Contribution of Disulfide Bonding to Viscoelastic Properties and Microstructures of 11S Globulin Gels from Soybeans: Magnesium Chloride-Induced Gels

Takao NAGANO

Department of Clinical Nutrition, Faculty of Health Science and Technology, Kawasaki University of Medical Welfare, 288, Matsushima, Kurashiki, Okayama 701-0193, Japan

Received June 18, 2012; Accepted October 3, 2012

The role of disulfide bonding in the structure of gels formed from soybean proteins was investigated. Heat-induced gels of 11S globulin (11S) and soybean protein isolate (SPI) were prepared using magnesium chloride as a coagulant, and the effects of 2-mercaptoethanol (2-ME), which prevents the formation of disulfide bonds, on the viscoelastic properties and microstructures of these gels were determined. The effects of 2-ME were quantified using dynamic viscoelastic measurement (DVM) and confocal laser scanning microscopy (CLSM). DVM indicated that 11S gels showed higher storage modulus (G') and lower mechanical loss tangent ($\tan \delta$) than SPI gels. CLSM was used to examine microstructures of 11S and SPI gels, and to determine their fractal dimension and the average density of network structures. The 11S gels showed lower fractal dimension and denser gel networks than SPI gels. These characteristic viscoelastic properties and microstructures of the 11S gel were lost with increasing 2-ME concentration, suggesting that the characteristic properties of 11S gels are due to disulfide bonding.

Keywords: 11S globulin, disulfide bond, heat-induced gel, dynamic viscoelastic measurement, confocal laser scanning microscopy

Introduction

Tofu is a traditional soybean-based food that is especially popular in Asian countries. Tofu is a gel of soybean proteins that is produced by adding coagulants to heated soybean milk (Saio, 1979). Magnesium chloride (MgCl_2), calcium sulfate (CaSO_4), and glucono- δ -lactone (GDL) are common coagulants used on an industrial scale for the preparation of tofu (deMan *et al.*, 1986). Although MgCl_2 is the most commonly used coagulant for tofu production in Japan (Toda *et al.*, 2003), there is little information concerning MgCl_2 -induced gels of soybean proteins. Most studies on tofu formation and properties have used GDL or CaSO_4 as the coagulant because the rates of gel formation are relatively slow and more homogeneous gels are formed (Saio *et al.*, 1969; Nishinari *et al.*, 1991; Kohyama and Nishinari, 1993; Kohyama *et al.*, 1995; Liu *et al.*, 2004; Tay and Perera, 2004; Guo and Ono, 2005; Ono, 2008).

The two dominant soybean storage proteins are 7S globu-

lin (7S) and 11S globulin (11S), which together constitute approximately 70% of the total storage protein at maturity (Nielsen, 1985). Soybean protein isolate (SPI) is prepared from water-extracted soybean proteins by acidic precipitation and consists mainly of 7S and 11S. Rheological properties of heat-induced 7S, 11S, and SPI gels have been extensively studied (Saio *et al.*, 1973; Hermansson, 1986; van Kleef, 1986; Nakamura *et al.*, 1986; Yamauchi *et al.*, 1991; Nagano *et al.*, 1994ab; Renkema *et al.*, 2001; Lakemond *et al.*, 2003; Renkema, 2004). Most studies have reported that of all the soybean proteins, 11S has the greatest ability to form heat-induced gels. However, the reasons why 11S proteins form harder and stiffer gels than other soybean proteins remain unclear.

The cross-link bonding of protein gel structures is believed to rely on hydrogen bonds, hydrophobic interactions, ionic attractions, and disulfide bonds (Nakai & Li-Chan, 1988; Utsumi *et al.*, 1997). The effects of salts, reducing agents, and denaturants on the formation of heat-induced gels have been investigated to clarify which intermolecular forces are involved in the formation of 11S gels. It is likely

---

*To whom correspondence should be addressed.

