Rheological Study on Gelation of Soybean 11S Protein by Glucono-δ-lactone

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Dynamic viscoelasticity studies on gelation of soybean 11S protein by glucono-δ-lactone have been done to analyze the gelation process of tofu. Observed gelation curves at constant temperatures were well approximated by first-order reaction kinetics. The saturated storage modulus depended mainly on the concentration of 11S protein. The saturated modulus was proportional to 3.4th power of 11S concentrations. The rate constant of the gelation increased with increasing gelling temperature and was mainly governed by the concentration of glucono-δ-lactone. The activation energy of the gelation was calculated to be 1.5×10^1 kJ/mol from an Arrhenius plot of the rate constants. The latent time at which the shear modulus began to deviate from the baseline became shorter with increasing concentration of glucono-δ-lactone. However, the latent time was not shortened by an increase in protein concentration, in contrast to previous findings for many other protein gels.

INTRODUCTION

From ancient times, soybeans have been utilized for food in many Asian countries. They have served as one of the most important protein sources in Japan and China. Tofu is a gellike food made from soybean milk and a coagulant. Soy milk has traditionally been coagulated with Nigari or Sumashiko. Nigari, also known in the West as bittern, is the residue after extraction of sodium chloride from sea water and consists of magnesium chloride with the trace minerals in sea water. Sumashiko is crude calcium sulfate prepared from gypsum. Recently, glucono-δ-lactone (GDL) became a more popular coagulant than refined calcium sulfate or natural Nigari, especially in tofu-processing factories. There are two types of tofu in Japan. One is Momen tofu, which means the tofu that is made by using a cotton-cloth, i.e., momen in Japanese, filter, and the other is softer Kinugoshi tofu or silken tofu. In both, soybean milk is boiled and mixed with coagulant solution. Kinugoshi tofu is allowed to stand until it becomes firm without removal of the whey. However, Momen tofu is made from soymilk curds. After the bulk of the whey is removed, the curds are scooped into a cloth-lined molding box with draining holes and pressed for around 30 min to remove the residual whey. Usually, the protein content of Japanese tofu is 5.0% for Kinugoshi and 6.8% for Momen tofu (Resources Council, Science and Technology Agency, 1982). These values are lower than those of tofu in the U.S. markets. Water content of tofu varies from 84 to 90%.

There have been many investigations on the processing of tofu, based on the measurements of the gel strength (Saio et al., 1969; Hashizume et al., 1975; Saio and Watanabe, 1978; Hara and Negishi, 1987). Gel strength is the breaking force for fully gelled samples. This method is widely used by tofu makers using a curdmeter or a texturometer.

It has been reported that 11S globulin, the major component in soybean protein, principally determines the hardness of tofu (Saio et al., 1969; Saio and Watanabe, 1978) by virtue of sulfhydryl bonds. Rheological studies by Mori and his co-workers (Utsumi et al., 1982; Nakamura et al., 1984, 1985; Mori et al., 1989) on heat-induced gels of soybean 11S globulin are also based on texturometer measurements. Since fracture phenomena are probabilistic, it is necessary to repeat the breaking force measurements; in addition, texturometer measurements cannot follow the gelation process in real time. In previous work, the gelation process of soy milk (Yoshida et al., 1990; Nishinari et al., 1991) or soybean 11S protein (Yoshida et al., 1990, 1992) by glucono-δ-lactone (GDL) was followed with dynamic viscoelasticity measurements. It was found that the storage modulus was mainly dependent on the concentration of 11S protein and the gelation rate was increased with increasing concentration of GDL (Yoshida et al., 1990, 1992).

Recently, gelation processes of some other protein gels such as heat-induced bovine serum albumin (Richardson and Ross-Murphy, 1981), egg albumen and whey protein (Beveridge et al., 1984), bovine globin (Autio et al., 1990), and enzyme-induced casein gel (Tokita et al., 1982a,b; Niki and Sasaki, 1987; Niki et al., 1991) were studied by dynamic viscoelasticity measurement.

The gelation kinetics of 11S–GDL systems was studied and compared to those of other protein gels in the present work. As mentioned above, Kinugoshi tofu is made without molding and pressing. Investigation of the kinetics of the system is expected to be useful for processing of Kinugoshi tofu.

