

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

FUH-JUIN KAO, NAN-WEI SU, AND MIN-HSIUNG LEE*

Graduate Institute of Agricultural Chemistry, National Taiwan University, Taipei 10617, Taiwan

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% $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ 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% $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$. The SEM observations suggested that the tofu made with 0.4% $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ 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%) $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ 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

* To whom correspondence should be addressed. Tel: +886-2-23630231 ext. 2490. Fax: +886-2-23632714. E-mail: mhlee@ccms.ntu.edu.tw.

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 $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ 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 ($13 \times 13 \times 5.5 \text{ cm}^3$) and then pressed at 21.8 g/cm² for 10 min, 43.6 g/cm² for 10 min, and 65.4 g/cm² 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 \times 120 mm). The sample weight was recorded after centrifugation at 120g 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:

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

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 \times 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 $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ 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_2EDTA , pH 8.0). The mixture was sonicated at 60 °C for 90 min to extract the proteins. The extract was then centrifuged at 12 000g 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 6000g 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 000g for 20 min at 20 °C. Polypeptides smaller than a molecular mass of 10 kDa in the supernatant were removed by ultrafiltration (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. TS-DS-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 $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ 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 $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ concentrations showed clearly different fine structures (Figure 1). The network of tofu obtained with 0.2% $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ revealed a coarse and discontinuous structure with many fragments and large pores (Figure 1A). On increasing the $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ 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% $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ 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% $\text{CaSO}_4 \cdot$

