

Homogeneity and Microstructure of Tofu Depends on 11S/7S Globulin Ratio in Soymilk and Coagulant Concentration

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The relationship between the coagulant concentration and soybean protein composition for the finer structure of tofu was investigated using its textural and microstructure data. The textural property of tofu with different ratios of 11S/7S globulin was measured at various concentrations of coagulant, and the tofu structure was observed by scanning electron microscopy. The tofu microstructure had the finest network near its change point of breaking stress (CaSO₄) or breaking strain (glucono- δ -lactone). Thus, the coagulant concentration for obtaining a finer tofu network is important for tofu preparation. At concentrations below and above the optimal coagulant concentration, the tofu network consisted of large cell-like units, while at the optimal concentration it consisted of small, uniform units. The structural change point of tofu rich in 11S globulin was at lower coagulant concentrations (0.15–0.2%) and that rich in 7S was at higher coagulant concentrations (0.3–0.4%)

Keywords: tofu, soymilk, microstructure, tofu, coagulant, SEM

Introduction

Soybean contains 30–40% protein and 20–30% lipid, making them an important dietary source of these components. The major soybean products are soymilk and tofu, which are used in many Asian countries as milk and cheese are used in western countries.

Tofu, a traditional curd-like food prepared from soymilk by the addition of a coagulant, has mild taste. Therefore, its sensory evaluation, especially of its physical property, is important for quality control. Many studies indicate that the factors that influence the physical properties of tofu depend on the differences in components of the soybean varieties and on cultivation conditions (Skurray *et al.*, 1980; Wang and Hesseltine, 1983; Taira, 1990). The use of soybean varieties with high protein content produces tofu with a firmer and springier texture (Wang and Hesseltine, 1983; Shen *et al.*, 1991). Tofu manufacturers in Japan have commonly used soybean varieties, such as Fukuyutaka and Enrei, containing high protein content. The gel hardness from purified soy

proteins is reported to be positively correlated with the 11S (glycinin) content (Saio *et al.*, 1969; Saio, 1979; Kang *et al.*, 1991). In the preparation of tofu curd, a coagulant is added to soymilk, which is comprised of many components including protein, lipid, and sugar. Therefore, the factors that influence curd firmness remain to be clarified (Skurray *et al.*, 1980; Taira, 1990; Murphy *et al.*, 1997). The main components involved in the formation of tofu curd were found to be particle and soluble proteins and lipid globules in the soymilk (Ono *et al.*, 1993; Guo *et al.*, 1999). Curd formation is able to explain a 2 step process: (1) conjugation of protein particles on the surface of lipid globules following the addition of the coagulant and (2) formation of a network of non-repulsive lipid globules covered with protein particles (Ono, 2000). Soybeans with different subunits, including 11S (glycinin) and 7S (β -conglycinin), have been used to produce soymilk with an increased ratio of 11S protein subunits, resulting in the increased number of protein particles and then firmer tofu due to these protein particles covering the oil globules (Tezuka *et al.*, 2000; Guo *et al.*, 2002; Guo and Ono, 2005). Tofu prepared from soybeans with high 11S content has higher breaking stress than that prepared from soybeans with low

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11S content (Cai and Chang, 1999; Yagasaki *et al.*, 2000; Cai *et al.*, 2002; Tay and Perera, 2004; Kim and Wicker, 2005).

Tofu firmness is also dependent on coagulant concentration, which is correlated with both protein concentration and protein composition at low concentrations, and each soybean has a particular coagulant concentration to obtain maximum tofu firmness (Toda *et al.*, 2003). Protein particles in soymilk aggregate at lower coagulant concentration than soluble protein (Ono *et al.*, 1993). Tofu curd from 11S-rich soymilk is formed at a low coagulant concentration due to the greater number of particles in 11S-rich soymilk, as tofu firmness increases relative to the protein particle content (Guo and Ono, 2005). Although coagulant concentration has a strong relationship with protein composition, its relationship with the optimal structure of tofu has not been elucidated. However, scanning electron microscopy (SEM) studies have found that tofu retaining the most soybean proteins and water has more homogeneous and finer microstructure (Kao *et al.*, 2003; Liu *et al.*, 2004).

This study investigated the optimal coagulant concentration relative to the soybean protein composition using textural data and SEM structural data of tofu. The textural property of tofu prepared from soybeans with different ratios of 11S/7S was measured at various concentrations of coagulant, and the tofu structure was observed by SEM.

Materials and Methods

Materials Two soybean (*Glycine max* L.) varieties (Suzukari (S) and Fukuyutaka (F)) (Satoumasayuki Seeds, Ltd., Morioka, Japan) and 2 soybean lines (Tosan 205 (T) and Yumeminori (Y)) containing different ratios of 11S/7S were used in this study. The T line (7S-rich) had a low content of glycinin and was bred as Tosan 205 from Tamahomare (Yagasaki *et al.*, 1997). The Y line (11S-rich) lacked α and α' subunits of β -conglycinin and was bred as Yumeminori (Ogawa *et al.*, 2000). The seeds of these lines were harvested at the Iwate University farm. These soybeans were stored at 5°C until use.

Calcium sulfate dihydrate ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$; Kanto Chemicals Co. Ltd., Tokyo, Japan) or glucono- δ -lactone (GDL; Nacalai Tesque Ltd., Kyoto, Japan) was used as the coagulant. Antifoam AF emulsion (Nacalai Tesque) and all other chemical reagents were of the highest purity available and were used without further purification.

