The localization of phytate in tofu curd formation and effect of phytate on
tofu texture

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Localization of phytate and tofu texture

JFS: Food Chemistry and Toxicology

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Abstract

The localization of phytate on tofu making and its effect on tofu texture was investigated. 38% and 3% of phytate in soymilk was bound to soluble protein and particulate protein respectively, and the others were in free form. In the early stage of curd formation, phytate bound to particulate proteins and then a large part of phytate was taken into the tofu curd.

Increase of phytate contents in soymilk resulted in increase of coagulant requirement to make firm tofu. Optimal coagulant concentration (OCC) for making tofu was influenced by phytate contents. The increase of phytate in tofu caused decrease of hardness at OCC and resulted in increase of brittleness and viscosity of tofu. We concluded that the phytate content is important as one of factors that contribute to tofu texture.

Key words: phytate, tofu, texture, breaking stress, soymilk

Introduction

Tofu has been a popular food in some Asian countries since ancient times. Its consumption is now increasing as it is recognized as a wholesome food all over the world. Tofu is made from soymilk by the addition of coagulants. Multivalent cations (Mg^{2+} , Ca^{2+}) are mainly used for the coagulation.

Tofu quality depends mainly upon its textural properties, including hardness. Components of soybean, such as protein and phytate, are known to influence tofu texture. Many studies have clarified that the protein content and composition (11S/7S ratio) has an influence on tofu texture. Soymilk protein is fractionated into particulate and soluble ones (Ono and others 1991). The particulate protein aggregates at a lower concentration of coagulant than the soluble protein and plays a preferential role in tofu curd formation (Ono and others 1993). An increase of particulate protein in soymilk has been reported to increase tofu hardness (Guo and Ono, 2005). Thus a protein form in soymilk influences the textural properties of tofu.

Phytate occurs in many grains and legumes. The phytate content of soybeans is known to vary with variety and growing condition (Ishiguro and others 2006). Binding of phytate to soy protein has been reported to influence the physicochemical properties of the protein. Katoh and others (2002) reported that the removal of phytate from soy protein increased the surface hydrophobicity and emulsifiability of the protein. Those phenomena suggest that the linkage of phytate to protein could change the existing form of protein in soymilk and then affect tofu texture.

A few studies have discussed the influence of phytate on tofu texture. Hou and Chang (2003) reported that phytate contents in soymilk have little effect on pressed tofu. Saio and others (1969) reported that increasing the phytate contents in soymilk caused a decrease in hardness and an increase in yield of tofu. However, the tofu is made from soymilks containing various phytate contents and by always using the same coagulant concentration. Because phytate can form a complex with minerals (such as the coagulant), it would call for an increasing amount of coagulant in tofu making. Liu and Chang (2004) have reported that phytate contents in soymilk have a correlation with optimal coagulant concentration. Toda and others (2006) showed addition of phytate to soymilk shifted MgCl_2 concentration that makes firm tofu which has maximum breaking stress. Therefore, it is advisable to choose a suitable coagulant concentration in order to obtain the most desirable tofu texture. "Optimal coagulant

concentration” was defined as concentration of coagulant that makes maximum breaking stress to tofu, in this paper.

Tofu texture has traditionally focused on hardness (breaking stress). However, it can be assessed with several other parameters, such as Young’s modulus, breaking strain, stress relaxation, and others. The use of these parameters for estimating tofu texture enables a comprehensive discussion of the topic.

We investigated the amount of phytate combined with protein in soymilk and in tofu curd, and its change during the curd formation process.

Furthermore, we make clear the relationship of phytate content to the coagulant quantity required for making a firm tofu, and also the effect of phytate on tofu textural parameters.

Materials and Methods

Materials

Soybeans (*Glycine max* var. Suzuyutaka) were harvested at Iwate University Experimental Farm located in Morioka, Japan, and stored at 4 °C until use.

