Rheological Characteristics and Gelation Mechanism of Tofu (Soybean Curd)

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Dynamic viscoelasticity measurements and compression tests of soybean proteins after the addition of glucono-δ-lactone (GDL) or calcium sulfate were carried out to analyze the gelation process of tofu (soybean curd). The gelation curve, viscoelasticity as a function of time, was analyzed and found to fit first-order reaction kinetics. The difference between GDL-induced and calcium-induced gelation was mainly in the kinetics; *i.e.*, gelation by calcium was faster. The structure of calcium gels inferred from the rheological properties was quite similar to that of GDL gels. It is suggested that the gelation of tofu is a two-step process: (1) protein denaturation by heat and (2) hydrophobic coagulation promoted by protons from GDL or by calcium ions. The addition of GDL or calcium induces gelation by promoting aggregation via hydrophobic interactions. Charge–charge interactions may also be subordinately involved, while formation of disulfide bonds was not involved in the second process.

Keywords: *Gel; soybean curd; soy protein; gelation; viscoelasticity; coagulant*

INTRODUCTION

Tofu (soybean curd) is one of the most important food products made from soybean protein. Soy milk is boiled and then treated with a coagulant. Kinugoshi-tofu is made without removing the whey, and the protein content is 5.0% (Resources Council, Science and Technology Agency, 1982). Recently, calcium sulfate and/or glucono-δ-lactone (GDL) have been mostly used as coagulant for the production of kinugoshi-tofu in Japan (private communication, Japan Tofu Association).

One of the authors studied the gelation process of soy milk and soybean 7S and 11S globulins (7S and 11S), which are two major components of soy protein (Fukushima, 1991), using GDL by dynamic viscoelasticity (Nishinari et al., 1991; Yoshida et al., 1992; Kohyama et al., 1992; Kohyama and Nishinari, 1992a,b, 1993). However, the effects of the other coagulant, such as calcium sulfate, on the gelation of tofu have not been investigated. Calcium effects may differ from GDL ones in the mechanism of the gel formation and the stabilizing of the gels.

Dynamic viscoelasticity measurements of the gelation process for soybean protein isolate (SPI) in the presence of calcium sulfate were carried out in the present study. The properties were compared to those with GDL as coagulating agent. Breaking properties of the gels were also measured. The mechanism of gel formation in the presence of GDL or calcium was discussed.

On the basis of the processes mentioned above, tofu could be an example of a protein gel formed by two-step process as in the case of bovine serum albumin (Murata et al., 1993), hen egg lysozyme (Tani et al., 1993), ovalbumin, etc. (Doi, 1993). In this paper, the two-step process model was applied to the gelation mechanism of tofu.

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MATERIALS AND METHODS

Materials. Soybean protein isolate (SPI) (var. Enrei) was kindly supplied by Fuji Oil Co. Ltd. (Osaka). The protein content determined according to the Kjeldahl method was 94%. The coagulants, GDL and calcium sulfate, were purchased from Wako Pure Chemicals Industries Ltd. (Osaka) and used without further purification.

Gelation Process. The measurement method, which has been described elsewhere (Kohyama and Nishinari, 1993), was partially modified. Soy protein was dispersed in distilled water and heated in boiling water for 10 min. About 50 μL of GDL solution freshly prepared or $CaSO_4$ suspension was mixed with about 2 mL of the protein solution. The final concentrations of GDL and calcium sulfate in the mixture were adjusted to 20 and 30 mM, respectively, unless otherwise stated. The mixture was immediately put into the cell of a Rheolograph Sol apparatus (Toyoseiki Seisakusho, Tokyo). Sinusoidal shear oscillations with a frequency of 1.0 Hz and an amplitude of 25 μm were applied, and the dynamic viscoelasticity at a constant temperature (5–90 °C) was recorded every minute as a function of time.

The observed gelation curve was fitted to an empirical formula as described in the previous papers (Kohyama et al., 1992; Kohyama and Nishinari, 1992a, 1993), since both moduli began to increase at a certain time (t_0) after the coagulant addition, and they reached saturation.

$$G'(t) = G'_{sat}\{1 - \exp[-k(t - t_0)]\} \quad (1)$$

$G'(t)$ is the storage modulus at time t, G'_{sat} is the saturation value of the storage modulus [$G'(\infty)$], k is the rate constant of gelation, and t_0 is called the gelation time. The starting time corresponded to the time when a coagulant was added to the protein solution. The gelation time was defined as the point corresponding to the time when G' began to deviate from the base line. Values of k and G'_{sat} were estimated from curve fitting by the least squares method.

