Rheological Studies on the Gelation Process of Soybean 7S and 11S Proteins in the Presence of Glucono-δ-lactone

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Dynamic viscoelastic study and compression testing on gelation of soybean 7S and 11S proteins by glucono-δ-lactone (GDL) were done to elucidate the gelation process of tofu (soybean curd). Gelation curves at constant temperatures observed in dynamic viscoelastic measurement were approximated by first-order reaction kinetics. Both storage and loss moduli at the final stage of the gelation for 7S–GDL gels showed almost the same value as those of 11S gels when concentrations of protein and GDL were the same. However, parameters obtained in the compression testing, i.e., breaking stress and breaking strain of fully gelled 7S, were smaller than those of 11S gels. The rate of gelation for 7S was much slower and the gelation time was longer than for 11S. The gelation rate increased and the gelation time decreased with increasing GDL concentration at a constant 7S concentration as observed for 11S–GDL systems. The gelation time of systems which consist of 7S and GDL at a fixed ratio of 10:1 decreased with increasing 7S concentration. The minimum concentration of 7S protein for the gelation in the presence of GDL was lower than that of 11S–GDL system.

INTRODUCTION

Soybeans are extensively utilized for food processing. They have served as one of the most important protein sources in Japan. Tofu (soybean curd) is a gel-like food made by addition of coagulant to heated soybean milk. Magnesium chloride or calcium sulfate was traditionally used as coagulant, but recently glucono-δ-lactone (GDL) has been widely used in tofu-processing factories because of the advantage of easily formed homogeneous gels.

Soybean proteins contain two major globulins, 7S and 11S (Wolf et al., 1961; Saio and Watanabe, 1978; Brooks and Morr, 1985), which show different thermal transition temperatures (German et al., 1982; Varfolomeyeva et al., 1986; Damodaran, 1988) and gel-forming properties (Hermansson, 1986; Morr, 1990). Most workers studying soybean protein gels have examined only "hardness" by a texturometer or the force required for breaking (breaking force). The hardness for gels made from 7S and 11S globulins was compared by several groups. Saio et al. (1969) reported that 11S gels made in the presence of calcium sulfate were much harder than crude 7S gels and that 11S mainly determines the hardness of tofu gels. Hashizume et al. (1975) also observed that 11S gels coagulated by GDL showed greater breaking force than crude 7S gels. Utsumi and Kinsella (1985) studied 7S and 11S gels made by heating at 80 °C for 30 min and reported that 7S formed harder gels. When solutions were heated at 100 °C for a short time (<5 min), 7S gels were harder than 11S; however, long heating time gave rise to opposite results according to Nakamura et al. (1986). Since large deformation used for the measurements of hardness or breaking force destroys the gel structure and, moreover, measurement of large deformation properties cannot follow the gelation process, the gelation mechanism and the kinetics of 7S and 11S globulins have not yet been clarified.

The method of Thanh and Shibasaki (1976) for isolation of 7S and 11S from soybeans has been adopted by many researchers. This method is based on the different solubilities of these globulins and can easily give several grams of both fractions (Brooks and Morr, 1985). However, the purity of 7S globulin was not very high (Damodaran, 1988), and further purification by chromatography was required to obtain pure 7S globulin. Since it is known that a complex of 7S subunits and 11S basic subunits is formed by heat (German et al., 1982), a small amount of 11S protein contained in the 7S fraction may affect gel properties. Generally, a larger amount of protein is needed to study gels than is required for solutions or crystals. The difficulty in the preparation of 7S globulin with high purity and quantity has hindered the investigation of gel properties and gelation.

The authors have studied the gelation process of soymilk (Nishinari et al., 1991; Yoshida et al., 1992) and soybean 11S globulin (Kohyama and Nishinari, 1992a; Kohyama et al., 1992; Yoshida et al., 1992) in the presence of GDL as coagulant by dynamic viscoelasticity measurements. We also discussed some conditions in a uniaxial compression testing of tofu (Kohyama and Nishinari, 1992b). Recently, soybean 7S globulin was isolated by a new method (Nagano et al., 1992), and the sample prepared by this method showed only one endothermic peak in a heating curve of differential scanning calorimetry. In the present study, the gelation of 7S globulin isolated according to the method of Nagano et al. in the presence of GDL is investigated by a dynamic viscoelastic measurement and a compression testing and discussed in comparison to the 11S–GDL systems.

MATERIALS AND METHODS

Preparation of 7S and 11S Globulins. The details of the isolation method were described elsewhere (Nagano et al., 1992). Defatted soybean meals (var. Enrei) were mixed with a 15-fold volume of distilled water, and then the pH was adjusted to 7.5. The water-extractable soybean protein was obtained by centrifugation. Sodium bisulfite was added to the supernatant and the pH adjusted to 6.4, and the mixture was kept in an ice bath overnight. The insoluble fraction of 11S globulin was separated as the precipitate by centrifugation. The supernatant was adjusted to contain 0.25 M NaCl and to be pH 5.0. The insoluble fraction was removed. The supernatant was diluted 2-fold with ice-cold water, adjusted to pH 4.8, and then centrifuged again. The 7S globulin was obtained as the precipitate. Both 11S and 7S fractions were washed, adjusted to pH 7.5, and then freeze-dried. The protein contents of these powders were 95% for 11S and 92% for 7S globulin determined according to the Kjeldahl method with a conversion factor of 6.25.