Preparation of soymilk

Soybean seeds (10.0 g) were soaked in deionized water for 18 h at 4 °C. The swollen beans were ground into a homogenate with 70 mL of water using an Oster blender (Oster Co., Milwaukee, Wis, USA) and the homogenate was filtered through a defatted cotton sheet. High-phytate soymilk was prepared by the addition of potassium phytate (pH 7.0) was added to the swollen beans instead of water.

Soymilk was prepared by heating the filtrate in a boiling bath for 5 min above 95 °C.

Fractionation of soymilk

Particulate protein (diameter larger than 40 nm) was precipitated by centrifugation at 156,000 x g for 30 min. The supernatant, containing soluble protein, was designated as soluble fraction. Protein content in the supernatant was measured by the method of Bradford (1976). Particulate protein content was calculated by subtraction of the protein concentration in the supernatant from that in the soymilk.

Measurement of phytate

The phytate content was measured by the method of Ishiguro and others (2005). Soymilk (1.0 mL) was mixed with 0.5 mL of 18% trichloroacetic acid for removing protein. The mixture was kept at room temperature for 20 min and was then centrifuged at 8000 x g for 3 min. The deproteinized supernatant (1.0 mL) was mixed with 0.5 mL of 1 N NaOH and 50 μ L of 1 M CaCl_2 to precipitate phytate as its calcium salt. The mixture was kept at room temperature for 10 min and then centrifuged at 8000 x g for 3 min. The precipitate was then dissolved in 0.5 mL of 0.5 M citrate buffer (pH 6.0). This solution was used as the sample for IR measurement (1070 cm^{-1}) of phytate.

Measurement of phytate bound to protein

In order to measure the amount of bound phytate, equilibrium dialysis was performed as follows: a cellulose tube (cut-off MW 12000-14000), filled with 5 mL of water, was immersed in 100 mL of the soymilk and kept at 4 °C for 24 h. The internal phytate in the tube was measured as dialyzable (non bound) phytate.

Measurement of protein and phytate solubilities after addition of calcium chloride

Various concentrations of calcium chloride (0-20 mM) were added to soymilk with settling out at room temperature for 10 min. The protein and phytate solubilities of the solutions were determined by measuring the protein and phytate contents of the supernatant after centrifugation at 8000 x g for 3 min.

Preparation of tofu

Soymilk (25 mL) was degassed by vacuum-aspiration and cooled to 4 °C. Each 1.0 mL of MgCl_2 solutions (29.5-148 mM) was mixed into the soymilk and the mixture was poured into a tofu making device (Ishiguro and others 2006). The device was covered with a glass ball as a lid and placed in a water bath (Isotemp Fisher general purpose water bath, Fisher Scientific, Boston, MA, USA) at 90 °C for 1 h.

Measurement of tofu texture

Tofu texture was measured by the method of Ishiguro and others (2006). The tofu was placed in a refrigerator (4 °C) for over 18 h. When the curd texture was measured, the temperature of the tofu curd was at room temperature. Four sections of tofu were taken from the tofu making device. Each tofu sample had a 13-mm height and a 20-mm diameter and was

placed on a measuring plate. Compression test and stress relaxation test were carried out with a Rheotech Fudoh Rheometer (Rheotech Co., Tokyo, Japan) at a compression rate of 2 cm/min.

5 Compression tests were carried out with 3 plungers; they were a 25-mm diameter plate, an 8-mm diameter plate, and a 10-mm diameter globule. Breaking stress, breaking strain, and Young's modulus values with each plunger were measured and calculated.

10 Stress relaxation tests were as follows: The tofu sample (13 mm in height and 20 mm in diameter) was compressed to 30% strain and stayed, and the change in force with time was measured. Plungers of 2 types (25-mm-diameter plate and 8-mm-diameter plate) were used. Relaxation value and relaxation ratio were calculated from the measurement data.

Statistical Analyses

15 Significant differences between group means were analyzed by a t-test ($p>0.05$) using the WinSTAT program.

Results

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Binding of phytate to protein in soymilk

25 Tofu texture is one of the important parameters for determining its quality. The textural properties depend mainly on its protein properties. Phytate has been reported to influence protein characteristics such as solubility and physicochemical properties (Saio and others 1968). Therefore, the amount of phytate bound to the particulate and the soluble proteins in soymilk were measured.