Compression Test. The experimental procedures were reported previously in detail (Kohyama and Nishinari, 1992b, 1993). An SPI solution preheated at 100 °C was cooled to room temperature, and a 2.5% volume of GDL solution or $CaSO_4$

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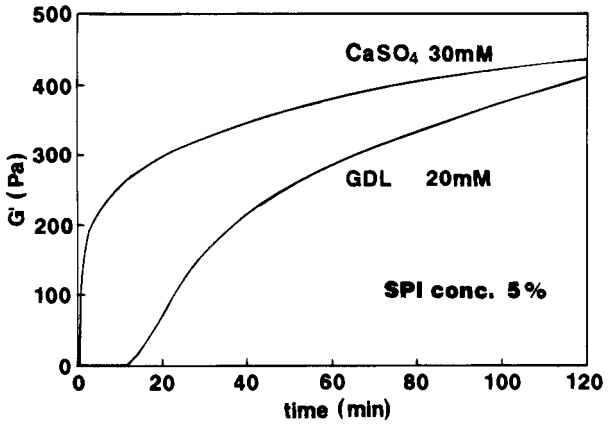

Figure 1. Typical gelation curves for 5% SPI at 70 °C.

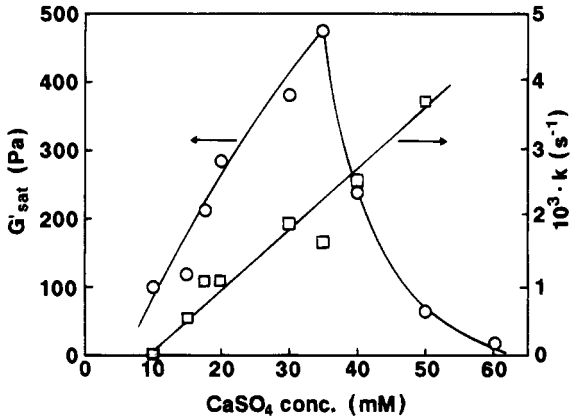

Figure 2. Dependence of G'_{sat} and rate constant of gelation k of 5% SPI on $CaSO_4$ concentration at 70 °C: (○) G'_{sat}; (□) k.

suspension was added to the SPI solution. Final concentrations of protein, GDL, and $CaSO_4$ were adjusted to 5.0%, 20 mM, and 30 mM, respectively. The mixture was immediately poured into glass molds 16 mm in diameter and 10 mm in height. They were allowed to stand for various periods of time at 70 °C, cooled, and then kept at room temperature (25 °C) for 60 min for aging. The compression test was carried out using a Rheoner RE-33005 (Yamaden Co. Ltd., Tokyo) with a flat plunger 40 mm in diameter. The compression rate was 1.0 mm/s. Breaking stress, breaking strain, and breaking energy were calculated without correction on the diameter change from four measurements.

RESULTS AND DISCUSSION

Gel Formation Induced by Calcium Sulfate. Figure 1 shows a typical gelation curve for 5% SPI at 70 °C. The concentrations of protein and coagulant GDL or calcium sulfate corresponded to the values for practical tofu-making. The values of G' increased with time and seemed to saturate. Estimated G'_{sat} values were almost the same for both cases, while the use of $CaSO_4$ resulted in faster gelation compared with the use of GDL. Values of the loss modulus (G'') also increased with time the same as the storage modulus; however, the values of loss tangent (G''/G') were decreased to about 0.1 as observed in the previous works on GDL gels (Nishinari et al., 1991; Yoshida et al., 1992; Kohyama et al., 1992; Kohyama and Nishinari, 1992a, 1993).