MATERIALS AND METHODS

Preparation of 11S Globulin. 11S globulin was isolated according to a method of Thanh et al. (1975) as follows. Soybeans (varieties Enrei and Tachisuzunari) were ground and defatted with *n*-hexane. Powders obtained were extracted with 65 mM tris(hydroxymethyl)aminomethane–hydrochloric acid (Tris-HCl) at pH 7.8 and centrifuged to remove insoluble residue for 15 min at 12 000 rpm at 20 °C. Then, 2 N hydrochloric acid was added to lower the pH to 6.6. The extract was dialyzed against Tris-HCl buffer at pH 6.6 for 3 h at 2–3 °C and centrifuged for 20 min at 12 000 rpm at 2 °C. The precipitate was freeze-dried. Powders obtained in this way were submitted to ultracentrifuge sedimentation analysis (Yoshida et al., 1992) and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Kitamura et al., 1984). The results confirmed that the precipitate was an 11S globulin fraction with purity of about 90%.

Rheological Measurements. 11S globulin was dissolved in deionized water and heated in boiling water for 5 min and then

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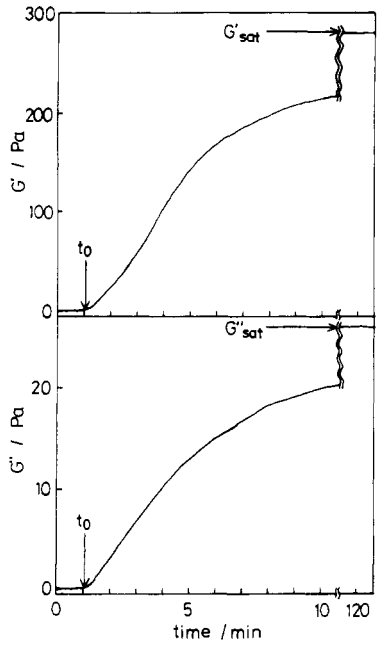

Figure 1. Typical gelation curves [the storage (G') and loss (G'') moduli as a function of time] for 4% 11S solution at 60 °C. 11S protein was separated from Enrei variety soybeans. GDL concentration was 0.4%. t_0 is the latent time. G'_{sat} and G''_{sat} are saturated values of the storage and loss moduli, respectively.

cooled to room temperature. The solution was heated to 5 °C below the measuring temperature, and 1.5 mL of the solution was injected into a cell of a Rheolograph Sol (Toyoseiki Seisakusho) (Kaibara and Fukada, 1976, 1983; Yoshida et al., 1990, 1992; Nishinari et al., 1991). The cell was heated to the same temperature beforehand. Then the solution was slowly heated to the measuring temperature, and 0.05 mL of glucono-δ-lactone solution freshly dissolved in ice-cold water was added. After the mixture was stirred quickly, the sample solution was subjected to 2-Hz sinusoidal shear oscillations with an amplitude of 125 μm. The surface of the sample was covered with silicone oil to prevent the evaporation of water. The storage and loss moduli were recorded as a function of time. The details of the apparatus were described elsewhere (Yoshida et al., 1992).

RESULTS AND DISCUSSION

Analysis of Gelation Curves. Figure 1 shows a typical gelation curve. Storage modulus was about 10 times larger than loss modulus. The origin of the time, $t = 0$, was taken as the time when GDL was added. Both storage and loss moduli began to rise after a certain time, which will be called latent time (t_0) hereafter. The latent time obtained from the curve of loss modulus vs time was almost the same as that obtained from the storage modulus. Storage and loss moduli increased with time and reached equilibrium values after a long time.

Gelation curves appear to fit first-order reaction kinetics. Therefore, the observed data were fitted to an empirical formula

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

where G_{sat} is the saturated value of storage or loss modulus, k is the rate constant of gelation, t_0 is the latent time, and t is time. The rate constant k was estimated from curve fitting by a least-squares method.

Saturated Modulus and 11S Concentration. Figure 2 shows the relationships between the saturated storage modulus of the gel, G'_{sat} and 11S concentration, C_p. Gelation of Tachisuzunari was studied at 60 °C (solid squares) and that of Enrei at 60 (open circles) and at 80 °C (solid circles); GDL concentration was 0.4% in all cases. Gelation properties of Enrei at 60 °C were similar to those of Tachisuzunari. Gelation did not occur when C_p was lower than 1%. The saturated storage modulus correlated well with gel strength measured by a curdmeter (Yoshida et al., 1990, 1992). The concentration dependence of G'_{sat} was more pronounced in the lower concentration range of C_p.

