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Coagulation of soymilk and quality of tofu as affected by freeze treatment of soybeans

E.J. Noh [a], S.Y. Park [b], J.I. Pak [c], S.T. Hong [d], S.E. Yun [a,*]

[a] Department of Food Science and Technology, Institute of Agricultural Science and Technology, Chonbuk National University, Chonbuk, Chonju 561-756, Republic of Korea

[b] Food Industrial Technology Research Center, Mokpo National University, Mokpo 534-721, Republic of Korea

[c] Department of Food Science and Technology in Animal Resourses, Kangwon National University, Chunchon 200-701, Republic of Korea

[d] Division of Food and Culinary Science, Howon University, Gunsan 573-718, Republic of Korea

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Abstract

The present study was carried out to investigate the effects of freezing of soybeans on the coagulation of soymilk and quality of tofu. Soymilk, prepared from frozen soybeans, was found to coagulate faster in the presence of coagulant and produced a more uniform-structured gel than that from unfrozen soybeans. Tofu, prepared from frozen soybeans, showed a more orderly and denser network structure than that from unfrozen soybeans, thereby inducing an increase in some textural parameters such as hardness, springiness, gumminess and chewiness as well as syneresis. Freezing also brought about some changes in tofu quality: lower yield, lower fat and higher protein contents. Results of sensory evaluation showed that tofu from frozen soybeans had better sensory properties in terms of flavour and mouthfeel. From these results, it was concluded that freezing promoted the coagulation process of soymilk and changed the quality of tofu in a positive way.

Keywords: Tofu; Freezing; Frozen soybeans; Texture; Sensory properties

1. Introduction

The quality and yield of tofu are influenced by cultivar of soybeans (Shen, de Man, Buzzel, & de Man, 1991; Sun & Breene, 1991), processing methods (Saio, 1979; Shih, Hou, & Chang, 1997) and type and concentration of coagulants (Lim, de Man, de Man, & Buzzell, 1990; Shen et al., 1991; Sun & Breene, 1991). Processing factors include heating rates and times (Beddows & Wong, 1987), stirring speeds and times for coagulation (Cai & Chang, 1998; Hou, Chang, & Shih, 1997; Shih et al., 1997), temperature for coagulation (Shih et al., 1997) and pressing time and weight (Gandhi & Bourne, 1988).

When a solution of soyproteins is frozen, the protein molecules become partially insoluble, due to the formation of intermolecular disulphide bonds (Hashizume, Kakiuchi, Koyama, & Watanabe, 1971). Lee, Choi, Kim, and Yun (1992) have shown that freezing is effective in improving the taste of soybeans, as well as reducing the cooking time to one-half. From these results, it can be expected that use of frozen soybeans may change the quality of soybean products. However, few researches have been carried out on the application of freezing of soybeans to modify the quality of soybean products. Some studies have been conducted to change the texture of tofu by freezing under high pressure (Fuchigami & Teramoto, 1997) and to develop a

* Corresponding author. Tel.: +82 63 270 2568; fax: +82 63 270 2572.

E-mail address: seyun@moak.chonbuk.ac.kr (S.E. Yun).

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textured protein from frozen tofu (Hashizume, Kosaka, Koyama, & Watanabe, 1974; Hashizume, Nakamura, & Watanabe, 1974).

The present study aims to investigate how the freezing of soybeans affects the coagulation of soymilk and quality of tofu prepared by a traditional method.

2. Materials and methods

2.1. Materials

Soybean (*Glycine max* Merr., cv Jang-yeob) used for tofu making was purchased from a local grower (Chonbuk province, Korea). All chemicals used were reagent grade and purchased from Sigma.

2.2. Methods

2.2.1. Preparation of soymilk coagulum

The soybeans (approximately 150 g) were soaked in 800 ml tap water at room temperature for 10 h (soybean:water = 1:2.2). The soaked beans were placed in a basket to remove excess water, followed by freezing to −20 °C for 5 h by air-blast freezing. The frozen bean was thawed and ground with 1650 ml tap water by using a Waring blender for 4 min at high speed. After grinding, the slurry was brought to the boil with stirring and held at 95 °C for 2.5 or 5 min. Then the slurry was squeezed manually with a muslin cloth to obtain soymilk (1700 ml). An aliquot of 50 ml of the soymilk was transferred to a rheometer cell (ID: 50 mm) and coagulated with 0.02 M calcium sulfate ($CaSO_4 \cdot 2H_2O$) at 72 °C to produce the soymilk coagulum. For comparison, soymilk coagulum was prepared by the procedure described above except for the freezing of soybeans. Unless otherwise stated, freezing refers to freezing of soybeans and heating represents heating of soy slurry.