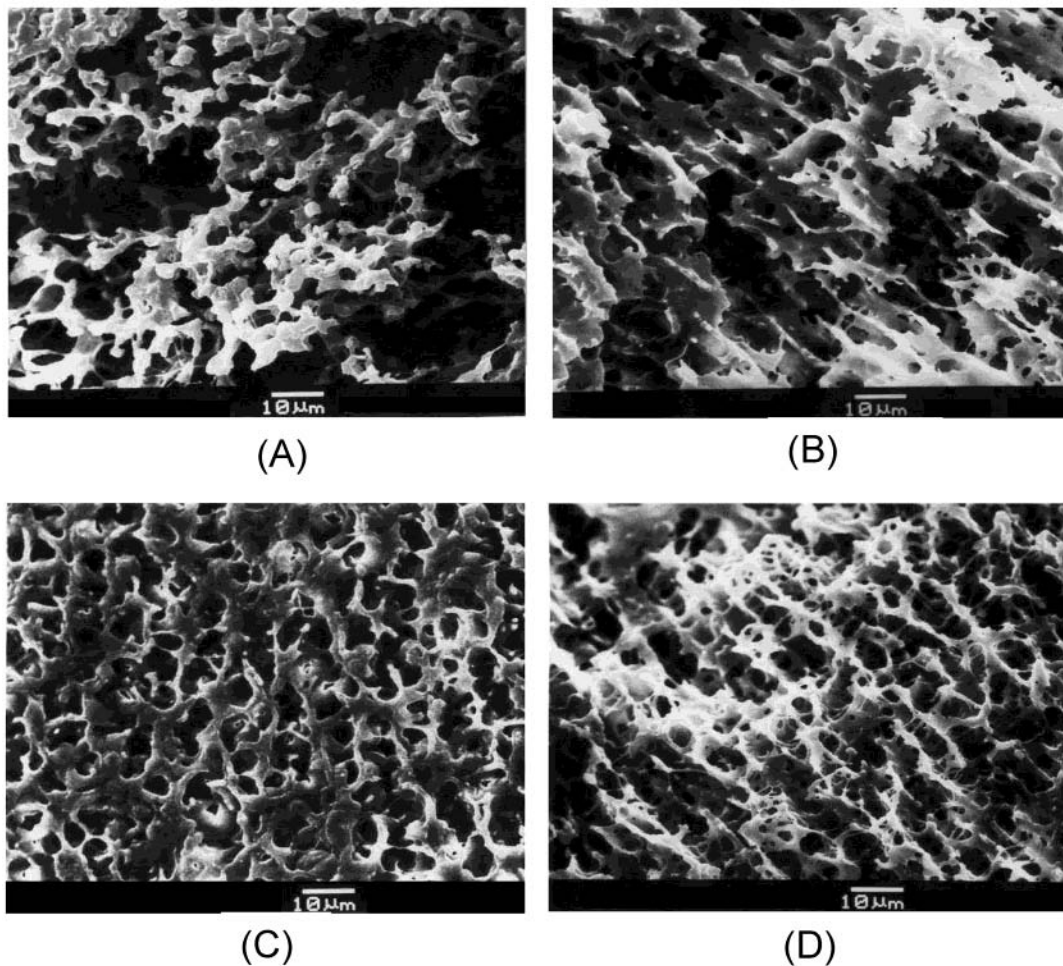


Figure 1. SEM images of freeze-dried tofu prepared with different concentrations of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ in soy milk; (A) 0.2, (B) 0.3, (C) 0.4, and (D) 0.5%.

$2\text{H}_2\text{O}$, and this was similar to that obtained with $\text{CaSO}_4 \cdot 1/2\text{H}_2\text{O}$ by deMan et al. (2). When the $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ 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% $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$.

Effect of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ Concentration on Yield, WRA, Protein, and Solid Recoveries of Firm Tofu. In this study, we investigated the effects of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ concentrations, from 0.2 to 0.5%, on the microstructure and physical properties and found that 0.4% $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ in soy milk 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 $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ concentration increased from 0.2 to 0.4% but decreased as the $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ 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% $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ (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% $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, 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% $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$. This, in turn, resulted in an increased syneresis and induced the loss of water, whey proteins,

Table 1. Effect of Calcium Sulfate Concentration in Soy milk on Moisture Content, WRA, Tofu Yield, Solid Recovery, and Protein Recovery of Firm Tofu^a

$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ (%, 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.

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% $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$), 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 2. Effect of Calcium Sulfate Concentration in Soymilk on the Textural Characteristics of Firm Tofu^a

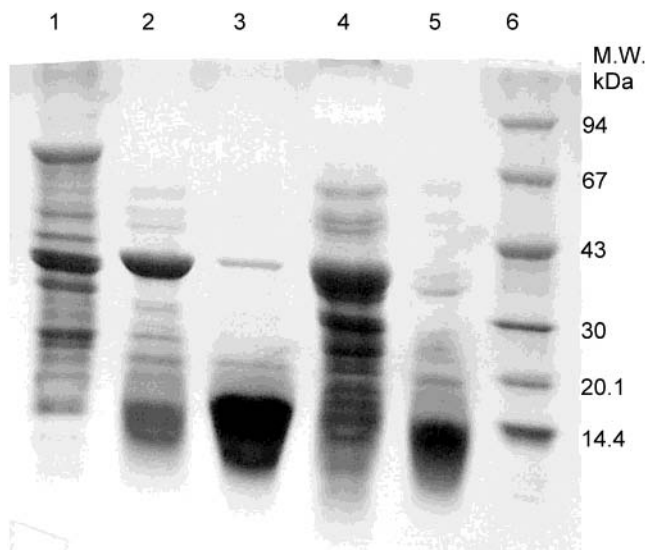
CaSO ₄ ·2H ₂ O (% w/v)	hardness (kg)	springiness (mm)	cohesiveness	gumminess (kg)	chewiness (kg mm)
0.20	0.40 b	7.84c	0.35 d	0.14 c	1.11 d
0.25	0.45 b	8.70 b	0.39 c	0.18 c	1.52 c
0.30	0.66 a	9.74 a	0.40 bc	0.26 b	2.57 b
0.35	0.68 a	9.76 a	0.41 b	0.28 b	2.72 b
0.40	0.71 a	9.76 a	0.45 a	0.32 a	3.11 a
0.45	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 CaSO₄·2H₂O 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 CaSO₄·2H₂O. Similar results were reported by Shih et al. (9). Scafer 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 CaSO₄·2H₂O 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 CaSO₄·2H₂O Concentration on the Constitution of Tofu Whey Proteins. The effect of CaSO₄·2H₂O 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% CaSO₄·2H₂O 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 CaSO₄·2H₂O 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 ·2H ₂ O (% 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 CaSO₄ 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% CaSO₄·2H₂O (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% CaSO₄·2H₂O (29.0 and 39.4%, respectively). This suggested that a homogeneous network can more efficiently retain the LMW proteins in tofu matrices.