30 In order to examine the amount of phytate bound to the particulate protein, both phytate contents in the soymilk and the supernatant fraction (centrifuged at 156,000 x g, 30 min) were measured. The phytate content in the supernatant fraction was 97% of the soymilk (Figure 1). The results show a little (3%) phytate binding to the particulate protein.

35 Then the amount of the phytate bound to whole protein was measured by using equilibrium dialysis, 59% of phytate was dialyzable (Figure 1). It indicates 41% of phytate was bound to whole protein in the soymilk.

Those results indicate that 38% of phytate was bound to soluble protein, 3% was to particulate protein, and the other 59% was in free form.

Figure 1 – localization of phytate in soymilk

Changes of protein and phytate solubilities in soymilk by addition of calcium chloride

Binding of phytate to protein may influence the textural properties of tofu. Changes of the protein and phytate solubilities by addition of calcium chloride were measured for the purpose of estimating incorporation of phytate into tofu during curd formation. “Solubility” was defined as relative concentration in supernatant after addition of calcium chloride.

As shown in Figure 2, protein solubility was lowest at 10 mM calcium concentration, indicating curd formation occurred. Phytate solubility decreased to 30% at 14 mM calcium chloride, indicating much phytate (70%) in soymilk was taken into the curd.

Changes of protein and phytate contents in the particulate protein fraction of soymilk by addition of calcium chloride

Incorporation of phytate into tofu curd took place as shown in Figure 3. To make clear the mechanism, we examined the protein and phytate contents of the particulate fraction in soymilk at a lower level of calcium addition (before coagulation).

As shown in Figure 3, about 50% of soymilk protein is in particulate form without addition of calcium. Addition of calcium caused an increase of particulate protein, which reached 70% at 6 mM of calcium addition. Phytate content in the particulate fraction was also increased by the addition of calcium as well as the protein content. Although a little phytate was fractionated to the particulate fraction in soymilk, about 40% of phytate was moved to the particulate fraction at 6mM of calcium addition.

The results indicated that new protein particles were formed from soluble protein in the early period of curd formation, and phytate was also taken into the particles during the formation.

Changes of protein and phytate solubilities in high-phytate (6.2 mM) soymilk by the addition of calcium chloride

Soybean phytate content has been reported to vary depending upon both variety and cultivation condition (Ishiguro and others 2006). In order to confirm the relationship of phytate content in soymilk with the incorporation amount into tofu curd, changes of the protein and phytate solubilities in high-phytate (6.2 mM) soymilk by addition of calcium chloride were examined.

Figure 2 - Changes in protein and phytate solubilities in soymilk by addition of CaCl_2

Figure 3 - Changes in the proportions of protein and phytate in the particulate protein fraction of soymilk after addition of CaCl₂

Protein solubility in the soymilk decreased from 10 mM calcium chloride, as shown in Figure 4, and was lowest at 20 mM calcium concentration. Change of phytate solubility showed the same pattern as the protein curve, and phytate solubility reached less than 20% at 20 mM calcium chloride.

The result shows that a large part of phytate in soymilk was taken into the curd, even in soymilk having a high phytate content.

Influence of phytate concentration on coagulant requirement for tofu making

There are MgCl_2 , CaSO_4 , and GDL (glucono- δ -lactone) as the coagulants for tofu making, and MgCl_2 is used most frequently in Japan. Phytate has been suspected to influence the requirement of coagulant. In order to examine the influence of phytate on MgCl_2 requirement, we measured the breaking stress of tofu made from soymilks containing various concentrations (3.3, 4.8, 6.2 mM) of phytate with various MgCl_2 concentrations (5-30 mM).