E-mail: naganot@mw.kawasaki-m.ac.jp

that disulfide bonding is a predominant force in the formation of 11S heat-induced gels (Utsumi and Kinsella, 1985ab; Mori *et al.*, 1986; Mori *et al.*, 1988).

In a previous study (Nagano and Tokita, 2011), we prepared heat-induced gels of 11S and SPI using MgCl_2 as a coagulant, and investigated their viscoelastic properties and microstructures using dynamic viscoelastic measurement (DVM) and confocal laser scanning microscopy (CLSM). DVM indicated that 11S formed stiffer and more solid gels than SPI. CLSM showed that the microstructures of 11S gels were coarser and more heterogeneous than those of SPI gels. The microstructures of 11S and SPI gels were also analyzed by determining the average density of gel networks, which revealed that 11S forms denser gel networks than SPI. It is highly likely that denser gel networks make 11S gels stiffer and more solid than SPI gels. To investigate the contribution of disulfide bonds to the formation of heat-induced protein gels, 2-mercaptoethanol (2-ME), a reducing agent of disulfide bonds, is often used (Utsumi and Kinsella, 1985ab; Mori *et al.*, 1986; Mori *et al.*, 1988). In this study, the contribution of disulfide bonding to gel structure was determined by examining the effects of 2-ME on the viscoelastic properties and microstructures of 11S and SPI gels. These effects were quantified using DVM and CLSM.

Materials and Methods

Materials Soybeans (Fukuyutaka variety) were used in this study. Rhodamine B was purchased from Sigma-Aldrich (St Louis, USA). All other chemicals used in this study were of reagent grade, and were used without further purification.

Preparation of soybean protein isolate (SPI) and 11S globulin (11S) SPI and 11S were prepared as described previously (Nagano *et al.*, 1992; Nagano *et al.*, 1996). The following procedures were performed at room temperature. Defatted soybean meal was prepared from ground soybean seeds by solvent extraction with *n*-hexane. The defatted meal was then extracted in 15 volumes of distilled water, adjusted to pH 7.5 with 2 N sodium hydroxide (NaOH), for 1 h. The extract was strained through a nylon mesh (180 mesh size), and defatted soymilk was obtained by removing the insoluble material by centrifugation ($9000 \times g$ for 30 min). To prepare SPI, the pH of the obtained defatted soymilk was adjusted to pH 4.8 with 2 N hydrochloric acid (HCl), and the insoluble fraction was collected by centrifugation ($6500 \times g$ for 20 min). The precipitate, yielding SPI, was washed three times with 10 volumes of distilled water adjusted to pH 4.8 using 2 N HCl, then recovered by centrifugation ($6500 \times g$ for 20 min), adjusted to pH 7.0 with 2 N NaOH, and then lyophilized. To prepare 11S, dry sodium bisulfate (SBS) was added to the defatted soymilk (0.98 g of SBS/L), the pH

was adjusted to pH 6.4 with 2 N HCl, and then the mixture was kept at 4 °C overnight. The precipitate was collected by centrifugation ($6500 \times g$ for 20 min) at 4 °C. The precipitate containing the 11S protein was washed three times with 10 volumes of distilled water adjusted to pH 5.5 with 2 N HCl, collected by centrifugation ($6500 \times g$ for 20 min), adjusted to pH 7.0 with 2 N NaOH, and then lyophilized.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was carried out on 11% polyacrylamide gels according to the method of Laemmli (1970). The amount of 11S in the prepared SPI (56% 11S) and the purity of the obtained 11S fraction (> 90%) were measured by densitometry.

Sample preparation Protein dispersions of 11S or SPI were adjusted to pH 6.8 with 0.5 N HCl, heated at 96 °C for 10 min in a water bath, and then immediately cooled in running water for 20 min. A solution containing MgCl_2 and sodium chloride (NaCl, 0.2 mL) was added to the protein dispersion (3.8 mL) in a test tube and vortexed for 10 s. An undiluted solution of 2-mercaptoethanol (2-ME) was added when MgCl_2 and NaCl were added. The effects of NaCl concentration (0.05 to 0.25 M) on 11S and SPI gels were examined at 3.3% (w/w) protein concentration and 0.25% (w/v) MgCl_2 . The effects of 2-ME (0 to 15 mM) on soybean protein gels were investigated at 0.25% (w/v) MgCl_2 and 0.2 M NaCl (for 11S gels) or 0.15 M NaCl (for SPI gels).