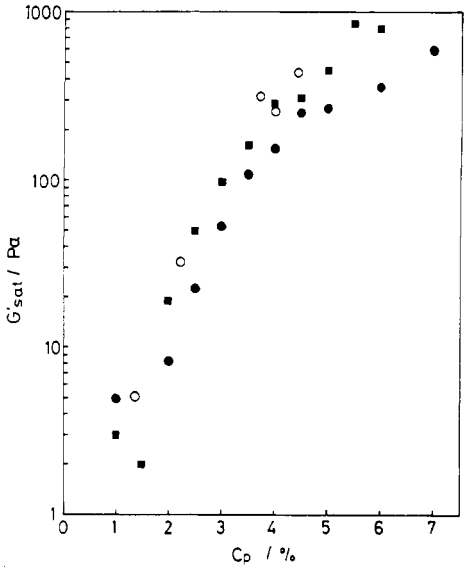

Figure 2. Semilog plot of saturated storage modulus vs 11S concentration for 11S gel containing 0.4% GDL. Tachisuzunari at 60 °C (■); Enrei at 60 (○) and 80 °C (●).

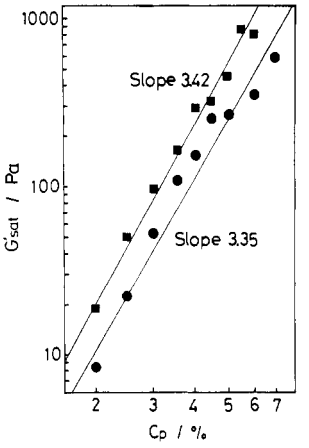

Figure 3. Log–log plot of saturated storage modulus vs 11S concentration for 11S gels containing 0.4% GDL. Tachisuzunari at 60 °C (■); Enrei at 80 °C (●).

Figure 3 shows the double-logarithmic plot of G'_{sat} and C_p. Gels, whose texture is within the range for ordinary Kinugoshi tofu, were obtained when the 11S concentration ranged from 2 to 6%. In this concentration range, the exponent 3.4 in the power law relation between the saturated modulus G'_{sat} and the polymer concentration C_p is rather larger than in the case of the well-known square power law which has been observed for many biopolymer gels (Hirai, 1955; Fukada and Kaibara, 1973; Nishinari and Watase, 1983). However, the exponent 3.4 is smaller than 5, which was reported by Bikbov et al. (1979) for heat-induced soybean globulin gels of concentration of 7.5–58.4%. If the relationship between G'_{sat} and C_p at higher concentrations by GDL could be examined, the concentration dependence of G'_{sat} should tend to be less pronounced. Since 11S globulin solutions of concentrations higher than 10% unfortunately formed heat-induced gels during heating in boiling water for 5 min, it is

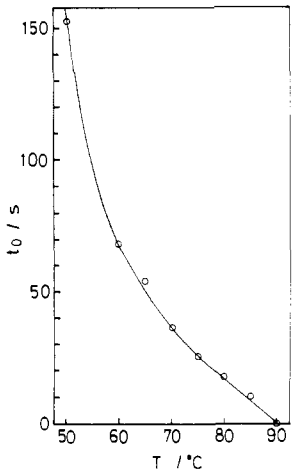

Figure 4. Temperature dependence of the latent time. Sample: 4% 11S protein separated from Enrei with 0.4% GDL.

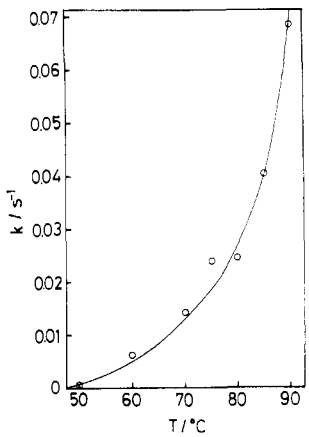

Figure 5. Temperature dependence of the rate constant of gelation. Sample: 4% 11S protein separated from Enrei with 0.4% GDL.

impossible to examine them experimentally. The cubic power relationship did not depend so much on the soybean cultivar or gelling temperature (60 and 80 °C). A similar tendency was also observed for the loss modulus. These findings will be useful to estimate the amount of water that should be added to soybeans in tofu processing.