Preparation of soymilk Different ratios of 7S-rich (T) : 11S-rich (Y) soybean seeds (4:0, 3:1, 2:2, 1:3, and 0:4; w/w) were used to prepare 5 types of soymilk with the same protein content but different protein composition. Each soybean mixture (20 g) was soaked in deionized water for 18 h at 4°C. The swollen beans were grounded into a homogenate with

140 mL water at 11,400 rpm for 2 min using a blender (Oster Co., Milwaukee, WI, USA) and then grounded again for 2 min following the addition of 0.02 g antifoam AF emulsion. The homogenate was heated in a boiling water bath for 5 min (> 95°C) and then filtered through a defatted cotton sheet. The filtrate was quickly cooled to 20°C in ice water and was used as soymilk for the subsequent experiments.

This method was also used to prepare soymilks from two soybean varieties (F and S).

Preparation of tofu curd Tofu curd (filled tofu) was prepared as previously reported (Ishiguro *et al.*, 2006). Briefly, GDL powder or freshly prepared 10% $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ suspension was added to 50 mL soymilk at 20°C or 13°C, respectively, in a beaker; the GDL or $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ concentration was adjusted to 0.15%, 0.20%, 0.30%, and 0.45%. The mixture of soymilk and coagulant was mixed quickly and immediately distributed into 2 25-mL plastic syringes (20 mm diameter and 90 mm height) and then allowed to stand in an 80°C water bath for 1 h. The coagulated soymilk (tofu curd) was then cooled at room temperature and stored in a refrigerator at 4°C overnight for aging of tofu curd.

Texture analysis of tofu curd Before the texture analysis, the tofu samples stored at 4°C were allowed to stand at room temperature for 1 h. The tofu curds in the plastic containers were carefully removed and cut into a columned sample of 13 mm height (20 mm diameter) with a thin copper wire. A penetration test was carried out at a compression rate of 2 cm/min using a rheometer (Fudoh, Rheotech Co., Tokyo, Japan) with an 8-mm diameter cylindrical plunger. Breaking stress, breaking strain, and Yang's modulus of the curds were obtained as mean values of 5 measurements with standard deviation using the analysis software of the instrument.

Determination of the water holding capacity of tofu The water holding capacity (WHC) of tofu was determined by a modification of the water retaining amount (WRA) method of Kao *et al.* (2003). A tofu disk (20 mm diameter and 13 mm height) weighing W_b was placed on three sheets of filter paper (No.2; Advantec MFS, Inc., Tokyo, Japan) in a plastic vessel, stored for 1 h after the vessel was covered, and the sample weight was then recorded (W_a). The tofu sample was subsequently dried at 105°C for 6 h to a constant weight (W_d). The WHC of tofu was calculated using the following equation:

$$\text{WHC (\%)} = (W_a - W_d) / (W_b - W_d) \times 100$$

Determination of the major components of soymilk The protein, lipid, and carbohydrate contents in each soymilk mixture were determined by the method of Nakasato *et al.* (2004) using an FT-IR spectrophotometer (Spectrum 2000,

Perkin-Elmer, Beaconsfield, England) equipped with a horizontal-ATR 45° ZnSe crystal cell (mirror angle, 45°). The protein, lipid, and sugar contents were calculated from the absorbance values at 1545, 1745, and 1000 cm^{-1} , respectively.

Scanning Electron Microscopy A 1-mm thick tofu sample from the plastic container was prepared for SEM. The sample was fixed at room temperature with 1% glutaraldehyde for 1 h, washed with deionized water, flash frozen in liquid nitrogen, and freeze-dried. The dried sample was mounted with a carbon adhesion tape on an aluminum stub and then post-fixed with 4% osmium tetroxide vapor for 1 h. This fixed sample was sputter coated with gold (10 nm thickness) and then analyzed with a JSM-5800LV scanning electron microscope (JEOL Datum, Ltd., Tokyo, Japan) at 7 kV. Magnification used for the observation ranged from 1000 to 10,000.

Electrophoresis To determine the relative ratio of 11S/7S, sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out on a 1-mm thick vertical slab gel using an alkaline discontinuous buffer (Laemmli, 1970). A broad range of molecular weight standard proteins was prepared from bovine serum albumin (66,000), ovalbumin (45,000), β -casein (24,000), and lysozyme (13,000). The protein bands stained with Coomassie brilliant blue G-250 in the gel were scanned with a CanoScan L50 instrument (Canon Co., Tokyo, Japan), and the stain intensities were then analyzed using Scion Image PC software (Scion Co., Frederick, MD, USA).

Results

Preparation of soymilk with adjusted protein composition Soymilk with various 11S/7S ratios was prepared from different ratios of 7S-rich (T) and 11S-rich (Y) soybeans (T/Y = 4:0, 3:1, 2:2, 1:3, and 0:4). The content of the major components (protein, lipid, and sugar) and 11S/7S ratios are shown in Table 1. The concentration of each component was not significantly different among the preparations (inside the

range of their standard deviations), while the difference in the 11S/7S ratio varied from 0.47 to 4.35. Therefore, they were all used for tofu preparation.

Textural property of tofu prepared from adjusted soymilks Tofu was prepared by adding a coagulant ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ or GDL) with a concentration of either 0.15%, 0.2%, 0.3%, or 0.45% to the adjusted soymilk preparations. Analysis of the texture of tofu using a rheometer measured the values of breaking stress, Yang's modulus, and breaking strain, as well as the WHC of tofu (Figs. 1 and 2). For $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ coagulant, the breaking stress of tofu increased up to a concentration of 0.3% and then reached a constant at concentrations more than 0.3% (Fig. 1a). This tendency was significant in 4:0 (T/Y) tofu, and the concentration of the changing point decreased gradually in the order of 3:1, 2:2, 1:3, and 0:4. As the 11S content increased (T/Y from 4:0 to 0:4), the maximum breaking stress was obtained quicker at low concentrations of coagulant. The Yang's modulus values of tofu of various T/Y ratios (Fig. 1b) showed a similar tendency to those of breaking stress. The breaking strain values of 4:0 and 3:1 tofu increased for $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ concentrations of up to 0.03% and maintained a constant value at concentrations > 0.3% (Fig. 1c); however, values of 2:2, 1:3, and 0:4 tofu maintained a constant value from a low coagulant concentration (0.05%). The width of 0:4 tofu was narrower than others, probably due to the absence of α , α' of 7S globulin. In addition, the WHCs of these tofu samples decreased with increasing coagulant concentration despite the T/Y ratio (Fig. 1d). The WHC of Yumeminori tofu (T/Y = 0:4) was the lowest, most likely due to the absence of α , α' of 7S globulin.