2.2.2. Preparation of tofu

The soymilk (1700 ml) from frozen or unfrozen soybeans was coagulated with 0.02 M calcium sulfate at 72 °C for 10 min. The resultant coagulum was transferred to a home-made mould (17.6 cm × 9.6 cm × 6.8 cm) lined with cheese cloth and pressed for 20 min by placing a weight of 8.120 kg to produce tofu.

2.2.3. Texture measurement of soymilk coagulum and tofu

Measurements on soymilk coagulum and tofu used a TA-XT2 Texture Analyzer (Stable Micro Systems, Goldaming, UK) fitted with a 5-kg load cell. For the soymilk coagulum, a back extrusion test was carried out. The soymilk coagulum was compressed by a plunger (D: 45 mm) until the coagulum flowed up through the annulus. The maximum force (g) required to accomplish extrusion was measured at regular time intervals. For tofu, texture profile analysis (TPA) was carried out. Cube samples of tofu (10 mm × 10 mm × 10 mm) were compressed to 80% deformation by a compression plunger (D: 25 mm). The pre-test, test and post-test speeds were set to 2, 1 and 2 mm s^{-1}, respectively. Ten replicate tests were conducted for each sample.

2.2.4. Scanning electron microscopic observation

The specimen was prepared by the method of Inoue and Osatake (1988). The tofu was cut into blocks (ca. 3 mm × 3 mm × 3 mm). To a block, 3 ml of 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) were added, pre-fixed at room temperature, and stored at 4 °C for 1 h. The pre-fixed sample was washed twice with 0.1 M phosphate buffer (pH 7.4), followed by post-fixing in 1% osmium tetroxide solution. Then the sample was dehydrated in a graded series of ethanol solutions (60%, 70%, 80%, 90%, 95% and 99.5%) and transferred to *t*-butyl alcohol. The sample in *t*-butyl alcohol was placed in a refrigerator to freeze. The frozen sample was then transferred into the bell jar of a vacuum evaporator to sublimate *t*-butyl alcohol. After sublimation, the dried sample was coated with Au–Pd to produce a specimen in an ion beam sputtering system (E-1030; Hitachi Co., Japan) using argon gas. The specimen was examined by a scanning electron microscope (model S-4700; Hitachi Co., Japan).

2.2.5. Yield and proximate analyses

Yield of tofu was expressed as weight of tofu obtained from 1700 ml of soymilk. Moisture content was determined by drying 0.9 g of fresh tofu at 105 °C in a drying oven to a constant weight. Total protein was determined by the microKjeldahl method (AOAC, 1995) and crude fat by the Soxhlet method (AOAC, 1995).

2.2.6. Syneresis

Syneresis was evaluated by employing the modified method of Amstrong, Hill, Schrooyen, and Mitchell (1994). Six pieces of tofu samples with diameter of 1.5 cm were weighed and filled into dialysis tube (D: 2.5 cm), bound and wrapped with plastic wrap to minimise evaporation. These cylinders were then tied to a wire frame placed over a 2-l beaker in a hanging position for 24 h at 4 °C. Percentage syneresis was calculated as the weight of water released from the sample in 24 h divided by the weight of sample and multiplied by 100.

2.2.7. Sensory evaluation

Ten panellists who trained to recognise and score the sensory attributes performed sensory evaluation. The attributes evaluated were colour, flavour and mouthfeel and the results were expressed on a 9-point hedonic scale (1 = excellent, 9 = not good) for each attribute.

2.2.8. Colour

Colour was evaluated on fresh tofu samples using a Colour and Colour Difference Meter Model TC-3600 (Tokyo Denshoku Co. Ltd., Japan). A standard white tile with reflectance values of $L = 90.2$, $a = 0.4$, and $b = 3.4$ was used as a reference. A representative sample was placed on a 3-cm petri dish. Hunter L (0.00 = dark black, 100.00 = white), a (+ = red, − = green), and b (+ = yellow, − = blue) were then determined for each sample. Each value represented a mean value of three replicate determinations.