Dynamic Viscoelasticity. 7S or 11S globulin was dissolved in distilled water and heated in boiling water for 10 min and then

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cooled to room temperature. About 2 mL of protein solution was heated at 60 °C for 10 min. Then, GDL (reagent grade, Wako Pure Chemicals Industries Ltd., Osaka) was dissolved in ice-cold water and immediately 0.05 mL of the solution was added to the protein solution. The mixture (1.6 mL) was injected into a cell of a Rheolograph Sol (Toyoseiki Seisakusho, Tokyo) (Nishinari et al., 1991; Yoshida et al., 1992), which had been heated to 60 °C beforehand. The surface of the sample was covered with silicone oil to prevent the evaporation of water. The sample solution was subjected to sinusoidal shear oscillations. A frequency of 1.0 Hz and an amplitude of 25 μm were taken as standard conditions so that the shear strain was 2.5%. The storage and loss moduli were recorded as a function of time. The details of the rheological apparatus were illustrated elsewhere (Yoshida et al., 1992).

Analysis of the Gelation Process. Zero time was taken as the time when GDL was added to the protein solution. The observed data were fitted to an empirical formula as used in the previous work (Kohyama et al., 1992)

$$G(t) = G_{sat}[1 - \exp\{-k(t - t_0)\}] \quad (1)$$

where G_{sat} is the saturated value of storage or loss modulus, k is the rate constant of gelation, t_0 is the gelation time, and t is time. The gelation time was defined as the time when the storage modulus began to rise from the baseline. The rate constant k was estimated from curve fitting by a least-squares method. Calculations were done with a SALS program (ver. 2.5) (Nakagawa and Oyanagi, 1980) on a time-sharing system at the Computer Center of the Agriculture Forestry and Fisheries Research Secretariat (Tsukuba, Japan).

Compression Testing. A 7S or an 11S protein solution preheated for 10 min at 100 °C in 20-mL vials was kept in a 60 °C bath. A 5% volume of GDL solution freshly prepared was added to the solution. Concentrations of protein and GDL were adjusted to 4.0% and 0.4%, respectively. The mixture was immediately poured into ring-shaped glass molds of 16-mm diameter and 10-mm height. They were allowed to stand for various periods after addition of GDL at 60 °C and cooled in an ice bath (3 °C) and tap water (26 °C) for 60 min each. Then, the gel was aged at the room temperature (27 °C) for 60 min before the measurement as described elsewhere (Yoshida et al., 1992). Compression testing of gels was carried out using a Rheoner RE-33005 (Yamaden Co. Ltd., Tokyo) attached a 2 kgf load cell. A cylindrical gel sample on the polyacetal stage was vertically compressed with a polyacetal flat plunger of 40-mm diameter at 27 °C. The compression rate was set to 1.0 mm/s. The testing conditions and gel sizes were discussed in the previous work for tofu (Kohyama and Nishinari, 1992b). Breaking stress of gels was calculated from the load value at a breaking point divided by the initial cross-sectional area of the gel. Breaking strain was determined as the ratio of the deformation at a breaking point to the initial height. The Young modulus was defined as the slope of each stress–strain curve at small strain range. The breaking energy was calculated from the area under the stress–strain curve, and it was normalized per unit volume (m^3). Mean value and standard deviation were adopted from four repeated experiments.

RESULTS AND DISCUSSION

True Gels Formed by 7S and GDL. The frequency dependence of the saturated storage (G'_{sat}) and loss (G''_{sat}) moduli at various amplitudes is shown in Figure 1. The amplitude did not influence either storage or loss moduli for 4.0% 7S gels (open circles, Figure 1) when applied strain was small. The storage modulus slightly increased with increasing applied frequency as usually observed in a linear region where the stress is proportional to the strain (Ferry, 1970). The strains induced by amplitude of 100 μm and frequency higher than 1 Hz seem to destroy the gel structure, since storage moduli became smaller than those observed at lower amplitudes. The decrease of storage modulus with increasing deformation indicates that the applied strain was beyond a linear region. The lower 7S concentration of 2.0% was also examined at a fixed amplitude (25 μm). The storage modulus increased

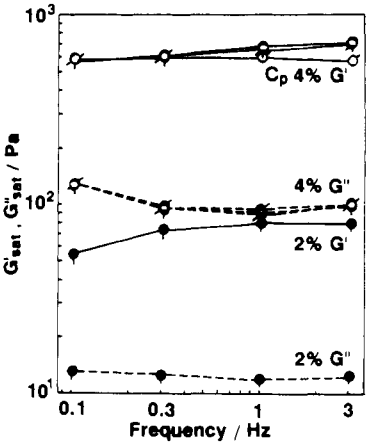

Figure 1. Saturated storage (G'_{sat}) and loss (G''_{sat}) moduli measured at various frequencies and amplitudes of 7S protein separated from Enrei in the presence of 0.4% GDL. 7S protein concentration C_p: (●) 2.0%; (○) 4.0%. Temperature: 60 °C. Amplitude: (ϙ) 25 μm; (ϙ) 50 μm; (ϙ) 100 μm.