A possible gelation mechanism for firm tofu in the presence of CaSO₄ is schematically represented in Figure 3. It has been suggested that a Ca²⁺ 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 CaSO₄ is added, and this reduces the electrostatic repulsion between the charged filaments, causing them to aggregate with the Ca²⁺ 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

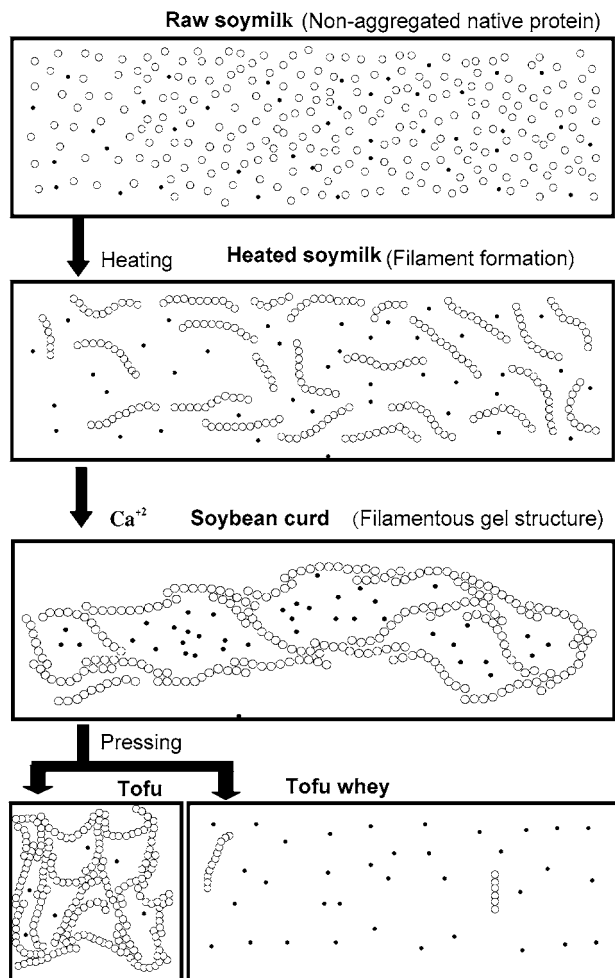


Figure 3. Gelation mechanism of firm tofu in the presence of CaSO_4 ; (○) representing HMW protein; (●) representing LMW protein. Each square area represents a relative volume.

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% $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$), 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 $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ 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 $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ concentration further increased to the optimum concentration (0.4% $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$), 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% $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ in soymilk was the most appropriate concentration for making firm tofu. The firm tofu made with 0.4% $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ had an excellent uniform and homogeneous microstructure that retained more water and more LMW proteins in tofu matrix,

