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The Effect of Chitosan on the Rheological Properties of Soymilk and Quality Characteristics of Tofu

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Abstract

The effects of low viscosity chitosan on the rheological properties of soymilk using a model system and on tofu qualities were examined. The flow behavior of soymilk with chitosan closed the Newtonian flow and stabilized according to increasing chitosan concentration. The soymilk containing glucono-δ-lactone exhibited a more pseudoplastic flow behavior compared with that of the control soymilk. The addition of low viscosity chitosan to the tofu preparation did not significantly affect its physicochemical properties. However, the results of the TEM image and instrumental textural properties showed that low viscosity chitosan affected the construction of the tofu structure. Chitosan tofu had low scores across the whole field of appearance in the sensory evaluation, and its overall eating quality was scored significantly lower. These results suggest that the addition of low viscosity chitosan affects the quality of tofu, which changes according to the degree of polymerization and concentration of chitosan.

Key words: soymilk, tofu, low molecular weight chitosan, rheology, texture, sensory evaluation

INTRODUCTION

Chinese, Japanese, Korean, macrobiotic, or vegetarian soybean dishes prepared with tofu (in Korean, *dubu*) and soymilk have become increasingly popular worldwide among people seeking to prevent illness, reduce cholesterol levels, and generally improve their health (1). Soybeans are a rich source of isoflavones which are the most common estrogenic compounds found in plants. Isoflavones have been shown to possess antimicrobial and insecticidal properties and to prevent and reduce the risk of various cancers (2,3). Tofu making is a complex interaction of various factors, including intrinsic characteristics (chemical composition) of the soybeans and processing conditions. Varieties and storage conditions of soybeans have been reported to affect the texture of tofu, but the results differ greatly (4,5). Processing conditions, such as type and concentration of coagulants (6,7), heating time and temperature of the soymilk (8), temperature and extent of stirring during coagulation (9), and molding time and pressure all affect the quality of the tofu. Soymilk which is a highly homogeneous emulsion of protein particles and oil droplets, is a food product in its own right whose quality is mainly determined by texture and sensory characteristics of the beverage. As tofu is prepared from soymilk, the physical properties of the tofu largely depend on the properties of the soymilk.

Polysaccharides have often been used to alter or control the functional properties of food. Chitosan, a natural amino polysaccharide, is a deacetylated chitin formed by the reaction of chitin with concentrated alkali, and is a readily available commercial product. Chitosan is charged with cations at low pH values (pH$<$6) due to the protonation of its amino groups. The application of chitinous polymers in food industries has capitalized on their functionalities such as hypocholesterolemic activity, lipid binding properties, antibiotic activities, water conservation, emulsion stability, and dietary fiber (10,11). Especially, there has been increasing interest in the application of chitosan as a food additive due to its antimicrobial activity (12). Chitosan has the potential to be used as an additive in tofu to prolong shelf life because of its antimicrobial activity. The antimicrobial activity of chitosan has been recognized against fungi and some bacteria and is influenced by a number of factors that include the type of chitosan (e.g. plain or derivative), the degree of chitosan polymerization, host natural nutrient constituency, substrate chemical and/or nutrient composition, and environmental conditions (e.g. substrate water activity and/or moisture) (13,14). It was reported that the addition of chitooligosaccharide and high

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viscosity chitosan to tofu slightly affected its physicochemical properties and sensory attributes, but it also produced a softer tofu and inhibited cross-linking between the protein granules (15,16).

This study was undertaken to examine the effect of low viscosity chitosan on the rheological properties of soymilk using a model system and to investigate how the qualities of tofu are affected by the addition of low viscosity chitosan during its preparation.

MATERIALS AND METHODS

Materials

Soybeans grown in Chungpook Province, Korea were purchased from Nong-Hyup market. Low viscosity chitosan (200 ~ 250 cps viscosity grades) was obtained from RC Bio Chemicals (Busan, Korea). All other chemicals and reagents were commercially available analytic grade.

