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Effect of Calcium Sulfate Concentration in Soymilk on the Microstructure of Firm Tofu and the Protein Constitutions in Tofu Whey

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The effects of calcium sulfate concentration in soymilk on the microstructure of tofu and the constitutions of protein in tofu whey were investigated. The firm tofu made with 0.4% $CaSO_4 \cdot 2H_2O$ was found to be most uniform and continuous in the microstructure. This tofu gave the maximal tofu yield, maximal solid and protein recoveries, and the best water retention ability. The results of gel electrophoresis and the ratio of amino nitrogen to total organic nitrogen indicated that the low molecular weight proteins in tofu whey were at their lowest when the tofu was made with 0.4% $CaSO_4 \cdot 2H_2O$. The SEM observations suggested that the tofu made with 0.4% $CaSO_4 \cdot 2H_2O$ has the most uniform and homogeneous microstructure and, consequently, can most efficiently retain soybean proteins and water in the tofu gel.

KEYWORDS: Firm tofu; microstructure; calcium sulfate; tricine SDS−PAGE

INTRODUCTION

Tofu is one of the most popular food items in the traditional oriental diet (*1*). Because of its bland taste, the textural properties markedly influence its food quality and consumer acceptability (*2*). Since the 1970s, the SEM and other analyses using instruments have been used to research tofu and have provided a more stereoscopic image of the fine structure of tofu. Saio studied the relationship between fine structure and texture of tofu and concluded that the microstructure of tofu varied with the density of the gel network, the size of protein granules in the network, and the size of coagulate prepared during the coagulating process (*3*). All of them correlated with the hardness of tofu. Furthermore, the fine structure of tofu is subject to change during subsequent processing, such as deep-fried tofu and frozen tofu (*3*). These processes lead to changes in the texture of the tofu. deMan et al. (*2*) reported that the texture and microstructure of the soybean curd were greatly influenced by the type of coagulant used. They found that curd produced by GDL and $CaSO_4$ gave a much finer and a more uniform honeycomb-like structure than the curd prepared with $CaCl_2$, $MgCl_2$, and $MgSO_4$ (*2*). Tsai et al. (*1*) considered that GDL and $CaSO_4$ were the most suitable coagulants for tofu making and gave a higher yield than other calcium salt coagulants, but the former was not suitable for Chinese style tofu due to its sour taste.

As with the type of coagulant, the concentration of the coagulant also affects the nature of tofu. The amount of coagulant added in tofu manufacturing is one of the critical control points, and it determines crucially the product's texture, flavor, and yield (*4*). The correlation of tofu properties and the coagulant concentrations has been reported by many research groups (*1*, *2*, *5*−*13*). However, no definite conclusion was drawn. Because of the limited solubility of calcium sulfate, it was reported that a proper coagulant concentration for tofu making depends not only on coagulation temperature (*7*), stirring speed, and time (*9*, *11*) but also on the stirring blade size, mixing tank dimension, and soybean variety (*10*). Various optimal concentrations from 9.5 to 58 mM (0.16−1.0%) $CaSO_4 \cdot 2H_2O$ in soymilk have been reported as appropriate for production of tofu (*1*, *2*, *5*−*13*). However, very few reports concerning the effects of coagulant concentration on the microstructure of tofu were reported. Additionally, the proteins in tofu whey are usually regarded as the LMW proteins (*14*, *15*); however, there were no direct evidences. In this study, we investigate the effects of coagulant concentration on the microstructure of tofu, as well as the protein constitutions in tofu whey.

MATERIALS AND METHODS

Materials. Soybeans of the Ohio FG1 cultivar were obtained from a local agency. Food grade calcium sulfate dihydrate (Ako Kasei Co., Ltd., Hyogo, Japan) was used as the coagulant in the tofu production and was obtained from Gemfont Corporation (Taipei, Taiwan).

