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Effects of Heating Milk on Viscoelastic Properties and Microstructure of Acid-induced Milk Gel

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When milk is acidified and its pH approaches the isoelectric point of casein (pH 4.6), casein micelles (CM) in milk aggregate and subsequently coagulate, and finally form a gel network. The objective of this study was to investigate the effects of heating milk on the viscoelastic properties and microstructure of acid-induced milk gelation. The samples used were skim milk (SM) and a CM suspension. The changes in storage modulus (G') and loss modulus (G") of the samples were measured using a rheological apparatus after addition of an acidulant, glucono-δ-lactone, in order to obtain the gelation curves. The gelation rate (GR), the maximum of G'(G'max) and the gelation time (GT) were obtained from the gelation curves. When SM was preheated above 70°C for 10 min, GR and G'max of the gels increased markedly due to the association between β-lactoglobulin and the casein micelles. Analysis by scanning electron microscopy (SEM) showed the presence of a three-dimensional network comprised of strands of casein micelles (average diameter: 150 nm) in all samples. Our findings demonstrate that acid-induced milk gels belong to the category of particle gels. The gel prepared from the heated sample exhibited a finer and more branched gel network composed of thinner aggregates compared to the gel prepared from the unheated sample.

Key words: acid-induced milk gel, gel microstructure, heating milk, viscoelastic properties of gel

1. INTODUCTION

The formation of a milk gel by acidification is the principle applied in production of yogurt. The physical properties of yogurt are influenced by various factors, for example, the concentration of milk protein (casein), the ionic strength, the kind of acidogen used, the composition of milk, homogenization of milk, etc. Among these factors, preheating of milk is the most important factor in the manufacture of yogurt, because it is known that preheating of milk at a relatively high temperature has a favorable effect on the texture of the final product. Usually milk for yogurt is pasteurized at a higher temperature (85 - 95°C for 10 - 40 min) than that applied in the case of milk used for other dairy products [1].

Although there have been a number of studies on the effects of preheating milk on the physical properties of yogurt or acid-induced milk gel, little is known about the effects of preheating milk on the viscoelastic properties and the microstructure of acid-induced milk gel. In this paper, skim milk was heated at various temperatures between 60°C and 90°C for 10 min and acidified by addition of glucono-δ-lactone (GDL). The changes in storage modulus and loss modulus after addition of GDL to the samples were examined to investigate the influence on the viscoelastic properties of acid-induced milk gel prepared from preheated milk. Furthermore, the microstructure of acid-induced gels prepared from heated and unheated samples was examined by scanning electron microscopy (SEM) to elucidate the relationship between the viscoelastic properties and the microstructure of the gel.

2. MATERIALS AND METHODS

Skim milk: Fresh milk was centrifuged at 1000 x g at 20°C for 30 min. Skim milk was then removed from the centrifugation tubes by aspiration.

Casein micelle (CM) suspension: Skim milk was centrifuged at 70,400 x *g* for 70 min at 20°C. After centrifugation, the sedimented casein micelles were collected as a pellet. The pellet was suspended in simulated milk serum ultrafiltrate (SMUF)[2] containing 0.02% sodium azide.

Heat treatment of samples: The samples, each in a bottle with a screw cap, were preheated using a water bath adjusted thermostatically to the required heating temperature. A thermocouple was mounted in the cap of the bottle to monitor the temperature.

Viscoelastic measurements: Viscoelastic experiments were carried out according to the methods of Niki et al. [3]. G' and G" of samples were determined as a function of time after the addition of of GDL, and the time course of changes in G', that of G" and that of tan δ of the samples were determined. Furthermore, from the results obtained concerning the time course of G', we determined the values of three parameters, namely, the maximum of storage modulus (G'max), the gelation time (GT), and the rate of gelation (GR). The gelation time was estimated as the time at which the shear modulus began to deviate from baseline. The GR value was calculated as follows: the value of one-half G'max was divided by the time elapsed from the gelation time (GT) to the time where G' reached the value of one-half G'max.

Electron microscopic experiments: The samples for the electron microscopic experiments were prepared by the methods of Park et al [4]. The specimens were examined using a scanning electron microscope (JSM 6301F, Nihondenshi Co. Ltd., Tokyo).

3. RESULTS AND DISCUSSION

Skim milk preparations preheated at various temperatures between 60°C and 90°C for 10 min were acidified by addition of GDL at 25°C to examine the effect of preheating on the viscoelastic properties of the acid-induced gels.