Dynamic Viscoelastic Measurement (DVM) The sample dispersion of 11S or SPI was prepared as described above and placed between parallel plates (60 mm in diameter), and then the gap between the two plates was set to 1 mm. The samples were covered with a thin layer of silicon oil to prevent evaporation during the experiment. The solution was heated at 70°C for 30 min, cooled to 25°C as soon as possible (approximately 3 min), kept at 25°C for 30 min, and then subjected to a strain sweep experiment at 1 Hz with an AR 550 rheometer (TA Instruments Japan, Tokyo, Japan). The values of G' and $\tan \delta$ were obtained where G' remained constant against strain (linear viscoelastic region). All measurements were conducted in at least triplicate.

Confocal Laser Scanning Microscopy (CLSM) The sample dispersions of 11S and SPI were prepared as described above. The protein was stained with the fluorescent dye rhodamine B (0.001% w/w). Dispersions were placed on glass slides with a shallow indent, the sample was covered with a glass cover slip, and then the cover slip was sealed with nail polish to prevent evaporation. The glass slides were heated in a water bath at 70°C for 30 min and then cooled to room temperature. The gels were observed using a Leica TCS MP2 confocal laser scanning microscope (Leica Microsystems, Baden-Württemberg, Germany). The excitation wavelength was 561 nm (delivered by a He-Ne laser), and

emission was measured at 580 – 690 nm. Images were obtained under an oil-immersed 100 × objective at a depth of 10 μm below the cover slip surface to minimize the disturbance from the glass. Digital images were acquired at 1024 × 1024 pixel resolution.

Image Analysis CLSM images were analyzed using Image-Pro Plus 5.0 (Nippon Roper, Tokyo, Japan). Binary images were obtained by thresholding using dynamic programming from two-dimensional projections of CLSM pictures. The fractal dimension was calculated using the box-counting method as described previously (Doi and Tokita, 2005). The average density of gel networks was calculated from the proportion of the total white region in each binary CLSM image.

Results and Discussion

Effects of NaCl concentration on viscoelastic properties of 11S and SPI gels In our previous study (Nagano and Tokita, 2011), addition of NaCl was required for both 11S and SPI to form MgCl_2 -induced gels. In that study, the structures of 11S and SPI gels containing different concentrations of protein were studied using CLSM, and the fractal dimension (D) was calculated from two-dimensional projection of CLSM images. The difference in D between 11S and SPI gels at the same protein concentration became larger with decreasing protein concentration. A protein concentration of less than 3.4% was preferable to compare gel structures of 11S and SPI gels. In addition, manufacturers prefer to use a concentration of 0.25% MgCl_2 as the coagulant in tofu production (Toda *et al.*, 2003). Therefore, for this study, 11S or SPI gels containing 3.3% (w/w) protein and 0.25% (w/v) MgCl_2 were used to determine the effects of NaCl concentration on viscoelastic properties of the gels. Viscoelastic properties of gels can be quantified by dynamic viscoelastic measurements (DVM), and are well characterized by the storage modulus (G'), loss modulus (G''), and mechanical loss tangent ($\tan \delta$) (Ferry, 1980). G' is proportional to the energy stored in a gel per cycle, while G'' is proportional to the energy lost per cycle. The ratio G''/G' is known as $\tan \delta$. A perfectly elastic material will show $\tan \delta = 0$, while $\tan \delta$ tends to infinity for purely viscous materials.