Preparation of soymilk

The soymilk was prepared as follows: 300 g of soybeans were washed and soaked in water at room temperature for 12 hr. The swollen beans were drained and ground for 10 min in a homogenizer (A76, Moulinex, Paris, France) with enough added water to give a water-dry beans ratio of 10:1 (weight basis), and the homogenate was heated to boiling in a stainless steel pot (18 cm diameter × 11.5 cm height) with manual stirring and maintained at 92 ~ 96°C for 15 min. The hot soy slurry was filtered through double layers of cheesecloth. When the soymilk was cooled to about 80°C, a solution of 0.1 or 0.2 g chitosan in 20 mL of 2% glucono-δ-lactone (GDL) solution was added (Table 1). The resulting solution was again filtered through double layers of cheesecloth to remove remaining large particles and cooled to 20°C in ice water. The total solid content of the suspension was adjusted to Brix 16% using a refractometer (Model PR-201, range Brix 0 ~ 60%, Atago, Japan).

Rheological measurements of soymilk

The rheological properties of the soymilk containing chitosan were measured using a viscometer (Model LVDVII+, Brookfield Inc., USA) with a concentric cylinder system (cup 27.5 mm dia. × 92.39 mm ht., bob 25.15 mm dia. × 90.74 mm ht.). By changing the rate of shear from 1 to 120 sec^{-1}, viscosity measurements were carried out at 25°C, which was controlled by a thermosel temperature controller and a water bath circulator. Each sample measurement was made with three replications. The shear rate vs. shear stress relationship was analyzed using the power law model (17).

$$\sigma = K\gamma^n$$

Where σ is the shear stress (mPa); K, a consistency index (mPa·s); γ, the shear rate (s^{-1}); and n, a dimensionless number that indicates the closeness to Newtonian flow.

Preparation of tofu

Tofu was prepared by the same method as previously reported (15). When the soymilk that was filtered through double layers of cheesecloth was cooled to 80°C, 0.1 or 0.2 g of low viscosity chitosan suspended in 20 mL of 2% GDL solution was added to 1700 mL of soymilk. Then, 9 g of calcium chloride in 20 mL distilled water was slowly added with stirring until the soymilk began to coagulate (Table 2). After settling for 10 min, the curd was transferred to a cheesecloth-lined wooden box (13 × 9 × 7 cm) and pressed by a 1 kg weight placed on the top for 30 min. The tofu was removed from the box and put in a container with tap water where it stood for 30 min, and then transferred to a container filled with 300 mL tap water. The tofu was sampled for chemical analysis, and the rest was stored in water at 4°C prior to textural analysis and sensory evaluation. The control tofu was prepared using the same procedure without the addition of chitosan and GDL.

Physicochemical properties of tofu

The moisture content of the tofu was determined by the AOAC method (18). Yield was calculated as the wet weight of fresh tofu obtained from 300 g of soybean. The color of tofu (L, a, and b value) was measured using a Whiteness checker RF-1 colorimeter (Nippon Denshoku

Table 1. Preparation conditions for tofu containing low viscosity chitosan

Tofu	Soymilk (mL)	Low viscosity chitosan (g)	2% Glucono-δ-lactone (mL)	$CaCl_2$ (g)
Control tofu[1)]	1700	0	0	9
GDL tofu[2)]	1700	0	20	9
Chito tofu-I[3)]	1700	0.1	20	9
Chito tofu-II[4)]	1700	0.2	20	9

[1)]Control tofu: tofu without glucono-δ-lactone.
[2)]GDL tofu: tofu containing glucono-δ-lactone.
[3)]Chito tofu-I: GDL tofu with 0.1 g low viscosity chitosan.
[4)]Chito tofu-II: GDL tofu with 0.2 g low viscosity chitosan.