Fig. 1 shows the time course of changes in G' of the samples after addition of GDL. It can be seen in Fig.1 that G' increased after the time of gelation and approached a maximum value (G'max).

The values of three parameters, G'max, GT and GR, were determined from the time course of changes in G' presented in Fig. 1 and summarized in Table 1. The values of these parameters in the case of the sample heated at 60°C were quite similar to those in the case of the unheated sample. However, when skim milk was heated above 70°C, G'max and GR increased with the preheating temperature, and GT decreased. The values of G'max and GR obtained in the case of the sample heated at 90°C were about 3 times higher than those in the case of the sample heated at 60°C. These findings indicated that acid-induced gelation of skim milk was enhanced when the skim milk was heated at temperatures higher than 70°C, suggesting that there was an increase in the number and/or strength of elastically effective bonds between casein particles (micelles) in the gel prepared from the heated skim milks as compared to the gel prepared from unheated milk.

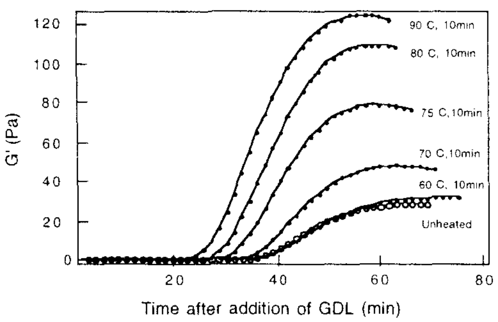

Fig. 1 Influence of heating skim milk on the time course of storage modulus of acid-induced milk gel

Skim milks were heated at 60°C -90°C for 10 min.
Gelation temperatture : 25°C

Table 1 Efffects of heating skim milk on GT, G'max and GR

Temp. of heat treatment(°C)	GT (min)	G'max (Pa)	GR (Pa/min)
Unheated (Control)	36.0	29	1.5
60	36.0	32	1.5
70	33.0	48	2.1
75	28.5	80	3.0
80	25.0	109	3.4
90	22.5	122	4.5

Fig. 2 shows the time course of changes in tan δ of gels prepared from skim milk preheated at various temperatures. The curves obtained showed a peak at about the gelation point, then, soon after the onset of gelation, tan δ decreased steeply and gradually reached a constant value. The final values were in the range of 0.32 to 0.36 and the values decreased with the temperature of preheating. Zoon et al. [5]have reported the tan δ of rennet-induced gel prepared from skim milk. According to them, higher values of tan δ are indicative of the formation of gels which are less elastic and more viscous. Roefs et al. [6] reported that there is a relationship between the value of tan δ and the relaxation behavior of the protein-protein bonds, and sugested that a high value of tan δ is indicative of easy breaking and formation of protein-protein bonds. On the basis of these assumptions, the gels prepared from skim milk heated at higher temperatures are likely to have more stable bonds as compared to gels prepared from skim milk preheated at lower temperatures.

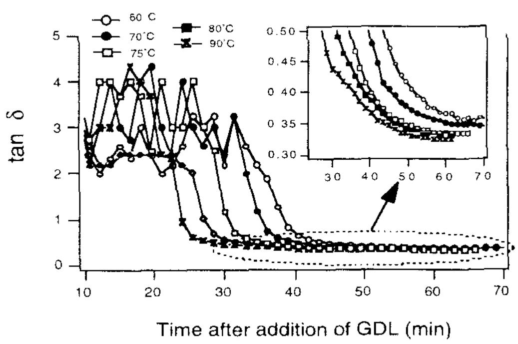

Fig. 2 Influence of heating skim milk on the time course of tan d of acidinduced milk gel

Skim milks were heated at 60°C -90°C for 10 min.
Gelation temperatture : 25°C

Various changes in milk during heat treatment have been reported by many researchers. Especially, the effects of heating on β-lactoglobulin (β-Lg) in milk and the association between heat-denatured β-Lg and casein micelles have been investigated extensively. For example, Kalab et al. [7] examined electrophoretically the supernatant obtained upon ultracentrifugation of yogurt prepared from heated milk and it was found to contain a negligible amount of β-Lg as compared to that in the case of yogurt prepared from unheated milk. These findings indicated that β-Lg becomes associated with casein micelles during heat treatment. Thus, it can be assumed that the association of this protein with micelles is an important determinant of the strength of the interaction between casein micelles in milk upon acidification of milk. On the basis of this assumption, we tried to