Figure 1 shows the G' and $\tan \delta$ of 11S and SPI gels as a function of the NaCl concentration. MgCl_2 -induced gels formed at NaCl concentrations exceeding 0.1 M for 11S and 0.05 M for SPI. At low NaCl concentrations, many large protein aggregates were observed in confocal laser scanning microscopy (CLSM) images for both 11S and SPI gels. These protein aggregates disappeared and network structures of the gel tended to form with increasing NaCl concentration (data not shown). These results suggest that MgCl_2 has a high ability to crosslink soybean proteins with ionic attractions and

its ability can be reduced or screened by increasing ionic strength; therefore, soybean proteins form the network structure of the gel. SPI formed gels at lower NaCl concentrations than 11S. The G' of 11S gels increased with increasing NaCl concentration up to 0.2 M and then decreased. In contrast, the G' of SPI gels increased with increasing NaCl concentration up to 0.1 – 0.15 M and then decreased. At NaCl concentrations higher than 0.15 M, 11S gels showed higher G' than SPI gels. The $\tan \delta$ of 11S and SPI gels was relatively insensitive to NaCl concentration. The $\tan \delta$ values of 11S gels were lower than those of SPI gels at comparable NaCl concentrations. These viscoelastic data indicate that 11S formed stiffer and more solid gels than SPI. These results are consistent with previous reports that of all the soybean proteins, 11S has the greatest ability to form heat-induced gels (Saio *et al.*, 1973; van Kleef, 1986; Nakamura *et al.*, 1986; Renkema *et al.*, 2001).

Effect of 2-mercaptoethanol (2-ME) on viscoelastic properties of 11S and SPI gels The reducing agent 2-ME prevents disulfide bond formation between protein molecules during the formation of heat-induced gels (Utsumi and Kinsella, 1985ab). Therefore, to examine the contribution of disulfide bonds to gel structure, the effects of 2-ME on

Fig. 1. Storage modulus G' (A) and mechanical loss tangent $\tan \delta$ (B) of 11S and SPI gels as a function of NaCl concentration. (●) 11S, (○) SPI. Data are expressed as the mean $\pm$ SD.

viscoelastic properties of 11S and SPI gels were investigated using DVM. The highest values of G' for 11S and SPI gels were observed at NaCl concentrations of 0.2 M and 0.1 – 0.15 M, respectively. The G' of SPI gels increased rapidly from 0.05 M to 0.1 M NaCl (Fig. 1). Thus, for 11S and SPI gels, NaCl concentrations of 0.2 M and 0.15 M, respectively, were chosen for further analyses.

We conducted DVM within the linear viscoelastic region where G' remained constant against strain or stress. The strain γ dependence of G' for 11S and SPI gels at different 2-ME concentrations was investigated (Fig. 2). In 11S gels, a linear response was observed at γ less than 0.008, where G' is independent of γ . Further increase in strain resulted in an increase in G' up to $\gamma \approx 0.02$ and then G' decreased, indicating irreversible breakage of bonds between clusters. In SPI gels, a linear response was observed at γ less than 0.02. At high strain values, the modulus of the gels sometimes increased more rapidly than would be expected for a linear relationship. This behavior is known as strain hardening, and indicates a stiffening of the gel network when it is deformed (Gisler *et al.*, 1999; Pouzot *et al.*, 2006).

The G' and $\tan \delta$ of 11S and SPI gels as a function of the 2-ME concentration is shown in Fig. 3. In 11S gels, G'

Fig. 2. Typical strain dependence of storage modulus G' for 11S (A) and SPI (B) gels with 2-ME at concentrations of 0 mM (●), 5 mM (○), 10 mM (◐), 15 mM (◑).

Fig. 3. Storage modulus G' (A) and $\tan \delta$ (B) of 11S and SPI gels as a function of 2-ME concentration. (●) 11S, (○) SPI. Data are expressed as the mean $\pm$ SD.

decreased and $\tan \delta$ increased with increasing 2-ME concentration. In contrast, G' and $\tan \delta$ of SPI gels were relatively insensitive to 2-ME concentration. In the absence of 2-ME, 11S gels showed higher G' and lower $\tan \delta$ than SPI gels. The higher G' and the lower $\tan \delta$ of 11S gels compared with SPI gels were lost with increasing 2-ME concentration. These results suggest that disulfide bonding is the predominant force responsible for the stiffness and solidness of 11S gels. These results are consistent with other reports that disulfide bonds play an important role in gelation of 11S (Utsumi and Kinsella, 1985ab; Mori *et al.*, 1986; Mori *et al.*, 1988).