These results show that the textural properties of tofu containing a higher 11S protein content reached the maximum value at lower coagulant ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) concentration; however, the WHC of tofu decreased with increasing coagulant concentration despite protein composition.

For GDL as the coagulant, the breaking stress (Fig. 2a) and Yang's modulus (Fig. 2b) values of tofu increased with increasing coagulant concentration, but the extent of increase

Table 1. Contents of major components (%) and 11S/7S ratios of soymilks prepared from mixed soybeans of Tosan 205 (T) and Yumeminori (Y), Suzukari (S), and Fukuyutaka (F).

T/Y	4:0	3:1	2:2	1:3	0:4	S	F
Protein	3.87 ±0.07	3.88 ±0.01	3.92 ±0.07	3.88 ±0.07	3.81 ±0.09	3.94 ± 0.05	3.93 ± 0.08
Lipid	1.71 ± 0.10	1.58 ± 0.09	1.61 ± 0.11	1.63 ± 0.09	1.77 ± 0.11	1.43 ± 0.10	1.76 ± 0.10
Sugar	1.47 ± 0.04	1.44 ± 0.02	1.48 ± 0.05	1.40 ± 0.03	1.42 ± 0.02	1.43 ± 0.03	1.32 ± 0.03
11S/7S	0.47	0.85	1.34	2.08	4.35	1.23	2.38

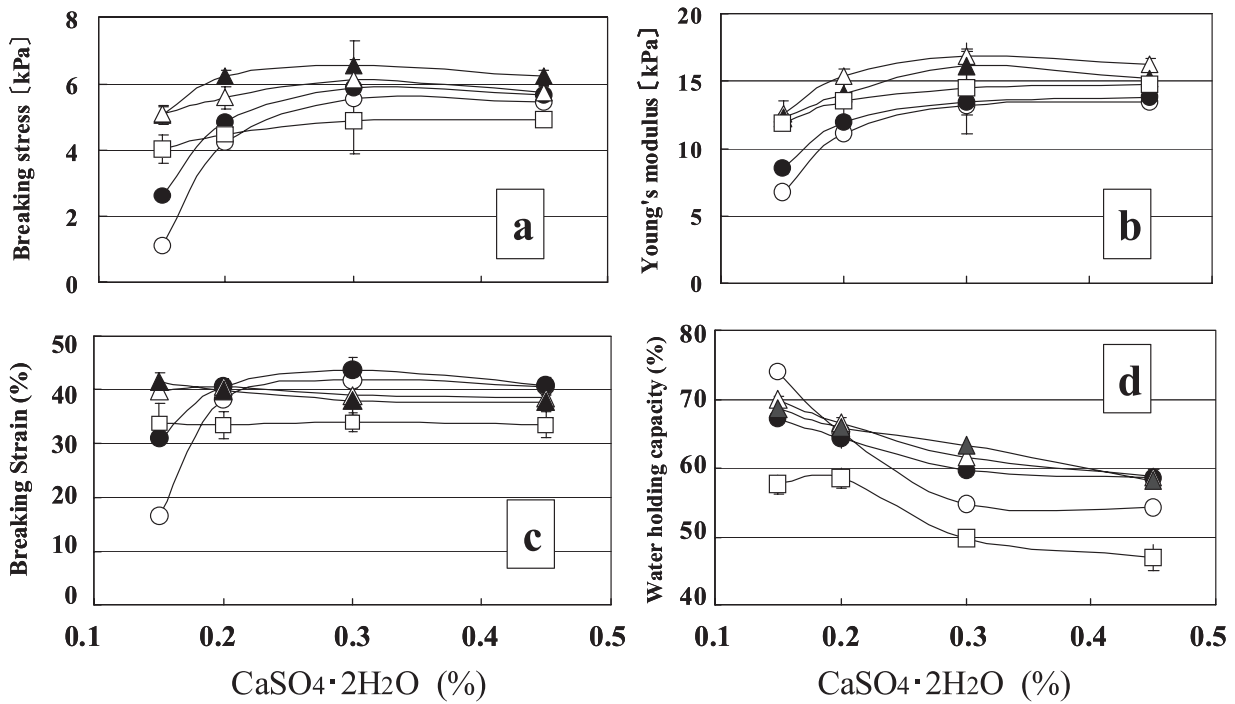


Fig. 1. Changes in the textural properties of tofu prepared from adjusted soymilk relative to the coagulant ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) concentration. a, breaking stress; b, Yang's modulus; c, breaking strain; d, water holding capacity. Adjusted soymilk from soybeans with different Tosan 205 (T):Yumeminori (Y) ratios; ○ is 4:0; ● is 3:1; △ is 2:2; ▲ is 1:3; ■ is 0:4.