2.2.9. Statistical analysis

Data were analysed by ANOVA or GLM using the Statistical Analysis System programme (SAS, 1990). Means comparisons were made by least significant difference (LSD) test for ANOVA and repeated measurement for GLM. A significant level was defined as a probability of 0.05 or less.

3. Results and discussion

3.1. Coagulation of soymilk

Coagulation of soymilk is an important step in the tofu manufacturing process because coagulation conditions greatly influence tofu yield and quality (Beddows & Wong, 1987; Cai & Chang, 1998; Hou et al., 1997; Saio, 1979; Shih et al., 1997; Sun & Breene, 1991). Fig. 1 shows the photograph of soymilk coagulums prepared from either frozen or unfrozen soybeans using calcium sulfate as coagulant. The soymilk from frozen soybeans was found to produce a more uniform-structured gel than that from unfrozen soybeans, suggesting that the freezing can control the coagulating process of soymilk in a positive way. To look into these differences in more detail, a back extrusion test was carried out for the soymilk coagulums. As shown in Fig. 2, the force to extrude

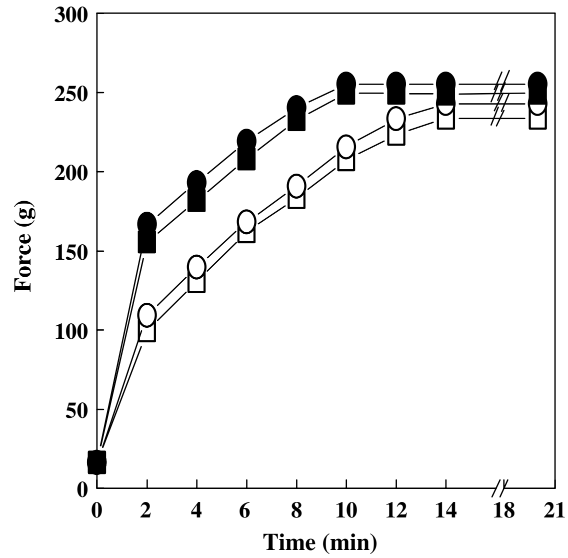

Fig. 2. Changes in the force obtained by a back extrusion test for the soymilk coagulum. The soymilk coagulum was prepared using soymilk heated for 2.5 or 5 min, made from frozen soybeans or unfrozen soybeans: □ unfrozen and 2.5-min heating; ○ unfrozen and 5-min heating; ■ frozen and 2.5-min heating; ● frozen and 5-min heating. The values were obtained from triplicate experiments and expressed as mean values.

the soymilk coagulums from frozen soybeans increased faster and reached the maximum value earlier than that from unfrozen soybeans (i.e., a faster coagulation). A difference in the force between the soymilk coagulums from the frozen and unfrozen soybeans was evident, regardless of heating time. It was also observed that the force for the coagulums obtained with 5-min heating was a little higher than that with 2.5-min heating, for all samples.

The gelation of tofu has been known to involve a two-step process: protein denaturation by heat, followed by hydrophobic coagulation promoted by coagulant (Kohyama, Sano, & Doi, 1995). At first, the hydrophobic

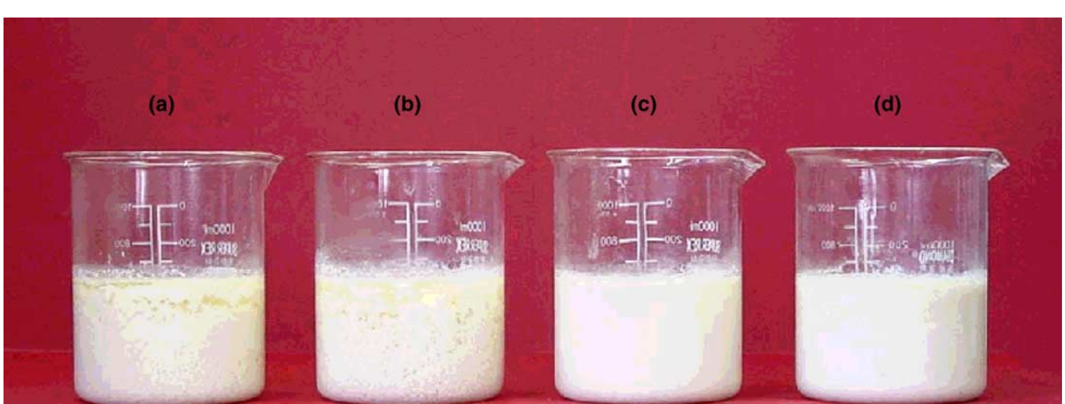

Fig. 1. Photograph of soymilk coagulum. The soymilk coagulum was prepared using soymilk heated for 2.5 or 5 min, made from frozen soybeans or unfrozen soybeans: (a) unfrozen and 2.5-min heating; (b) unfrozen and 5-min heating; (c) frozen and 2.5-min heating; (d) frozen and 5-min heating.

