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Rennet-induced gels and their mechanical properties

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우유의 렌넷 젤에 대한 기계적 특성

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Abstract : Casein micelles are the basic building block of rennet-induced gels. The stiffness of these gels is increased with reaction time. This is probably due to the continuous participation of activated casein micelles into growing network. Dual binding model of casein micelles, which explains assembly of casein and colloidal calcium phosphate, can provides fairly reasonable explanation for the changes in mechanical properties of rennet-induced gels made from different milk pHs and varying colloidal calcium phosphates. The changes in stiffness of these gels would be used for controlling textural properties of cheeses.

Key words **:** Rennet-induced gels, Dual binding model, Mechanical properties of gels

I. Introduction

The principal components of bovine milk include water (87.1%), fat (4.0%), protein (3.3%), lactose (4.6%), and mineral substances (0.7%) (Walstra et al., 1999). Two types of milk proteins are found in milk. Caseins (CN) are the major type of milk proteins and account for ~80% of total milk protein. The globular whey proteins make up the rest (20%). CN are not distributed as individual molecules in solution rather, they exist in a stable colloidal suspension of aggregates known as casein micelles (CM). CM are largely composed of water and CN but it also contains a small but essential amount of inorganic constituents. On the dry matter, CM is composed of ~6.6% salts and 93% CN.

Surface properties of CM are very important for the stability or coagulability of milk. Van der Waals attractive force is ubiquitously present for all molecules to which CM is not an exception. CM in milk solution are extremely stable if repulsive forces are not removed. It is almost accepted that the repulsive forces, which dominate over van der Waals attraction, are steric stabilization and electrostatic repulsion. Macropeptide, the C-terminal region of κ-CN on the surface of CM, that sticks out into the milk serum exerts steric hindrance, and the negative charge on this peptide, as well as some β-CN peptides on the surface of CM, are responsible for electrostatic repulsion (Walstra et al., 1999). The destabilization of CM can be created with one of four methods that differ in their mechanism; 1) enzyme (rennet) action: cheese, 2) acidification: yoghurt, 3) ethanol: cream liqueurs, and 4) combination of acid and heat: Ricotta cheese. It must be realized that the surface properties are important just before milk coagulation. Once milk is destabilized, internal properties dominate overall textural properties of coagulated milk such as cheese and yogurt.

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In the recent dual binding model for the structure and formation of CM (Horne, 1998), detailed internal structures are well documented. Individual CN molecules are considered as block copolymers. Micellar assembly is viewed as a polymerization process occurring as a result of hydrophobic interactions or by bridging via colloidal calcium phosphate (CCP) (Horne, 1998). The balance between electrostatic repulsion and attractive hydrophobic interaction controls the degree of incorporation of individual CN molecules in the assembly of CM. Thus, the dual binding model for CM views CCP as a key bridging material. It is well known that CCP is solubilized as milk pH decreases (Dalgleish and Law, 1989). This model dictates the assembly of CM, and therefore, it could predict a consequences resulting from changes in the internal structure of CM during milk processing.

Gelation of milk proteins is the first key step in cheese manufacture. The addition of chymosin to milk initiates the destabilization of CM via hydrolysis of κ-CN, which is the first phase of rennet coagulation (Dalgleish, 1992). Once a sufficient degree of destabilized paracasein micelles are produced, aggregation begins, which is the second phase (Hyslop, 2003), and this leads to the formation of a three-dimensional space-filling gel, which is the third phase (Horne and Banks, 2004). In contrast to the first and second stages of rennet coagulation, there have been fewer studies on the third stage of curd development. In this article, we review the third stage of curd firming with reaction time and dual binding model is employed to predict a mechanical properties of rennet-induced gels.