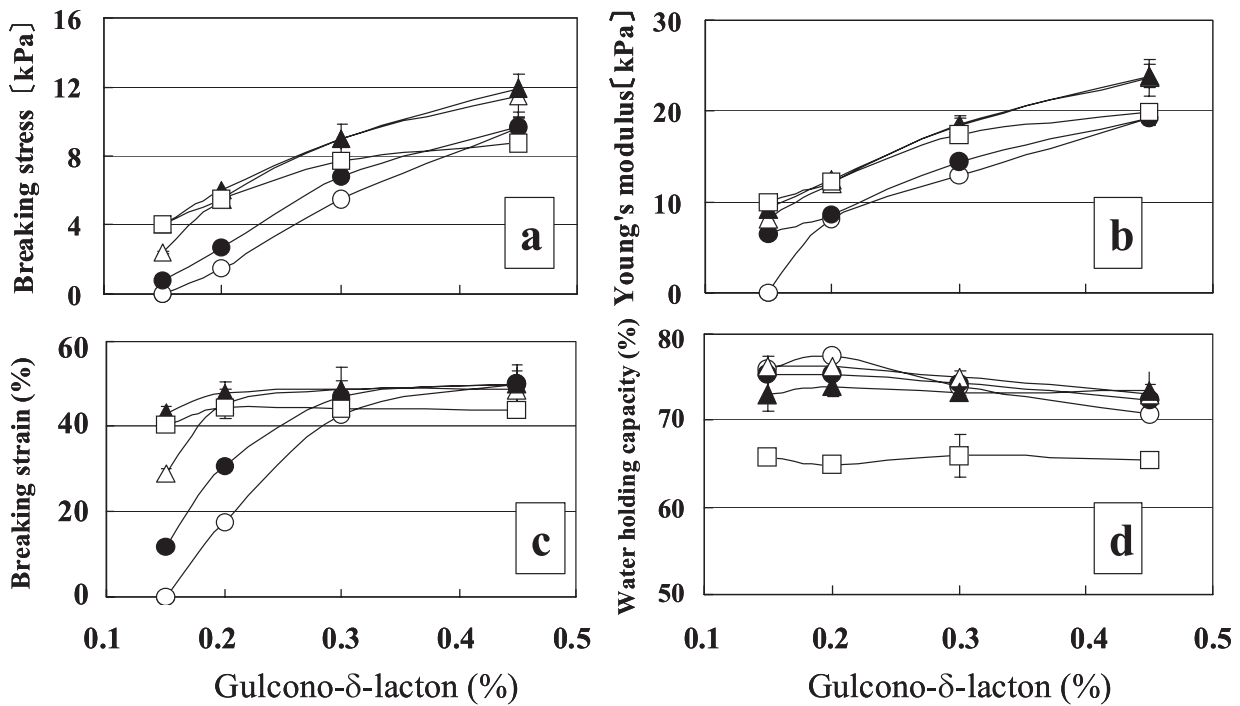


Fig. 2. Changes in the textural properties of tofu prepared from adjusted soymilks against coagulant (glucono- δ -lactone) concentration. a, breaking stress; b, Yang's modulus; c, breaking strain; d, water holding capacity. Adjusted soymilk from soybeans with different Tosan 205 (T):Yumeminori (Y) ratios; [is 4:0; ● is 3:1; r is 2:2; s is 1:3; □ is 0:4.

decreased with increasing 11S content (T/Y from 4:0 to 0:4). The breaking strain values increased up to 0.3% GDL concentration and maintained a constant value at concentrations >0.3% (Fig. 2c). This tendency was again significant in 4:0 tofu, and the concentration of the changing point decreased gradually in the order of 3:1, 2:2, 1:3, and 0:4. The increase in 11S protein content resulted in the maximum value at a low coagulant concentration. The width of 0:4 tofu was also the narrowest. The WHCs of these tofu samples showed no change despite increasing coagulant concentration and T/Y ratio (Fig. 2d). The WHC of Yumeminori tofu (T/Y = 0:4) was also the lowest (Fig. 1d).

SEM of tofu at various coagulant concentrations Figures 3 and 4 show the SEM of microstructures of tofu prepared from soymilk with different 11S/7S ratios and 0.15%, 0.3%, and 0.45% $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ and GDL, respectively. As shown in Fig. 3a, 4:0 tofu had large cells (>10 μm) with a flat wall at 0.15% $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$. The structure showed fine uniform cells (< 5 μm) at 0.3% (Fig. 3b), and larger non-uniform cells (about 5 – 15 μm) at 0.45% (Fig. 3c). On the other hand, 2:2 tofu also had large cells (> 10 μm), but not a flat wall at 0.15% $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ (Fig. 3d), fine uniform cells at 0.3% (Fig. 3e), and large non-uniform cells at 0.45% (Fig. 3f). As shown in Fig. 3g-i, the structure of 0:4 tofu had fine uniform cells, large non-uniform cells, and larger non-

uniform cells (about 5 – 20 μm) at 0.15%, 0.3%, and 0.45% $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, respectively.

As shown in Fig. 4a, 4:0 tofu had large cells (> 10 μm) with a flat wall at 0.15% GDL. The structure of this tofu showed fine uniform cells (< 5 μm ; Fig. 4b) and larger non-uniform cells (about 5 – 15 μm ; Fig. 4c) at 0.3% and 0.45% GDL, respectively. On the other hand, 2:2 tofu also had large cells but not so thin wall at 0.15% GDL (Fig. 4d), fine uniform cells at 0.3% (Fig. 4e), and large non-uniform cells (> 10 μm) at 0.45% (Fig. 4f). As shown in Fig. 4g-i, the structure of 0:4 tofu had fine uniform cells, larger non-uniform cells, and large non-uniform cells at 0.15%, 0.3%, and 0.45% GDL, respectively.

