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Components in Soybean Seeds Affecting the Consistency of Tofu

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Abstract

We examined the relationship between the breaking stress of tofu made with 0.25% $MgCl_2$ and protein content, the ratio of 11S protein to 7S protein (11S/7S ratio), calcium content, and phytic acid content using six soybean cultivars: Enrei, Sachiyutaka, Fukuyutaka, Tachinagaha, Hatayutaka, and Ayakogane. Among the four components, protein content, 11S/7S ratio and calcium content showed cultivar differences, while the phytic acid content showed little cultivar variation. No single component was found for explaining the breaking stress of tofu and its cultivar difference. Among the six cultivars, only Ayakogane showed a significant correlation between the protein content and the breaking stress of tofu (r = 0.72*), which might be due to high variation of the protein content within Ayakogane. For the 11S/7S ratio, each cultivar showed a positive correlation, however, correlation coefficient was 0.56 at most. Tachinagaha, showing the highest calcium content, showed the lowest coefficient of determination for the relationship between the phytic acid content and the breaking stress of tofu. In contrast, Sachiyutaka, showing the lowest calcium content, showed the highest coefficient of determination. These results implied that the calcium affects the relationship between the phytic acid content and the breaking stress of tofu, however, further study is required to clarify the mechanism of the effect of the phytic acid and relationship between phytic acid and calcium.

Discipline: Food
Additional key words: β-conglycinin, calcium, *Glycine max*, glycinin, phytic acid

Introduction

Tofu is the main processed soybean product in East Asian countries such as China, Japan and Korea. The processing properties of soybeans for tofu are an important concern in these countries. Among factors such as taste, color, consistency, and texture relating to the processing properties of tofu, consistency is the most critical since it influences both handling stability and production yield. Thus, to produce quality tofu, it is important to understand how the consistency of tofu is affected by soybean seed components.

It is generally accepted that protein content is an important factor for consistency. However, tofu manufacturers sometimes fail to produce tofu that has the necessary consistency even when using soybean cultivars with high protein content, indicating that other components may be involved.

Glycinin and β-conglycinin are major storage proteins in soybeans and correspond to the 11S and 7S fractions, respectively[20]. Many researchers have pointed out the importance of protein composition for tofu texture. Saio et al.[10] reported that tofu gel made from 11S protein was significantly harder than that from 7S protein, and that 11S gel also had a greater cohesiveness and elasticity than 7S. It was also reported that the 11S/7S ratio in soymilk strongly affected the textural properties of tofu[3].

Phytic acid, which is the storage form of phosphorus (P) and accounts for 70% of total soybean seed P, has six phosphate groups which can bind divalent metal ions such as magnesium and calcium to act as a buffer against the coagulation reaction[2]. Prattley and Stanley[9] reported that phytic acid can bind both protein and calcium ions *in situ*. Saio et al.[11] reported that softer tofu was produced from soybeans containing larger amounts of phytic acid. In our previous report, we showed that the phytic acid content had a negative correlation with the breaking stress

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of tofu made with 0.25% $MgCl_2$, which is an indicator of tofu consistency [16]. Obtained data supported the idea that variations in the content of phytic acid, which can act as a buffer against coagulation of soy proteins, could account for the fluctuation in the consistency of tofu made with soybeans of the same cultivar grown under different cultivation conditions. Among the three soybean cultivars tested, Sachiyutaka contained the least calcium, and showed an especially high correlation between the phytic acid content and the breaking stress of tofu made with 0.25% $MgCl_2$. These results indicated that calcium is another component affecting the consistency of tofu. Since calcium ions can bind phytic acid to diminish its buffering effect, unbound phosphate groups of phytic acid due to the low content of calcium in soymilk of Sachiyutaka may have a greater effect on coagulation for the consistency of tofu.

Although many studies have been reported about the effect of the soybean components described above on the consistency of tofu, there are few reports analyzing the effect of those components using the same soybean samples. In addition, fewer studies have been conducted using magnesium chloride as a coagulant than other coagulants, although, at present, magnesium chloride is mainly used by tofu manufacturers in Japan.

