

Seed Protein Content and Consistency of Tofu Prepared with Different Magnesium Chloride Concentrations in Six Japanese Soybean Varieties

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The relationship between the protein content of soybean seeds and the consistency of tofu was examined for six Japanese soybean varieties, Enrei, Fukuyutaka, Sachiutaka, Ayakogane, Hatayutaka and Tachinagaha. The seed protein content was estimated by determining the nitrogen content using the Dumas method. Tofu was prepared from a raw homogenate of water-soaked soybeans by heating and by the addition of MgCl₂ as a coagulant. The tofu consistency was evaluated by measuring the breaking stress of tofu curd using a Creep meter. The breaking stress of tofu increased when the concentrations of MgCl₂ in soymilk increased above 0.20%. The breaking stress reached a maximum value at concentrations of around 0.40%, with differences among soybean varieties and cultivation conditions of the soybeans. There was a significant positive correlation ($r=0.87$) between the maximum breaking stress of tofu and the seed protein content for the six varieties. In contrast, the breaking stress of tofu prepared with 0.25% MgCl₂ did not show a significant correlation ($r=0.27$) with the seed protein content for the six varieties but was significantly correlated ($r=0.52$), when the data of Sachiutaka were excluded. Fukuyutaka and Ayakogane required a lower MgCl₂ concentration for the maximum breaking stress of tofu than Sachiutaka, Enrei, Tachinagaha and Hatayutaka, which required a MgCl₂ concentration above 0.40% for the maximum breaking stress of tofu. Especially, Sachiutaka required the highest MgCl₂ concentration, 0.45% on the average, for the maximum breaking stress of tofu among the six varieties. Sachiutaka-tofu showed the lowest breaking stress on the average at a concentration of 0.25% MgCl₂, which is the concentration generally used by manufacturers, in spite of its high content in seed protein. In contrast, Fukuyutaka required the lowest MgCl₂ concentration, 0.34% on the average, for the maximum breaking stress and the highest breaking stress of tofu prepared with 0.25% MgCl₂. That is one of reasons why manufacturers prefer to use Fukuyutaka for producing tofu. Concentration of a coagulant for the maximum breaking stress as well as seed protein con-

tent could become criteria for quality evaluation of soybeans for tofu processing.

Key Words: soybean, tofu, protein content, breaking stress, magnesium chloride concentration.

Introduction

Tofu is the gel-like precipitate obtained from heated soymilk by the addition of coagulants, such as magnesium salt. Soymilk is obtained by squeezing the homogenate of water-soaked soybean. Tofu has been widely consumed in the Orient for more than 2000 years. It is used in other regions because it is low in calories and cholesterol-free. In Japan, tofu is the main traditional food derived from soybeans. Although tofu consumed in Japan is mainly made from imported soybeans, recently tofu made from domestic soybeans and organic soybeans has become popular with the Japanese consumers. However, it is difficult to mass-produce tofu of uniform quality from Japanese domestic soybeans because of the variations in soybean quality due to the small-scale production, the use of different varieties or differences in the cultivation environments (Ohmura and Ozaki 2000).

A typical soft tofu is characterized by a bland taste and fine texture with a moisture content of 84–90% (Kohyama *et al.* 1993). The quality of tofu is determined by its color, taste and texture. These parameters are affected by many soybean components that change depending on the varieties and cultivation conditions (Wang *et al.* 1983, Skurray *et al.* 1980, Taira 1992). Appropriate consistency is important not only for the consumers but also for the manufacturers because the consistency is related to the taste, appearance and handling. Among the various characteristics of tofu, consistency is the key parameter for good quality tofu. It is generally recognized that the use of soybean varieties with a high content of protein results in the production of a firm tofu. Wang *et al.* (1983) and Shen *et al.* (1991) reported that varieties with a higher protein content produced a firmer and more springy tofu texture. Thus, one objective for breeders is to develop soybean varieties with a high protein content. However, Lim *et al.* (1990) who studied the relationship of consistency of tofu to the protein content of beans and tofu using nine soy-

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bean varieties, failed to detect any significant correlation between these parameters. Tofu manufacturers sometimes can not produce consistent tofu-gel in spite of using a soybean variety with a high protein content. It is, therefore, necessary for breeders and manufacturers to develop methods for evaluating the suitability of soybeans for tofu production.