The value of G'_{sat} of 5% SPI increased with $CaSO_4$ concentration up to 35 mM and then decreased as shown in Figure 2. This figure also shows the dependence of the rate constant of the gelation k on the concentration of $CaSO_4$. The coagulant accelerated the gelation as

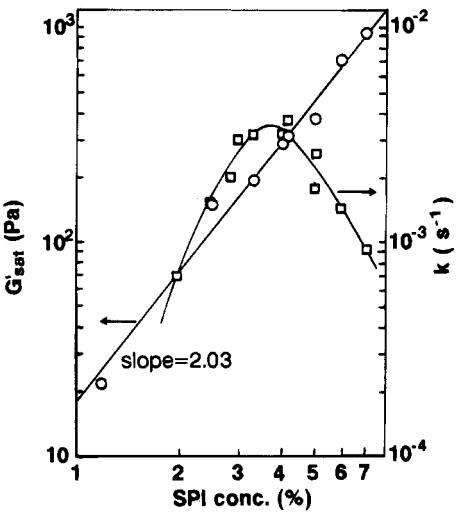

Figure 3. Dependence of G'_{sat} and rate constant k on SPI concentration in the presence of 30 mM $CaSO_4$ at 70 °C: (○) G'_{sat}; (□) k.

observed with GDL (Yoshida et al., 1992; Kohyama et al., 1992; Kohyama and Nishinari, 1992a, 1993). Concentrations of $CaSO_4$ higher than 35 mM lead to larger aggregates and syneresis of the gels; therefore, the apparent values of G'_{sat} decreased.

The effect of protein concentration was studied for systems containing 30 mM calcium sulfate. Figure 3 shows the dependence of the values of G'_{sat} and rate constant k on the SPI concentration. G'_{sat} values increased with increase of the protein concentration as in the case of GDL gels (Yoshida et al., 1992; Kohyama et al., 1992; Kohyama and Nishinari, 1992a, 1993). The slope (2.03) of the double-logarithmic plot indicated that the G'_{sat} value was proportional to the square of the SPI concentration ranging from 1.2 to 7.1%. The relationship between the G'_{sat} value of gels and polymer concentrations, which is represented by the square power law, has been generally observed for biopolymer gels (Ross-Murphy, 1994). This law will be practically useful to estimate the soybean/water ratio in tofu processing. The exponent 2 was almost the same as that for the 7S gels in the presence of GDL (Kohyama and Nishinari, 1993), but smaller than the 3 exponent for 11S GDL gels (Kohyama et al., 1992; Kohyama and Nishinari, 1993) and the exponent 5 for the heat-induced SPI gels (Bikbov et al., 1979). The structure of SPI gels induced by calcium was evidently different from that of the heat-induced gels.

The rate constant showed a maximum value at an SPI concentration of 4% (Figure 3). As commonly observed, the gelation proceeded more rapidly with increasing SPI concentration when SPI concentrations were lower than 4%. However, the opposite relationships were observed at the higher concentrations, presumably due to the lack of coagulant for protein.

Temperature Dependence of Gel Viscoelasticity. Figure 4 shows the temperature dependence of G'_{sat} values of SPI gels coagulated with 20 mM GDL and 30 mM $CaSO_4$. Under these conditions, the time required for reaching the saturation level was shorter for the calcium gels than for the GDL gels, as shown in Figure 1. Since the gelation in the presence of GDL was slow, the G'_{sat} value could not be calculated at lower temperatures. However, the G'_{sat} value obtained for the GDL gel was similar to that for calcium gel formed at the same temperature, and G'_{sat} decreased with higher coagulation temperatures. Since the modulus value of

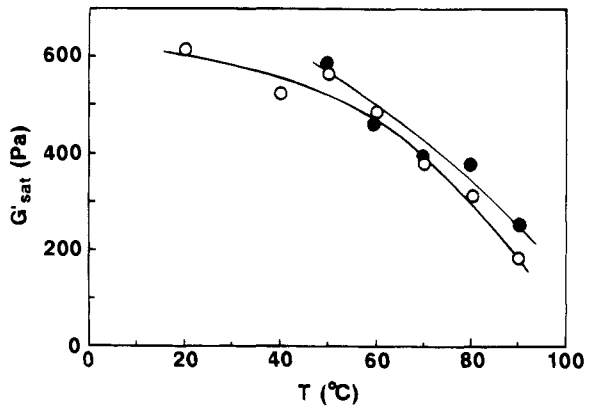

Figure 4. Temperature dependence of G'_{sat} of SPI gels coagulated with GDL and $CaSO_4$: (●) 20 mM GDL; (○) 30 mM $CaSO_4$. SPI concentration was 5.0%.