residues of the protein molecules which are located inside are exposed to the outside by heating (Koshima, Hamano, & Fukushima, 1981; Matsudomi, Mori, Kato, & Kobayashi, 1985). Since calcium ion reduces the electro-negativity of the heat-denatured soybean protein (Kohyama & Nishinari, 1993), the hydrophobic interactions between the protein molecules become more feasible, causing an extensive aggregation. In the present study it appears that the freezing promotes the hydrophobic coagulation of soyproteins.

Cotyledon of soybean seeds is composed of Palisade-like cells in which spherical protein bodies adhered to lipid bodies (also known as spherosomes) (Synder & Kwon, 1987). Lee et al. (1992) examined the effect of freezing on the microstructure of soybeans using an optical microscope. It was found that freezing ruptured cell membranes and the lipids were pushed out of the cells when the frozen soybeans were heated, while any leakage of lipid bodies was not observed in unfrozen soybeans. It seems that, by freezing, the protein bodies are easily separated from the lipids as they become freeze-denatured, thereby being partially insolubilised (Hashizume et al., 1971). With the leakage of the lipid bodies induced by freezing, the protein molecules in the frozen soybeans may become closer to neighbouring protein molecules. This can in turn facilitate the formation of intermolecular disulphide bonds, as reported by Hashizume et al. (1971). In addition, results of separate experiments (Noh, Kang, Hong, & Yun, 2004) in our laboratory show that freezing in combination with heat treatment, induces a considerable increase in hydrophobicity of soyprotein. It may therefore be expected that, in the presence of coagulant, the soymilk from the frozen soybeans coagulates faster (by enhanced hydrophobic interactions and intermolecular disulphide linkages) than that from unfrozen soybeans, resulting in a more uniform-structured gel (or tofu) network.

3.2. Scanning electron microscopic structure

The scanning electron microscopic (SEM) observation was carried out to explore whether the SEM image would differentiate the network structure of tofu prepared under different conditions. Fig. 3 shows the SEM image of tofu samples, a typical view of a particulate protein gel. It appears that the particulate network structure is composed of protein aggregates held together (forming the threads of the network). In addition,