Tofu Making. Tofu was produced by the traditional method as follows. Beans (300 g) were soaked in tap water (three times bean weight) at room temperature for 9 h to bring the soaked beans weight to approximately 2.2 times their initial weight. Hydrated beans were drained and ground with an amount of tap water to make the total weight of 3300 g in a grinder (Pineapple grinder, Great Yen Electric Food Grinder Co., Ltd., Taipei, Taiwan) equipped with an automatic centrifugal filter to separate raw soymilk from the residue. This corresponded to a water:soybean ratio of 10:1 by weight for extracting solids from soybean into the raw soymilk and brought the total solid content of soymilk to approximately 6%. After adding 1 g of antifoaming agent (containing 90% glycerin fatty acid ester, 5% calcium

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carbonate, 4.3% soybean phospholipids, and 0.7% silicone resin), the raw soymilk was heated to 95 °C and held at this temperature for 5 min. After the soymilk was cooled to about 73 °C, 50 mL of various concentrations of calcium sulfate solution was added to soymilk with stirring at a speed of 250 rpm for 10 s and then incubated for 20 min to form bean curd. The final $CaSO_4 \cdot 2H_2O$ concentrations used in the soymilk were 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, and 0.50% (w/v). The bean curd was then broken slightly and transferred into a muslin cloth-lined stainless steel mold ($13 \times 13 \times 5.5$ cm^3) and then pressed at 21.8 g/cm^2 for 10 min, 43.6 g/cm^2 for 10 min, and 65.4 g/cm^2 for 30 min. At the end of pressing, the cloth was removed and the weight of tofu and the volume of drained whey were recorded. The tofu yield was expressed as grams of fresh tofu per 100 g of soybeans. The tofu was transferred into a plastic bag and stored at 4 °C overnight for texture measurement.

Determination of the Protein and Solid Contents and Their Recoveries. The protein contents in soybean, soymilk, tofu, and tofu whey were determined in triplicate by the micro-Kjeldahl method (*16*). The solid content of tofu was determined by drying 5 g of homogenized tofu in an oven at 105 °C until constant weight was obtained. Protein recovery in tofu was expressed as the amount of protein in tofu divided by the amount of protein in raw soybeans multiplied by 100% on a dry weight basis. The same calculation was applied to tofu solid recovery.

Determination of WRA of Tofu. WRA was determined by a modification of the water holding capacity method (WHC) of Puppo and Añón (*17*). About 5 g (w_1) of tofu was placed on a cotton cloth membrane maintained in the middle position of a 250 mL centrifuge tube (62 mm × 120 mm). The sample weight was recorded after centrifugation at 120*g* for 5 min at 15 °C (w_2) and subsequently heated to a constant weight (w_3) at 105 °C. The WRA of tofu was calculated as the following equation:

$$\mathrm{WRA} = ((w_2 - w_0)/(w_1 - w_0)) \times 100\%$$

Determination of Textural Properties. Textural properties of tofu were measured by a texture analyzer (model TA-XT2, Stable Micro systems, Haslemere, Surrey, U.K.). A 5 kg load cell was used with the crosshead controlled at 1.5 mm/s. A cylindrical plunger with a diameter of 35 mm was used to compress the tofu cakes (35 mm diameter × 22 mm height) to 50% deformation. Hardness, cohesiveness, gumminess, springiness, and chewiness were calculated using the textural profile analysis curve (*18*). Hardness was defined as the height of the force peak on the first compression cycle, which was the force necessary to attain a given deformation. The ratio of the positive force areas under the second and the first compression was defined as cohesiveness. Gumminess was defined as the product of hardness and cohesiveness. The springiness was the degree to which the tofu returned to its original shape after it had been decompressed and was expressed as the horizontal distance (mm) between the point when the second curve started and the point when the second curve reached a peak. Chewiness was defined as the product of gumminess and springiness.

SEM. A JEOL JSM-6300 scanning electron microscope (JEOL USA, INC., Peabody, MA) was used to examine the fine structure of tofu coagulated with different $CaSO_4 \cdot 2H_2O$ concentrations. The procedure used for sample preparation was that of deMan et al. (*2*) with some modifications. A small piece of each tofu sample was fixed at room temperature with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.1) for 2 h. After five washings with 0.1 M phosphate buffer (pH 7.1) at 10 min intervals, it was then postfixed with 1% osmium tetroxide in the same buffer for 90 min at room temperature. The fixed sample was rinsed five times with phosphate buffer at 10 min intervals. The sample was frozen in liquid nitrogen and freeze-dried. The dried sample was finally mounted on an aluminum stub and sputter-coated with gold/palladium (60/40). The observations were carried out at 20 kV.