Effects of 2-ME on microstructures of 11S and SPI gels
The effects of 2-ME on microstructures of 11S and SPI gels were investigated using CLSM. Figure 4 shows CLSM images of 11S and SPI gels at different 2-ME concentrations. The fluorescent dye rhodamine, which stains the protein phase, was used to stain gels. Thus, brighter regions on the image represent areas of higher protein density, while dark regions represent areas with lower protein density. CLSM images showed that the structure of the 11S gel was coarser and more heterogeneous than that of the SPI gel in the absence of 2-ME. Upon addition of 2-ME, the characteristic structure of the 11S gel became more like that of the SPI

Fig. 4. CLSM images of 11S and SPI gels containing different concentrations of 2-ME. Width of each image = 75 μ m.

gel, i.e., more dispersed protein networks. In contrast, the structure of the SPI gel was relatively insensitive to the addition of 2-ME. It is likely that the differences in gel structures are a result of the different types of aggregates formed. Ono (2008) indicated the important role of protein particles (> 40 nm in diameter) in soymilk for the formation of tofu curd. The protein particle content and 11S content of the particle increased with increasing 11S content of soymilk (Guo *et al.*, 2002). The breaking stress of tofu curd was higher with the increase in the protein particle content (Guo and Ono, 2005).

The structure of particulate gels has been described using the fractal concept (Hagiwara *et al.* 1997; Marangoni *et al.*, 2000). Microscopic image analyses are the most direct method for evaluating fractal dimension. Thus, the fractal dimension D was calculated from two-dimensional projections of CLSM images. Figure 5 shows D of 11S and SPI gels as a function of the 2-ME concentration. The D for 11S was between 1.75 and 1.79, and for SPI gels was between 1.79 and 1.81. The D of the 11S gel was lower than that of the SPI gel. In 11S gels, D increased markedly with increasing 2-ME concentration. In contrast, D of SPI gels was relatively insensitive to 2-ME concentration. The difference in D between 11S and SPI gels was lost with increasing 2-ME concentration. It should be noted that the fractal dimension remains unchanged upon projection, providing $D \leq 2$ (Mandelbrot, 1983). Thus, the values of D in this study were close to the value that is expected for the cluster-cluster aggregation model (Weitz and Huang, 1984; Weitz and Huang, 1986; Meakin, 1998; Ikeda *et al.*, 1999). The structure of opaque and particulate gels was previously analyzed in terms of the fractals using CLSM (Hagiwara *et al.*, 1997; Doi and Tokita, 2005). Hagiwara *et al.* (1997) studied the gel structures of β -lactoglobulin, a globular protein and a major component of milk whey proteins, containing CaCl_2 (referred to as a weak-link type), and calculated a D value of 1.70. Doi and Tokita

(2005) investigated opaque poly(acrylamide) gels at high cross-linker concentrations (> 0.3 mole fraction) and showed that D ranged from 1.5 to 1.7.

The proportion of the bright region in the CLSM image was calculated from the two-dimensional projection of CLSM pictures, and gave a value for the average density of gel networks. A lower proportion of bright region indicates that protein aggregates are denser. Therefore, the lower the proportion of the bright region, the higher the average density of the gel networks. Figure 6 shows the average density of gel networks, as calculated from images of 11S and SPI gels, as a function of the 2-ME concentration. In the absence of 2-ME, the average density of gel networks was higher in the 11S gel than in the SPI gel. The average density of gel net-

Fig. 5. Fractal dimensions of 11S and SPI gels as a function of 2-ME concentration. (●) 11S, (○) SPI. Data are expressed as the mean $\pm$ SD. Fractal dimensions were determined from CLSM images.