Latent Time and Gelation Rate. The plot of the latent time against temperature is shown in Figure 4. As mentioned above, tofu-like gels were formed when 11S concentration was between 2 and 6%. The middle concentration, 4%, was chosen to do this experiment. At low temperatures, it takes a long time for gelation to commence. Gelation began immediately after GDL was added to an 11S solution at 90 °C.

Figure 5 shows the plot of rate constants against temperature. Contrary to the case of t_0, k increased with increasing temperature. In comparison with the temperature dependence of the latent time (Figure 4), the gelation rate seems to be estimated by the latent time instead of the rate constant.

The rate constants at various temperatures were logarithmically plotted against the reciprocal of the absolute temperature (Figure 6, solid circles). The rate constants lie on a straight line except for one measured at 50 °C. Because the gelation rate is very slow at such a low temperature, it is difficult to obtain saturated shear moduli from a rheological measurement. A large error in the k value may result. The other seven points lie on a straight line (upper solid line shown in Figure 6). The activation energy for the gelation can be estimated from the slope.

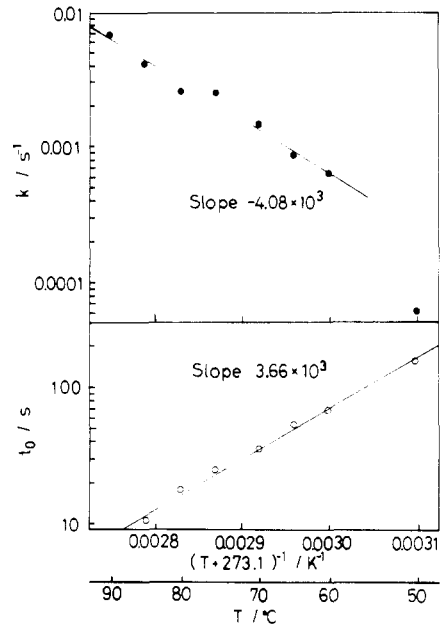

Figure 6. Arrhenius plot for 4% 11S protein separated from Enrei with 0.4% GDL. Rate constant (●) and latent time (○). Two straight lines are obtained with the least-squares method.

From the slope of this Arrhenius plot -4.08×10^3, the activation energy, E_a, was calculated as 1.5×10^1 kJ/mol. This value is rather small compared to 3.81×10^2 kJ/mol for the heat insolubilization of 11S globulin (Watanabe, 1988) or 1.13×10^2 kJ/mol for the heat denaturation of soybean 7S globulin kept at high temperatures (Iwabuchi et al., 1991). However, it is of the same order as those reported for clotting of casein micelles by rennet [3.4×10^1 kJ/mol (Tuszyński, 1971); 4.2×10^1 kJ/mol (Tokita et al., 1982a); and 8.8×10^1 kJ/mol (Niki and Sasaki, 1987)]. The small activation energy results from the fact that the gelation process does not depend so much on temperature. GDL in the present system may play a similar role as an enzyme (rennet) does in the clotting of casein. The gelation process was not induced by heat. Richardson and Ross-Murphy (1981) plotted the logarithm of the latent time (they call it gel time) against the reciprocal absolute temperature. A similar plot is shown in Figure 6 with open circles. We omitted a latent time obtained at 90 °C, because it was too short to calculate a log value. The other seven latent times lie on a straight line (lower line in Figure 6). The slope was 3.66×10^3 by least-squares method. It was close to the absolute value (4.08×10^3) of the slope from logarithm of k against the reciprocal of the absolute temperature. Therefore, the rate constant of this system would be inversely proportional to the latent time. If we assume that one slope of the Arrhenius plot represents one process (Richardson and Ross-Murphy, 1981), the gelation consists of a single process between 50 and 90 °C. Higher temperatures increase the gelation rate but would not change the gelation mechanism in this temperature range. The temperature at which a coagulant is added to soy milk does not greatly affect the properties of their final product, tofu, even though it changes the gelation rate. The thermal denaturation temperature of soybean 11S globulin in water was reported as 84.5 °C and the denaturation completed at around 93 °C by differential adiabatic scanning calorimetry (Bikbov et al., 1983). All 11S globulin molecules in our system should be denatured below 100 °C. Since the 11S protein was heated in boiling water before GDL was added, the denaturation by heat was completed during heating. At 11S concentration lower than 7%, the preheated protein solution did not form a