Preparation of Soymilk Protein and Tofu Whey Protein. The preparation of soymilk protein was based on a method modified from Cai and Chang (*19*). Fifty milligrams of freeze-dried soymilk was dispersed in 1 mL of buffer (86 mM Tris-90 mM glycine-4 mM Na_2·EDTA, pH 8.0). The mixture was sonicated at 60 °C for 90 min to extract the proteins. The extract was then centrifuged at 12 000*g* for 20 min. The supernatant was collected and regarded as the soymilk protein extracted. Tofu whey protein was extracted according to a method modified from Sorgentini and Wagner (*20*). Tofu whey was centrifuged at 6000*g* for 20 min at 20 °C. The clarified supernatant was adjusted to pH 8.0 with 2 N NaOH, kept for 1 h at room temperature, and then centrifuged at 12 000*g* for 20 min at 20 °C. Polypeptides smaller than a molecular mass of 10 kDa in the supernatant were removed by ultrafilteration (Centricon-10, Millipore Corp., Bedford, MA). The retentate was collected and regarded as tofu whey protein extract. The protein concentrations in soymilk protein and tofu whey protein extract were both determined by the method of Bradford (*21*).

Electrophoresis. TSDS−PAGE was performed according to the method of Schägger and Gagow (*22*). The protein extract was adjusted to 2 mg/mL with double-distilled water. An equal volume of the SDS sample buffer containing 10% 2-mercaptoethanol was added and then was boiled for 5 min. An aliquot of sample solution containing about 35 μg of proteins was loaded. The run was performed in 12% polyacrylamide gel in a Hoefer Mighty Small II SE-250 mini vertical gel electrophoresis unit at a 135 V constant voltage. After the electrophoresis, the gel was stained in a solution of methanol, acetic acid, and water (5:1:4) containing 0.25% Coomassie Brilliant Blue R-250. The Pharmacia LMW kit (α-lactalbumin, 144 000; trypsin inhibitor, 20 100; carbonic anhydrase, 30 000; ovalbumin, 43 000; bovine serum albumin, 67 000; and phosphorylase b, 94 000) was employed as a standard to determine the molecular masses of the extracted proteins. Protein quantification of the electrophoresis gels was carried out by direct densitometric scanning. The gels were scanned and analyzed by a Kodak EDAS 290 scanner equipped with Kodak Digital Science 1D 3.5 image analysis software.

Determination of FN, Ammonium Nitrogen, and AN. FN and ammonium nitrogen in the soymilk and tofu whey were determined by titration according to the Chinese National Standards method (*23*). To determine the content of FN, 5 g of soymilk or clarified tofu whey was diluted to 250 mL with distilled water and adjusted to pH 8.5 with 0.1 N NaOH. Forty milliliters of sample solution was mixed with an equal volume of 37% formaldehyde solution (previously adjusted to pH 8.5) and then titrated to pH 8.5 with 0.1 N NaOH. To determine the content of ammonium nitrogen, 10 g of soymilk or clarified tofu whey was mixed with 25 mL of 40% NaOH and distilled for 5 min. A conical flask containing 40 mL of 4% boric acid was placed under the condenser outlet to receive the distillate. The ammonium borate solution formed was titrated to pH 8.5 with 0.1 N NaOH. The amount of FN and ammonium nitrogen was expressed as g of nitrogen/100 g of sample. AN was determined by subtraction of the ammonium nitrogen from FN.

Data Analysis. All of the results were analyzed by ANOVA using the general linear model (*24*). Duncan's multiple range test was used to determine differences among the samples. Significant levels were defined as probabilities of 0.05 or less. All processing treatments were in triplicate.

RESULTS AND DISCUSSION

Effect of $CaSO_4 \cdot 2H_2O$ Concentration in Soymilk on Microstructure of Firm Tofu. A better understanding of the changes in the microstructure of tofu that corresponded to the coagulation conditions would provide a more rational basis to determine the suitable concentration of coagulant. In this study, the SEM images of the tofu prepared with different $CaSO_4 \cdot 2H_2O$ concentrations showed clearly different fine structures (Figure 1). The network of tofu obtained with 0.2% $CaSO_4 \cdot 2H_2O$ revealed a coarse and discontinuous structure with many fragments and large pores (Figure 1A). On increasing the $CaSO_4 \cdot 2H_2O$ concentration to 0.3%, there was a trend toward decreasing pore size and increasing regularity and uniformity (Figure 1B). The microstructure of tofu with 0.4% $CaSO_4 \cdot 2H_2O$ showed the most continuous and uniform honeycomb-like structure (Figure 1C). This structure was very uniform with smaller holes than those prepared with 0.2 and 0.3% $CaSO_4 \cdot$

