Research Note

Changes in Quality Characteristics of Tofu with Freezing Treatment of Soybeans

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Abstract The aim of this study was to investigate the influence of length of freezing time (-18°C for 5 or 24 h) on the quality characteristics of tofu. The yield and moisture content of tofu obtained from frozen soybean were lower than control tofu. Tofu made from frozen soybeans showed higher hardness, gumminess and chewiness than the control. Freezing treatment also made the microstructure of the tofu more uniform than the control. However, after 24-h freezing, hardness decreased and the microstructure of tofu appeared to form pores. Sensory evaluation showed that mouth-feel decreased with an increase of freezing time. Among all of tofu frozen at -18°C , 5-h freezing treatment showed the highest overall acceptability. These results indicate that the length of time that soybeans are frozen before making tofu affects the quality of tofu.

Keywords: tofu, soybean, freezing treatment, textural properties, sensory evaluation

Introduction

Tofu, also known as gel-type soybean food, has increased interests for important and popular food items throughout the world. Based on producing process, textural characteristics, and moisture content, there are various kind of tofu such as dry tofu, firm tofu, or soft tofu (1,2). Tofu making properties such as solid extractability, tofu color, tofu yield, and texture is closely related to quality attributes affecting consumer acceptability (3). These properties of tofu are dependent on type and concentration of coagulants (4), method and condition of processing (5) as well as cultivar and storage condition of soybeans (3,6-7). In particular, several processing factors such as hydrothermal treatment (8), freeze treatment of soybean, (9) and hydrostatic pressure (10) have been used to improve the texture and quality of tofu.

The freeze treatment of soybean plays an important role in the taste of tofu and cooking time (11). In soybeans that have never been frozen, lipids are well-incorporated into the soy protein structure (11). However, when the soybean is frozen, the lipids are easily expelled from the protein bodies and become freeze-denatured so that they are partially insoluble. This may be related to be polymerization of protein molecules via formation of intermolecular disulfide bonds (12). Noh *et al.* (13) has confirmed that tofu made from soybeans frozen to -20°C for 5 h coagulates faster than that from unfrozen soybeans by heating. However, there is little information about changes in quality characteristics of tofu when the soybeans are frozen for different lengths of time. Thus, the aims were to investigate the effects of a freezing treatment (-18°C) for different times (5 or

24 h) on soybeans and the quality characteristics of tofu made from these soybeans.

Materials and Methods

Materials Soybeans were obtained from Nonghyup (Yeongju, Korea). Food-grade calcium sulfate ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) was purchased from TAEJIN GNS (Seoul, Korea).

Freezing treatment of soybeans Soybeans were soaked in distilled water for 10 h at room temperature (22°C). The soaked soybeans were drained in a basket, followed by freezing at -18°C for 5 or 24 h. After freeze treatment, soybeans were thawed with tap water for 5 min.

Preparation of soybean flours The frozen soybeans were dried at 40°C , ground and passed through 100 mesh sieve. The soybean flour was then stored at 4°C until analysis.

Hydration property of soybean flours The hydration properties (i.e., water absorption index [WAI] and water solubility [WS]) of soybean flours were measured according to a method described by Lee and Inglett (14). Soybean flours (2.5 g) were dispersed in distilled water (10 mL) and placed at room temperature for 30 min. After centrifugation at $3,000 \times g$ for 15 min, the supernatant was dried at 105°C in an oven until it was a constant weight, and the wet

sediment of the soybean flours was weighed. Then, the WAI and WS were calculated as follows:

$$\text{WAI} = \text{wet sediment weight} / \text{dry sample weight}$$

$$\text{WS (\%)} = \text{dry supernatant weight} / \text{dry sample weight} \times 100$$

Preparation of tofu Freeze-treated soybean (90 g, dry basis) were ground with 1,530 mL distilled water for 15 min and then heated for 8 min to produce soymilk using a tofu maker (IOM-201A). Then, the soymilk (1,350 mL) was coagulated with 1.8 g (2% of the soybean weight) of calcium sulfate to produce smoother tofu in an 80°C water bath for 10 min. The coagulum obtained was placed in a mold (9 cm × 5 cm × 7 cm) lined with cheesecloth and pressed with a 1 kg weight for 20 min to produce tofu. The tofu was then left in the distilled water for 1 h. Tofu that made from unfrozen soybeans was used as control.

Yield and moisture Yield of tofu was expressed as g tofu obtained from 1,350 mL of soymilk. Moisture content was measured by drying 2 g of fresh tofu at 105°C in a dry oven to a constant weight.

Color The colors of fresh tofu were measured using a CR-400 Chroma Meter (Minolta Co. Ltd., Osaka, Japan). The Minolta calibration plate was applied for calibration. The value of L^* (lightness), a^* (red-green), and b^* (yellow-blue) were expressed for each sample. Each value represented a mean value of nine replicate.