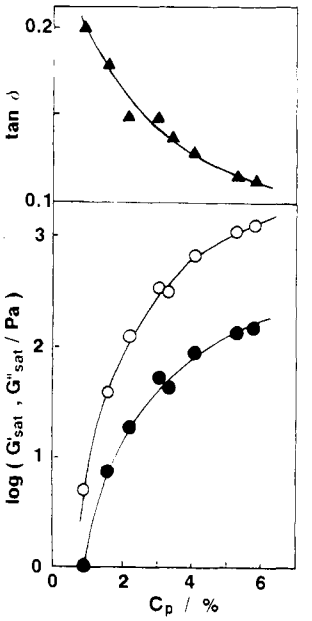

Figure 2. Semilog plot of saturated storage (○) and loss (●) moduli vs 7S concentration for 7S gels containing 0.4% GDL at 60 °C. The values of mechanical loss tangent at the final stage of gelation are also shown in solid triangles.

slightly with increasing frequency (solid circles, Figure 1). Therefore, an amplitude of 25 μm and a frequency of 1 Hz were adopted hereafter. Since the loss modulus at 0.1 Hz was larger than those at other frequencies, the gel showed liquid-like behavior at lower frequencies. These tendencies were also observed in 11S–GDL gels (Kohyama and Nishinari, 1992a). The details of decreasing G'' with frequency in a certain range of frequency were discussed elsewhere (Nishinari, 1976; Kohyama and Nishinari, 1992a).

Figure 2 shows the 7S concentration dependence of the saturated storage and loss moduli, G'_{sat} and G''_{sat}, for gels containing 0.4% GDL. Heat-set gels were formed at 60 °C after heating in boiling water for 10 min without coagulant at a 7S concentration higher than 6.2%. Since the gelation mechanism of heat-induced gels is expected to be different from that of 7S–GDL gels, only the gelation process of 7S at concentrations lower than 6% was studied. The dependence was more pronounced at lower concentrations of 7S, as observed in heat-induced gels of soybean protein (Bikbov et al., 1979), soymilk and soybean 11S gels in the presence of GDL (Kohyama and Nishinari,

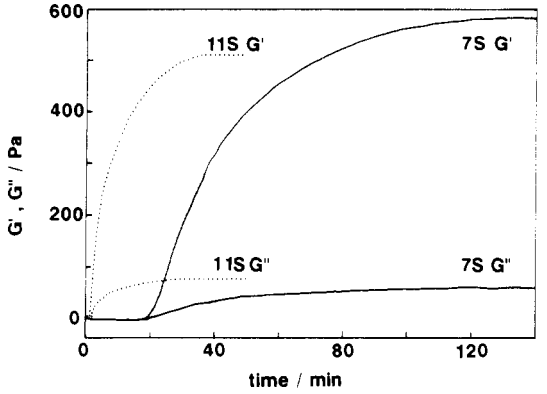

Figure 3. Typical gelation curves for a 7S (solid lines) and an 11S (dotted lines) protein in the presence of 0.4% GDL. Protein concentration: 4.0%. Temperature: 60 °C.

1992a; Kohyama et al., 1992; Yoshida et al., 1992), and many other gels (Clark and Ross-Murphy, 1987). Values of tan $\delta = G''/G'$ at the final stage of the gelation decreased with increasing protein concentration C_p (solid triangles in Figure 2). This indicated that the gels prepared from higher C_p were more solid-like. The three-dimensional networks formed by the polymers become denser with increasing C_p.

Since tan δ was larger than 0.1 even at higher C_p, the 7S–GDL gel was more liquid-like than other typical biopolymer gels such as agar (Nishinari, 1976; Nishinari et al., 1980), gelatin (Laurent et al., 1980; Nishinari et al., 1980), and ovalbumin (van Kleef, 1986). Values of tan δ for the present system were almost the same as observed in the 11S–GDL system (Kohyama and Nishinari, 1992a) and in heat-induced soybean protein isolate and 11S globulin gels of 25% protein (van Kleef, 1986). Many systems that have tan δ values of about 0.1 belong to the so-called "weak gels" or colloidal dispersions (Clark and Ross-Murphy, 1987). In weak gels, the storage and loss moduli as a function of frequency increase monotonously with increasing frequency, and the storage modulus decreases steeply with increasing shear strain because the structure is destroyed by the strain (Clark and Ross-Murphy, 1987). In comparison to weak gels, the typical "true gels" show a small frequency dependence of storage modulus, minimum loss modulus at a certain frequency, and little decrease in shear modulus even at large strain ranges (Clark and Ross-Murphy, 1987). The 7S–GDL gel was considered to be a true gel as was 11S–GDL gel (Kohyama and Nishinari, 1992a) because the storage modulus slightly increases with increasing frequency and the loss modulus as a function of frequency showed a minimum at around 1 Hz as shown in Figure 1.