Kogyo Co., Osaka, Japan). The pH of the soaking solution of the tofu was determined using a Toldedo 340 pH meter (Mettler, Leicester, England).

Determination of textural properties of tofu

The texture of the tofu was measured using a rheometer (COMPAC-100, Sun Scientific, Tokyo, Japan). Cylindrical samples (2.5 cm dia. × 2.0 cm ht.) were cut with a stainless steel cylindrical cutter. The tofu was cut into six pieces, and three central pieces were used for texture measurement and one was evaluated for sensory quality. The tofu samples were compressed to 30% deformation with a 5 kg loading cell and 300 mm min^{-1} crosshead speed. Textural properties including hardness, cohesiveness, and springiness were measured.

Transmission electron microscopy (TEM)

Microstructures of the tofu were observed using a transmission electron microscope (TEM, Hitachi-H-7100, Nakashi, Japan). The tofu samples were serially dehydrated with 50%, 60%, 70%, 80%, 90%, and 95% ethanol for 15 min, respectively, and the samples were immersed in isoamyl acetate for 1 hr at room temperature. Then dehydrated samples were treated with propylene oxide, embedded in epon 812 (Embed-812, EMS Co. Ltd., Washington, USA), sliced with an ultramicrotome (Sapernova, Leica, Austria), and electrostained with 2% uranyl acetate and 2% lead acetate.

Sensory evaluation

The sensory quality of the tofu was evaluated using a line-scale method with a 15-cm line anchored from none to extremely intense for each attribute. The overall eating quality of the tofu was evaluated separately during another session. Ten panelists who were graduate students in the Department of Food Science and Nutrition, Kyungpook National University performed the sensory evaluations. The panel was trained in 3 training sessions with commercial and control tofu until the sensory scores were consistent. The sample was cut into 3×4×1 cm blocks and presented in petri dishes with covers. The tofu was stored at 4°C for 30 min prior to serving. Reference samples were labeled and unknown samples were coded with random three digit codes using a random number table. Replicates of the treated samples were evaluated on different days.

Statistical analysis

The experiments for analyses of physicochemical properties were carried out in triplicate and the textural properties measured six times. Sensory evaluation was performed with four replications for each sample. Statistical analyses of collected data were evaluated using SAS software (19). The results were reported as mean values with standard deviation. A two-way ANOVA was conducted and Duncan's Multiple Range Test was performed to separate differences among the group means. Significance was established at $p<0.05$.

RESULTS AND DISCUSSION

Rheological properties of soymilk

Although soymilk solutions containing low viscosity chitosan showed nearly identical Newtonian behaviors (Fig. 1), the calculated rheological parameters of these solutions using the power law equation revealed changes in flow behavior with GDL and low viscosity chitosan (Table 2). With increasing chitosan concentrations, soymilk showed higher values in the flow behavior index and lower values in the consistency coefficient, suggesting that the flow behavior of soymilk closed the Newtonian flow and stabilized. The stabilizing effect of chitosan on soymilk may be due to differences in conformational structure rather than their charged amino groups. Soymilk is a colloidal dispersion consisting of lipid, protein, etc. The protein content of soymilk accounts for about 50% of its total solids (20). Therefore, proteins in soymilk play an important role in controlling the flow behavior of soymilk. The rheology of a protein solution can be a function of the geometry and interaction of the particles. The geometry, both the size and shape of the proteins in a solution, may vary due to conformational changes and also more predominantly