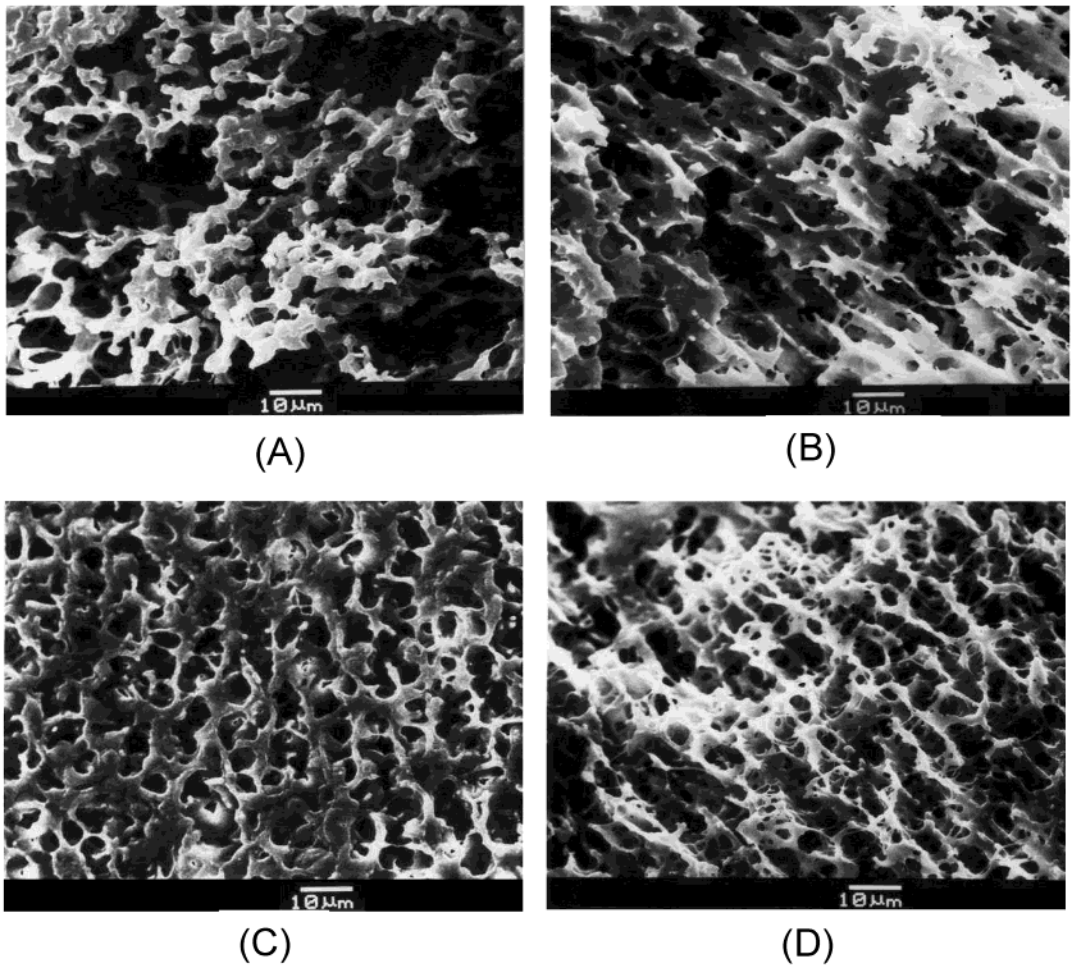

Figure 1. SEM images of freeze-dried tofu prepared with different concentrations of $CaSO_4 \cdot 2H_2O$ in soymilk; (A) 0.2, (B) 0.3, (C) 0.4, and (D) 0.5%.

$2H_2O$, and this was similar to that obtained with $CaSO_4 \cdot 1/2H_2O$ by deMan et al. (*2*). When the $CaSO_4 \cdot 2H_2O$ concentration was increased to 0.5%, the network of tofu became too porous and too compact (Figure 1D) and not as uniform and smooth as that obtained with 0.4% $CaSO_4 \cdot 2H_2O$.