Factors Affecting Saturated Shear Modulus. Typical gelation curves for 4.0% 7S and 11S solutions in the presence of 0.4% GDL at 60 °C are shown in Figure 3. The saturated storage modulus for 4% 7S seemed slightly larger than that for 4% 11S, while the saturated loss modulus for 7S was almost the same as that of 11S. Therefore, the tan δ of completely gelled 7S (G''_{sat}/G'_{sat}) became a little smaller than that of 11S.

GDL was added to 7S protein solution to adjust the ratio of 7S and GDL to 10:1. Figure 4 shows the dependence of G'_{sat} and G''_{sat} on the C_p for this system. It was difficult to determine the saturated moduli experimentally for concentrations of 7S lower than 2.5% because it took a long time (>3 h) for saturation. Saturated storage and loss moduli increased with increasing 7S concentration; however, 7S concentration dependence of both moduli was less pronounced than that observed at a constant GDL condition (Figure 2).

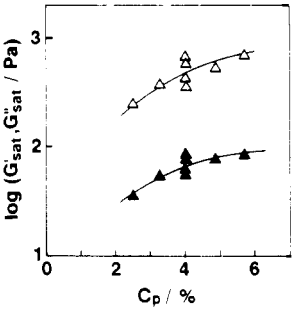

Figure 4. Semilog plot of saturated storage (△) and loss (▲) moduli vs 7S concentration. Each sample contains 7S and GDL at a fixed ratio of 10:1.

The shear modulus G of polymer gels is often described by an exponential function of polymer concentration C_p.

$$G \propto C_p^{\,m} \qquad m = \text{constant} \tag{2}$$

In many cases, the exponent becomes 2 (Hirai, 1955; Fukada and Kaibara, 1973; Nishinari and Watase, 1983). However, in our previous work, the G'_{sat} of 11S gels containing 0.4% GDL was proportional to 3.4th power of 11S concentration, when 11S concentration ranged from 2% to 6% (Kohyama et al., 1992). The relationships between G'_{sat} and 7S concentration shown in Figures 2 and 4 are examined in the same concentration range (2–6%). The exponent m for 7S gels with 0.4% GDL was 2.43 and that for gels containing C_p:C_g = 10:1 was 1.89, which was smaller than the former. Here, C_g stands for the concentration of GDL. Exponent values for 11S gels with 0.4% GDL and 11S gels containing C_p:C_g = 10:1 are also calculated. In the former case, m became 3.52, which is not so different from that obtained (3.4) in the previous work (Kohyama et al., 1992). In the latter case, m was also smaller (3.19) than the former one. The protein concentration dependence of G'_{sat} for both 7S and 11S was more pronounced in gels with 0.4% GDL than in those containing a fixed ratio of GDL to protein. This fact suggests that GDL decreases the saturated modulus slightly. Since m values for 7S were smaller than those for 11S, the saturated storage modulus of 7S gels was larger than that for 11S at lower C_p than 4–5% and the inverse relationship was observed at higher C_p. Since the protein content of Kinugoshi tofu is 5.0% (Resources Council, Science and Technology Agency, 1992), tofu-like gels are made in the presence of GDL, when protein concentration is around 5%. Both 7S and 11S gels containing about 5% protein showed almost the same value for the saturated modulus.

Gelation Rate. A large difference in the rate of gelation was observed between 7S and 11S as shown in Figure 3. The gelation time t_0 of 7S was much longer than that of 11S at the same protein concentration C_p, GDL concentration C_g, and temperature. The gelation rate of 7S was also slower than that of 11S; therefore, it took a long time before storage and loss moduli of 7S reached equilibrium values.

Figure 5 shows the relationships between the rate constant of gelation k and C_g at a constant C_p (4.0%) for both 7S and 11S proteins. Gelation proceeded faster in systems of higher GDL concentrations, and a similar tendency was observed for 11S systems. However, the value of k for 11S was 2–3 times larger than that for 7S.

The relationships between k and protein concentration at a constant C_g (0.4%) are shown in Figure 6. Gelation rate decreased with increasing protein concentrations for both 7S and 11S systems. A slower gelation rate was observed again for 7S than for 11S in the fixed GDL concentration systems.

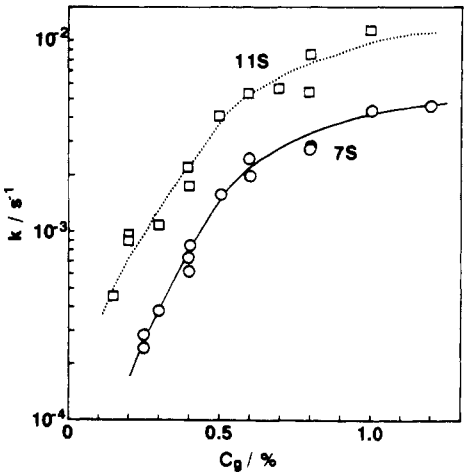

Figure 5. GDL concentration dependence of the rate of gelation k for 4.0% 7S (○ and solid line) and 11S (□ and dotted line) protein at 60 °C.