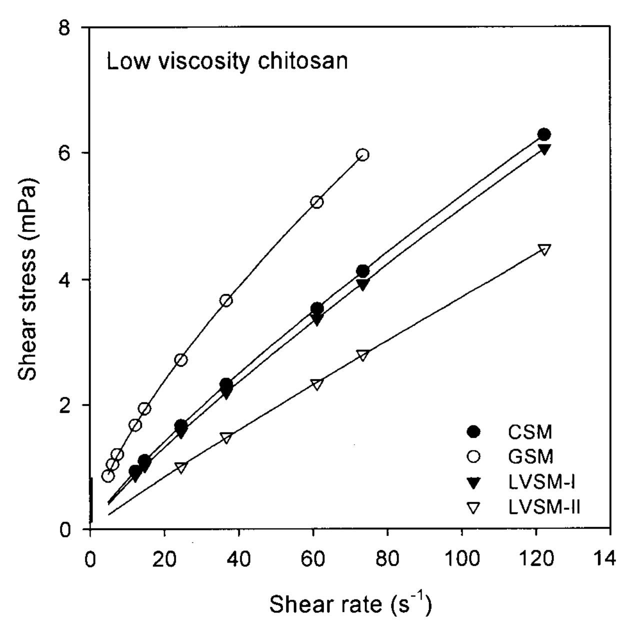

Fig. 1. The flow behavior of soymilk with added low viscosity chitosan as shear stress versus shear rate. CSM, control soymilk; GSM, control soymilk containing glucono-δ-lactone; LVSM-I, GSM with 0.1 g chitosan; LVSM-II, GSM with 0.2 g chitosan.

Table 2. Rheological constants for soymilk containing chitosan calculated by the power equation

Soymilk	2% Glucono-δ-lactone (mL)	Chitosan (g)	Flow behavior index (n)	Consistency coefficient (K)	R^2
CSM[1)]	0	0	0.827	0.287	0.97
GSM[2)]	20	0	0.706	0.104	0.95
LVSM-I[3)]	20	0.1	0.845	0.104	0.98
LVSM-II[4)]	20	0.2	0.926	0.052	0.98

[1)]CSM: Control soymilk.
[2)]GSM: Control soymilk containing glucono-δ-lactone.
[3)]LVSM-I: GSM with 0.1 g chitosan.
[4)]LVSM-II: GSM with 0.2 g chitosan.

due to the association or dissociation of the protein molecules due to intermolecular interactions (21). GSM exhibited a more psuedoplastic flow behavior compared with that of control soymilk. Changes in the flow behavior of GSM were probably due to the association of proteins by release of gluconic acid from GDL (7). The association and dissociation, in addition to changing in the hydrodynamic volume, because of the change in the exposed surface groups also leads to changes in the interaction potential.

Physicochemical properties of tofu

Chitosan is soluble in organic acids, so we used the GDL solution (2%) to dissolve low viscosity chitosan. GDL solution has a milder acidic taste than other acids and accordingly has no effect on the taste of tofu, and also acts as a coagulant. Two kinds of tofu, tofu based on water (control tofu) and tofu based on GDL solution (GDL tofu), were prepared to examine the additional effects of low viscosity chitosan on tofu (Table 1). The physicochemical properties of tofu are presented in Table 3. For all treatments, there were no significant differences in the yield, but the water content was significantly reduced in GDL-based chitosan tofu because GDL acts as a co-coagulant in soymilk. The pH of tofu ranged from 5.65 to 5.71, lower than pH 6.0, in all the groups. It was reported that the coagulation of soymilk of lower than pH 6.0 was a more important factor than the addition of calcium or magnesium ions to soymilk (22). If calcium or magnesium ions are bound to protein particles, the proteins coagulate with the lowering of the pH and start to form tofu. The action of GDL was probably due to the isoelectric precipitation of the protein by release of gluconic acid from GDL. Therefore, the addition of the GDL solution with coagulant accelerated the formation of tofu curd by decreasing the pH. Tofu made from each treatment showed no significant changes ($p<0.05$) in Hunter L and a values with respect to treatments. There were no significant differences in color among the other tofu samples. All tofu prepared in this study had a pale yellow color, indicating that the addition of low viscosity chitosan does not deteriorate the color of tofu.