Fig. 6. Average density of gel networks for 11S and SPI gels as a function of 2-ME concentration. (●) 11S, (○) SPI. Data are expressed as the mean $\pm$ SD. Density data were obtained from CLSM images.

works decreased with increasing 2-ME concentration in the 11S gel, but was relatively insensitive to 2-ME concentration in the SPI gel. The difference in the average density between 11S and SPI gels decreased with increasing 2-ME concentration. These results suggested that the denser gel networks of 11S gels are due to disulfide bonds. In addition, Ono *et al.* (1991) investigated the size distribution of the protein particles in soymilk heated in boiling water for 5 min using differential centrifugation. Although large-sized particles (LSP) greater than 100 nm in diameter constituted 40% of the total protein in soymilk, 70% of the protein components were 11S. LSP was not formed in the presence of 2-ME. Therefore, LSP might be correlated with the formation of denser networks in 11S gels.

In this study, the effects of 2-ME on viscoelastic properties and microstructures of 11S and SPI gels were investigated. 11S formed stiffer and more solid gels than SPI, and showed denser network structures. These characteristics of the 11S gel are most likely due to disulfide bonding. One might wonder why the viscoelastic properties and microstructures of SPI gels were relatively insensitive to 2-ME concentration when SPI contains more than 50% 11S protein. This might be because MgCl_2 -induced gels are formed mainly by ionic attractions between protein molecules and magnesium ions. Gelation of SPI and 11S is also affected by other molecular forces such as hydrophobic interactions and hydrogen bonds. Because gel formation is a very complex phenomenon, further research is required to fully elucidate all the intermolecular forces involved in the formation and structure of soybean 11S and SPI gels.

Conclusions

In this study, the effects of 2-ME on viscoelastic properties and microstructures of 11S and SPI gels were determined. DVM indicated that 11S formed a stiffer and more solid gel than SPI. CLSM revealed that 11S formed gels with lower fractal dimension and higher average density of gel networks than SPI. These characteristic viscoelastic properties and microstructures of the 11S gel decreased with increasing 2-ME concentration. These results suggest that disulfide bonding is an important intermolecular force for the stiffness and solidness of 11S gels, and for the high density of their protein networks.

Acknowledgments This investigation was supported by the Fuji Foundation for Protein Research. The author thanks Emi Moriyasu and the staff at the Physiological Function Research Center of Kawasaki Medical School for technical assistance.