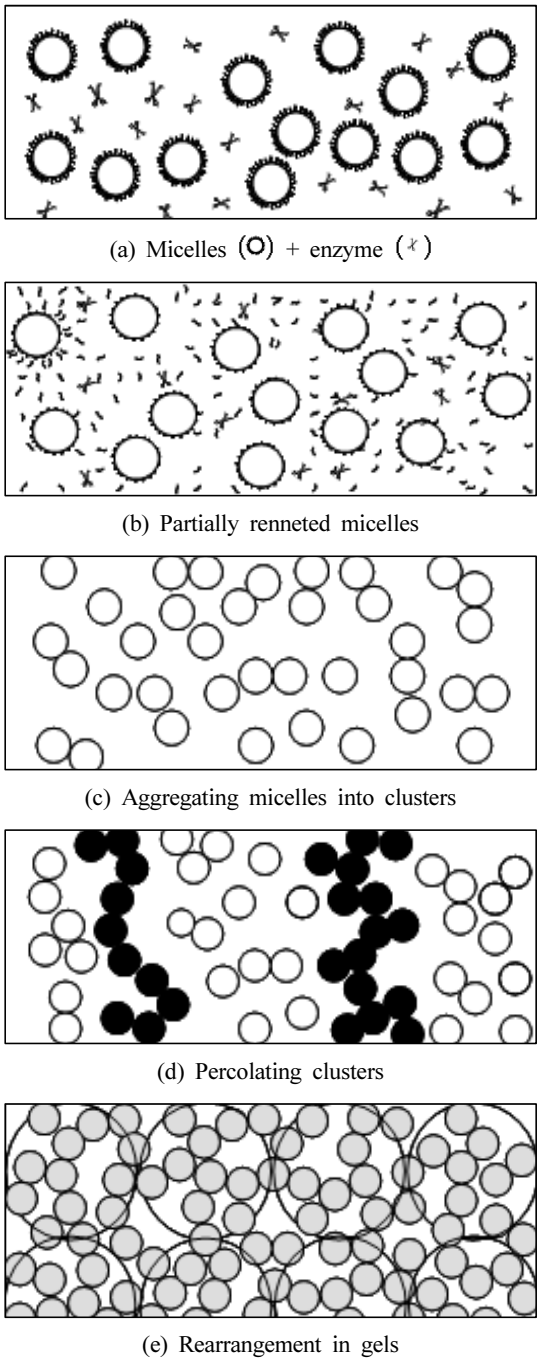

Fig. 1. Schematic description of the various stages envisaged in the enzymatic coagulation of milk, starting from (a) the initial mixture of casein micelles and enzyme and proceeding through (b) proteolysis, (c) initial aggregation into small clusters and reaching (d) a gelation time at percolation. (d) and (e) are two different scenarios defining the occurrence of the gelation time. (d), the percolation cluster model where gelation is sensed by the measuring device whenever clusters extend across the container; (e), the formation of a weak gel where most particles are weakly linked into the network but reversibility allows rearrangement. The larger circles depict the fractal blob concept where the system is fractal within but close packed outside (Horne and Bank, 2004).

II. Rennet-induced coagulation of milk

1. Gel development

Conversion of milk into cheese starts with the destabilization of CM via limited proteolysis by rennet

or milk-clotting enzyme. In the enzymatic reaction (that is the first of three gelation stages; Fig. 1), rennet cleaves the peptide bond at Phe_{105}-Met_{106} of κ-CN, and results in removal of macropeptides from CM and the macropeptide is now located in the serum. The subsequent changes (Horne and Banks, 2004) involve the aggregation of rennet-altered CM (the second stage; Fig. 1c and d) and the formation of three-dimensional, space-filling gels (the third stage; Fig. 2). The first and second stages have received many studies, but the third phase, gel development, has drawn relatively less attention.

Visually it is easy to recognize the transition from first to third stage as milk changes from a liquid to a semi-solid gel. Probably the best way to monitor this change (gel formation) is to use rheometry (Horne and Banks, 2004). Typical development of viscoelasticity during rennet-induced gel formation as a function of reaction time is shown in Fig. 3. Although not visible on the scale plotted, storage modulus (G′) initially lags lower than the growth of the loss modulus (G″), but quickly crosses over G″ and dominates as gel elasticity rapidly develops, the transition point (gelation time; Fig. 1d) occurring at loss tangent (LT) = 1. Because of the limit of rheometer instruments, gelation time (t_g) is usually defined as a time at which G≥0.1 Pa. Both dynamic moduli evolve as sigmoidal curves which tend to reach maximum, and eventually decrease with much longer reaction time due to macro/microsyneresis and proteolysis (Roefs et al., 1990). The LT value remains relatively constant after t_g, which may indicate that the type/strength of bonds created among rennet- modified CM remains constant (Horne and Banks, 2004). An obvious question is "why do both moduli increase with reaction time?" Particle gel theory and scaling behavior of rennet gels (Horne, 1995 and 1996) have something to do with this question.