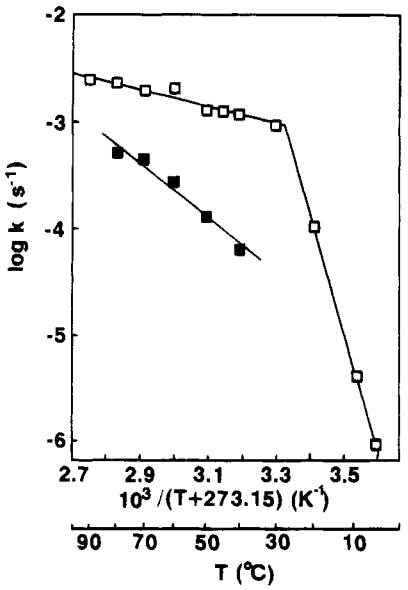

Figure 5. Arrhenius plot for SPI gelation: (■) 20 mM GDL; (□) 30 mM $CaSO_4$. SPI concentration was 5.0%.

a gel heated for a long time, *i.e.*, complete gelation, decreased at a high temperature (data are not shown), and the initial slope of stress–strain curves, which represents the Young's modulus, for commercial tofu was decreased with higher test temperatures (Kohyama and Nishinari, 1992b), additional cross-links of the gels were unlikely to be formed at a high temperature. The decrease in modulus with higher temperatures is commonly observed in many viscoelastic materials (Ferry, 1980).

Figure 5 is the Arrhenius plot, in which k is logarithmically plotted against the reciprocal of the absolute temperature. The value of the rate constant k for the calcium system was much larger than that for the GDL systems. The k values for the $CaSO_4$ system are located on two straight lines, with slopes of -7.78×10^2 for temperatures higher than 25 °C and -1.12×10^4 for lower ones, suggesting that the gelation involves at least two processes as pointed out (Richardson and Ross-Murphy, 1981). If the absolute value of the slope was smaller above a certain temperature, the first process would be rate-determining below this temperature, so the reaction may be considered to be promoted by heat above the temperature. As SPI and $CaSO_4$ were mixed at room temperature, the mixture was cooled to the test temperature in the cell of the apparatus in three cases at lower temperatures. This effect may influence the temperature dependence on k. For the GDL systems, the five points were located on a straight line, the slope value of which was -2.61×10^3. The apparent activation energies, which can be calculated from the slopes of the Arrhenius plot, were 14.9 and 214.5 kJ/mol for the $CaSO_4$ system at high and low temperatures, respectively, and 50.1 kJ/mol for the GDL system. Except for the low-temperature region, both systems showed smaller values for the activation energy. The value for the calcium-induced system was lower than that for the GDL ones. Recently, Takahashi et al. (1994) also reported a similar behavior for gelation of soy milk. Values of apparent activation energy for SPI–GDL gels were lower than that for 11S GDL (Kohyama et al., 1994), probably due to the lower GDL amount to protein. [We reported that the activation energy was 15 kJ/mol in a previous paper (Kohyama et al., 1992). This value was incorrect due to an error in the calculation. The correct value was 78 kJ/mol (Kohyama et al., 1994).] Smaller amounts of GDL would result in coarser coagulation and more flexible network structures of the gels.

It is suggested that gelation with both of the coagulants is not induced by heat above 30 °C. As in the case of the gelation of soybean 11S by GDL, it is considered that the induction of gelation of SPI is promoted by protons produced by GDL or cations from calcium sulfate, which lead to the decrease of negatively charged groups of the protein molecules for coagulation.

Breaking Properties of Gels. As observed in the 7S and 11S gels coagulated by GDL, two gels that have similar storage moduli do not always show the same breaking stress, strain, or energy (Kohyama and Nishinari, 1993). Although G'_{sat} values highly correlated with the breaking force in the GDL gels (Yoshida et al., 1992; Kohyama and Nishinari, 1993), breaking stress is another important parameter of tofu gel, because it better reflects its texture. To compare the calcium- and GDL-induced gels, breaking properties should also be tested. Figure 6 shows the breaking energy, breaking stress, and breaking strain for SPI gels as a function of heating time at 70 °C. The values of the breaking energy and breaking stress for the GDL gels became almost similar to those for the calcium gels, after the gels were heated for a sufficiently long period of time. The saturated calcium gel seemed to exhibit a slightly higher value for the breaking strain than the GDL gel. This result was the same as recent report that the breaking stress and breaking energy were greater in tofu coagulated with GDL than in tofu coagulated with calcium sulfate, when values of the breaking strain for both gels were similar (Takahashi et al., 1994). However, difference in the breaking strain was not significant in the present case; it can thus be concluded that both gels display similar properties rheologically. This is consistent with the observation by scanning electron microscopy (SEM) that the microstructures of tofu coagulated with GDL and with calcium sulfate were quite similar (deMan et al., 1986).

