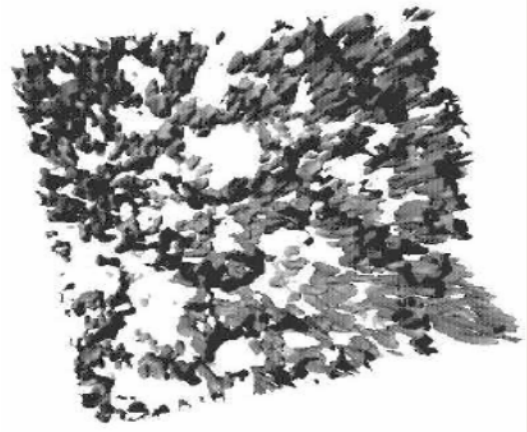

Figure 9. A 3D projection of a stack of confocal laser scanning images of an acid casein gel. Protein network appears gray.
(Source: Færgemand and Andresen, 2003; http://www.mli.kvl.dk/dairy/special/microstructure/microstr.htm)

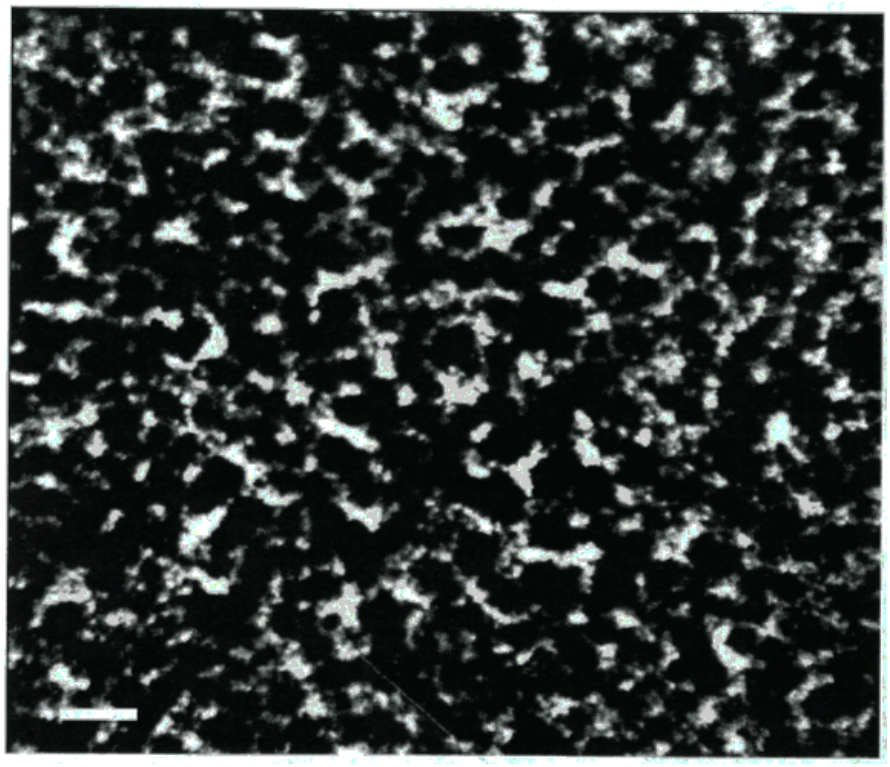

Figure 10. Confocal laser scanning micrographs of Quarg gel made from skim milk fermented with 1%(w/w) of a mesophilic starter culture at 22ºC. Milk was pre-heated at 90ºC for 5 min. The pH of the gels was approximately 4.7. Scale bar represents 20 μm. (Source: Lucey, 2004)

aggregated protein, whereas DWP gels appeared much finer networks with smaller pores. CSLM showed that increasing the SMUF concentration decelerated the onset of gelation. It was also shown that pre-denatured whey proteins interacted with casein micelles at comparatively low temperatures (25-40ºC) with the presence of SMUF, causing the initiation of gelation at a higher pH (6.2). These results indicated that gel microstructure and viscoelastic properties can be controlled by the inclusion of denatured whey proteins and manipulation of ionic environment. These findings will help food technologists to create novel textures (Auty and O'Kennedy, 2001; http://www.irishscientist.ie/2001/).