Textural properties of tofu prepared from commercial soybeans Soymilk prepared from Suzukari or Fukuyutaka soybeans were examined for the content of their major components (protein, lipid, and sugar) (Table 1). Both soybean varieties had similar protein content. The 11S/7S ratios in Suzukari and Fukuyutaka were 1.23 and 2.38, respectively. Tofu was prepared from Suzukari or Fukuyutaka soymilk with 0.15%, 0.2%, 0.3%, and 0.45% coagulant concentrations ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ or GDL). The texture of these tofu samples was analyzed using a rheometer. The breaking stress and strain values for $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ coagulant are shown in Fig. 5a and 5b. The breaking stress of tofu prepared from Suzukari

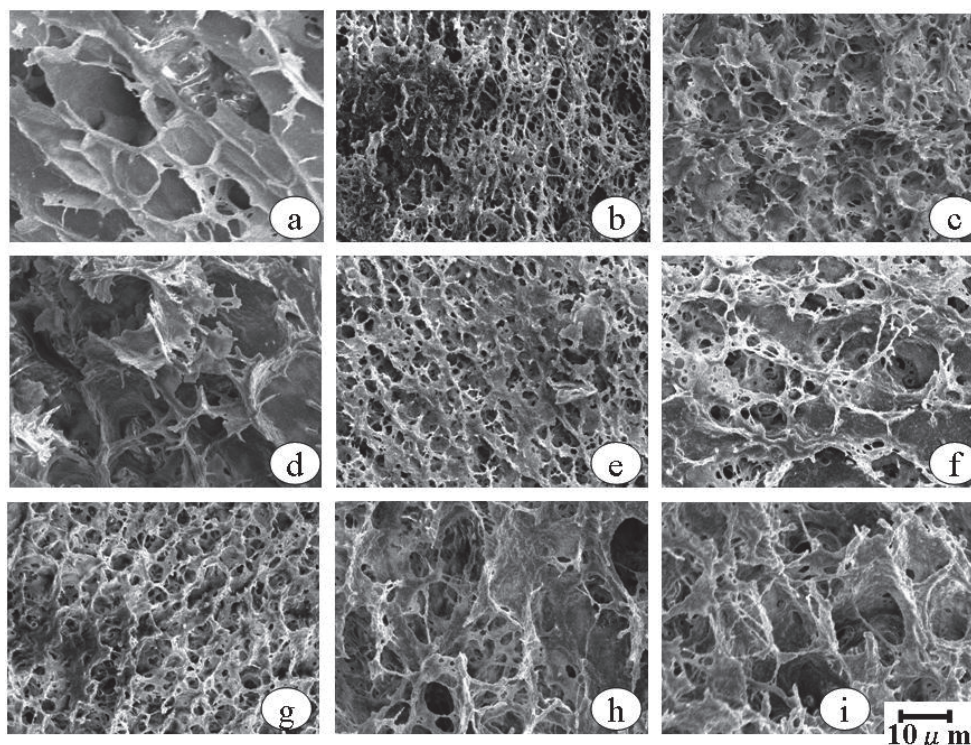


Fig. 3. Scanning electron micrographs of tofu prepared from adjusted soymilks and various coagulant ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) concentrations.

Adjusted soymilks were prepared from soybeans with different Tosan 205:Yumeminori ratios: 4:0 (a-c); 2:2 (d-f); 0:4 (g-i). Coagulant concentrations are 0.15% (a, d, and g), 0.3% (b, e, and h), and 0.45% (c, f, and i).

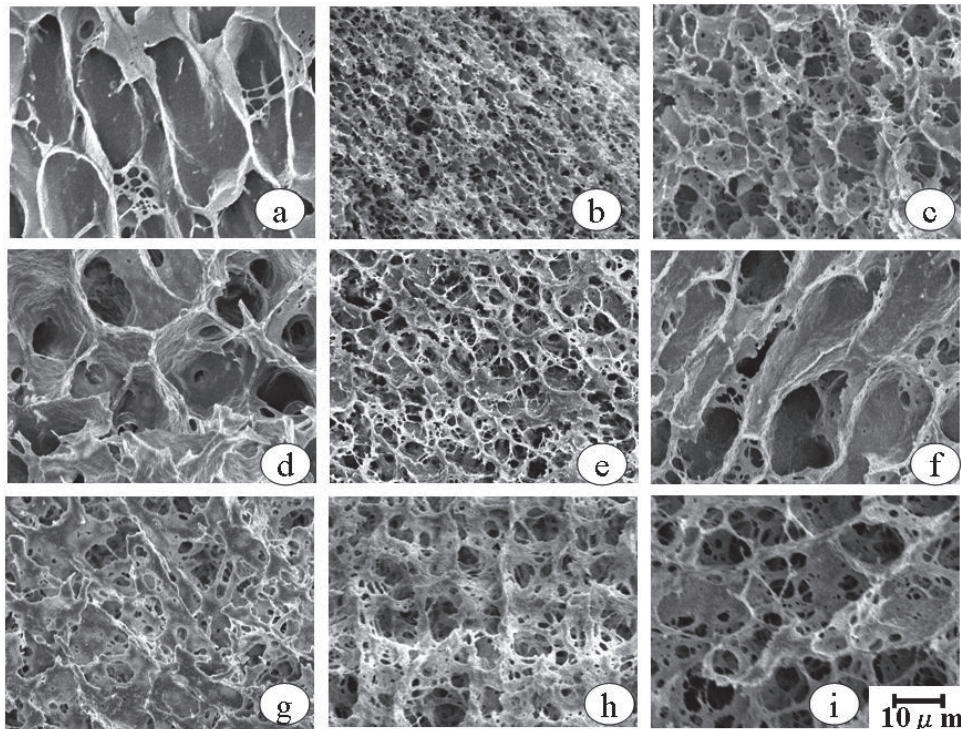


Fig. 4. Scanning electron micrographs of tofu prepared from adjusted soymilks and various coagulant (glucono- δ -lactone) concentrations.

Adjusted soymilks were prepared from soybeans with different Tosan 205:Yumeminori ratios; 4:0 (a,b,c); 2:2 (d-f); 0:4 (g-i). Coagulant concentrations: 0.15% (a, d, and g), 0.3% (b, e, and h), and 0.45% (c, f, and i).

increased up to 0.3% coagulant concentration and attained a constant value at concentrations more than 0.3% (Fig. 5a). On the other hand, the breaking stress of tofu prepared from Fukuyutaka increased until 0.2% coagulant concentration and attained a constant value at higher concentrations. The breaking strain values of Suzukari tofu increased up to 0.3% coagulant concentration and attained a constant value at concentrations above 0.3% (Fig. 5b), but the breaking strain values of Fukuyutaka tofu were constant right from 0.15% coagulant concentration. These results show that the textural properties of tofu with more 11S protein content attained the maximum value at lower coagulant ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) concentration.