TEM images of tofu

To study the network of the tofu matrix and its behavior after adding low viscosity chitosan, we analyzed the tofu structure using the TEM images. They showed the network structures constructed with small protein granules and oil drops (Fig. 2). The GDL tofu (B) and Chito tofu-I (C) also had well-developed protein aggregations, but the connections between the proteins were less developed when compared with those of the control tofu. This may be due to the calcium binding affinity in the GDL tofu being weaker than that in the control

Table 3. Physicochemical properties of tofu with low viscosity chitosan

Properties	Control tofu[1)]	GDL tofu[2)]	Chito tofu-I[3)]	Chito tofu-II[4)]
Yield (g/g bean)	$1.47\pm0.14^{5)a6)}$	1.51 ± 0.20^{a}	1.45 ± 0.20^{a}	1.60 ± 0.15^{a}
Water content (%)	83.40 ± 0.39^{a}	81.50 ± 1.25^{b}	79.19 ± 0.22^{c}	79.02 ± 0.15^{c}
pH	5.70 ± 0.03^{a}	5.65 ± 0.00^{b}	5.71 ± 0.05^{a}	5.71 ± 0.02^{a}
Color				
L	78.61 ± 0.22^{a}	78.71 ± 1.74^{a}	79.37 ± 0.12^{a}	79.28 ± 0.52^{a}
a	1.90 ± 0.22^{a}	1.81 ± 0.45^{ab}	1.56 ± 0.11^{b}	2.06 ± 0.28^{a}
b	11.22 ± 0.60^{ab}	11.03 ± 0.25^{b}	10.91 ± 0.20^{b}	11.44 ± 0.43^{a}

[1)]Control tofu: tofu without glucono-δ-lactone.
[2)]*GDL tofu: tofu containing glucono-δ-lactone.*
[3)]Chito tofu-I: GDL tofu with 0.1 g low viscosity chitosan.
[4)]Chito tofu-II: GDL tofu with 0.2 g low viscosity chitosan.
[5)]Values are the means of three replications.
[6)]Means within a row in each basic solution followed by the same letter are not significantly different ($p<0.05$).

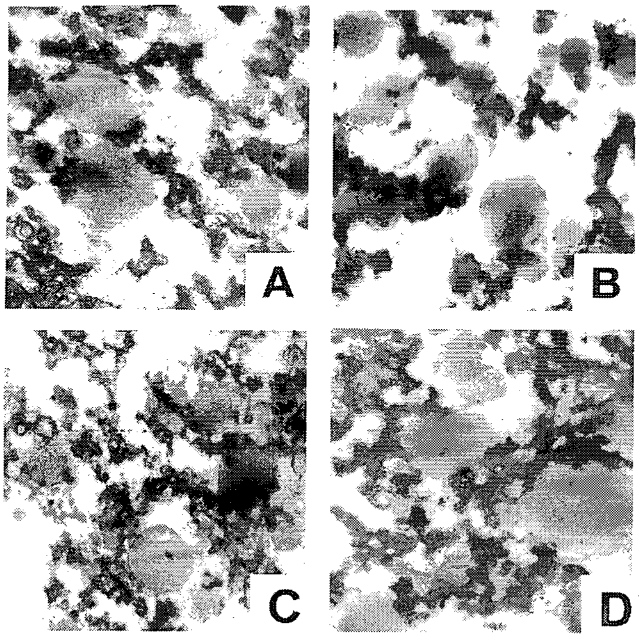

Fig. 2. Transmission electron microscope images (× 10,000) of tofu with low viscosity chitosan. A, control tofu (tofu without glucono- δ -lactone); B, GDL tofu (tofu containing glucono- δ -lactone); C, Chito tofu-I (GDL tofu with 0.1 g low viscosity chitosan); D, Chito tofu-II (GDL tofu with 0.2 g low viscosity chitosan).