Schorsch *et al*. (2002) studied micellar casein gelation at high sucrose content by investigating 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 complete portrayal of the structures formed. CLSM was used to visualize the microstructures of the gels. To stain the proteins, Rhodamine B (0.001% wt/wt) was added to the dispersions, and then GDL or rennet was added and mixed. A small amount of the sample was placed on a microscope slide with a cavity and then covered with a cover slip. The samples were stored for 24 h at 20ºC, and then placed on a temperature-controlled stage. Visualization was carried out with laser excitation of 488 nm, provided by CLSM. The microstructures of casein gels produced by acidification, renneting, or by a combination of acidification and renneting, in the

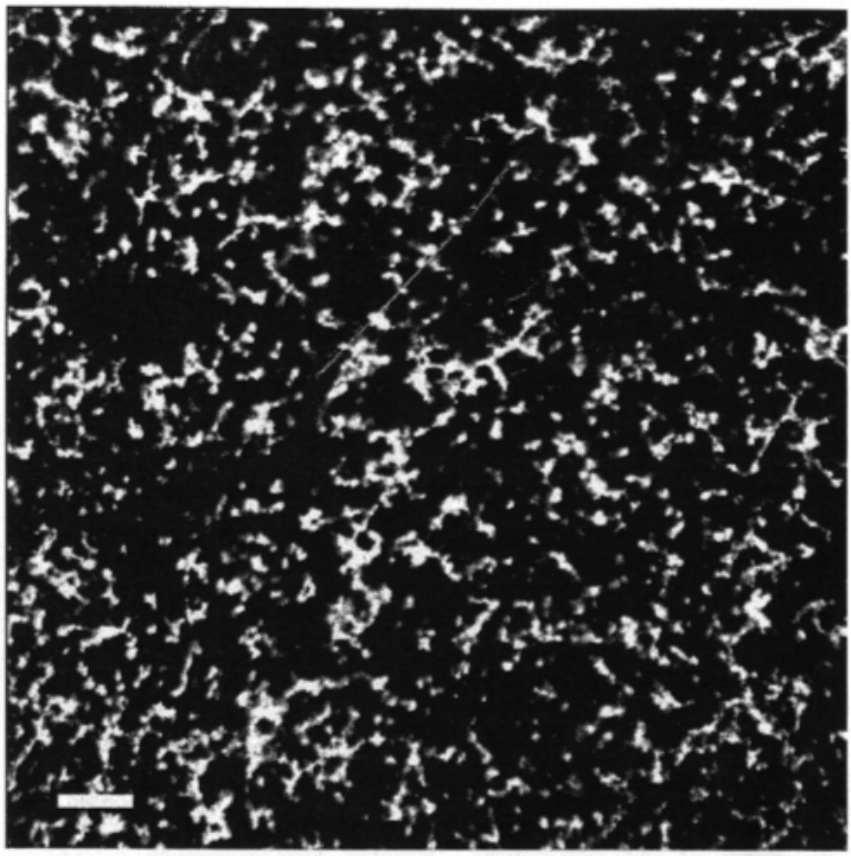

Figure 11. Confocal laser scanning micrographs of Cottage cheese gels made from skim milk fermented with 5% of mesophilic starter culture at 32ºC. Standard strength rennet was added 1 ml/ 45 kg milk immediately after culture addition. The pH of gel was approximately 4.7. Scale bar represents 20 µm. (Source: Lucey, 2004)

presence or absence of sucrose, were compared. Figure 12 shows 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, indicating that sucrose can prevent the local phase separation normally present in acid- or rennet-induced casein gels.

The textural properties of acidified milk gels are influenced by several factors, such as casein concentration, pH, temperature, the history of pH and temperature (Walstra, 1997), total solid contents (Lucey and Singh, 1998), and processing parameters such as homogenization pressure and incubation temperature (Phadungath, 2003). An extremely firm texture can be caused by a very high total solid content of the mix (both casein and fat) or an excessive amount of added stabilizers. On the contrary, the weak texture can be because of factors such as a low total solids (fat content) in the mix, deficient heat treatment of the milk, low acidity (high pH value), and too low gelation temperature (Phadungath, 2003; Lucey, 2004). Table 1 summarizes the effects of some processing factors on the acid coagulation of milk and properties of acid gels.

Table 1. Summary of the effects of some processing conditions on the acid coagulation of milk and properties of acidified milk gels.