The textural properties of tofu when GDL was used as a coagulant are shown in Fig. 5c and 5d. The breaking stress (Fig. 5c) of Suzukari tofu (11S/7S ratio = 1.23) increased with increasing GDL coagulant concentration, but the extent of increase was decreased in Fukuyutaka tofu with more 11S content (11S/7S ratio = 2.38). The breaking strain values of Suzukari tofu increased at concentrations up to 0.3% GDL and then maintained at a constant value (Fig. 5d), but those of Fukuyutaka tofu increased up to 0.2% concentration. Thus, Fukuyutaka tofu containing a higher 11S protein content reached stable values of breaking stress and strain at lower coagulant concentrations.

SEM of tofu prepared from commercial soybeans at various coagulant concentrations Figure 6 shows the microstructures of tofu prepared from soymilk of Suzukari and Fukuyutaka with 0.15%, 0.30%, and 0.45% $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$. Suzukari tofu had large cells ($> 10 \mu\text{m}$) with a flat wall at 0.15% $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ (Fig. 6a). This tofu showed large cells (about 5 – 15 μm) at both 0.3% and 0.45% $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ (Fig. 6b and 6c, respectively). Fine uniform cells may be observed between 0.3% and 0.4% concentration. Fukuyutaka tofu had fine uniform cells ($< 5 \mu\text{m}$) at 0.15% $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ (Fig. 6d) and large cells at 0.3% (Fig. 6e) and 0.45% (Fig. 6f). The microstructures of tofu from Suzukari and Fukuyutaka at various GDL concentrations showed similar tendency as those for $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ (data not shown).

Discussion

Tofu is prepared from soymilk by the addition of a coagulant. The initiation of tofu curd formation involves 2 steps: the conjugation of protein particles on the surface of lipid globules following the addition of a coagulant and the formation of a network of retained water molecules with the non-repulsive lipid globules covered with protein particles (Ono, 2000).

Protein in soymilk is in particle and soluble forms (Ono *et al.* 1991); the particle protein aggregates at lower con-

centrations of coagulant than the soluble protein (Ono *et al.*, 1993). The increase in 11S globulin content induces an increase in the particle protein content in soymilk, and 11S-rich soymilk form harder tofu curd at low coagulant concentration (Guo and Ono, 2005). On the other hand, 7S-rich soymilk requires more coagulant to form a hard tofu curd (Fig. 1). The breaking stress (using CaSO_4) or breaking strain (using GDL) of tofu from Tosan 205 (T:Y = 4:0) with poor

11S content compared to that from tofu with a T:Y ratio of 3:1 increased significantly at coagulant concentrations between 0.15% and 0.3% and reached a maximum at concentrations above 0.3%. The tofu from Yumeminori (T:Y = 0:4) without α and α' subunits had inadequate hardness but hardened at low coagulant concentrations in a manner similar to tofu prepared from 11S-rich soymilk. As the increase in 11S globulin content induces an increase in the particle protein content in

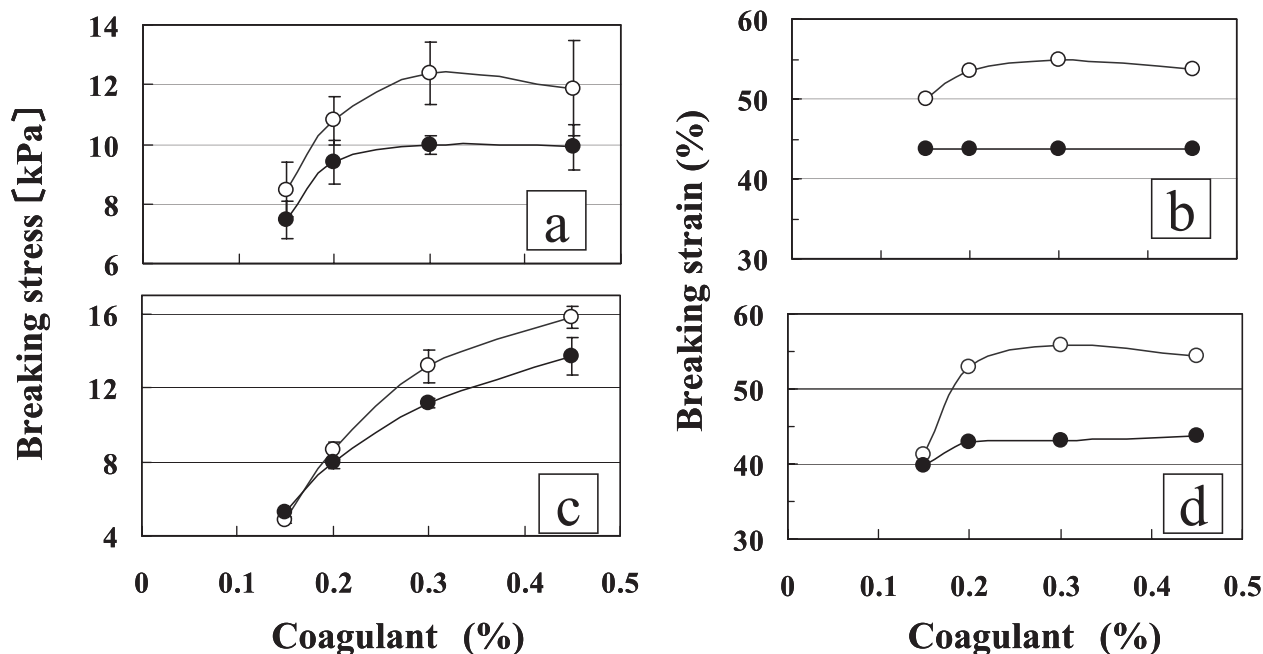


Fig. 5. Changes in the textural properties of tofu prepared from commercial soybeans (Suzukari and Fukuyutaka) against various coagulant ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ and GDL) concentrations.