2. Rennet gels as particle gels

Rennet gels, cheese, and yoghurt are examples of particle gels (Dickinson, 1990). In rennet gels, particles refer to the activated CM via the hydrolysis of κ-CN. Interparticle attractive forces are somehow created with time, leading to the three-dimensional network. Three theories (Horne and Banks, 2004) are available that explain how particles are crosslinked and cause the observed increase in shear moduli.

First, in adhesive hard sphere model (De Kruif, 1998), it is assumed that all of the CM would be considered as a network from time at which the proteolysis of

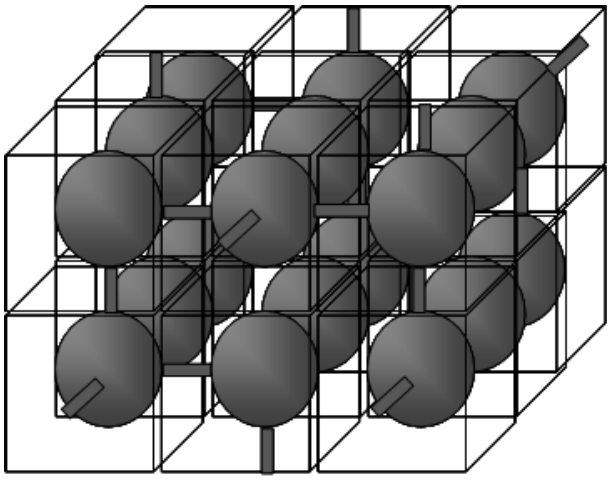

Fig. 2. Representation of renneted casein micelles at volume fraction 0.1, distributed on a cubic lattice, with network formation underway. Coordination number, *k*, would be 6 in this example (Horne, 2002 American Dairy Science Association meeting).

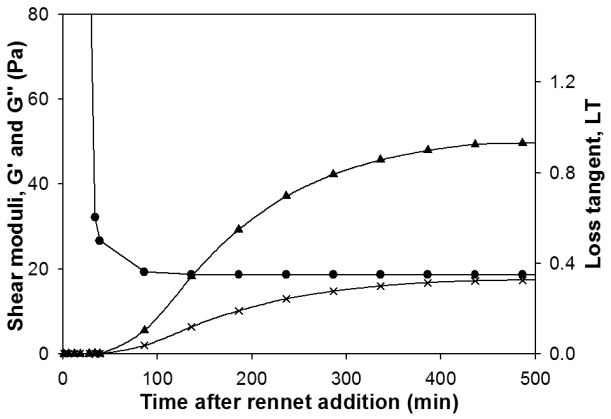

Fig. 3. A typical example of the change in viscoelastic parameters observed during gel formation, showing changes in shear moduli, G′ (▲) and G″ (×), and loss tangent (●) with time after rennet addition.

κ-CN was initiated and the attractive well began to deepen. Well-depth increases as proteolysis progresses, and bonds become increasing stronger. Because bond lifetime in this model is changing smoothly during gelation from very short to very long, the gelling system passes monotonically from a viscous liquid to a stiff elastic solid. The LT values therefore more sensitively reflect changes in the nature of the bonds. As shown Fig. 3 however, the LT value drops rapidly in the early stages through t_g but remains substantially constant throughout the main growth period in the shear moduli. This indicates that the nature and strength of the bonds do not change significantly during this growth. It is therefore unlikely that the increase in shear moduli is caused by an increase (change) in some type of bond strength with time.

Second, in rearrangement theory (Bremer et al., 1989; Mellema et al., 2002) bonds are weak, aggregation is reversible, and clusters are breaking up as rapidly as they form. The critical gelation condition is reached when the clusters achieve a pseudo-close-packed arrangement as depicted in Fig. 1e. The majority of potential bonds are satisfied at this juncture and increases in shear moduli occur as the system rearranges itself in its quest for an equilibrium structure. However, the scaling behavior model of Horne (1996) indicates that the gel development kinetics clearly depends on the rate of the proteolysis reaction, the rate controlling the achievement of t_g. Thus, rearrangement is very unlikely to be responsible (We think that the proteolysis itself could interfere with rearrangement so do not rule this out).