Soy protein consists of two main proteins, glycinin and conglycinin, and their coagulation profiles differ from each other (Saio *et al.* 1969a, Johnson and Wilson 1984). Glycinin forms larger and more protein particles and is more sensitive to calcium and magnesium ions compared with conglycinin, which results in the production of a firmer tofu (Tezuka *et al.* 2000). The glucono- δ -lactone induced tofu-gel increases the breaking stress with the increase in the proportion of glycinin to conglycinin (Yagasaki *et al.* 2000). Among the glycinin subunits, group IIB glycinin subunit plays an important role in increasing the gel consistency, whereas group IIA glycinin subunit is closely related to the gel formation rate in the heat-induced gel (Nakamura *et al.* 1984, 1985). Glycinin contains more SH groups in a molecule than conglycinin. The consistency of tofu is affected by the contents of SH groups, and when soy protein contains more SH groups, a firmer tofu can be obtained (Saio *et al.* 1971). Oil bodies bind with protein particles and form a core of tofu curd upon coagulation with calcium chloride (Guo *et al.* 1999). Phytic acid can bind with magnesium and calcium ions, which affects the optimum amount of coagulants and makes tofu curd soft (Saio 1979, Prattley and Stanley 1982).

In spite of the large amount of information, it remains to be determined why the use of soybeans with a high protein content sometimes results in soft tofu, as indicated by the tofu manufacturers. In recent years, processing conditions such as temperature, type and concentration of coagulants, rate of stirring, and water to bean ratio that affect the quality and yield of tofu have been investigated (Saio 1979, Tsai *et al.* 1981, Wang and Hesseltine 1982, deMan *et al.* 1986, Sun and Breene 1991, Beddows and Wong 1987a, 1987b, 1987c, Shen *et al.* 1991). It is generally recognized that the relation between the concentration of coagulants and consistency of tofu is variable due to the differences in soybean varieties or cultivation conditions. However, to our knowledge, no report has been published on the correlation between the protein content of the soybeans and the consistency of tofu produced by the use of variable concentrations of coagulants for many soybean samples.

The objective of this study was to reexamine the correlation between the protein content of soybean seeds and the consistency of resulting tofu. We also aimed at comparing the characteristics of the relationship between coagulant concentrations and tofu breaking stress among six Japanese soybean varieties.

Materials and Methods

Soybean samples

Six Japanese varieties of soybeans, Enrei, Fukuyutaka,

Sachiyutaka, Ayakogane, Hatayutaka and Tachinagaha, produced both in 2000 and 2001, were obtained from several locations. The numbers of samples were nine or ten for each soybean variety. Details about these soybean samples are given in Table 1. Fukuyutaka, Tachinagaha and Enrei are the main soybean varieties in the Kyushu, Kanto and Hokuriku regions, respectively, in Japan. Sachiyutaka is a new variety for the Kyushu and the Chugoku regions. Hatayutaka and Ayakogane are relatively new varieties for the Kanto and the Hokuriku regions, respectively. They were stored in plastic boxes at 5°C until they were used for tofu preparation.

Soymilk preparation and tofu making

Soybeans (50 g each batch) were washed three times with distilled water and soaked in distilled water at 20°C for 18 h. Hydrated seeds were drained and ground into homogenates with distilled water equivalent to six times the weight of dry seed. Raw soymilk was separated from the homogenate by centrifugation using a centrifugal filter (120 mesh). Soymilk was prepared by incubating raw soymilk in boiling water for 6 min and was cooled in flowing water. MgCl₂ was added as a coagulant after soymilk was chilled in ice cold water for 30 min. The coagulated soymilk was incubated at 80°C for 1 h and cooled in flowing water. Two cylindrical cakes of tofu 26 mm in diameter and 80 mm in length were prepared for each soybean sample with the same MgCl₂ concentration. They were incubated at 20°C until the measurement of the tofu texture was performed.