Effect of $CaSO_4 \cdot 2H_2O$ Concentration on Yield, WRA, Protein, and Solid Recoveries of Firm Tofu. In this study, we investigated the effects of $CaSO_4 \cdot 2H_2O$ concentrations, from 0.2 to 0.5%, on the microstructure and physical properties and found that 0.4% $CaSO_4 \cdot 2H_2O$ in soymilk produced tofu with a maximum tofu yield (253 g tofu/100 g soybean), maximum protein recovery (70.9%), maximum solid recovery (55.2%), and a maximum WRA (77.2%). All four parameters increased significantly as the $CaSO_4 \cdot 2H_2O$ concentration increased from 0.2 to 0.4% but decreased as the $CaSO_4 \cdot 2H_2O$ concentration increased from 0.4 to 0.5% (Table 1). At the lower calcium sulfate concentrations, the interaction of the protein molecules with calcium ion was not strong enough to form a firm tofu gel. There were many discontinuous fragments and large holes in the microstructures of tofu made with 0.2 or 0.3% $CaSO_4 \cdot 2H_2O$ (Figure 1A,B). The exclusion of noncoagulated proteins and other soluble solids with the water during the pressing step resulted in a lower tofu yield and the lower protein and solid recoveries (Table 1). On the contrary, at a high concentration of 0.5% $CaSO_4 \cdot 2H_2O$, too many cross-linkages were formed and resulted in a too compact and too porous structure (Figure 1D). This structure was less uniform and less homogeneous than that with 0.4% $CaSO_4 \cdot 2H_2O$. This, in turn, resulted in an increased syneresis and induced the loss of water, whey proteins, and other soluble substances. These events would seem to account for the lower yield of tofu (240 g tofu/100 g soybean), lower protein recovery (68.3%), and lower solid recovery (51.5%) at this concentration. At the optimum coagulant concentration (0.4% $CaSO_4 \cdot 2H_2O$), a uniform, continuous tofu network was formed (Figure 1C), and it was able to trap more water and soluble substances. Therefore, it could obtain the highest tofu yield, protein and solid recoveries, and WRA (Table 1). These observations were in agreement with the findings of Puppo and Añón (*17*), who showed that a protein gel with a homogeneous and fine structure gave high WRA, when compared to the gel with a nonhomogeneous structure, that had a high degree of syneresis.

Table 1. Effect of Calcium Sulfate Concentration in Soymilk on Moisture Content, WRA, Tofu Yield, Solid Recovery, and Protein Recovery of Firm Tofu[a]

$CaSO_4 \cdot 2H_2O$ (%, w/v)	moisture content (%)	WRA[b] (%)	tofu yield (g tofu/ 100 g soybean)	solid recovery (%)	protein recovery (%)
0.20	80.2 a	67.5 c	205 e	45.9 c	62.6 e
0.25	80.2 a	67.7 c	230 d	49.7 b	67.0 cd
0.30	80.9 a	70.3 b	237 cd	51.2 b	67.9 bcd
0.35	80.8 a	75.6 a	243 bc	51.9 b	68.4 bc
0.40	80.5 a	77.2 a	253 a	55.2 a	70.9 a
0.45	80.7 a	76.9 a	247 ab	53.7 ab	69.5 ab
0.50	81.0 a	76.0 a	240 bc	51.5 b	68.3 bc

[a] Mean scores bearing the same letters among the same column are not significantly different ($p < 0.05$). [b] WRA of tofu.

Table 2. Effect of Calcium Sulfate Concentration in Soymilk on the Textural Characteristics of Firm Tofu[a]

$CaSO_4 \cdot 2H_2O$ (%, w/v)	hardness (kg)	springiness (mm)	cohesiveness	gumminess (kg)	chewiness (kg mm)
0.20	0.40 b	7.84c	0.35 d	0.14 c	1.11 d
0.25	0.45 b	8.70 b	0.39 c	0.18 c	1.52 c
0.30	0.66 a	9.74 a	0.40 bc	0.26 b	2.57 b
0.35	0.68 a	9.76 a	0.41 b	0.28 b	2.72 b
0.40	0.71 a	9.76 a	0.45 a	0.32 a	3.11 a
0.45	0.73 a	9.84 a	0.45 a	0.33 a	3.21 a
0.50	0.74 a	10.32 a	0.45 a	0.33 a	3.39 a

[a] Mean scores bearing the same letters among the same column are not significantly different ($p < 0.05$).