Third, Horne and Banks (2004) proposed that the continuous participation of potential particles into percolation strands with reaction time leads to increase in shear moduli. As in Fig. 1, particles (i.e. rennet-altered CM) get closer and touch one another, forming clusters. At t_g a percolation cluster spanning the container results (Fig. 1d). Gel evolution continues as the remaining free particles and clusters are incorporated into the particle gel network, creating more stress carrying strands. At maximum G′ most of the particles take part in network formation as shown in Fig. 2. We think that this explanation is very likely to be the cause for the increase in the shear moduli of rennet gels with time.

III. Mechanical properties of rennet gels

Van Vliet and Walstra (1985) derived the relationship between shear modulus and deformation of the network following the application of a shear force as [Equation 1]:

$$G = CN\frac{d^2F}{dx^2} \quad (1)$$

where N is the number of stress-bearing strands per unit area in a cross-section perpendicular to x that is the direction of the external force or stress, C is a characteristic length determining the geometry of the network, dF is the change in Gibbs free energy when the elements are moved apart over a distance dx, and is therefore related to the bond strength in systems where enthalpic effects dominate over entropic. In the case of particles that are homogeneously distributed over the available space, or at least homogeneously on the length scales of the experimental measuring device, all particles involved in the network will contribute to the network modulus to the same extent. N will then be directly proportional to the volume fraction of particles in the network. In the fully developed network of particles, such as rennet-induced gels (Fig. 2), the measured G′ or G″ is proportional to the number of bonds involved times the bond strength of each (Horne, 1996), that is;

$$G' \text{ or } G'' \propto \sum_{i=\text{bond type}}^{n} (\text{Bond}_i \times \text{Strength}_i) = (\text{N}_k \times \text{S}_k) + (\text{N}_{ccp} \times \text{S}_{ccp}) + (\text{N}_{HI} \times \text{S}_{HI}) \quad (2)$$

where N_k, N_{ccp}, and N_{HI} are the number of coordinations, CCP crosslinks, and hydrophobic interactions, respectively, and S_k, S_{ccp}, and S_{HI} are the strength of coordination, CCP crosslinks, and hydrophobic interactions, respectively.

At the normal pH of milk where integrity of CM is assured, there is no need to consider internal bonds, i.e. N_{ccp}, N_{HI}, S_{ccp}, and S_{HI}, of CM in rennet gels. However, we may have a different situation. CN carries negative charges at normal milk pH (~6.7) and the solubilization of CCP also depends on pH of milk. It is also known that the overall shape of CM, sphere, does not change much even if most of the CCP cross-links are removed (Roefs et al., 1985). [Equation 2] then gives approximately what impact the loss of CCP at variable pH and constant pH of milk can have on mechanical properties of rennet gels. It can be deduced that as pH is lowered from ~6.7 to ~5.0, the number of *k* and hydrophobic interactions would not change, but at the same time the number of crosslinks decreases. However, the strength of *k* is stronger as a result of lower negative charge on CM. Since newly exposed multiphosphorylated serine clusters are titrated, the strength of CCP crosslinks and hydrophobic interactions would remain the same. On the other hand, if CCP crosslinks are removed with a chelating agent, such as EDTA, at constant pH, then the number of CCP crosslinks only decreases (the number of *k* and hydrophobic interactions would remain the same). The strength of CCP crosslinks and hydrophobic interactions are likely to be weaker due to neighboring electrostatic repulsion, but the strength of *k* would remain the same since pH is constant. Choi et al. (2007) found that the loss of CCP crosslinks from the internal structure of CM resulted in lower G′ of rennet-induced gels at constant pH.

IV. Conclusions

In this review, an adhesive hard sphere model, a rearrangement of activated casein micelle in the network, and a continuous participation of available micelles are critically evaluated for explaining the curd firming of rennet-induced gels. The first two theories more unlikely occur than a continuous participation of available micelles into network, as given in the text. The dual binding model helps predict the mechanical properties of these gels made from different milk pH and varying concentration of colloidal calcium phosphate.

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