In this study, we examined the correlation of breaking stress of tofu with protein content, 11S/7S ratio, phytic acid content and calcium content using six soybean cultivars and 0.25% $MgCl_2$ as a coagulant. We discuss the cultivar differences of these four components and their effects on the breaking stress of tofu.

Materials and methods

1. Soybean materials

Six Japanese cultivars of soybeans (*Glycine max*), Enrei, Sachiyutaka, Fukuyutaka, Tachinagaha, Hatayutaka, and Ayakogane were used. These harvested seeds were obtained over several years from several locations. There were 11 samples for Enrei and Sachiyutaka, 10 for Fukuyutaka, Tachinagaha and Ayakogane, and 7 for Hatayutaka. Cultivar, location and cultivation year of soybean samples are shown in Table 1. Seeds were stored in plastic boxes at 5ºC until they were used for tofu preparation, which was done within one year after harvest.

2. Tofu making and texture profiling

Tofu was prepared as previously described[16]. Seeds (50 g) for each batch were washed and soaked in distilled water at 20ºC for 18 h. Water content of seeds was 10%. Hydrated seeds were drained and ground into homogenates with 265 g of distilled water. Raw soymilk was separated from the homogenate by centrifugation at 3,000 rpm using a centrifugal separator equipped with a nylon filter (120 mesh). Raw soymilk was heated by incubating in boiling water for 6 min and cooling in flowing water at 20ºC. Hexahydrate food grade 0.25% $MgCl_2$ was added as a coagulant after the soymilk was chilled in ice-cold water. The coagulated soymilk was incubated at 80ºC for 1 h using a water bath and cooled in flowing water at 20ºC. Cylindrical cakes of packed tofu 26 mm in diameter and 80 mm in thickness were prepared, and then incubated at 20ºC until the tofu texture was measured.

Three cylindrical pieces of tofu (10 mm in height and 26 mm in diameter) were cut from the middle part of the tofu sample and used for the texture analysis. The texture profiles of the tofu samples were investigated using a RHEONER (RE3305, Yamaden Co., Ltd., Japan) with a cylindrical plunger 11 mm in diameter at a compression rate of 1 mm sec^{-1}, according to the method of Kohyama and Nishinari[6].

3. Determination of soybean components

Components of soybeans were determined as previously described[16]. Nitrogen content of seed flour was determined by the combustion method. Protein values of soybeans on a dry matter basis were calculated by multiplying the nitrogen content by 6.25. Phytic acid in soymilk was precipitated by adding 20 mM calcium chloride at pH 11.5 according to the method of Ishiguro et al.[4]. Precipitated phytic acid was degraded to phosphate by heating with sulfuric acid and hydrogen peroxide, followed by phosphorus assay using a modification of the Fiske and Subbarow methods[1]. Calcium content in soymilk was measured after removing protein by precipitation with 3% trichloroacetic acid using a Calcium E-Test Wako kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan). A total globulin fraction was prepared by the method of Yagasaki et al.[20]. The protein was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)[7] and stained by coomassie brilliant blue G-250. The stained protein bands in the gel were scanned with a scanner and then the stained intensities were analyzed using PC software (Lane Analyzer, ATTO Co., Tokyo, Japan).

4. Statistical Analysis

Data on soybean components and breaking stress of tofu were obtained from at least two individual experiments and analyzed with the statistical package SPSS 12.0 (SPSS Inc, Chicago, USA).