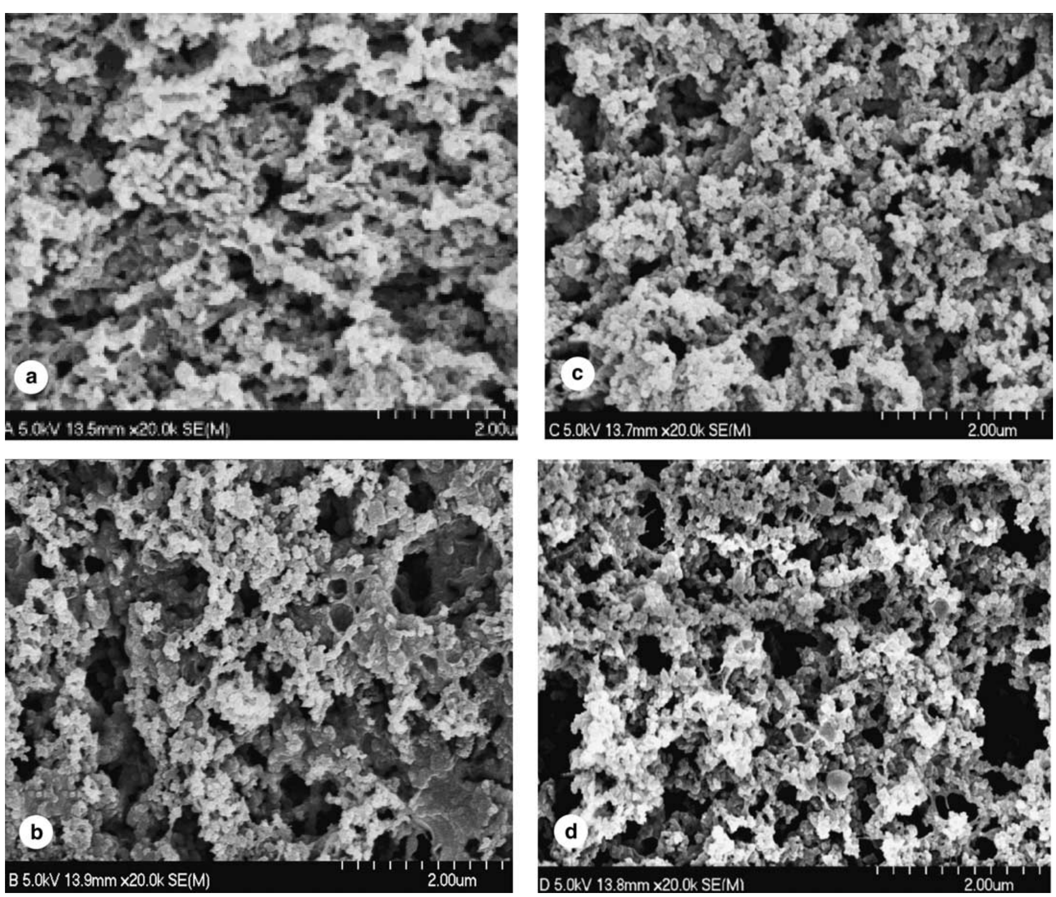

Fig. 3. Scanning electron microscopic image of tofu (20.0×). Tofu was prepared using soymilk heated for 2.5 or 5 min, made from frozen soybeans or unfrozen soybeans: (a) unfrozen and 2.5-min heating; (b) unfrozen and 5-min heating; (c) frozen and 2.5-min heating; (d) frozen and 5-min heating.

Table 1
Texture profile analysis of tofu

Sample	Hardness (g)	Cohesiveness	Springiness (mm)	Gumminess (g)	Chewiness (g mm)
$TSN_{2.5}$ [A]	511.2[aB]	0.289[a]	7.498[a]	118.5[a]	888.4[a]
$TSF_{2.5}$	852.3[b]	0.350[b]	7.906[bc]	259.2[bc]	2047.3[bc]
TSN_5	625.6[ab]	0.360[b]	7.717[b]	198.4[ab]	1533.4[ab]
TSF_5	1175.9[c]	0.344[b]	7.819[c]	324.5[c]	2537.7[c]

[A] $TSN_{2.5}$, tofu from soymilk heated for 2.5 min, made from the unfrozen soybeans; TSN_5, tofu from soymilk heated for 5 min, made from the unfrozen soybeans; $TSF_{2.5}$, tofu from soymilk heated for 2.5 min, made from the frozen soybeans; TSF_5, tofu from soymilk heated for 5 min, made from the frozen soybeans.

[B] Means with the same letter within columns are not significantly different from each other ($P \leqslant 05$).

Table 2
Syneresis, yield and contents of moisture, protein and fat in tofu prepared under different conditions

Sample	Yield (g)	Water (%)	Protein (%)	Fat (%)	Syneresis (%)
$TSN_{2.5}$ [A]	301.8[aB]	82.6[a]	9.6[d]	2.208[a]	2.9[a]
$TSF_{2.5}$	284.9[a]	79.1[b]	16.2[b]	1.451[b]	4.4[b]
TSN_5	289.8[a]	79.2[b]	14.0[c]	2.206[a]	3.3[a]
TSF_5	279.7[a]	77.2[c]	19.3[a]	1.597[b]	4.9[b]

[A] Sample designations are the same as in Table 1.

[B] Means with the same letter within columns are not significantly different from each other ($P \leqslant 0.05$).

there were differences in size and density of the particles among the tofu samples: tofu from frozen soybeans showed more orderly, denser, and smaller particles, as shown in Fig. 3(c) and (d) and more space was observed in the longer heating-time samples (Fig. 3(b) and (d)). As described earlier, it is likely that enhanced hydrophobic interactions (Noh et al., 2004) and intermolecular disulphide linkages (Hashizume et al., 1971) are responsible for the more orderly and denser network of tofu from frozen soybeans. It has been reported (Koshima et al., 1981; Matsudomi et al., 1985) that heat treatment makes the molecules of globular proteins unfold, exposing hydrophobic residues to the surface. In tofu making, therefore, with a longer heating time, more hydrophobic residues of the protein molecules would be exposed, resulting in enhanced hydrophobic interactions among the protein molecules. Water trapped in the gel network formed under these circumstances can easily be removed out by pressing. This may cause the gel structure to be more spacious (see also Table 2).