Texture measurements

The texture profiles of the tofu samples were investigated using a RHEONER II (YAMADEN company 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 (1992). 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 breaking stress of tofu was expressed based on three measurements from one tofu sample, namely, based on six measurements for each soybean sample at the same MgCl₂ concentration.

Determination of protein content

Soybean seeds were ground into flour using an ultra centrifugal mill (ZM100, F. Kurt Retsch GmbH & Co. KG, Germany). Moisture content of seed flour for the calculation on a dry matter basis was determined by heating the flour samples for 3 h at 130°C. Soymilk was dried at 105°C for 10 h and broken into small pieces. Nitrogen content of seed flour and dried soymilk was determined by the Dumas combustion method using rapidN (Elementar Analysensysteme GmbH, Germany) and aspartic acid as a standard. Protein values of soybean and soymilk on a dry matter basis were calculated by multiplying the nitrogen content by 6.25.

Table 1. Variety, location and cultivation year of soybean samples

Variety	Production area	Year	Variety	Production area	Year
Enrei	Shiojiri, Nagano pref.	2000	Ayakogane	Furukawa, Miyagi pref.	2000
	Fukui, Fukui pref.	2001		Nagaoka, Niigata pref.	2000
	Tsukuba, Ibaraki pref.	2001		Shiojiri, Nagano pref.	2000
	Shintone, Ibaraki pref.	2001		Fukui, Fukui pref.	2001
	Kouzaki, Chiba pref.	2001		Furukawa, Miyagi pref.	2001
	Nagaoka, Niigata pref.	2001		Hara, Nagano pref.	2001
	Shiojiri, Nagano pref.	2001		Shiojiri, Nagano pref. (C) ²⁾	2001
	Tottori, Tottori pref.	2001		Shiojiri, Nagano pref. (D) ²⁾	2001
	Toyama, Toyama pref.	2001		Suzaka, Nagano pref.	2001
Fukuyutaka	Asakura, Fukuoka pref.	2000	Hatayutaka	Nagaoka, Niigata pref.	2001
	Fukuyama, Hiroshima pref.	2000		Hitachinaka, Ibaraki pref.	2000
	Tsukushino, Fukuoka pref.	2000		Mito, Ibaraki pref. (A) ¹⁾	2000
	Asakura, Fukuoka pref.	2001		Mito, Ibaraki pref. (B) ¹⁾	2000
	Tsukuba, Ibaraki pref.	2001		Toukai, Ibaraki pref.	2000
	Kikuchi, Kumamoto pref. (A) ¹⁾	2001		Tsukuba, Ibaraki pref.	2001
	Kikuchi, Kumamoto pref. (B) ¹⁾	2001		Mito, Ibaraki pref. (A) ¹⁾	2001
	Shiojiri, Nagano pref.	2001		Mito, Ibaraki pref. (B) ¹⁾	2001
	Tsukushino, Fukuoka pref.	2001		Kariwano, Akita pref.	2001
Zentsuui, Kagawa pref.	2001	Oomagari, Akita pref.	2001		
Sachiyutaka	Akaiwa, Okayama pref.	2000	Tachinagaha	Mito, Ibaraki pref. (A) ¹⁾	2000
	Fukuyama, Hiroshima pref.	2000		Furukawa, Miyagi pref.	2001
	Houjou, Ehime pref.	2000		Tsukuba, Ibaraki pref.	2001
	Kikuchi, Kumamoto pref.	2000		Mito, Ibaraki pref. (A) ¹⁾	2001
	Akaiwa, Okayama pref.	2001		Mito, Ibaraki pref. (B) ¹⁾	2001
	Houjou, Ehime pref.	2001		Hara, Nagano pref.	2001
	Tsukuba, Ibaraki pref.	2001		Shiojiri, Nagano pref. (C) ²⁾	2001
	Tsukushino, Fukuoka pref.	2001		Shiojiri, Nagano pref. (D) ²⁾	2001
	Tottori, Tottori pref.	2001		Kariwano, Akita pref.	2001
Zentsuui, Kagawa 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

Protein concentration of soymilk and protein content of soybean

Protein concentration of soymilk was determined from part of the soybean samples used for tofu making. Nineteen samples of four soybean varieties, Sachiyutaka, Hatayutaka, Ayakogane and Tachinagaha were randomly selected. The protein concentration of soymilk was significantly correlated with the protein content of soybeans, with a correlation coefficient of 0.90 (Fig. 1). There was no difference in the yield of protein from soybean seeds to soymilk among the nineteen samples. The protein yield from soybean to soymilk was estimated at about 80%, a value similar to that estimated by Nakayama *et al.* (1960). These results suggested that the concentration of protein in soymilk can be predicted from the protein content of soybeans.