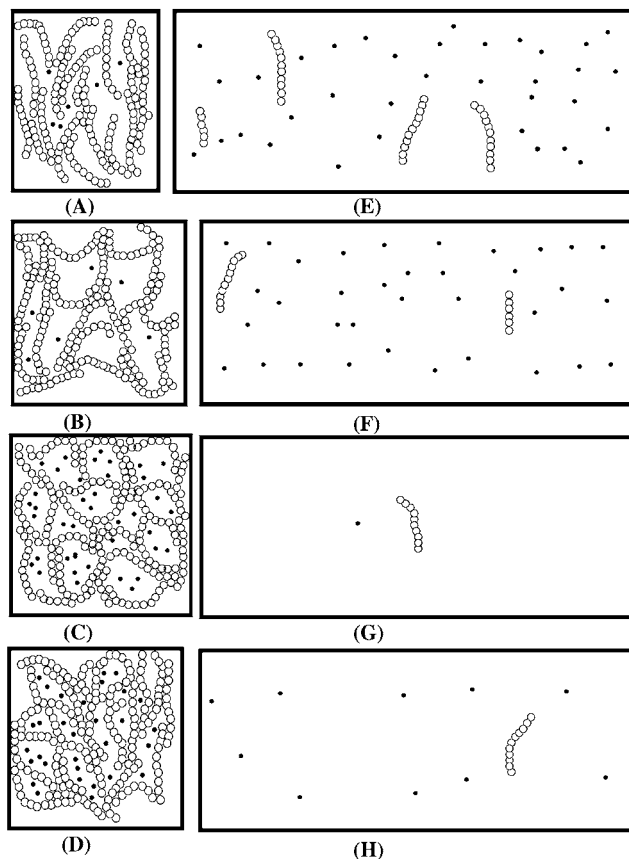


Figure 4. Representation (highly schematic) of likely states of constitution of protein filaments and LMW proteins in the tofu network and in the tofu whey obtained with different concentrations of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ in soymilk; (○) representing HMW protein; (●) representing LMW 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.

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.

ACKNOWLEDGMENT

We are grateful to Miss Chien Lin for technical assistance with picture drawing.

LITERATURE CITED

- (1) Tsai, S. J.; Lar, C. Y.; Kao, C. S.; Chen, S. C. Studies on the yield and quality characteristics of tofu. *J. Food Sci.* **1981**, *46*, 1734–1737.
- (2) deMan, J. M.; deMan, L.; Gupta, S. Texture and microstructure of soybean curd (tofu) as affected by different coagulants. *Food Microstruct.* **1986**, *5*, 83–89.
- (3) Saio, K. Tofu-relationships between texture and fine structure. *Cereal Foods World* **1979**, *24*, 342–354.