References

- deMan, J.M., deMan, L. and Gupta, S. (1986). Texture and microstructure of soybean curd (tofu) as affected by different coagulants. *Food Microstr.*, **5**, 83-89.
- Doi, Y. and Tokita, M. (2005). Real space structure of opaque gel. *Langmuir*, **21**, 5285-5289.
- Ferry, J.D. (1980). "Viscoelastic properties of polymers, 3rd ed." John Wiley & Sons, Chichester.
- Gisler, T., Ball, R.C., and Weitz, D.A. (1999). Strain hardening of fractal colloidal gels. *Phys. Rev. Lett.*, **82**, 1064-1067.
- Guo, S.T., Tsukamoto, C., Takahashi, K., Yagasaki, K., Nan, Q.X., and Ono, T. (2002). Incorporation of soymilk lipid into soy protein coagulum by the addition of calcium chloride. *J. Food Sci.*, **67**, 3215-3219.
- Guo, S.-T. and Ono, T. (2005). The role of composition and content of protein particles in soymilk on tofu curdling by glucono- δ -lactone or calcium sulfate. *J. Food Sci.*, **70**, C258-262.
- Hagiwara, T., Kumagai, H., and Matsunaga, T. (1997). Fractal analysis of the elasticity of BSA and β -lactoglobulin gels. *J. Agric. Food Chem.*, **45**, 3807-3812.
- Hermansson, A.-M. (1986). Soy protein gelation. *J. Am. Oil Chem. Society*, **63**, 658-666.
- Ikeda, S., Foegeding E.A., and Hagiwara, T (1999). Rheological study on the fractal nature of the protein gel structure. *Langmuir*, **15**, 8584-8589.
- van Kleef, F.S.M. (1986). Thermally induced protein gelation: gelation and rheological characterization of highly concentrated ovalbumin and soybean protein gels. *Biopolymers*, **25**, 31-59.
- Kohyama, K. and Nishinari, K (1993). Rheological studies on the gelation process of soybean 7S and 11S proteins in the presence of glucono- δ -lactone. *J. Agric. Food Chem.*, **41**, 8-14.
- Kohyama, K., Murata, M., Tani, F., Sano, Y., and Doi, E. (1995). Effects of protein composition on gelation of mixtures containing soybean 7S and 11S globulins. *Biosci. Biotechnol. Biochem.*, **34**, 240-245.
- Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680-685.
- Lakemond, C.M.M., de Jongh, H.H.J., Paques, M., van Vliet, T., Gruppen, H., and Voragen, A.G.J. (2003). Gelation of soy glycinin; influence of pH and ionic strength on network structure in relation to protein conformation. *Food Hydrocolloids*, **17**, 365-377.
- Liu, Z.S., Chang, S.K.C., Li, L.T., and Tasumi, E. (2004). Effect of selective thermal denaturation of soybean proteins on soymilk viscosity and tofu's physical properties. *Food Res. Int.*, **37**, 815-822.
- Mori, T., Nakamura, T., and Utsumi, S. (1986). Behavior of intermolecular bond formation in the late stage of heat-induced gelation of glycinin. *J. Agric. Food Chem.*, **34**, 33-36.
- Mori, T., Mohri, M., Artik, N., and Matsumura, Y. (1988). Rheolog-