Coagulant are $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ (a and b), and glucono- δ -lactone (c and d). \circ is Suzukari and \bullet is Fukuyutaka commercial soybeans.

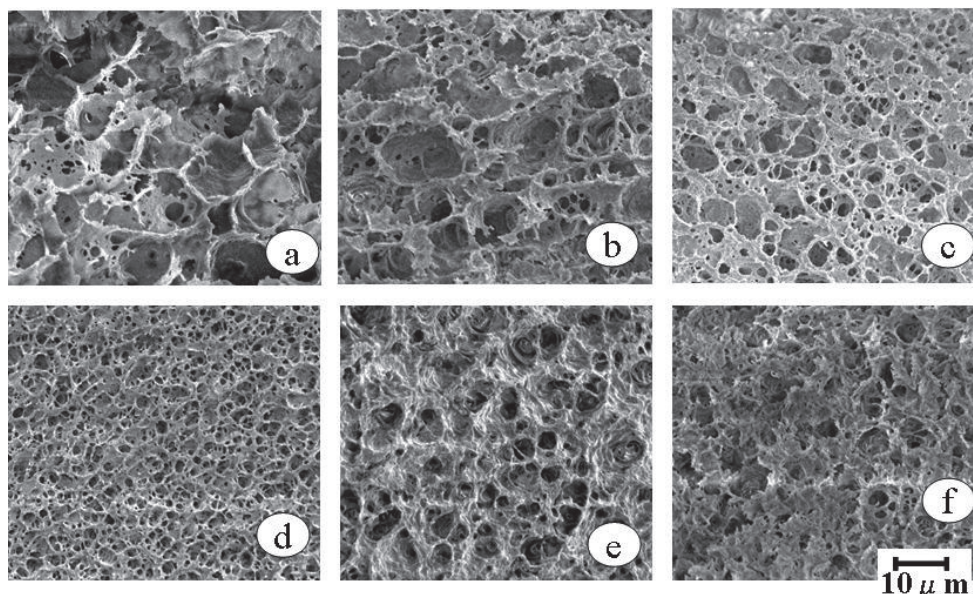


Fig. 6. Scanning electron micrographs of tofu prepared from soymilks of commercial soybeans (Suzukari and Fukuyutaka) and various coagulant ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) concentrations.

Suzukari: a, b, c; Fukuyutaka: d, e, f. Coagulant concentrations are 0.15% (a and d), 0.3% (b and e), and 0.45% (c and f).

soymilk, a firmer tofu curd is formed at low coagulant concentration due to the sensitivity of the particle protein to the coagulant (Ono *et al.*, 1993).

Whey protein aggregated by heating also plays a role in the formation of protein particles in soymilk (Ren *et al.*, 2009). The ratio of whey protein in soymilk is less than 10% of total protein (Rackis *et al.*, 1971) and changes slightly with the change in the 11S/7S ratio. Therefore, it has no effect on variations in particle protein content in soymilk relative to the ratio of 11S/7S globulin, suggesting at potential use of soybean protein and whey protein.

Tofu curd forms through a heterogeneous concentration system that dissolves CaSO_4 (suspended reagent) or through a homogeneous concentration system that suspends and dissolves GDL homogeneously in soymilk. When more CaSO_4 is added to soymilk, the tofu becomes more heterogeneous, causing more cracks in the curd. Therefore, the breaking stress of CaSO_4 -tofu does not increase after the network formation at the optimal coagulant concentration. Since GDL-tofu does not form cracks in the curd, the breaking stress increases relative to a decrease in pH at coagulant concentration higher than optimum. The breaking strain may be due

to the bonding of the tofu network that is dependent on the solubility of proteins relative to pH. The lowest solubility region of soybean proteins has a wide range of pH (Tandang *et al.*, 2005). Therefore, the breaking strain of GDL-tofu may reach a maximum plateau at higher concentrations than optimum.

We used a simple procedure for SEM sample preparation in which a glutaraldehyde fixation of 1-mm thick samples, flash frozen by liquid nitrogen and freeze-drying, instead of the critical point drying, to remove water from the tofu specimen. The results indicate that this procedure is suitable for drying samples containing many oil bodies for examination by SEM and does not cause the tofu network structure to disrupt. The flash freezing by liquid nitrogen is used for cryo-SEM by which the structure containing water is observed.

Review of the SEM results showing the tofu network structure relative to the coagulant concentrations (Fig. 7) shows that at low coagulant concentration, the tofu network has a large cell-like structure and at the change point of tofu hardness (from increase to constant), the network has fine uniform cells. At higher coagulant concentrations, the tofu cells increase in size with a loss in smoothness of the