Textural properties analysis Measurements of textural properties of tofu were made using a TA-XT2i Texture Analyzer (Stable Micro Systems, Goldaming, UK) fitted with a 5-kg load cell. The samples of tofu (10 mm × 10 mm × 10 mm) were compressed twice by a cylinder probe (D: 35 mm) to 80% deformation of their original height. The pre-test, test and post-test speeds were all set to 1 mm/s. The following parameters of the samples were measured: hardness, springiness, cohesiveness, gumminess and chewiness. All experiments were conducted in six replicate tests.

Microstructure analysis A scanning electron microscope (NOVA NANO SEM 450) was used to examine the microstructure of the tofu. Tofu was cut into 2 mm × 2 mm × 1 mm pieces. Samples were frozen in liquid nitrogen and freeze-dried. The microscope was operated at 3.0 kV for observation.

Sensory evaluation The tofu samples were evaluated by 35 untrained panelists using the method as described by Kim *et al.* (15). Samples were cut into 10 mm × 10 mm × 10 mm pieces. The panelists evaluated the sensory attribute of the tofu in respect to mouth-feel, firmness, flavor and overall acceptability, and the results were recorded using a 5-point scale (1 point=very poor to 5 points=very good). All samples were coded and presented in a randomized arrangement.

Statistical analysis All analyses were carried out triplicate unless otherwise stated. SPSS (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Significant difference was determined using analysis of variance (ANOVA) and followed by Duncan's multiple range tests at a significant level of 5%.

Results and Discussion

Hydration property of soybean flours Table 1 shows the hydration property of soybean flours made from control, 5-h-frozen soybeans and 24-h-frozen soybeans. After the freezing treatment, WAI of soybean flours increased for both treatment times. As observed by Akingbala *et al.* (16), fat can restrict swelling of soy flour. Therefore, these results are consistent with the observations that fat can leach out of the soybean during frozen storage (11,12) and, in turn, increase the WAI of the resultant matrix. However, after 24-h freezing, the WAI of the soybean flour was less than that at 5-h freezing, although still greater than that of the control. WS of frozen soybean flours was lower than the control, indicating that freezing treatment decreased the WS of soybean flour. Compared to 5-h-frozen soybean flours, the WS of 24-h-frozen soybean flours was higher.

Physicochemical properties of tofu Yield and moisture of tofu made with unfrozen soybeans (control), 5-h-frozen soybeans and 24-h-frozen soybeans are shown in Table 1. The yield of tofu made with 5-h-frozen soybeans was lower than control. However, after 24-h freezing treatment, the yield increased again, though not quite to the value obtained with unfrozen soybeans. The order was as followed: control (195.67 g) > -18°C, 24 h (190.39 g) > -18°C, 5 h (186.31 g). The moisture content of tofu was significantly decreased by freezing ($p < 0.05$). Consistent with the yield trends, tofu made from 5-h-frozen soybeans showed the least amount of moisture, and the moisture content of tofu made from 24-h-frozen soybeans was between that of the control and 5-h-frozen soybeans. The significant decrease yield was possibly attributed to the lower moisture content. This result is consistent with Cai and Chang (17) who reported that higher moisture content correlated with higher tofu yield. The lower yield and moisture content of tofu made with frozen soybeans may be responsible for the denser and more compact structure (Fig. 1), which facilitates water release from the curd during pressing. These results are also consistent with results obtained by Noh *et al.* (13).

Tofu color is white or light yellow, suggesting it is good quality. As shown in Table 1, all the tofu color showed a light yellow (L : 82.66–83.03; b : 10.98–11.39). Hunter lab value indicate no significant differences in L , a , and b values between treatment groups ($p > 0.05$). This indicates that freezing of the soybeans had little effect on the color of tofu.

The texture properties of tofu made from soybean with and without freezing treatment are shown in Table 1. Freezing treatment

Table 1. Physicochemical properties of flour and tofu made from unfrozen soybeans (control), 5-h-frozen soybeans (−18°C, 5 h) and 24-h-frozen soybeans (−18°C, 24 h)

Sample ^a	Parameters	Control	−18°C, 5 h	−18°C, 24 h
Flour	Technological parameters			
	Water absorption index (WAI)	2.97±0.15 ^{ab1)}	3.03±0.18 ^a	2.78±0.17 ^b
	Water solubility (%)	42.72±1.11 ^a	25.71±0.39 ^c	30.16±0.88 ^b
Tofu	Technological parameters			
	Yield (g)	195.67±1.64 ^a	186.31±2.74 ^c	190.39±0.74 ^b
	Moisture (%)	82.59±0.71 ^a	80.08±0.63 ^c	81.31±0.70 ^b
	Color parameters			
	L*	83.03±0.41 ^a	82.66±0.25 ^a	82.85±0.29 ^a
	a*	−1.12±0.05 ^a	−1.13±0.02 ^a	−1.18±0.02 ^a
	b*	10.98±0.04 ^a	11.07±0.13 ^a	11.39±0.29 ^a
	Textural parameters			
	Hardness (g)	612.33±62.68 ^c	1087.49±56.59 ^a	744.51±31.13 ^b
	Springiness	0.91±0.07 ^a	0.94±0.03 ^a	0.92±0.03 ^a
	Cohesiveness	0.44±0.02 ^a	0.43±0.02 ^a	0.43±0.02 ^a
	Gumminess (g)	0.64±0.02 ^c	0.64±0.02 ^a	0.66±0.05 ^b
	Chewiness (gs)	0.40±0.01 ^c	0.41±0.04 ^a	0.42±0.01 ^b