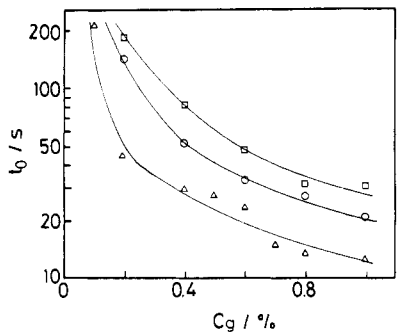

Figure 7. Relationships between the latent time and concentration of GDL. Measuring temperature was 60 °C. Sample: 11S protein separated from Tachisuzunari (▲) 2%, (○) 4%, (□) 6%.

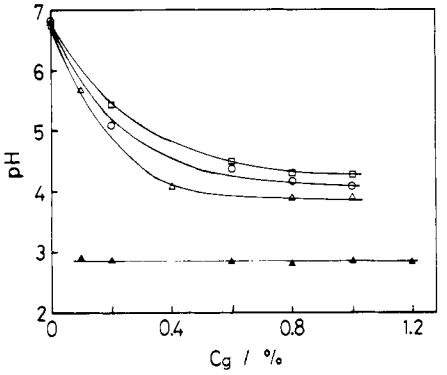

Figure 8. Relationships between the pH of the gel and concentration of GDL. Tachisuzunari 11S: (▲) 0%, (△) 2%, (○) 4%, (□) 6%.

gel without GDL below 90 °C. Then, the gelation in the present system would not be induced by heat. The gelation is thus chemically induced by GDL.

Latent Time and GDL Concentration. The plot of the latent time against the concentration, C_g, of GDL is shown in Figure 7. The test temperature was 60 °C to extend latent time. The latent time became shorter with increasing concentration of GDL, and it became shorter with decreasing 11S concentration at the same GDL concentration.

Since the pH of the solution decreases on addition of GDL, the pH of the gel was measured. The results are shown in Figure 8. The pH of the GDL solution did not depend on GDL concentration. However, because of the buffering action of the protein, the pH value of the gel was higher than 3.9. The dependences of t_0 (Figure 7) and the pH (Figure 8) on C_g are similar. This suggests that the gelation of 11S is promoted by acidification, which is induced by the addition of GDL. GDL is partially cleaved into gluconic acid in water, and then some gluconic acid molecules dissociate. Each chemical species of GDL exists in chemical equilibrium, and the amount of each species is controlled by various conditions such as the temperature, the concentration of GDL, and the other component in the system. Since a lactone is chemically inactive, it should not react with 11S protein. The lowered pH shows the existence of dissociated gluconic acid. It is clear that the acidic form of this coagulant is important to the gelation of 11S protein.

The latent time decreased slightly with increasing concentration of GDL at high GDL concentration range, $C_g > 0.8\%$ (Figure 7). This might be attributed to a slight drop in pH, which in turn might cause greater effect on the latent time. At higher concentrations of GDL, the pH seems to decrease only slightly with increasing concentration of GDL, although it is not clearly seen. Since it is difficult to measure the pH of gels accurately within the error of ±0.1, this must be explored in the future.

In many cases, the gelation rate increases with increasing protein concentration. This is reasonable because the density of reaction sites, which can form three-dimensional networks, becomes higher with increasing polymer concentration. Nakamura et al. (1984) reported that the minimum time for thermal gelation of soybean 11S globulin decreased with protein concentration. A similar tendency was also observed for an enzymatically induced gelation, casein micelle clotting by rennet (Tokita et al., 1982b). However, as mentioned above (Figure 7), the latent time for higher 11S concentrations was longer than that for lower 11S concentrations. At higher C_p, the drop in pH at any given GDL concentration was smaller than that at lower C_p (Figure 8). Therefore, the increase in the latent time with increasing C_p might be related to pH. Gluconic acid produced by the hydrolysis of GDL generates protons to lower the pH to initiate coagulation of the proteins. The more protein that is present, the more acid is required to lower the pH to a given value. As the concentration of GDL is increased, more acid is generated in a given time, and hence the latent time for initiation of coagulation will be shorter.

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