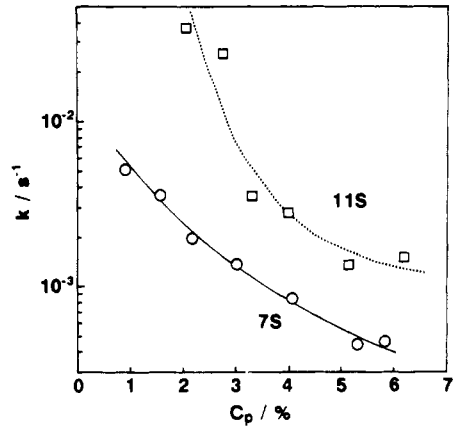

Figure 6. Protein concentration dependence of the rate of gelation k for 7S (○ and solid line) and 11S (□ and dotted line) protein in the presence of 0.4% GDL at 60 °C.

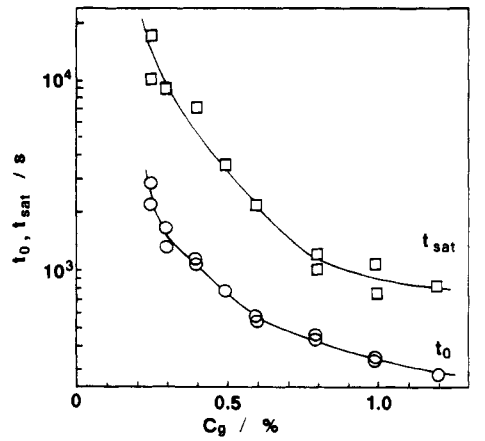

Figure 7. GDL concentration dependence of the gelation time (t_0, open circles) and the time (t_{sat}, open squares) at which the gelation curve becomes saturated for the gel of 4.0% 7S protein.

The gelation time t_0 decreased with increasing GDL concentration at a constant 7S concentration (4.0%) as shown in Figure 7. Gelation was not observed at concentrations of GDL (C_g) lower than 0.2%. If t_{sat} was taken as the time at which the gelation curve became saturated, t_{sat} also decreased with C_g as shown in Figure 7. The gelation time for 7S was almost 10 times longer and t_{sat} for 7S was much longer than those of 11S–GDL gel (Kohyama and Nishinari, 1992a) when C_p, C_g, and testing conditions were the same.

The enzyme chymosin determines the gelation kinetics in the clotting of casein micelles (Tokita et al., 1982a,b).

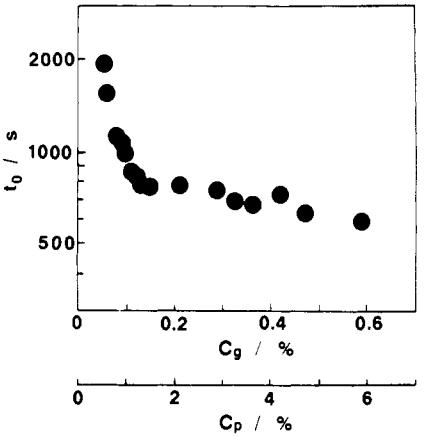

Figure 8. Relationships between the gelation time and concentration of 7S protein at 60 °C. The ratio 7S:GDL is 10:1.

The substrate casein micelle concentration does not affect the gelation kinetics, even though it greatly influences the saturated shear modulus of milk gel. Since casein excessively exists against chymosin, the rate constant of the gelation is a function of only chymosin concentration. In our system, the gelation rate did depend on not only C_g but also C_p. It suggests that GDL is insufficient for 7S protein in the present system. The gelation rate in the presence of a fixed GDL concentration thus decreased with increasing protein concentration as shown in Figure 6. When the ratio of 7S and GDL was fixed to 10:1, the gelation time decreased with increasing 7S polymer concentration C_p (Figure 8) as commonly observed in many other gelation systems (Ross-Murphy, 1991a,b). However, the decrease was less pronounced at higher C_p range. Higher C_p made the density of reaction sites of protein which form a three-dimensional network higher, and then it shortened the gelation time. The effect of lacking GDL to 7S could be deleted in the fixed ratio of 7S to GDL system.