^{1)a-c}Values with different subscripts within the same row are significantly different among samples at $\alpha=0.05$ level by Duncan's multiple range test.

significantly ($p<0.05$) increased the hardness, gumminess and chewiness while having little effect on the springiness and cohesiveness. Hashizume *et al.* (12) also indicated that stronger hydrophobic interactions and more intermolecular disulfide linkages are present in soymilk from frozen soybeans. Thus, these proteins coagulate faster than those from unfrozen soybeans, and result in harder tofu. The results of this study show the same trend. The hardness of tofu with frozen soybeans (mean of 5 and 24 h) was 300 g higher than control. Compared to tofu made from soybeans with 5-h freezing treatment, the hardness of tofu from soybeans with the 24-h freezing treatment decreased from an average of 1087.49 to 744.51 g, suggesting that excessive freezing may limit the formation of a good gel network. All tofu samples were similar ($p>0.05$) in regard to springiness and cohesiveness. The gumminess and chewiness parameters showed the same trend as the hardness values.

Microstructure analysis of tofu To further confirm the change of gel network by freezing treatment, the microstructures of tofu made from unfrozen soybeans, 5-h-frozen soybeans and 24-h-frozen soybeans were investigated by SEM (Fig. 1). SEM investigation showed that the different freezing treatments caused changes in the microstructure of tofu. Control tofu exhibited a discontinuous network with big and irregular pores. However, after freezing, the structure of tofu became continuous and uniform. Consistent with our observations, Hu *et al.* (18) observed that the increased surface hydrophobicity of soy protein isolate (SPI) could lead to formation of more compact aggregates, resulting in a more uniform network. Therefore, the freezing treatment may enhance hydrophobic interactions of soy protein, thereby making the structure of the tofu more uniform. Hashizume *et al.* (12) also suggested that more orderly and denser network of tofu made with frozen soybeans would be possibly due to

enhanced hydrophobic interactions and intermolecular disulfide linkages. The tofu made from 5-h-frozen soybeans showed the most orderly, most continuous, and densest structure. After freezing for 24 h, microstructure of tofu showed a larger pore, which indicate that the quality of tofu decreased (19). This result is consistent with the changes in textural analysis (Table 1). Thus, we were able to correlate characteristics of the microstructure and textural properties of the tofu: the hardness, gumminess, and chewiness were increased due to the more orderly and denser the structure by freezing treatment. Similarly, Saio (5) reported that the density of the tofu strongly influences the hardness of tofu. Moreover, Shen *et al.* (6) also confirmed that the hardness of the tofu would be related to the denser microstructure and the larger the protein aggregates in the structure.

Sensory characteristics The sensory analysis of tofu are shown in Fig. 2. The quality attributes that were assessed were mouth-feel, flavor, firmness and overall acceptability on a 5-point scale. Mouth-feel was decreased with an increase of freezing time up to 24 h. However, no significant difference ($p>0.05$) was found between the control tofu and the tofu made from 5-h-frozen soybeans. All of tofu samples had similar flavor score. After freezing for 24 h, there was a significant decrease in perceived firmness ($p<0.05$). However, tofu made from 5-h-frozen soybeans showed the highest firmness score. In all of the tofu samples, tofu made with 5-h-frozen soybeans showed the highest score of overall acceptability. The order of overall acceptability score was as follows: −18°C, 5 h>−18°C, 24 h>control. These results agree with results of Lee *et al.* (11) that freezing soybeans improved the taste of tofu.

In this study, soybean was subjected to freezing treatment at −18°C for two different storage times (5 or 18 h) and investigated for its

Fig. 1. Scanning electron micrographs of tofu. The tofu was made from (A) unfrozen soybeans (control), (B) 5-h-frozen soybeans (-18°C , 5 h) or (C) 24-h-frozen soybeans (-18°C , 24 h). The length of scale bar represents 10 μm .

effects on flour and tofu characteristics. The freeze treatment influenced hydration properties (e.g. WAI, WS, and SP) of the flour, which varied dependent on the length of freezing time. The tofu prepared from soybean frozen at -18°C for 5 h showed highest hardness among all tested samples. Also, the microstructure of the tofu from soybean frozen at -18°C for 5 h showed a denser structure, much more continuous and uniform than tofu from soybean frozen at -18°C for 24 h. The results indicated that tofu made from 5 h-frozen soybeans showed the best quality in both textural and

Fig. 2. Sensory properties of tofu made from unfrozen soybeans (control), 5-h-frozen soybeans (-18°C , 5 h) and 24-h-frozen soybeans (-18°C , 24 h).

sensory properties, confirming that appropriate freezing treatment notably improved the tofu quality.

Disclosure The authors declare no conflict of interest.

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