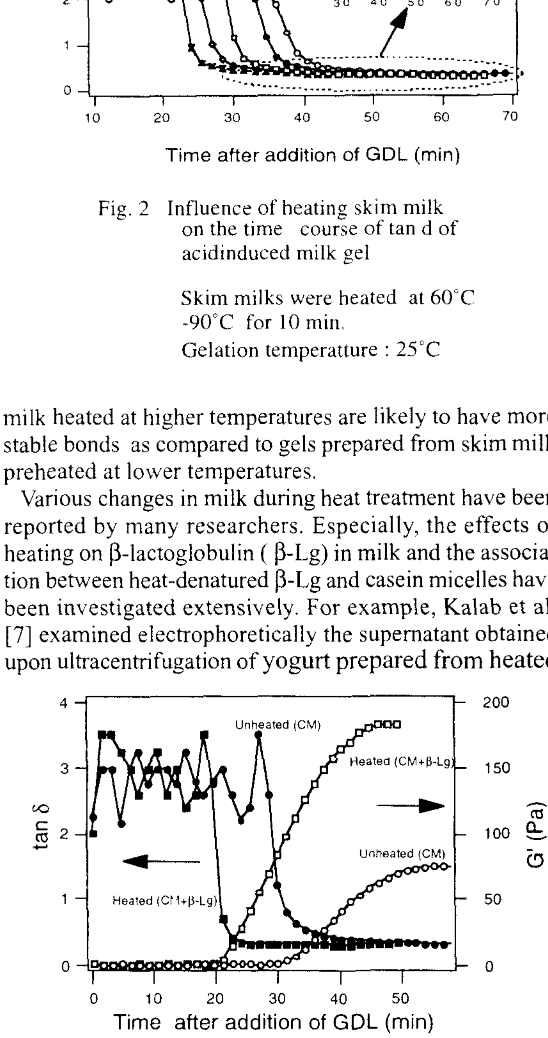

Fig. 3 Time course of storage modulus of acid-induced CM gel prepared from CM suspensions unheated and heated in the presence of 0.5% β-Lg.

Gelation temperature: 25°C
Sample was heated at 80°C for 10 min.

investigate the effects of β-Lg on the viscoelastic properties of acid-induced milk gel, as follows. Milk or skim milk was mostly used as samples in the experiments examining the effects of heat treatment on β-Lg in milk. We used CM suspended in SMUF and purified β-Lg as samples, to determine more precisely the influence of the interaction between casein micelles and β-Lg during heat treatment on the viscoelastic properties of acid-induced gel.

Fig.3 shows the time course of changes in G' of the CM suspension, unheated and heated, in the presence of 0.5% β-Lg after addition of GDL. The gelation curves (G' vs. time) of the unheated sample and the sample heated in the presence of β-Lg are markedly different. The G'max and GR of the gel prepared from the sample heated in the presence of β-Lg were about 2.3 times and 2.6 times larger than that in the case of the unheated sample, respectively. These findings indicated that the gelation of the CM suspension upon acidification was enhanced by preheating the suspension above the temperature at which denaturation of β-Lg and association of the denatured β-Lg with the casein micelle occurred. Fig. 3 shows the time course of changes in tan δ of samples after addition of GDL. In the case of both samples, the tan δ values decreased steeply and gradually reached a constant value. In the case of the heated sample, the tan δ values decreased more steeply after GT and reached a constant value early compared to the unheated sample. However, the final va'ue of tan δ was about 0.3 in the case of both samples.

The microstructure of acid-induced CM gel was investigated by SEM. Fig. 4 shows the microstructure of acid-induced gel prepared from CM suspension unheated (Fig. 4a) or heated in the presence of 0.5% β-Lg (Fig. 4b). The electron micrographs of the gels showed the presence of a three-dimensional network comprised of clusters and chains of casein micelles (average diameter: 150 nm). The form and size of the casein micelles in the gel network were not altered upon acidification [8]. According to the classification of Flory [9], acid-induced milk gel is a typical particle gel.