Gelation Mechanism. Since the breaking stress of heat-induced gels decreased by cleavage of intermolecular disulfide bonds or blockage of free SH groups, and the treatment affected more than 11S gels than the 7S ones, it appears that a disulfide bond newly formed by interchange reaction with a free SH during heating is required for 11S but not for 7S (Utsumi and Kinsella, 1985; Wang and Damodaran, 1990). Nakamura et al. (1984) reported that heat-denatured 11S formed soluble

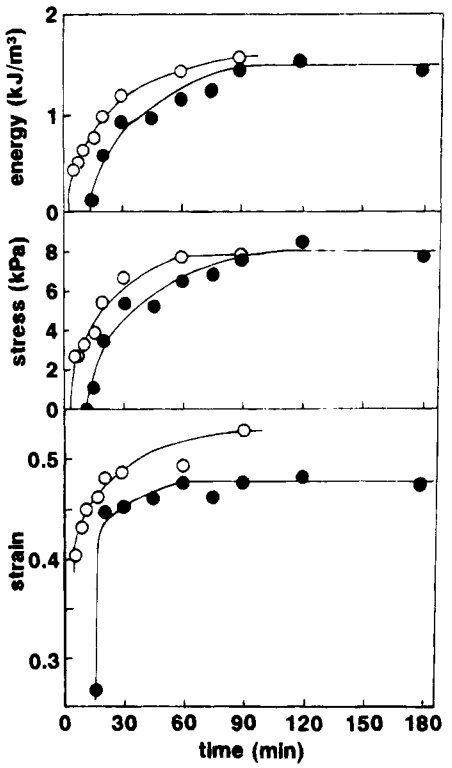

Figure 6. Dependence of the breaking energy, breaking stress, and breaking strain on heating time (at 70 °C) for SPI gels coagulated with GDL and $CaSO_4$: (●) 20 mM GDL; (○) 30 mM $CaSO_4$. Sample size was 16 mm diameter × 10 mm. Compression rate was 1.0 mm/s. Symbols represent the mean value.

aggregates with a disulfide exchange reaction and hydrophobic interaction at pH 7.6. Previous studies on heat-induced gels were carried out under alkaline conditions [pH 8.0 (Utsumi and Kinsella, 1985; Wang and Damodaran, 1990) or 7.6 (Nakamura et al., 1984)], where the interchange reaction easily occurs.

It is known that 11S globulin contains a larger amount of sulfhydryl (SH) groups than 7S globulin (Fukushima, 1991). Saio et al. (1971) reported that the value of the breaking force of tofu gels made from 11S proteins increased with the amount of SH groups and was higher than that of 7S globulin gels. They suggested a contribution of disulfide bonds in 11S globulin gels to the breaking stress. Hashizume et al. (1978) observed that the oxidation of the SH groups during heating decreased the value of the breaking stress of the tofu gel.

Since the pH value of the present protein solution in distilled water was about 6.8 and did not depend on the protein concentration (Kohyama et al., 1992; Kohyama and Nishinari, 1992a, 1993), the disulfide bonds were more stable than those in alkaline buffers. As the first step of the present gelation is similar to the formation of a heat-induced gel, such an interchange reaction may also occur during heating. The reaction could not be detected, because it may occur very rapidly and the total number of SH groups does not change. However, such an interchange reaction may not occur during the coagulation process or the second step of the gelation, since the pH decreased to the acidic region, where the cleavage of disulfide bonds becomes slow. We could not determine the presence of an interchange reaction after the formation of a firm gel due to problems in sampling. It is difficult to consider that a disulfide bond could be formed after the development of a three-dimensional network.