The microscopic images shown in Fig. 3 look a little different from those obtained from previous studies. The SEM image taken by Lee and Rha (1978) revealed well-defined three dimensional honeycomb structures with larger holes. The result of Saio (1979) showed a sponge-like structure with holes of different sizes. Different heating times may cause these differences.

3.3. Textural properties

The textural properties of tofu play an important role in terms of quality and consumer acceptability. Since microscopic examination revealed a clear difference in the structures of tofu samples (Fig. 3), a mechanical test was carried out to reveal any link between the structural characteristics and the textural properties. As shown in Table 1, tofu texture varied with the processing methods. Highest values for hardness, gumminess and chewiness were observed in tofu from soy slurry (using frozen soybeans) heated for 5 min (TSF_5), followed by $TSF_{2.5}$, tofu from soy slurry (using unfrozen soybeans) heated for 5 min (TSN_5) and $TSN_{2.5}$ in that order. Those values increased upon increasing the heating time, though only the hardness of tofu made with frozen soybeans and the springiness of tofu made with unfrozen soybeans were significantly different. Excessive heating for soy slurry prepared from frozen soybeans (e.g. 10 min) resulted in tofu with a ragged texture (data not shown). It is interesting to note in Table 1 that values for hardness, springiness, gumminess and chewiness are higher for the $TSF_{2.5}$ than for the TSN_5, although the differences are not significant. This suggests that freezing is more influential than heating in causing such textural properties.

We have shown in Figs. 1–3 that freezing may have a positive influence on the quality of tofu in various aspects. This speculation extends to this section. It appears that there is a positive correlation between the network structure and textural properties of tofu: the more orderly and denser is the structure, the higher are the values of textural parameters. Similarly, Saio (1979) also reported that density of the network of tofu correlates with the hardness of tofu. It should be point out, however, that, without heat treatment, soymilk made with frozen soybeans in the presence of coagulant only produced a paste-like gel (data not shown).

3.4. Yield and composition of tofu

Yield and composition of tofu varied with the processing methods, as shown in Table 2. The yield of tofu prepared from frozen soybeans was lower than that from unfrozen soybeans and the yield decreased with increase of the heating time. In addition, both freezing and prolonged heating also resulted in tofu with low moisture content. Decrease in the yield was reflected by the lower moisture content. From the results in Figs. 1 and 2, the lower yield and moisture content of tofu from frozen soybeans in Table 2 may be ascribed to the denser and more compact structure, which made water easily release from the curd during pressing.

Protein content of tofu from frozen soybeans was found to be higher than that of tofu from unfrozen soybeans: 19.3% for the TSF_5, 16.3% for the $TSF_{2.5}$, 14.1% for the TSN_5 and 9.65% for the $TSN_{2.5}$. In contrast, tofu from frozen soybeans contained less fat than that from unfrozen soybeans: 1.597% for the TSF_5, 1.45% for the $TSF_{2.5}$, 2.206% for the TSN_5 and 2.208% for the $TSN_{2.5}$. These results indicate that fats in the coagulum from frozen soybeans are more easily released during pressing, probably suggesting that freezing considerably decreases the fat-binding capacity of protein. Similar results have also been reported (Lee et al., 1992) that some fats in frozen soybean are pushed out of the cells during heating. As described earlier, freezing of soybeans may enhance the aggregation of the protein molecules during heating. This may in turn lead to an increased participation of the soyprotein in the gel network, thereby resulting in tofu with higher protein content.

3.5. Syneresis

As shown in Table 2, syneresis was found to be higher in tofu from frozen soybeans (4.9% for the TSF_5 and 4.4% for the $TSF_{2.5}$) than in that from unfrozen soybeans (3.3% for the TSN_5 and 2.9% for the $TSN_{2.5}$). It is generally accepted that syneresis in the protein gel during storage is caused by an increased cross-linking among protein molecules through various interactions, making the protein gel matrix denser. At higher protein concentrations, one might expect an increased number of cross-linking in the gel network. This may induce an increase in syneresis.