Soymilk containing 3.3 mM phytate reached maximum breaking stress at 15 mM of magnesium concentration, while soymilk containing 6.2 mM phytate reached maximum breaking stress at 26 mM of magnesium concentration (Figure 5). Magnesium concentration for maximum breaking stress shifted from 15 mM to 26 mM depending upon increase of phytate content from 3.3mM to 6.2 mM (Figure 5). This coagulant concentration is recognized as optimal. It showed that higher phytate contents of soymilk required more MgCl_2 for making firm tofu.

Influence of phytate concentration on textural parameters of tofu

The amount of phytate bound to protein in tofu curd is dependent on the phytate content of soymilk, almost all the phytate in soymilk is bound to tofu curd (already shown in Fig. 4). In order to examine the influence of phytate on tofu texture, we analyzed textural properties of 2 types of tofu in with phytate contents of optimal coagulant concentration.

The results of compression tests are shown in Table 1. The breaking stress of the high-phytate tofu was significantly lower than that of the low-phytate tofu, which was the case when using the 8-mm-diameter plate, and the 10-mm-diameter globular plungers. The breaking strain of the high-phytate tofu was significantly lower than that of the low-phytate when using all 3 plungers. There were no significant differences in Young's modulus values accompanying phytate content when using all 3 plungers. Those results indicate that high-phytate tofu tends to have softer curd than low-phytate tofu.

Figure 4. Changes in protein and phytate solubilities in high-phytate soymilk by addition of CaCl₂

Figure 5. Breaking stress of Mg-tofu prepared from soymilks having various phytate contents

Table 1. Compression test parameters of Tofu prepared from
soymilks having different phytate contents

	Breaking stress (kPa)	Breaking strain (–)	Young’s modulus (kPa)
8–mm plunger			
Phytate 3.3mM	9.85*	0.55*	17.8
6.2 mM	8.35	0.50	16.7
10–mm plunger			
Phytate 3.3mM	5.10*	0.57*	8.8
6.2 mM	4.43	0.51	8.7
25–mm plunger			
Phytate 3.3mM	5.57	0.41*	13.7
6.2 mM	5.33	0.39	13.7

* Significantly different at p <0.05.
8– and 25–mm plungers are plate type, 10–mm is globule type.

The results of stress relaxation tests are shown in Table 2. The relaxation ratio of the high-phytate tofu was significantly higher than that of the low-phytate tofu with optimal coagulant concentration. The results indicate that an increase of phytate content increased the viscosity of tofu.

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Discussion

Binding of phytate to protein is known to affect physicochemical properties of protein (Kato and others 2002). In our soymilk, 40% of phytate was bound to protein (Figure 1). Since pH of the soymilk was around 6.7, both the protein and phytate had a negative charge. It suggests that the multivalent cations Ca^{2+} and Mg^{2+} are serving as factors linking protein and phytate. Therefore, the amount of bound phytate may be influenced by the calcium and magnesium contents of soymilk. Saio and others (1968) have reported that addition of CaCl_2 increased the amount of phytate bound to protein in soymilk.

While the phytate bound to soluble protein accounted for 38%, a little phytate was bound to particulate protein (Figure 1). Proteins in soymilk differ in size and composition. Since diameter of the protein particle was estimated to be 80 nm (Ono and others 1991), its molecular weight amounts to more than 100,000,000. Since molecular weights of 7S and 11S globulins are about 190,000 and 320,000, respectively, the soluble protein is much smaller than that of the protein particle. Therefore, the soluble protein should have much larger surface area than the particle. The large surface probably induces an increase in the bound phytate. Furthermore, Ono and others (1991) reported the proportion of α , α' subunits of the soluble protein in soymilk to be much than those of the particulate protein. Phytate probably has a higher affinity to these subunits. (Okubo and others 1976)

The addition of 10 mM CaCl_2 curdled the soymilk, and the phytate in the soymilk was incorporated into the curd (Figure 2). At lower concentration of CaCl_2 an incorporation of the phytate into the particulate fraction was observed (to 6 mM, in Figure 3). Since phytate solubility (Figure 2) was about 100% between 0 and 6 mM of CaCl_2 , the incorporation was not caused by simple precipitation, such as calcium phytate, but was caused by phytate binding to the protein particle. The result shows that phytate binding to protein occurred at the early stage of curd formation.