Table 1. Cultivar name, location and cultivation year of soybean samples

Cultivar	Production area	Year	Cultivar	Production area	Year
Enrei	Shiojiri, Nagano Pref.	2000	Ayakogane	Miyagi Pref.	2000
	Shiojiri, Nagano Pref.	2001		Niigata Pref.	2000
	Shiojiri, Nagano Pref.	2002		Shiojiri, Nagano Pref.	2000
	Fukui Pref.	2001		Fukui Pref.	2001
	Tsukuba, Ibaraki Pref.	2001		Miyagi Pref.	2001
	Tsukuba, Ibaraki Pref.	2002		Hara, Nagano Pref.	2001
	Tsukuba, Ibaraki Pref.	2003		Shiojiri, Nagano Pref. (C)[2)]	2001
	Tsukuba, Ibaraki Pref.	2004		Shiojiri, Nagano Pref. (D)[2)]	2001
	Chiba Pref.	2001		Suzaka, Nagano Pref.	2001
	Tottori Pref.	2001		Niigata Pref.	2001
	Toyama Pref.	2001			
			Hatayutaka	Hitachinaka, Ibaraki Pref.	2000
Fukuyutaka	Asakura, Fukuoka Pref.	2000		Mito, Ibaraki Pref. (A)[1)]	2000
	Tsukushino, Fukuoka Pref.	2000		Mito, Ibaraki Pref. (A)[1)]	2001
	Tsukuba, Ibaraki Pref.	2001		Mito, Ibaraki Pref. (B)[1)]	2001
	Tsukuba, Ibaraki Pref.	2002		Kariwano, Akita Pref.	2001
	Tsukuba, Ibaraki Pref.	2003		Oomagari, Akita Pref.	2001
	Kagawa Pref.	2004		Tsukuba, Ibaraki Pref.	2001
	Shiojiri, Nagano Pref.	2001			
	Shiojiri, Nagano Pref.	2002	Sachiyutaka	Tsukushino, Fukuoka Pref.	2000
	Kumamoto Pref.	2001		Ohita Pref.	2001
				Okayama Pref.	2001
Tachinagaha	Mito, Ibaraki Pref. (A)[1)]	2000		Kumamoto Pref.	2001
	Mito, Ibaraki Pref. (A)[1)]	2001		Ehime Pref.	2001
	Mito, Ibaraki Pref. (B)[1)]	2001		Tsukuba, Ibaraki Pref.	2001
	Miyagi Pref.	2001		Tsukuba, Ibaraki Pref.	2002
	Tsukuba, Ibaraki Pref.	2001		Tsukuba, Ibaraki Pref.	2003
	Hara, Nagano Pref.	2001		Kagawa Pref.	2001
	Shiojiri, Nagano Pref. (C)[2)]	2001		Ibaraki Pref.	2004
	Shiojiri, Nagano Pref. (D)[2)]	2001		Shiojiri, Nagano Pref.	2002
	Kariwano, Akita Pref.	2001			
	Oomagari, Akita Pref.	2001			

1) Different fields were used for A and B.
2) Seeding date of D was later than that of C.

Results and discussion

Variations of protein content, 11S/7S ratio, calcium content, phytic acid content, and the breaking stress of tofu made with 0.25% $MgCl_2$ for six cultivars are shown in Table 2. Among the four components of soybeans, protein content, 11S/7S ratio and calcium content showed cultivar difference. In contrast, for the phytic acid content, a significant difference was shown only between Ayakogane and Tachinagaha, indicating little cultivar variation. It is generally accepted that Enrei and Sachiyutaka are of high protein content, Fukuyutaka is of relatively high protein content, and Hatayutaka, Tachinagaha and Ayakogane are of moderate levels of protein content. The present results were consistent with the generally accepted contents except that Fukuyutaka and Enrei showed a similar level of protein content. The coefficients of variations for the phytic acid content were higher than those for the protein content for each cultivar, indicating higher variation of the phytic acid content within the same cultivar, as we already reported[16]. Fukuyutaka showed the highest breaking stress of tofu while Sachiyutaka showed the lowest breaking stress of tofu. No single component was found for explaining cultivar difference of the breaking stress of tofu in this study.