3.6. Sensory characteristics

Table 3 shows results of sensory evaluation of tofu for some quality attributes such as colour, flavour and mouthfeel. The results were expressed on a 9-point hedonic scale. For all the attributes, except colour evaluated, tofu from frozen soybeans was found to have lower (better) scores than that from unfrozen soybeans. The lowest (best) scores were observed in TSF_5, while the highest (worst) scores were observed in $TSN_{2.5}$. It seems that heating for 2.5 min is not sufficient for the soyproteins to thermally denature in tofu making, thereby resulting in poor sensory properties. It has been reported (Saio, 1979) that limited heating for soymilk resulted in a paste-like gel, while excessive heating led to a decrease in cohesiveness of the gel. In commercial processing, heating at 95 °C for 7 min is adopted to ensure the destruction of trypsin inhibitor and the inactivation of lipoxygenase (Moizuddin, Harvey, Fenton, & Wilson, 1999; Wilkens, Mattick, & Hand, 1967). As with the case of textural properties, $TSF_{2.5}$ showed better sensory properties than TSN_5. These results are in line with the results of Lee et al. (1992) that freezing not only reduces the cooking time of soybean to one-half, but improve the taste of soybean.

Table 3
Effect of freezing of soybeans on sensory characteristics of tofu

Sample	Colour	Flavour	Mouthfeel	Overall acceptability
$TSN_{2.5}$ [A]	2.5^{aB}	5.1^{a}	6.2^{a}	7.1^{a}
$TSF_{2.5}$	2.8^{a}	4.0^{bc}	4.0^{bc}	4.7^{c}
TSN_5	2.5^{a}	4.8^{ab}	5.0^{b}	6.0^{b}
TSF_5	2.6^{a}	3.5^{c}	3.1^{c}	3.6^{d}

[A] Sample designations are the same as in Table 1.
[B] Means with the same letter within columns are not significantly different from each other ($P \leqslant 0.05$).

Table 4
Hunter colorimetric readings for tofu

Sample	Hunter values		
	L	*a*	*b*
$TSN_{2.5}$ [A]	82.7	1.4	11.8
$TSF_{2.5}$	83.2	1.3	12.5
TSN_5	83.3	1.4	12.0
TSF_5	82.8	1.6	12.0

[A] Sample designations are the same as in Table 1.

3.7. Colour

Good quality tofu is white or light yellow in colour. All of the tofu samples prepared in this experiment were light yellow in colour. Hunter colorimetric readings also indicate no differences in *L*, *a* and *b* values as shown in Table 4. This means that freezing of soybeans, as well as length of heating, had little effect on the colour of tofu.

4. Conclusions

It has been demonstrated that freezing of soybeans affects both the coagulation of soymilk and the quality of tofu. Freezing enhances some textural parameters of tofu such as hardness, springiness, gumminess and chewiness, as well as the rate of coagulation of soymilk in the presence of coagulant. Tofu from frozen soybeans has been evaluated to be better in some sensory attributes, such as flavour and mouthfeel. Freezing also makes it possible to produce a tofu with high protein and low fat content. In addition, heating time needed for tofu making may be reduced to some extent without impairing the quality of tofu if frozen soybeans are used. From these results, it is concluded that freezing of soybeans could be one practical way to modify the texture and sensory properties of a tofu though further research is much needed in this field.

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References

Amstrong, H. J., Hill, S. E., Schrooyen, P., & Mitchell, J. R. (1994). A comparison of the viscoelastic properties of conventional and Maillard protein gels. *Journal of Texture Study, 25*, 286–298.

AOAC. (1995). *Official methods of analysis*. (16th ed.). Arlington, VA.

Beddows, C. G., & Wong, J. (1987). Optimization of yield and properties of silken tofu from soybeans. II. Heat processing. *International Journal of Food Science Technology, 22*, 23–27.

Cai, T. D., & Chang, K. C. (1998). Characteristics of production-scale tofu as affected by soymilk coagulation method: Propeller blade size, mixing time and coagulant concentration. *Food Research International, 31*, 289–295.

Fuchigami, M., & Teramoto, A. (1997). Structural and textural change in kinu-tofu due to high-pressure-freezing. *Journal of Food Science, 62*, 828–832.

Gandhi, A. P., & Bourne, M. C. (1988). Effect of pressure and storage time on texture profile parameters of soybean curd (tofu). *Journal of Texture Study, 19*, 137–142.