Tofu Texture. The increase of $CaSO_4 \cdot 2H_2O$ concentration from 0.2 to 0.4% resulted in a steady increase in the hardness, springiness, cohesiveness, gumminess, and chewiness of tofu (Table 2). Texture is considered to be a direct consequence of microstructure as determined by chemical composition and the physical forces involved (*25*). A more dense and compact structure maintained by the Ca−protein bridges and hydrogen bonds might account for the higher hardness and higher springiness of the tofu at higher concentrations of $CaSO_4 \cdot 2H_2O$. Similar results were reported by Shih et al. (*9*). Scaefer and Love (*26*) also reported a significant correlation between calcium concentration and hardness ($r = 0.73$) and elasticity ($r = 0.83$) for tofu. When the concentration of the coagulant was increased from 0.2 to 0.4%, cohesiveness increased significantly but remained constant at concentrations between 0.4 and 0.5% (Table 2). Wang and Hesseltine (*7*) found that cohesiveness increased with increasing $CaSO_4 \cdot 2H_2O$ concentration from 10 to 20 mM (0.17−0.34%) and remained the same when beyond the range. Tofu with a less intensive network gave less cohesiveness. Because gumminess was defined as the product of hardness and cohesiveness and chewiness was the product of hardness, springiness, and cohesiveness, they all had similar trends (Table 2).

Effect of $CaSO_4 \cdot 2H_2O$ Concentration on the Constitution of Tofu Whey Proteins. The effect of $CaSO_4 \cdot 2H_2O$ concentrations on the constitution of tofu whey proteins was analyzed by TSDS−PAGE (Figure 2), a method that had been shown to have an improved resolution for LMW proteins (*22*). The TSDS−PAGE pattern of soymilk protein showed that the protein bands with molecular masses in the range of 16−40 and 42−80 kDa were the two major proteins of soybean, i.e., 11S (glycinin) and 7S (β-conglycinin) globulins. Glycinin is composed of acidic (34−45 kDa) and basic (17−20 kDa) polypeptide chains paired by a disulfide bond (*27*, *28*). β-Conglycinin consists of α, α', and β subunits, wherein the molecular masses of α and α' subunits are reported to be in the range of 57−83 kDa, while that of the β subunit is 42−53 kDa (*29*).

As compared with soymilk protein composition, the tofu whey protein contained higher amounts of LMW proteins with a molecular mass below 16 kDa (Figure 2). The protein bands on the gel were densitometrically scanned, and the relative ratio of bands was estimated. The percentages of LMW proteins in soymilk and tofu whey that were discharged from the pressed tofu made with 0.2, 0.3, 0.4, and 0.5% $CaSO_4 \cdot 2H_2O$ were 1.8, 41.1, 81.0, 29.0, and 53.7%, respectively. A similar trend was found in the ratio of AN to TON in soymilk and tofu whey (Table 3), with the values of 0.93, 43.1, 66.7, 39.4, and 57.5%, respectively. These results confirmed the suggestion that the proteins remaining in tofu whey were mainly LMW proteins (*15*).

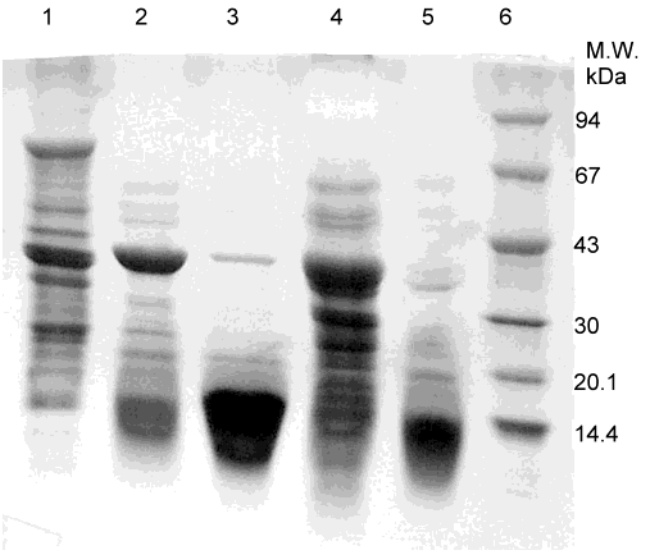

Figure 2. TSDS−PAGE profile of proteins of soymilk and tofu whey obtained during tofu making with different $CaSO_4 \cdot 2H_2O$ concentrations in soymilk. Lane 1, soymilk; lane 2, 0.2%; lane 3, 0.3%; lane 4, 0.4%; lane 5, 0.5%; and lane 6, protein molecular mass markers.