Comparison between Saturated Storage Modulus and Breaking Stress. Saio et al. (1969) reported that the hardness of 11S gels was several times larger than that of 7S gels from a fracture measurement using a texturometer. They used calcium sulfate as a coagulant and heated the mixture of the protein and the coagulant at 70 °C for 10 min. Hashizume et al. (1975) also reported a similar tendency, although they used GDL as coagulant and heated samples at 60 °C for 90 min. However, our findings from the dynamic viscoelasticity measurement suggest that both 7S and 11S proteins form true gels, and the saturated storage modulus for 7S–GDL gel was almost the same as that of 11S. We also studied fracture phenomena of 4.0% protein gels with 0.4% GDL by a uniaxial compression testing. From the result of dynamic viscoelasticity, it seems that 7S protein requires a longer heating time to form a gel than 11S. Then, the compression testing was carried out on gels made after various heating periods at 60 °C. The breaking stress and the breaking strain, the Young modulus E, and the breaking energy for 7S and 11S gels as a function of heating time are shown in Figures 9, 10, 11, and 12, respectively. Mean values and standard deviations were plotted against heating time in each figure. All four parameters increased with time and then seemed to level off. However, the breaking strain for 7S gels was slightly increasing even after heating for 240 min at 60 °C. Faster gelation in 11S than in 7S was clearly shown again in those figures. We examined the breaking stress of tofu prepared from soymilk with GDL using a curdmeter in the previous work and reported that the breaking stress and the saturated storage modulus at 80 °C observed in the present method were correlated

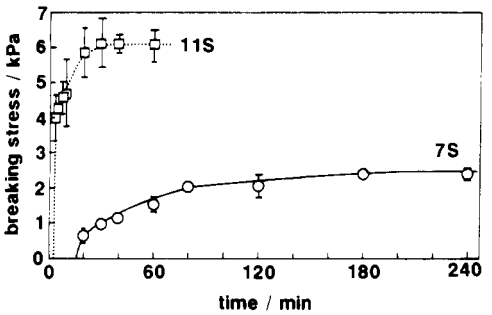

Figure 9. Heating time dependence of the breaking stress for 7S (○ and solid line) and 11S (□ and dotted line) protein. Concentrations of protein and CDL are 4.0% and 0.4%, respectively. Heating temperature: 60 °C. Sample size: 16 mm diameter × 10 mm. Compression rate: 1.0 mm/s. Test temperature: 27 °C. Polyacetal stage and plunger are used. Bars attached to symbols represent the standard deviation.

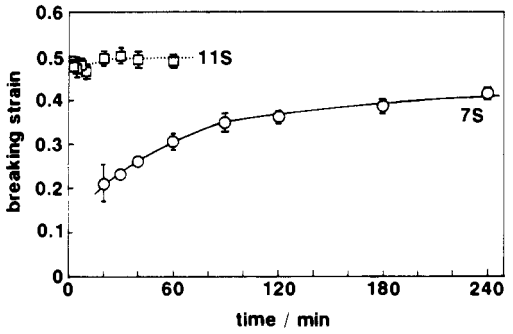

Figure 10. Heating time dependence of the breaking strain for 4.0% 7S and 11S protein gels in the presence of 0.4% GDL. Symbols and test conditions are the same as Figure 9.

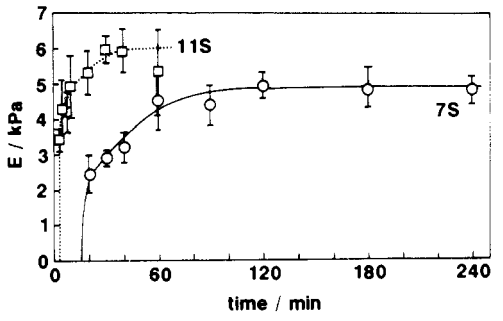

Figure 11. Heating time dependence of the Young modulus E for 4.0% 7S and 11S protein gels in the presence of 0.4% GDL. Symbols and test conditions are the same as Figure 9.

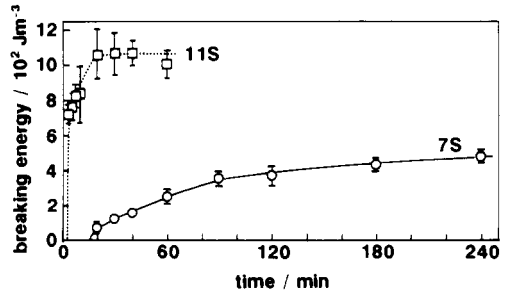

Figure 12. Heating time dependence of the breaking energy for 4.0% 7S and 11S protein gels in the presence of 0.4% GDL. Symbols and test conditions are the same as Figure 9.

well with $r = 0.998$ (Yoshida et al., 1992). In the present study, curve shapes for the breaking stress, and also the Young modulus and the breaking energy were similar to the storage or loss modulus curve (Figure 3) for both 7S and 11S proteins. Even though both 7S and 11S proteins show such a relationship individually, the ratio of the saturated storage modulus to the breaking stress was not the same.

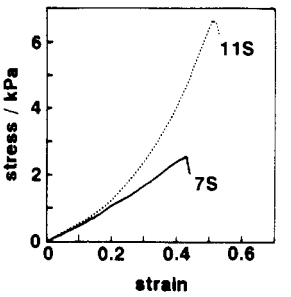

Figure 13. Typical stress–strain curves for 7S (solid line) and 11S (dotted line) protein gels in the presence of 0.4% GDL. Protein concentration: 4.0%. Heating conditions are 60 °C for 240 min in 7S and for 60 min in 11S.