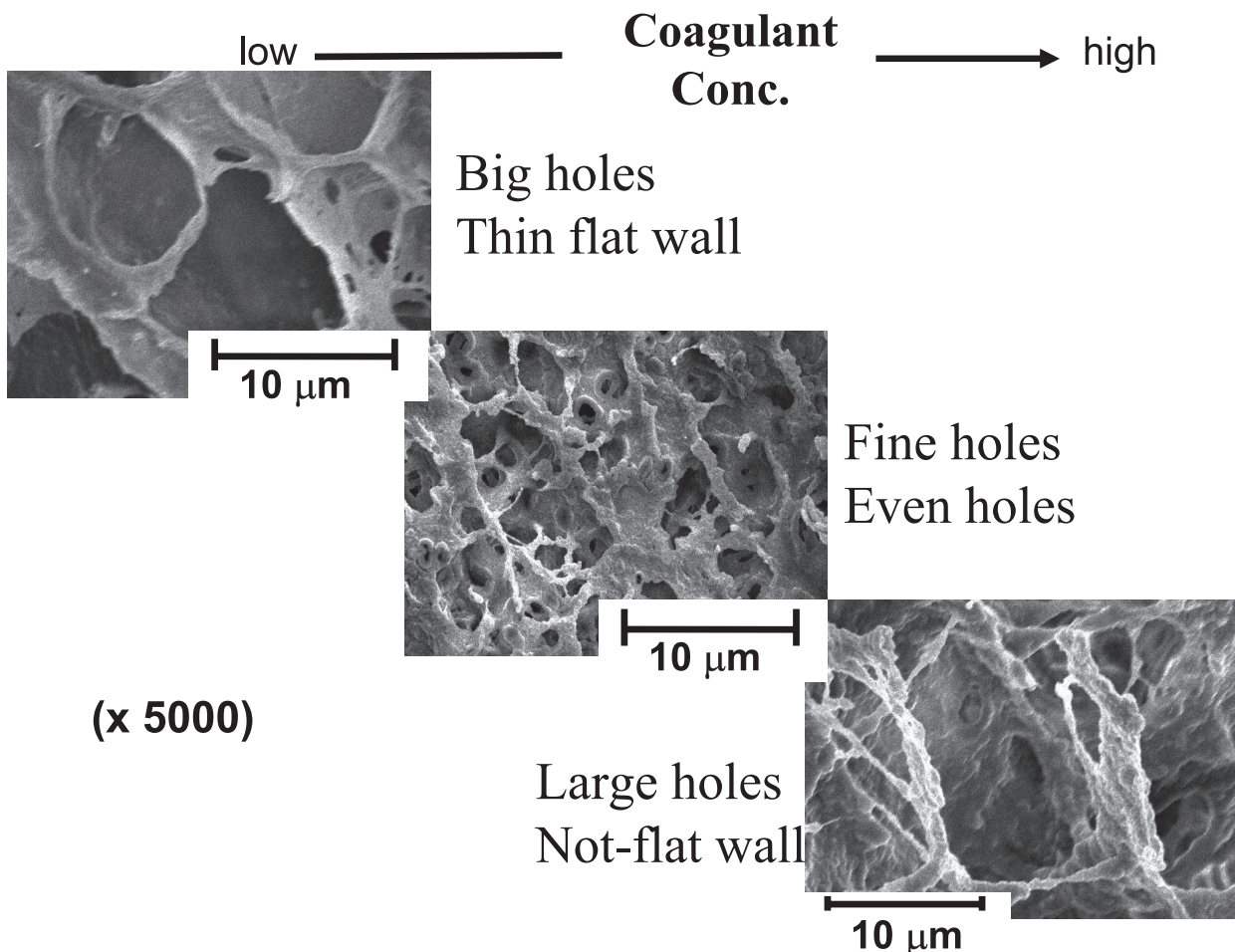


Fig. 7. Review of SEM photographs of the tofu network structure against coagulant concentrations compiled from Figs. 3 and 4.

cell wall. In the case of tofu containing more 11S protein, the change point of tofu hardness was at a lower coagulant concentration, with a subsequent shift in the formation of a network of fine uniform cells (Figs. 3 and 4). Therefore, the tofu network has a uniform finest structure at the change point. Kao *et al.* (2003) and Liu *et al.* (2004) reported that tofu retains the most soybean proteins and water content in homogeneous, finer microstructures using SEM, suggesting that almost all proteins in soymilk are incorporated into the tofu network at the change point of tofu breaking stress (using CaSO_4) or breaking strain (using GDL) and that the finer tofu network is formed by bonding of small uniform cells. The WHC of tofu decreased with increasing coagulant (CaSO_4) concentration (Fig. 1) and did not reach the maximum at the change point of tofu breaking stress. The WHC of tofu should increase at values farther from the isoelectric point of the protein due to the hydrophilic characteristics. The addition of CaSO_4 coagulant may decrease the pH of soymilk near the isoelectric point of protein (Ono *et al.*, 1993), resulting in a decrease in WHC of filled tofu with decreasing pH. On the other hand, the WHC of tofu with GDL hardly changed by the addition of the coagulant, indicating a more homogeneous tofu network formed by GDL than that formed by CaSO_4 , as the tofu network is formed after the coagulant is mixed and solubilized homogeneously in soymilk. The tofu network even for filled tofu forms some cracks using CaSO_4 but forms few cracks using GDL.

Toda *et al.* (2003) found that each soybean variety requires a particular coagulant concentration to achieve the maximum tofu firmness. Fukuyutaka variety reaches maximum tofu firmness at a lower coagulant concentration than Sachiyutaka variety. Furthermore, the breaking stress of Fukuyutaka at 0.25% MgCl_2 has been reported to be higher than that of Sachiyutaka, but lower at 0.40% MgCl_2 . We found that the 11S protein content of Fukuyutaka was higher than that of Sachiyutaka (data not shown). In this study, the 11S/7S ratios of Fukuyutaka and Suzukari are 2.38 and 1.23, respectively (Table 1), and Fukuyutaka contains more 11S protein than Suzukari. The change points of breaking stress of Fukuyutaka and Suzukari tofu were at 0.2% and 0.3% coagulant concentrations, respectively (Fig. 5). The Fukuyutaka tofu network formed a fine structure at 0.15% coagulant concentration (Fig. 6), while that of Suzukari tofu formed a fine structure between 0.3% and 0.45% coagulant concentrations.

Conclusions Tofu microstructure has the finest network near the change point of breaking stress (using CaSO_4 coagulant) or breaking strain (using GDL coagulant) of tofu. The change point of tofu rich in 11S globulin occurs at lower coagulant concentrations (0.15~0.2%) and that of tofu rich in 7S occurs at higher concentrations (0.3~0.4%).

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