Hashizume, K., Kakiuchi, K., Koyama, E., & Watanabe, T. (1971). Denaturation of soybean protein by freezing. *Agricultural and Biological Chemistry, 35*, 449–459.

Hashizume, K., Kosaka, K., Koyama, E., & Watanabe, T. (1974). Studies on the changes of the properties of soybean protein by freezing. IV. Production of new textured protein by freezing. *Journal of Japanese Society of Food Science and Technology, 21*, 136–140 (in Japanese).

Hashizume, K., Nakamura, N., & Watanabe, T. (1974). Studies on the changes of the properties of soybean protein by freezing.V. Improved production of new textured protein by freezing. *Journal of Japanese Society of Food Science and Technology, 21*, 141–145 (in Japanese).

Hou, H. J., Chang, K. C., & Shih, M. C. (1997). Yield and textural properties of soft tofu as affected by coagulation method. *Journal of Food Science, 62*, 824–827.

Inoue, T., & Osatake, H. (1988). A new drying method of biological specimens for scanning electron microscopy: The *t*-butyl alcohol freeze drying method. *Archives of Histology and Cytology, 51*, 53–58.

Kohyama, K., Sano, Y., & Doi, E. (1995). Rheological characteristics and gelation mechanism of tofu. *Journal of Agricultural and Food Chemistry, 43*, 1808–1812.

Koshima, I., Hamano, M., & Fukushima, D. (1981). A heat denaturation study of the 11S globulin in soybean seeds. *Food Chemistry, 6*, 309–322.

Kohyama, K., & Nishinari, K. (1993). Rheological studies on the gelation process of soybean 7S and 11S proteins in the presence of glucono-δ-lactone. *Journal of Agricultural and Food Chemistry, 41*, 8–14.

Lee, C. H., & Rha, C. K. (1978). Microstructure of soybean protein aggregates and its relation to the physical and textural properties of the curd. *Journal of Food Science, 43*, 79–84.

Lee, D. R., Choi, Y. H., Kim, M. K., & Yun, S. E. (1992). Influence of freezing upon the cooking time and eating quality of beans. *Journal of Korean Agricultural and Chemical Society, 35*, 219–226 (in Korean).

Lim, B. T., de Man, J. M., de Man, L., & Buzzell, R. I. (1990). Yield and quality of tofu as affected by soybean and soymilk characteristics. Calcium sulphate coagulant. *Journal of Food Science, 55*, 1088–1092.

Matsudomi, N., Mori, H., Kato, A., & Kobayashi, K. (1985). Emulsifying and forming properties of heat-denatured soybean 11S globulins in relation to their surface hydrophobicity. *Agricultural and Biological Chemistry, 49*, 915–919.

Moizuddin, S., Harvey, G., Fenton, A. M., & Wilson, L. A. (1999). Tofu production from soybeans or full-fat soyflakes using direct and indirect heating process. *Journal of Food Science, 64*, 145–148.

Noh, E. -J, Kang, C., Hong, S. T., & Yun, S. E. (2004). Freezing of soybeans influences on hydrophobicity of soyproteins. *Food Chemistry* (to be submitted).

Saio, K. (1979). Tofu-relationships between texture and fine structure. *Cereal Foods World, 24*, 342–354.

SAS Institute Inc. (1990). *SAS/STAT user's guide* (4th ed.). Cary, NC: SAS Institute Inc.

Shen, C. F., de Man, L., Buzzel, R. I., & de Man, J. M. (1991). Yield and quality of tofu as affected by soybean and soymilk charactreistics: Glucono delta lactone coagulant. *Journal of Food Science, 56*, 109–112.

Shih, M. C., Hou, H. J., & Chang, K. C. (1997). Process optimization for soft tofu. *Journal of Food Science, 62*, 833–837.

Sun, N., & Breene, W. M. (1991). Calcium sulphate concentration influence on yield and quality of tofu from five soybean varieties. *Journal of Food Science, 56*, 1604–1607.

Synder, H. E., & Kwon, T. W. (1987). Morphology and composition. In H. E. Synder & T. W. Kwon (Eds.), *Soybean utilization* (pp. 19–31). New York: Van Nostrand Reinhold.

Wilkens, W. F., Mattick, L. R., & Hand, D. B. (1967). Effect of processing method on oxidative off-flavors of soybean milk. *Food Technology, 21*, 1630–1633.