It seems that covalent bonds including disulfide bonding contributed to the large breaking properties of 11S–GDL gels. However, hydrogen bonds play an important role in gel formation of 7S and 11S in the presence of GDL, and it affects small deformation properties, such as the Young modulus and storage and loss moduli. If 7S forms a gel only by secondary bonds such as hydrogen bonds and hydrophobic interactions and 11S makes a true gel with three-dimensional networks with covalent bonds such as disulfide bonds, the breaking properties of 11S would become larger than those of 7S as suggested by Saio et al. (1969). The consideration seems to be reasonable because 7S does not contain any sulfhydryl groups while 11S has some (Peng et al., 1984). Utsumi and Kinsella (1985) reported that electrostatic interactions and disulfide bonds were involved in gelation of 11S by heat, while hydrogen bonding was mainly responsible for formation of heat-induced 7S gel.

Utsumi and Kinsella (1985) observed that the breaking force of heat-induced 7S gels was larger than that of 11S gels. It is well-known that the denaturation temperature of 7S globulin is lower than that of 11S (German et al., 1982; Varfolomeyeva et al., 1986). Therefore, 11S globulin requires a higher heating temperature to form a gel than 7S globulin. The heating temperature (80 °C) adopted by Utsumi and Kinsella (1985) may be too low for 11S to form a gel with consistency and resulted in stronger 7S and weaker 11S gels. The observation by Nakamura et al. (1986) that a shorter heating time at 100 °C made 7S gels with a larger breaking force than 11S gels also may be attributed to less thermal stability of 7S globulin.

As shown in Figures 3, 5, 6, and 9–12, the gelation time for 7S was much longer than that for 11S and the gelation rate for 7S was from half to one-third of that for 11S. Therefore, the time required to saturate mechanical properties for 7S gels became longer by several to 10 times than that for 11S. When enough time had passed and mechanical properties were almost saturated, 11S gel showed larger breaking stress, breaking strain, and Young modulus than 7S gels. Breaking stress and breaking energy of 11S gels after 60 min of heating were more than twice larger than those of 7S after 240 min of heating. Typical stress–strain curves for fully gelled 7S and 11S are shown in Figure 13. Stress values of both gels were almost the same at small strain, but 11S gels showed larger stress than 7S when a large strain was applied. 7S gels were more brittle than 11S gels, even though the 7S gels had a Young modulus similar to that of the 11S gels as observed in the dynamic viscoelasticity measurement. The result that 11S gels showed larger breaking stress than 7S is the same as previous studies (Saio et al., 1969; Hashizume et al., 1975). However, the earlier authors observed larger differences, whereas the difference between both protein gels was at most 2–3 times in the present study. This may be caused by the different times elapsed before measure-

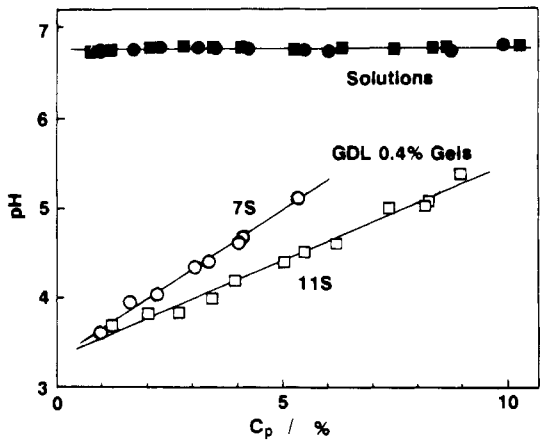

Figure 14. Relationships between pH and concentration of protein. (●) 7S solutions without GDL; (■) 11S solutions without GDL; (○) 7S gels with 0.4% GDL; (□) 11S gels with 0.4% GDL.

ment. Previous researchers adopted a short processing time, while 7S needs a longer time for gelation. The same 7S system heated longer would show larger breaking stress, and the difference would become smaller. The differences in coagulant materials and/or in the amount of coagulant may also cause different results; we will confirm this in the future.

Relationships between pH and Gelation Rate. Figure 14 shows the relationships between C_p and pH for 7S and 11S solutions with 0.4% GDL and without GDL. Both 7S and 11S solutions without GDL ($C_g = 0$) showed a constant pH around 6.8, which was independent of protein concentration. Gels formed in the presence of the acidic coagulant GDL showed lower values of pH. 7S gels showed a smaller pH depression than 11S gels containing the same concentrations of protein and GDL. Because of a buffering effect of the protein, the pH of the gels remained higher with increasing protein concentration in both cases.