- (4) Wilson, L. A. Soy foods. In *Practical Handbook of Soybean Processing and Utilization*; Erickson, D. R., Ed.; AOCS Press: Champaign, IL, 1995; pp 428–459.
- (5) Beddow, C. G.; Wong, J. Optimization of yield and properties of silken tofu from soybean. III. Coagulant concentration, mixing and filtration pressure. *Int. J. Food Sci. Technol.* **1987c**, *22*, 29–34.
- (6) Moizuddin, S.; Harvey, G.; Fenton, A. M.; Wilson, L. A. Tofu production from soybeans or full-fat soyflakes using direct and indirect heating process. *J. Food Sci.* **1999**, *64*, 145–148.
- (7) Wang, H. L.; Hesselatine, C. W. Coagulation conditions in tofu processing. *Process Biochem.* **1982**, *1*, 7–12.
- (8) Sun, M.; Breene, W. M. Calcium sulfate concentration influence on yield and quality of tofu from 5 soybean varieties. *J. Food Sci.* **1991**, *56*, 1604–1607.
- (9) Shih, M. C.; Hou, H. J.; Chang, K. C. Process optimization for soft tofu. *J. Food Sci.* **1997**, *62*, 833–837.
- (10) Cai, T. D.; Chang, K. C. Characteristics of production-scale tofu as affected by soymilk coagulation method: propeller blade size, mixing time and coagulant concentration. *Food Res. Int.* **1998**, *31*, 289–295.
- (11) Hou, H. J.; Chang, K. C.; Shih, M. C. Yield and textural properties of soft tofu as affected by coagulation method. *J. Food Sci.* **1997**, *62*, 824–827.
- (12) Lu, J. Y.; Carter, E.; Chung, R. A. Use of calcium salts for soybean curd preparation. *J. Food Sci.* **1980**, *45*, 32–34.
- (13) Lim, B. T.; deMan, J. M.; deMan, L.; Buzzell, R. I. Yield and quality of tofu as affected by soybean and soymilk characteristics: calcium sulfate coagulant. *J. Food Sci.* **1990**, *55*, 1088–1092.
- (14) Beddow, C. G.; Wong, J. Optimization of yield and properties of silken tofu from soybean. I. The water: bean ratio. *Int. J. Food Sci. Technol.* **1987a**, *22*, 15–21.
- (15) Karium, A. Abd.; Sulebele, G. A.; Azhar, M. E.; Ping, C. Y. Effect of carrageenan on yield and properties of tofu. *Food Chem.* **1999**, *66*, 159–165.
- (16) AOAC. *Official Methods of Analysis*, 13th ed.; Association of Official Analytical Chemists: Washington, DC, 1980.
- (17) Puppo, M. C.; Añón, M. C. Structural properties of heated-induced soy protein gels as affected by ionic strength and pH. *J. Agric. Food Chem.* **1998**, *46*, 3583–3589.
- (18) Bourne, M. C. Texture profile analysis. *Food Technol.* **1978**, *32*, 62–66.
- (19) Cai, T. D.; Chang, K. C. Processing effect on soybean storage proteins and their relationship with tofu quality. *J. Agric. Food Chem.* **1999**, *47*, 720–727.
- (20) Sorgentini, D. A.; Wanger, J. R. Comparative study of structural characteristics and thermal behavior of whey and isolate soybean proteins. *J. Food Biochem.* **1999**, *23*, 489–507.
- (21) Bradford, M. M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254.
- (22) Schägger, H.; von Gagow, G. Tricine-sodium dodecyl sulfate–polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal. Biochem.* **1987**, *166*, 368–379.
- (23) Chinese National Standard. *Method of Test for Soy Sauce*; N6008; Central Standard Bureau, Ministry of Economics, Executive Yuan: Taiwan, ROC, 1976.
- (24) SAS Institute. *SAS/STAT User's Guide, version 6.03*; Cary, NC, 1990.
- (25) Stanley, D. W.; Tung, M. A. Microstructure of food and its relation to texture. In *Rheology and Texture in Food Quality*; deMan, J. M., Voisey, P. W., Rasper, V. F., Stanley, D. W., Eds.; AVI Publishing: Westport, CT, 1976; pp 28–78.
- (26) Schaefer, M. J.; Love, J. Relationships between soybean components and tofu texture. *J. Food Qual.* **1992**, *15*, 53–66.
- (27) Kitamura, K.; Takagi, T.; Shibasaki, K. Subunit structure of soybean 11S globulin. *Agric. Biol. Chem.* **1976**, 1837–1844.
- (28) Staswick, P. E.; Hermodson, M. A.; Nielsen, N. C. Identification of the cysteines which link acidic and basic components of the glycinin. *J. Biol. Chem.* **1984**, *259*, 13431–13435.
- (29) Brooks, J. R.; Morr, C. V. Current aspects of soy protein fractionation nomenclature. *J. Am. Oil Chem. Soc.* **1985**, *62*, 1347–1354.
- (30) Frank, J. M.; German, J. B.; Kinsella, J. E. Effect of pH and temperature on protein unfolding and thiol/disulfides interchange reaction during heat-induced gelation of whey proteins. *J. Agric. Food Chem.* **1995**, *43*, 46–52.
- (31) Kohyama, K.; Sano, Y.; Doi, E. Rheological characteristics and gelation mechanism of tofu (Soybean curd). *J. Agric. Food Chem.* **1995**, *43*, 1808–1812.
- (32) McClements, D. J.; Keogh, M. K. Physical properties of cold setting gels formed from heat-denatured whey isolate. *J. Sci. Food Agric.* **1995**, *69*, 7–14.
- (33) Bryant, C. M.; McClements, D. J. Molecular basis of protein functionality with special consideration of cold-set gels derived from heat-denatured whey. *Trends Food Sci. Technol.* **1998**, *9*, 143–151.
- (34) Roff, C. F.; Foegeding, E. A. Dicationic-induced gelation of pre-denatured whey protein isolate. *Food Hydrocolloids* **1996**, *10*, 193–198.
- (35) Totosaus, A.; Montejano, J. G.; Salazar, J. A.; Guerrero, I. A review of physical and chemical protein-gel induction. *Int. J. Food Sci. Technol.* **2002**, *37*, 589–601.

Received for review February 27, 2003. Revised manuscript received July 16, 2003. Accepted August 8, 2003. We thank the National Science Council of the Republic of China, Taipei, Taiwan, for the financial support of this research under the Project NSC-89-2214-E-002-076 and NSC-90-2214-E-002-028.

JF0342021