Urea (8 M) completely dissolved the gels or aggregate, whose protein concentration was 5% at any time after the addition of coagulant. Urea is known to break up hydrogen bonds and to affect the water structure around the protein molecules; however, it cannot break covalent bonds. It is assumed that hydrogen bonds, hydrophobic interaction, and also charge–charge interaction may be involved in the network structure formed by both GDL and $CaSO_4$. The contribution of disulfide bonds on gel networks is considered to be negligible, because its formation is very slow at the low-pH regions. The stabilizing mechanism of the gels in the present cases is different from that for heat-induced 11S globulin gels, in which both disulfide bonding and noncovalent bonds are involved in their junction points (Nakamura et al., 1984; Mori et al., 1986).

Since the pH of the gel was not equal to the isoelectric point (Kohyama and Nishinari, 1993) and the SPI itself contained some components having different isoelectric points (Fukushima, 1991), charge–charge interaction may also contribute to the gelation. However, it would be a minor effect, since exposure of hydrophobic regions induced by heat treatment was prerequisite for the

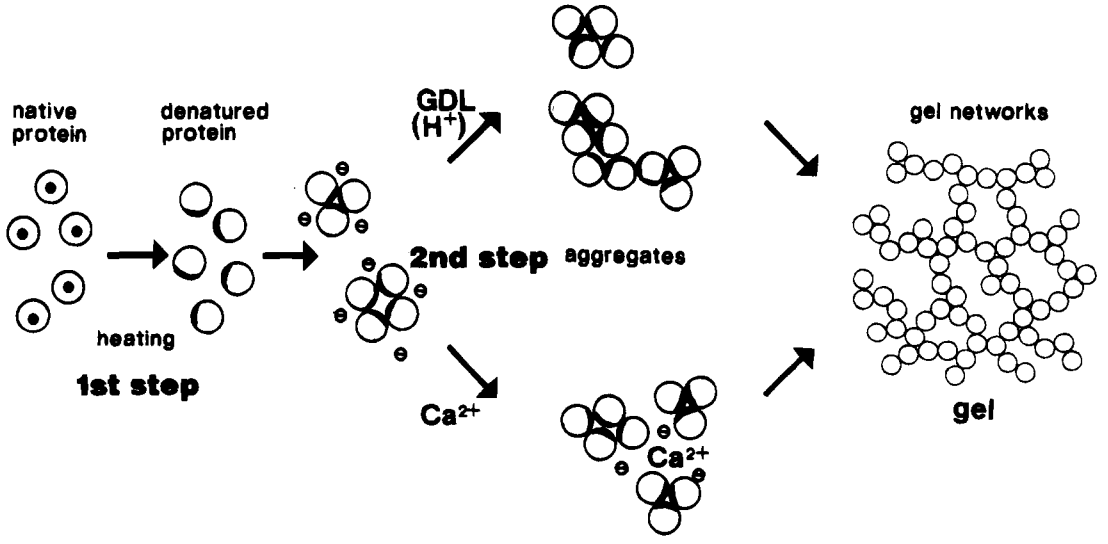

Figure 7. Gelation mechanism of soybean proteins in the presence of GDL or $CaSO_4$: (circles) protein molecules; (black areas) hydrophobic regions.

coagulation. We speculate, therefore, that hydrophobic interaction mainly plays a role in the gel structure.

The differences in the gelation rates between GDL- and calcium-induced gelation were due to slower cleavage of GDL. The k value was higher in the 11S system than in the 7S one in the presence of GDL (Kohyama and Nishinari, 1993). This is mainly due to the differences in the isoelectric points, because a larger amount of protons or calcium ions is required to remove the negative charge on 7S than on 11S. The kinetics data also support the hypothesis that hydrophobic interaction is a major factor for the gelation in those systems.

The gelation mechanism is schematically represented in Figure 7. The gel formation consists of the following two steps: protein denaturation and hydrophobic coagulation. At first, the hydrophobic regions of the protein molecules in the native state are located inside and are exposed to the outside by heat denaturation as shown by fluorescence studies (Koshiyama et al., 1981; Matsudomi et al., 1985). Since the denatured soybean protein is negatively charged (Kohyama and Nishinari, 1993), the formation of protons induced by GDL or calcium ions neutralizes the net charge of the protein in the second step. As a result, the hydrophobic interaction of the neutralized protein molecules becomes more predominant and induces the aggregation. It is considered that the gels are formed by random aggregation as imaged by SEM observation (deMan et al., 1986) and become turbid as generally developed near the isoelectric point (Doi, 1993).

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