- ical properties of heat-induced gel of soybean 11S globulin under high ionic strength. *J. Texture Stud.*, **19**, 361-371.
- Mandelbrot, B.B. (1983). "The fractal geometry of nature." New York: Freeman.
- Marangoni, A.G., Barbut, S., McGauley, S.E., Marcone, M., and Narine, S.S. (2000). On the structure of particulate gels-the case of salt-induced cold gelation of heat-denatured whey protein isolate. *Food Hydrocolloids*, **14**, 61-74.
- Meakin, P. (1998). "Fractals, scaling and growth far from equilibrium." Cambridge University Press, Cambridge.
- Nakamura, T., Utsumi, S., and Mori, T. (1986). Interactions during heat-induced gelation in a mixed system of soybean 7S and 11S globulins. *Agric. Biol. Chem.*, **50**, 2429-2435.
- Nagano, T., Hirotsuka, M., Mori, H., Kohyama, K., and Nishinari, K. (1992). Dynamic viscoelastic study on the gelation properties of β -conglycinin. *J. Agric. Food Chem.*, **40**, 941-944.
- Nagano, T., Mori, H., and Nishinari, K. (1994a). Rheological properties and conformational states of β -conglycinin gels at acidic pH. *Biopolymers*, **34**, 293-298.
- Nagano, T., Akasaka, T., and Nishinari, K. (1994b). Dynamic viscoelastic properties of glycinin and β -conglycinin gels from soybeans. *Biopolymers*, **34**, 1303-1309.
- Nagano, T., Fukuda, Y., and Akasaka, T. (1996). Dynamic viscoelastic study on the gelation properties of β -conglycinin-rich and glycinin-rich soybean protein isolates. *J. Agric. Food Chem.*, **44**, 3484-3488.
- Nagano, T., and Tokita, M. (2011). Viscoelastic properties and microstructures of 11S globulin and soybean protein isolate gels: magnesium chloride-induced gels. *Food Hydrocolloids*, **25**, 1647-1654.
- Nakai, S. and Li-Chan, E. (1988). "Hydrophobic interactions in food systems." CRC Press, Boca Raton.
- Nishinari, K., Kohyama, K., Zhang, Y., Kitamura, K., Sugimoto, T., Saio, K., and Kawamura, Y. (1991). Rheological study on the effect of the A₃ subunit on the gelation characteristics of soybean proteins. *Agric. Biol. Chem.*, **55**, 351-355.
- Nielsen, N.S. (1985). Structure of soy proteins. In "New Protein Foods," ed. by A.M. Altschul and H.L. Wilcke. Academic press, New York, pp. 27-64.
- Ono, T., Choi, M.R., Ikeda, A., Odagiri, S. (1991). Changes in the composition and size distribution of soymilk protein particles. *Agric. Biol. Chem.*, **55**, 2291-2297.
- Ono, T. (2008). The mechanism of soymilk and tofu formation from soybean, and the factors affecting the formation. *Nippin Shokuhin Kagaku Kogaku Kaishi*, **55**, 39-48 (in Japanese).
- Pouzot, M., Nicolai, T., Benyahia, L., and Durand, D. (2006). Strain hardening and fracture of heat-set fractal globular protein gels. *J. Colloid Interface Sci.*, **293**, 376-383.
- Renkema, J.M.S., Knabben, J.H.M., and van Vliet, T. (2001). Gel formation by β -conglycinin and glycinin and their mixtures. *Food Hydrocolloids*, **15**, 407-414.
- Renkema, J.M.S. (2004). Relations between rheological properties and network structure of soy protein gels. *Food Hydrocolloids*, **18**, 39-47.
- Saio, K., Watanabe, T., and Kaji, M. (1969). Food processing characteristics of soybean 11S and 7S proteins. Part I. Effect of difference of protein components among soybean varieties on formation of tofu-gel. *Agric. Biol. Chem.*, **33**, 1301-1308.
- Saio, K., Watanabe, T., and Kaji, M. (1973). Food use of soybean 7S and 11S proteins. Extraction and functional properties of their fractions. *J. Food Sci.*, **38**, 1139-1144.
- Saio, K. (1979). Tofu-relationships between texture and fine structure, *Cereal Foods World*, **24**, 342-354.
- Tay, S.L. and Perera, C.O. (2004). Physicochemical properties of 7S and 11S protein mixtures coagulated by glucono- δ -lactone. *J. Food Sci.*, **69**, FEP139-143.
- Toda, K., Ono, T., Kitamura, K., Hajika, M., and Takahashi, K. (2003). Seed protein content and consistency of tofu prepared with different magnesium chloride concentrations in six Japanese soybean varieties. *Breed. Sci.*, **53**, 217-223.
- Utsumi, S. and Kinsella, J.E. (1985a). Structure-function relationships in food proteins: subunit interactions in heat-induced gelation of 7S, 11S, and soy isolate proteins. *J. Agric. Food Chem.*, **33**, 297-303.
- Utsumi, S. and Kinsella, J.E. (1985b). Forces involved in soy protein gelation: effect of various reagents on the formation, hardness and solubility of heat-induced gels made from 7S, 11S and soy isolate. *J. Food Sci.*, **50**, 1278-1282.
- Utsumi, S., Matsumura, Y., and Mori, T. (1997). Structure-function relationships of soy protein. In "Food proteins and their applications," ed. by S. Damodaran and A. Paraf. Marcel Dekker, New York, pp. 257-291.
- Weitz, D.A. and Huang, J.S. (1984). Self-similar structures and the kinetics of aggregation of gold colloids. In "Kinetics of aggregation and gelation," ed. by F. Family and D.P. Landau. Elsevier Science Publishers B.V., New York, pp. 19-28.
- Weitz, D.A. and Lin, M.Y. (1986). Dynamic scaling of cluster-mass distributions in kinetic colloid aggregation. *Phys. Rev. Lett.*, **57**, 2037-2040.
- Yamauchi, F., Yamagishi, T., and Iwabuchi, S. (1991). Molecular understanding of heat-induced phenomena of soybean protein. *Food Rev. Int.*, **7**, 283-322.