As shown in Fig.4, there was no significant difference

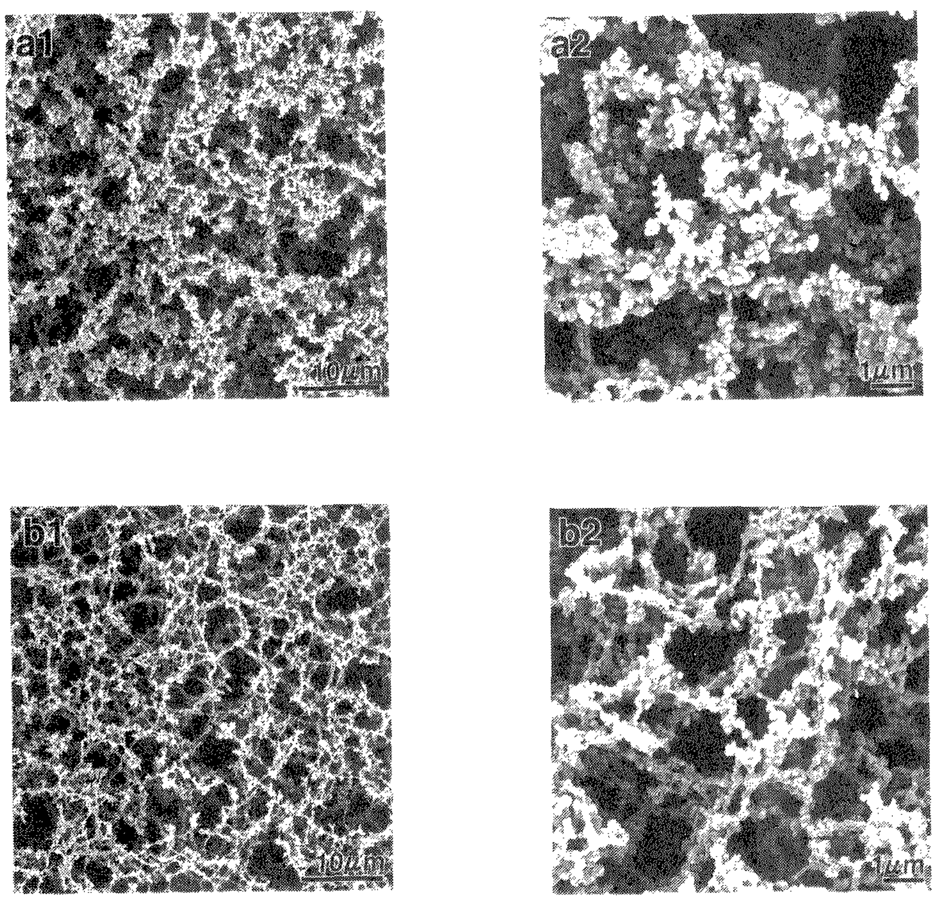

Fig. 4 Scanning electron micrographs of acid-induced CM gel prepared from CM suspensions unheated and heated in the presence of 0.5% β-Lg

Samples for the observation of electron microscopy were taken at the time where the G' showed the maximum values (in Fig. 3)
The pictures of a1 and a2 : unheated sample
The pictures of b1 and b2: heated sample

in the size or appearance of the casein particles (micelles) comparing the gels prepared from unheated CM suspension or CM suspension heated at 80°C for 10 min. However, a great difference in the network structure was found comparing the gels prepared from heated and unheated samples. In the case of acid-induced gel prepared from the unheated sample, the clusters of casein micelles were larger and thicker, that is, the gel prepared from the unheated sample formed a coarse gel network composed of relatively large clusters or aggregates, whereas the gel prepared from the heated sample exhibited a finer and more branched gel network composed of thinner aggregates compared to the gel prepared from the unheated sample.

The differences in microstructure between the gels prepared from samples unheated or heated in the presence of β-Lg (Fig. 4) may reflect differences in viscoelastic properties between the preheated and unheated samples (Fig. 3). In other words, the finer and more branched gel network prepared from preheated milk results in a stronger gel as compared to the gel prepared from unheated CM suspension.

Davis et al. [10] have investigated the effects of heat treatment on gel formation in yogurt by transmission electron microscopy and they observed the presence of filamentous appendages on casein micelles in yogurt prepared from heated milk. These were considered to be attributable to the formation of a complex between denatured β-Lg and k-casein on the casein micellar surface during heat treatment,. They assumed that the association of denatured β-Lg with casein micelles is an important determinant of micelle fusion and gel strength in yogurt, when milk is heated.

Although it is very difficult to explain the relationship between the viscoelastic properties of the gel and its microstructure, it may be concluded from the results of these experiments that the strong acid-induced milk gels with a higher G' value have a microstructure consisting predominantly of linear and more branched chains of casein micelle aggregates, compared to the weaker acid-induced milk gels, and that the denatured β-Lg which becomes associated with the casein micelles upon heating the milk may play an important role in forming the linear and branched chains in the gel network. It should be noted also that the denatured β-Lg molecules bound to the surface of the casein micelles may serve to limit the number of sites through which the casein micelles associate with each other upon acidification, and consequently this may inhibit the clustering of casein micelles and may promote the formation of linear and branched chains of casein micelle aggregates.

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