Correlations between the breaking stress of tofu and protein content, 11S/7S ratio, calcium content, and the phytic acid content for each soybean cultivar and for all samples (six cultivars) are shown in Table 3. Protein content showed a slight correlation with the breaking stress of tofu for all samples (r = 0.26, $p < 0.05$). Among the six cultivars, only Ayakogane showed a significant correlation between the protein content and the breaking stress of tofu (r = 0.72, $p < 0.05$). The high correlation for Ayakogane might be due to high variation of the protein content, which is shown by the higher SE than others (Table 2). Wang et al. [18] (1983) and Shen et al.[13] (1991) reported that cultivars with higher protein content produced firmer and more springy tofu texture. In contrast, Lim et al.[8] (1990) studied the relationship of hardness and firmness of tofu to protein content of beans and tofu using 9 soybean cultivars, and found no significant correlation. In this study, we used no cultivars with low protein content. Small variation of the protein content may be one of reasons for low correlation between the protein content and the breaking stress of tofu.

The 11S/7S ratio showed a significant positive correlation with the breaking stress of tofu for all samples (r = 0.39, $p < 0.01$, Table 3). The 11S/7S ratio also showed a positive correlation with the breaking stress of tofu for each cultivar. These results indicated that a higher 11S/7S ratio results in a higher breaking stress of tofu made with 0.25% $MgCl_2$.

When $MgCl_2$ concentration was changed, Sachiyutaka showed the highest $MgCl_2$ concentration for the maximum breaking stress of tofu and the lowest breaking stress of tofu made with 0.25% $MgCl_2$ among six soybean cultivars[15]. In contrast, Fukuyutaka showed the lowest $MgCl_2$ concentration for the maximum breaking stress of tofu and the highest breaking stress of tofu made with 0.25% $MgCl_2$[15]. These results implied different coagulation reactivities among soybean cultivars. In addition, the 11S/7S ratio was significantly correlated with $MgCl_2$ concentration for the maximum breaking

Table 2. Average of protein content, 11S/7S ratio, calcium content, phytic acid content and the breaking stress of tofu for each soybean cultivar and for all samples

Cultivar \ Component	Protein (%)	11S/7S ratio	Calcium (mg dL^{-1})	Phytic acid (mM)	Breaking stress of tofu (x $10^2 Nm^{-2}$)
Enrei	45.2 ± 1.5^{ab}	1.64 ± 0.26^{c}	20.3 ± 3.2^{ab}	3.40 ± 0.39^{ab}	77.6 ± 19.3^{ab}
Sachiyutaka	45.8 ± 1.0^{a}	1.53 ± 0.31^{c}	16.1 ± 3.4^{c}	3.32 ± 0.58^{ab}	41.2 ± 19.9^{d}
Fukuyutaka	45.2 ± 1.7^{ab}	2.76 ± 0.33^{a}	21.9 ± 4.6^{a}	3.17 ± 0.54^{ab}	91.9 ± 25.0^{a}
Tachinagaha	42.3 ± 1.2^{c}	2.34 ± 0.21^{b}	22.5 ± 4.1^{a}	3.05 ± 0.28^{b}	57.8 ± 18.7^{cd}
Hatayutaka	43.6 ± 1.3^{bc}	1.41 ± 0.11^{c}	17.2 ± 2.7^{bc}	3.05 ± 0.43^{ab}	71.6 ± 11.2^{abc}
Ayakogane	44.3 ± 2.7^{b}	2.34 ± 0.21^{b}	19.9 ± 4.0^{ab}	3.47 ± 0.25^{a}	72.2 ± 30.8^{bc}
All samples (six cultivars)	44.5 ± 2.0	2.02 ± 0.56	19.7 ± 4.3	3.26 ± 0.44	68.2 ± 26.8

Values represent the means ± SE. Those with different letters are significantly different among cultivars at $p<0.05$ by Tukey's least significant difference (LSD) test.