Guo and others (2002) reported as follows: Soymilk contains soluble and particulate proteins and oil globules. When a coagulant is added, the oil

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Table 2. Stress relaxation test parameters of tofu prepared from soymilks having different phytate contents.

	Relaxation value (mJ)	Relaxation ratio (—)
8-mm plunger		
Phytate 3.3mM	3.40	66.1*
6.2 mM	3.36	67.3
25-mm plunger		
Phytate 3.3mM	1.49	70.4*
6.2 mM	1.54	72.8

* Significantly different at $p < 0.05$

globules are at first wrapped with the original glycinin-rich particles and then with β -conglycinin-rich particles newly formed from soluble protein. After all that, oil is wrapped with triple layers of proteins, that is, oleosin (oil body protein), protein particles, and new β -conglycinin-rich particles from the soluble protein. Since β -conglycinin has sugar moieties in the molecule, the coagulum holds much water surrounding it and forms a curd. In this curdling mechanism of tofu, phytate must combine with the newly formed particles as described above (Figures 2 and 3). Therefore, the textural properties of tofu will be affected by phytate content.

Phytate content in soybeans is known to vary with variety and growing condition (Ishiguro and others 2006). Almost all phytate was incorporated with protein coagulation in both low (3.3 mM) and high (6.2 mM) phytate soymilks (Figures 2 and 4). These results show that the amount of phytate incorporated into tofu curd (the final product) is proportional to the phytate content in soybean (the ingredient). Variation of phytate content may affect the texture of tofu, because the combination of phytate with protein influences its physicochemical properties (Saio and others 1969, Katoh and others 2002).

An increase of phytate concentration in soymilk resulted in an increase of coagulant requirement for tofu formation (Figure 5). Liu and Chang (2003) have developed the facile titration method for the examination of soymilk coagulant requirement, and they observed that phytate content in soymilk was correlated to coagulant requirement. We confirmed this phenomenon with actual tofu and its texture.

A decrease of breaking stress (hardness) was observed in high-phytate tofu with optimal coagulant concentration, in particular when using the small plunger (Table 1). Breaking strain was also decreased in high-phytate tofu, while Young's modulus (firmness) showed no significant difference. Thus, the decrease in breaking stress was caused by the decrease of breaking strain. These results showed that tofu containing high phytate was broken at smaller deformation than tofu of low phytate. It suggests that phytate resulted in an increase brittleness of tofu.

The decrease of the breaking stress and breaking strain was significant on using the small plungers (ϕ 8 mm plate, ϕ 10 mm globular), while no significant difference was observed by using the big plunger (ϕ 25 mm plate). Since tofu used for the breaking test had ϕ 20 mm, small plungers broke the tofu by depression, such as cut and shear. These textual parameters were influenced by the addition of phytate. On the other hand, phytate had little effect on the total compression strength when using big plunger.

The results of the stress relaxation test showed that phytate increased the relaxation ratio of tofu significantly (Table 2). The relaxation value was also increased in the high-phytate tofu with optimal coagulant concentration. These results show that the phytate increased the viscosity properties of tofu.

Influence on eating quality of the changes in these tofu textural parameter is still unknown. A method of sensory evaluation is needed for assessing tofu eating quality.

Conclusion

In the soymilk, 38% of phytate was bound to soluble protein, 3% was to particulate protein, and the other 59% was in free form. During curd formation, phytate bound to particulate protein increased gradually with progression of curd formation. In tofu curd, a large part of phytate was taken into the curd. Those results show that coagulants cause binding of protein with phytate.

An increase in phytate content of soymilk resulted in increasing coagulant requirement to make firm tofu. Phytate caused a decrease in hardness due to an increase in the brittleness and viscosity of tofu. We concluded that phytate content of soybean and soymilk is important as one of the factors influencing tofu texture.

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