It is well-known that the isoelectric point of 7S globulin (4.5–5.0) is lower than that of 11S (6.3–7.0) (Brooks and Morr, 1985). Therefore, the 7S protein contains more negatively charged groups ($-COO^-$) in excess of positively charged groups at pH higher than the isoelectric point (4.8). However, anionic groups in 11S are balanced with cationic groups at the isoelectric point (6.4). The buffering effect against protons is stronger in 7S than in 11S in the pH range from 4 to 5. When the same amount of protons was added to both 7S and 11S solutions, the pH of the 7S systems remained higher than that of 11S. The slower gelation in 7S may be attributed to the smaller pH depression as shown in Figure 14. Since the higher protein concentration inhibits the tendency of the system to go to a lower pH, it slows down the gelation process, even though it increases the density of reaction sites in the protein. Therefore, a decrease in pH by the addition of GDL greatly promotes the gelation kinetics. The gelation of soybean 7S protein in the presence of GDL was also a process of acidic coagulation as observed in 11S systems (Kohyama and Nishinari, 1992a; Kohyama et al., 1992).

The gelation time decreased with coagulant concentration as shown in Figure 7. Globulin molecules have already been denatured and unfolded during heating in boiling water for 10 min before the addition of GDL (Morr, 1990). As shown in Figure 14, GDL decreases pH and promotes aggregation of the unfolded protein by increasing hydrophobicity and insolubilization. The phenomenon is similar to the aggregation of casein micelles by chymosin, which cleaves κ-casein from the micelle and makes the surface of casein more hydrophobic (Dalgleish, 1986). It is known that 7S globulin precipitates at the pH range from 4.5 to 5.0, while 11S precipitates at the pH range from 6.3 to 7.0 (Brooks and Morr, 1985). Thanh and Shibasaki (1976) utilized the solubility difference in Tris buffer to isolate 7S and 11S globulins. The present method (Nagano et al., 1992) is also based on the different solubilities of 11S and 7S globulins at pH 6.4 and 4.8. It suggests that more GDL is required to increase hydrophobicity and insolubilize 7S protein than 11S if the system contains the same amount of protein. Therefore, the gelation of 11S–GDL is much faster than that of 7S as clearly shown in Figures 3, 5, 6, and 9–12.

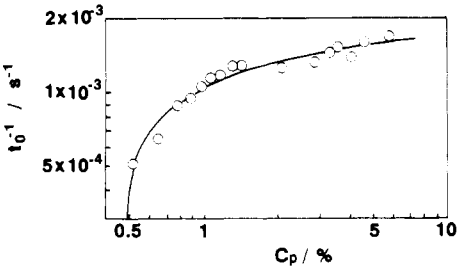

Figure 15. Double-logarithmic plot of reciprocal gelation time against 7S concentration. Experimental data are shown in open circles. The solid line shows the calculated curve using eq 3 with $n = 0.0192$, $p = 0.346$, $C_0 = 0.479$, and $K = 221$.

Critical 7S Concentration. Assuming that the reciprocal of the gelation time was proportional to the storage modulus, Ross-Murphy (1991a) proposed the following equation for the gelation time as a function of the polymer concentration C_p

$$t_0 \approx K/\{[(C_p/C_0)^n - 1]^p\} \quad (3)$$

where K is a proportionality constant, C_0 is the critical polymer concentration for gel formation, n is the number of cross-linking loci which form a junction zone, and p (>0) is the critical exponent. According to this equation, an increase in polymer concentration shortens the gelation time.

Figure 15 shows the relationship between C_p and t_0 shown in Figure 8 when fitted to eq 3 by a least-squares method with a SALS program (Nakagawa and Oyanagi, 1980). The critical concentration of 7S was calculated to be 0.479%. This critical value is considered to be acceptable, and it is lower than that of 11S (1.03%) (Kohyama and Nishinari, 1992a). This is opposite to the observation for heat-induced gelation of 7S and 11S globulins by Nakamura et al. (1986), who reported the minimum concentration of protein for gelation was 7.5% for 7S and 2.5% for 11S.

The calculated n value became very small (0.019) and the critical exponent p was 0.346 for the best fit. From the definition of n, n chains make a junction zone of the gel; therefore, a small value such as 0.019 indicates that the system cannot make a gel or a three-dimensional network. It is contrary to the observation that the system actually did form gels. The theoretical value for p is 3 in the classical theory of percolation on a Bethe lattice (Gordon and Ross-Murphy, 1975) and around 1.8 for percolation on a cubic lattice (Stauffer et al., 1982). A value of 0.346 seems to be unreasonable. The gelation time for a fixed ratio of 7S and GDL system did not decrease as much at higher concentrations of C_p. If the gelation kinetics was governed by the structural change of polymers such as unfolding, the p value would be unity because the gelation reaction should be written by a linear function of the polymer concentration. The gelation rate in the system with excess polymers to coagulant might be determined by the amount of coagulant as observed in the enzymatic clotting of casein micelles by chymosin (Tokita

et al., 1982a,b). In such a case, p would tend to zero. However, polymers would not be excessive relative to GDL in the system examined because it contained 7S protein and GDL in a fixed ratio. Therefore, the gelation of 7S protein by GDL cannot be treated by using eq 3.

Currently, we cannot explain the entire mechanism of the gelation process. Further structural studies are required to understand the gelation process.

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