Table 3. Effect of Calcium Sulfate Concentration during Tofu Making on TN and AN of Tofu Whey[a]

sample	TN[b] (%)	AN[b] (%)	AN/TON[b] (%)
soymilk	0.47 a	0.004 e	0.93 e
tofu whey $CaSO_4 \cdot 2H_2O$ (%, w/v)			
0.20	0.14 b	0.053 a	43.1 c
0.30	0.08 c	0.040 b	66.7 a
0.40	0.04 e	0.013 d	39.4 d
0.50	0.06 d	0.027 c	57.5 b

[a] Mean scores bearing the same letters among the same column are not significantly different ($p < 0.05$). [b] TON, subtraction of ammonium nitrogen from TN.

The increase of $CaSO_4$ concentration from 0.2 to 0.4% in tofu making resulted in a decrease in TN and AN in tofu whey and an increase in TN and AN when beyond 0.4% $CaSO_4 \cdot 2H_2O$ (0.04 and 0.013%, respectively). The results supported the previous finding that tofu with a homogeneous and uniform network gave the highest protein recovery at the optimum coagulant concentration (Table 1). Among the four tofu whey samples, the percentages of LMW proteins (Figure 2, lane 4) and the ratio of AN to TON (Table 3) were the lowest in tofu whey obtained from the soymilk with 0.4% $CaSO_4 \cdot 2H_2O$ (29.0 and 39.4%, respectively). This suggested that a homogeneous network can more efficiently retain the LMW proteins in tofu matrixes.

A possible gelation mechanism for firm tofu in the presence of $CaSO_4$ is schematically represented in Figure 3. It has been suggested that a Ca^{2+} gel is induced in the denatured protein solution to form a filamentous gel structure (*30*−*35*). Heating a soybean protein solution causes the proteins to unfold and form protein filaments due to the formation of disulfide bonds and hydrophobic interactions. A filamentous gel structure is formed when $CaSO_4$ is added, and this reduces the electrostatic repulsion between the charged filaments, causing them to aggregate with the Ca^{2+} ions acting as bridges between the adjacent charged carboxylic groups on neighboring protein molecules. At the end of pressing, the ratio of tofu whey volume

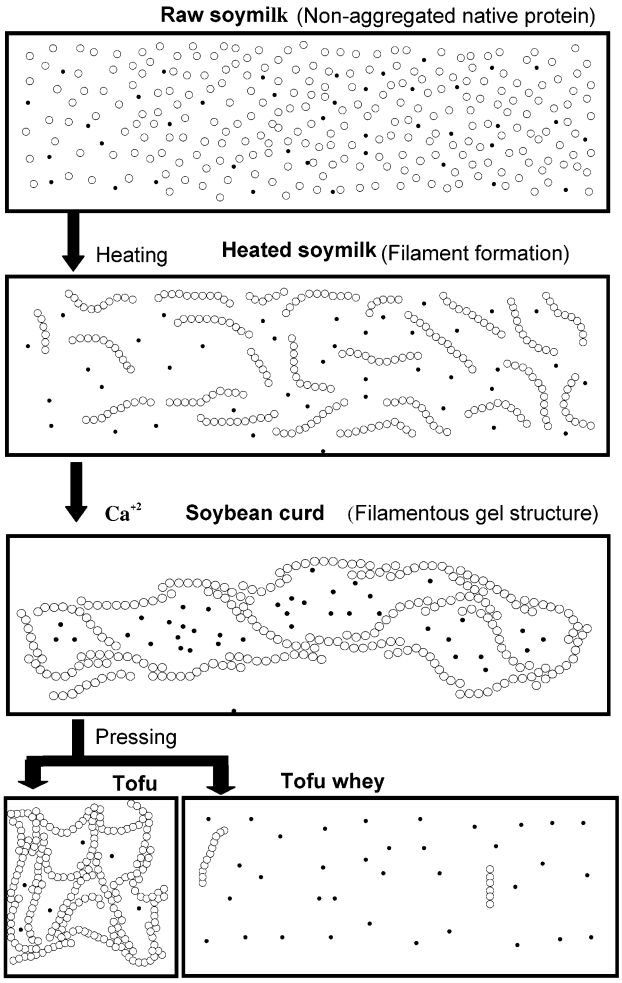

Figure 3. Gelation mechanism of firm tofu in the presence of $CaSO_4$; (○) representing HMW protein; (●) representing LMW protein. Each square area represents a relative volume.