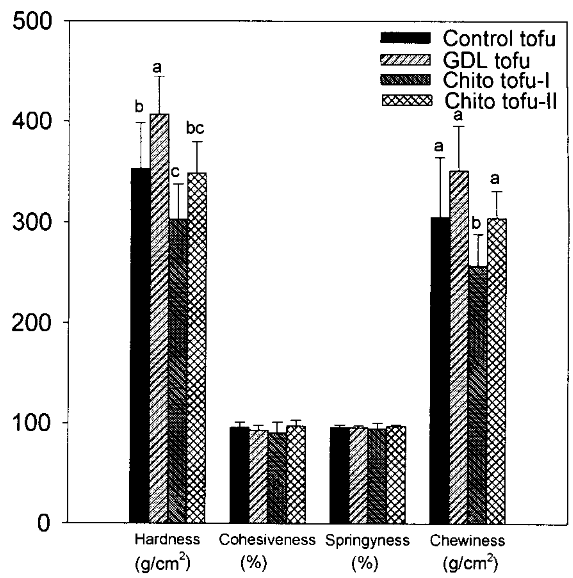

Fig. 3. The instrumental measurements of textural properties of tofu with low viscosity chitosan. Control tofu, tofu without glucono- δ -lactone; GDL tofu, tofu containing glucono- δ -lactone; Chito tofu-I, GDL tofu with 0.1 g low viscosity chitosan; Chito tofu-II, GDL tofu with 0.2 g low viscosity chitosan.

tofu because of the decreased pH by GDL (Table 3). A small change in pH produces a large change in the amount of Ca^{2+} binding capacity with protein between pH 3 and 7 because hydrogen ions compete with calcium ions for the same binding sites on the protein molecule (22). In the case of Chito tofu-I and-II, the addition of chitosan inhibited the connection between aggregated proteins and had a detrimental effect on gel formation. Otherwise, the control (A) had well-developed protein aggregations and connections between protein granules that surrounded the oil droplets. This suggests that low viscosity chitosan has an effect on the formation of tofu structure. In a previous study, tofu with high viscosity chitosan and chitooligosaccharide had loose, intermittent connections between protein granules and protein granules did not effectively surround the oil droplets in the TEM images (15,16).

Textural properties of tofu

The instrumental measurements of textural properties of tofu are shown in Fig. 3. The highest hardness appeared in GDL tofu, which indicates that GDL increased bridging. The resulting compaction of the protein matrix caused increased hardness. It has been reported that GDL tofu has a firm and somewhat rubbery texture (7). The textural properties of Chito tofu-II were similar to those of the control tofu, while Chito tofu-I had somewhat different textural properties with the control tofu. Chun et al. (23) have also reported that chitosan tofu has a low failure stress and lower inner hardness and is more fragile than tofu coagulated with $CaCl_2$. The Chito tofu-I had softer and somewhat more elastic properties compared with those of the control tofu. The differences in textural properties between GDL tofu and control tofu were probably due to the formation of protons induced by GDL. However, this effect was not found in tofu made with low viscosity chitosan in GDL solution, which suggests that the effect of positive charged groups in low viscosity chitosan is stronger than protons of GDL. It has been recognized that the coagulation of soy protein by the addition of salt is the most difficult step in making tofu. The amount of the salt and shear rate of its addition are two important factors necessary to make a good tofu. The hydrophobic regions of the native protein molecules are exposed on their surface to heat denaturation and the denatured soy protein has a negative charge. The phospholipids in oil droplets express an amphiphilic property in an aqueous solution. Therefore, phospholipids can enhance the stability of the dispersion of a protein or lipid. The ions on the surface of oil droplets and protein molecules will be suppressed and the droplets will come closer to one another and flocculate when calcium chloride is added. Calcium chloride and GDL provided sufficient positive charges to effectively neutralize the negatively charged proteins under our experimental condition. Han & Kim (15) reported that the positive charges of amine and amide groups in the chi-