Condition	Impact on acid coagulation and gel properties
Incubation temperature	Faster acid production at higher incubation temperatures leads to shorter gelation times. At a higher temperature (e.g. 30ºC) there are more re-arrangements of casein particles in the network leading to weaker gel and an increased possibility of whey separation than gels made at lower temperature (e.g. 23ºC). The gelation pH may increase at very high temperature, whereas no casein coagulation occurs even at isoelectric pH (pH 4.6) at very low temperature (e.g. 4ºC).
Heat treatment	Heat treatment of milk prior to gelation process at a temperature $\geq$ 78ºC for $\geq$ 5 min can cause whey protein to denature, resulting in increased gelation pH, decreased gelation time, and increased viscosity/firmness. This effect is caused by the high isoelectric pH (pH 5.3) of β-lactoglobulin. The explanation of this incident is that disulfide cross-linking of casein strands increases gel firmness but solubilization of CCP happens in casein particles already incorporated in the gel matrix, resulting in greater rearrangements, which is responsible for the increase in loss tangent observed in rheological tests.
pH	Aggregation takes place as the isoelectric pH of casein ($\leq$ 4.9) is reached. Maximum gel stiffness occurs around pH 4.6.
Casein content	Gel firmness is proportional to casein concentration

Adapted from: Lucey, 2004

Summary

Although acidified milk gels have been made for thousands of years, the microbiological and technological features of the products have not been thoroughly studied until recently. During the past two decades, extensive research has been done on the formation and physical properties of acidified milk gels. Recent developments regarding possible mechanisms involved in the formation of acid-induced milk gels were discussed. Modern techniques were also reviewed, containing the use of dynamic low amplitude oscillatory rheology to observe the process of gel formation, and confocal laser scanning microscopy to non-destructively monitor the gel microstructure. The author believes that future work is still needed in many areas; for instance, comparison of acidified milk gels made by different acidification techniques and development or modification of existing theoretical models for the formation of acidified milk gels.

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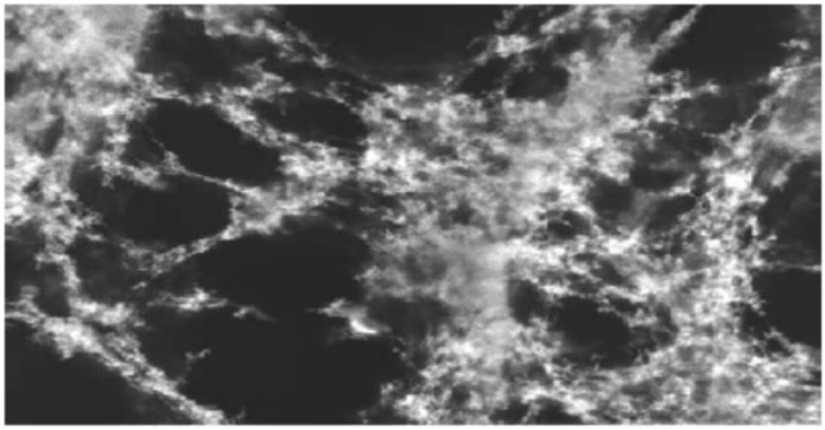

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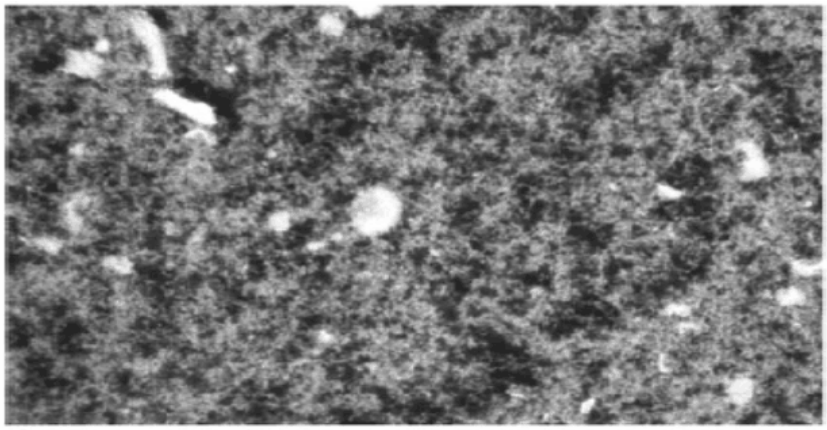

b

Figure 12. Confocal laser scanning 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 μm. (Source: Schorch *et al.*, 2002)

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