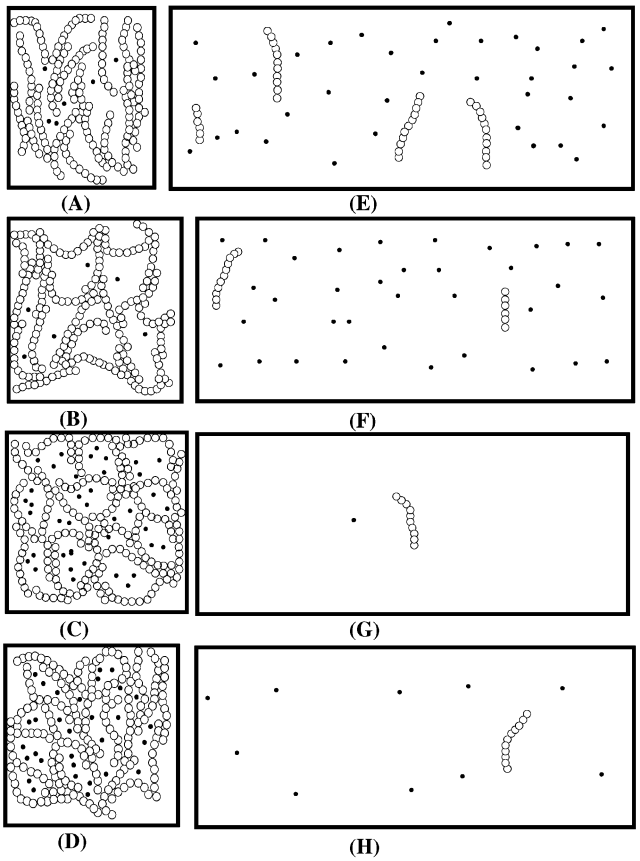

Figure 4. Representation (highly schematic) of likely states of constitution of protein filaments and LMW proteins in the tofu network and in the tofu whey obtained with different concentrations of $CaSO_4 \cdot 2H_2O$ in soymilk; (○) representing HMW protein; (●) representing LMW protein. The eight states were as follows: (A) 0.2% (tofu network), (B) 0.3% (tofu network), (C) 0.4% (tofu network), (D) 0.5% (tofu network), (E) 0.2% (tofu whey), (F) 0.3% (tofu whey), (G) 0.4% (tofu whey), and (H) 0.5% (tofu whey). The left-hand square areas represent the relative volumes of tofu, and the right-hand square areas represent the relative volumes of tofu whey.

to firm tofu volume was in the range of 2.4−3.2. An increase in the $CaSO_4$ concentration can promote gelation of the protein filaments. At a low $CaSO_4$ concentration (0.2% $CaSO_4 \cdot 2H_2O$), the protein filaments failed to form a firm tofu gel (Figure 4A), with many individual protein filaments and LMW proteins dispersed in tofu whey (Figure 4E). When the $CaSO_4 \cdot 2H_2O$ concentration was increased to 0.3%, the extent of aggregation of the protein filaments in tofu gel increased remarkably. However, the gel was still not homogeneous and exhibited a fragmentary network with a relatively low retention of water and LMW proteins (Figure 4B) and resulted in a maximum percentage of LMW proteins dispersed in tofu whey (Figure 4F). As the $CaSO_4 \cdot 2H_2O$ concentration further increased to the optimum concentration (0.4% $CaSO_4 \cdot 2H_2O$), the LMW proteins were well-dispersed in the spaces (pores) of the homogeneous network structure (Figure 4C) with only a few protein filaments and LMW proteins dispersed into tofu whey (Figure 4G). As the $CaSO_4$ concentration was further increased to 0.5%, the ability of the gel to retain water and LMW proteins adversely decreased due to the formation of a too compact network; therefore, the percentage of LMW proteins dispersed into tofu whey increased (Figure 4H).

In this study, we found that the coagulant of 0.4% $CaSO_4 \cdot 2H_2O$ in soymilk was the most appropriate concentration for making firm tofu. The firm tofu made with 0.4% $CaSO_4 \cdot 2H_2O$ had an excellent uniform and homogeneous microstructure that retained more water and more LMW proteins in tofu matrix, and consequently, resulted in the maximal tofu yield, maximal protein and solid recoveries, and the best WRA.

ABBREVIATIONS USED

SEM, scanning electron microscopy; GDL, glucono-δ-lactone; LMW, low molecular weight; HMW, high molecular weight; WRA, water retention ability; TSDS−PAGE, tricine sodium dodecyl sulfate−polyacrylamide gel electrophoresis; TN, total nitrogen; FN, formol nitrogen; AN, amino nitrogen; TON, total organic nitrogen; ANOVA, analysis of variance.

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