Table 4. Sensory characteristics of tofu with low viscosity chitosan

Sensory attributes	Control tofu[1)]	GDL tofu[2)]	Chito tofu-I[3)]	Chito tofu-II[4)]
Aroma				
Roasted nutty	$8.15\pm1.93^{5)a6)}$	6.70 ± 1.92^{b}	6.18 ± 2.19^{b}	6.68 ± 2.02^{b}
Bean	6.29 ± 2.73^{a}	6.03 ± 2.78^{a}	6.07 ± 2.66^{a}	5.92 ± 3.06^{a}
Taste				
Roasted nutty	7.86 ± 2.44^{a}	7.09 ± 2.84^{a}	6.87 ± 2.60^{a}	6.14 ± 2.89^{a}
Bean	6.87 ± 2.73^{a}	5.53 ± 2.86^{a}	5.86 ± 2.74^{a}	5.71 ± 2.96^{a}
Sour	5.45 ± 3.31^{a}	6.61 ± 3.29^{a}	5.95 ± 3.43^{a}	6.84 ± 3.43^{a}
Texture				
Hardness	6.70 ± 1.92^{a}	6.60 ± 1.63^{a}	5.96 ± 2.00^{a}	7.28 ± 2.68^{a}
Springiness	7.34 ± 2.88^{a}	6.89 ± 2.02^{a}	7.37 ± 2.11^{a}	7.62 ± 2.19^{a}
Adhesiveness	6.96 ± 2.27^{a}	6.96 ± 2.03^{a}	6.65 ± 2.47^{a}	7.53 ± 2.69^{a}
Appearance				
Color	8.67 ± 1.70^{a}	8.27 ± 1.51^{ab}	8.12 ± 1.78^{ab}	7.39 ± 1.88^{b}
Homogeneity	8.13 ± 2.14^{a}	7.96 ± 2.16^{ab}	8.50 ± 1.87^{a}	6.76 ± 2.25^{b}
Overall eating quality	8.50 ± 2.32^{a}	8.47 ± 1.98^{a}	8.54 ± 2.77^{a}	5.78 ± 1.89^{b}

[1)]Control tofu: tofu without glucono-δ-lactone.
[2)]GDL tofu: tofu containing glucono-δ-lactone.
[3)]Chito tofu-I: GDL tofu with 0.1 g low viscosity chitosan.
[4)]Chito tofu-II: GDL tofu with 0.2 g low viscosity chitosan.
[5)]Values are the means of responses for ten panelists with four replications.
[6)]Means within a row in each basic solution followed by the same letter are not significantly different ($p<0.05$).

tooligosaccharide inhibit protein aggregations that occur via hydrophobic interactions and create open spaces in the structure.

In this study, only enough $CaCl_2$ was used to coagulate the soymilk. Therefore, further addition of chitosan might cause a restabilization of the coagulated solids, resulting in decreased protein aggregation. Additionally, this effect can be expected to change according to the degree of polymerization and the concentration of chitosan (15).

Sensory evaluation

The sensory attributes of tofu are shown in Table 4. The roasted nutty aroma scores of GDL tofu and Chito tofu were significantly lower than those of control tofu and the other aroma and taste attributes scores were in the same range of good as the control tofu. The panelists did not detect any textural difference among any of the tofu groups. However, there was a statistically significant difference among the tofu samples when the textural properties of the tofu were measured using the instrumental method, as shown in Fig. 3. As previously mentioned, the colorimetric readings showed that the L value of tofu with chitosan was similar to that of control tofu, but the panelists evaluated the tofu with chitosan to be more yellowish than the control tofu. It was observed that the Chito tofu-II had a low score on the whole field of appearance in the sensory evaluation, especially on the homogeneity and color factors. Additionally, the overall eating quality of Chito tofu-II scored significantly lower. These results show that the visual sensory attributes in this experiment were an important factor in determining tofu quality, and also that the addition of low viscosity chitosan affected the quality of tofu.

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