Table 3. Correlatiion between the breaking stress of tofu made with 0.25% $MgCl_2$ and protein content, 11S/7S ratio, calcium content and phytic acid content for each soybean cultiver and for all samples

Cultivar \ Component	protein content	11S/7S ratio	calcium content	phytic acid content
Enrei	0.39	0.36	0.51	0.03
Sachiyutaka	0.10	0.06	–0.15	–0.59
Fukuyutaka	0.16	0.32	–0.27	–0.38
Tachinagaha	0.10	0.33	0.42	–0.02
Hatayutaka	–0.05	0.56	–0.41	–0.41
Ayakogane	0.72*	0.56	0.31	–0.59
All samples (six cultivars)	0.26*	0.39**	0.28*	–0.24

*: Siginificant at $p < 0.05$. **: Siginificant at $p < 0.01$.

stress of tofu[17], which suggested that the 11S/7S ratio is one of the important factors affecting coagulation reactivity. Therefore our studies support the idea that the cultivar difference of the 11S/7S ratio is correlated with the cultivar difference of breaking stress of tofu made with 0.25% $MgCl_2$ by affecting the coagulation reactivity.

However, Skurray et al.[14] (1980) reported that using 15 soybean cultivars, a slight variation in the texture of tofu was due to the 7S and 11S proteins in soybean, but an important factor affecting the texture of tofu was found to be the amount of calcium ions as a coagulant. The effect of the 11S/7S ratio might be reduced by changing the concentration of the coagulant. In our present study, the 11S/7S ratio was more correlated with the breaking stress of tofu than the previous report by Skurray et al.[14], possibly due to the constant concentration of $MgCl_2$ used as the coagulant. However, the correlation coefficient was 0.56 at most, implying that the 11S/7S ratio may partly affect the breaking stress of tofu.

For the calcium content, three cultivars showed positive correlations with the breaking stress of tofu while the other three cultivars showed negative correlations (Table 3). No significant correlation was found for each cultivar. Although calcium content showed a significant correlation with the breaking stress of tofu for all samples ($r = 0.28$, $p < 0.05$), the effect of calcium content remains to be clarified.

In our previous study, Sachiyutaka, which showed less calcium content than Enrei and Fukuyutaka, showed a higher correlation between the phytic acid content and the breaking stress of tofu than others[16]. In our present study, the correlation coefficients between the phytic acid content and the breaking stress of tofu were –0.59, –0.59, –0.41, –0.38, –0.02, and 0.03 for Sachiyutaka, Ayakogane, Hatayutaka, Fukuyutaka, Tachinagaha, and Enrei, respectively (Table 3). Tachinagaha, showing the highest calcium content (Table 2), showed the lowest coefficient of determination for the relationship between the phytic acid content and the breaking stress of tofu. In contrast, Sachiyutaka, showing the lowest calcium content (Table 2), showed the highest coefficient of determination. Although it was implied that the calcium content affects the relationship between the phytic acid content and the breaking stress of tofu, further study is required to clarify the mechanism of the effect of the phytic acid and relationship between phytic acid and calcium. The correlation coefficient between the phytic acid content and the breaking stress of tofu was –0.24 for all samples (six cultivars) but not significant. Some researchers, who mainly focused on the cultivar difference of the effect of the phytic acid content, found no effect of phytic acid on tofu consistency[14,12]. Other components such as protein and 11S/7S ratio, showing cultivar characteristics (Table 2), may overcome the effect of the phytic acid among cultivars.

In the present study, we found no single soybean components for explaining the variation of breaking stress of tofu made with 0.25% $MgCl_2$. The four components examined in this study could not explain the breaking stress of tofu even by a multiple correlation (data not shown). One of the possible reasons is interaction among these components. Interaction among 7S protein, calcium and phytic acid has been reported[2,9]. Ishiguro et al.[5] reported that 59% of phytic acid was in free form, but 41% of phytic acid in soymilk was bound to soluble or particulate protein. However, it remains to be clarified how their interaction affects the breaking stress of tofu. Understanding the interaction of these components may be required, although there is still the possibility of other components affecting the breaking stress of tofu.

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