Coagulant concentration and tofu breaking stress

We determined the breaking stress of tofu prepared by the addition of MgCl₂ at different concentrations. The dependency of the breaking stress of tofu on the concentrations

of MgCl₂ varied with the soybean samples. For example, tofu made from Fukuyutaka produced in Asakura, Fukuoka in 2000 showed the maximum breaking stress at a concentration of 0.35% MgCl₂ (Fig. 2). In contrast, tofu made from Sachiyutaka produced in Akaiwa, Okayama in 2000 showed a lower breaking stress than that of tofu made from Fukuyutaka at concentrations of 0.20% through 0.35% MgCl₂, but showed a higher value for the maximum breaking stress at a concentration of 0.40% MgCl₂ than that of Fukuyutaka.

Correlation between seed protein content and tofu breaking stress

The correlation between the protein content of seed and the tofu breaking stress was examined. We selected the maximum breaking stress and also the breaking stress of tofu prepared with 0.25% MgCl₂, which is commonly used to evaluate tofu quality and is similar to the concentration used by tofu manufacturers (deMan *et al.* 1986 and personal communication from a tofu manufacturer). The seed protein content was not correlated with the breaking stress at 0.25% MgCl₂ ($r=0.27$) while it exhibited a strong positive correlation ($P < 0.001$, $r = 0.87$) with the maximum breaking

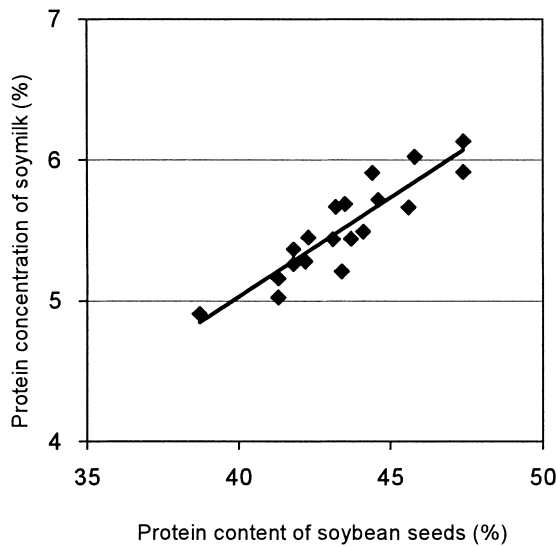


Fig. 1. Correlation between the protein content of soybean seeds and the protein concentration of soymilk. Approximate linear equation ($r=0.90$) shows that the protein yield from soybean to soymilk was 80% in this study.

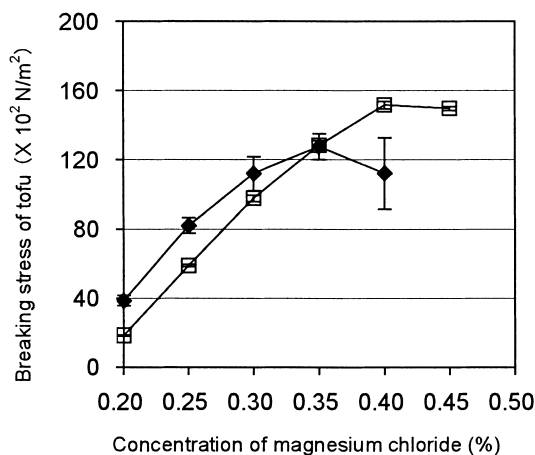


Fig. 2. Relationship between the $MgCl_2$ concentrations and the breaking stress of tofu. \blacklozenge denotes the plot of the breaking stress of tofu made from Fukuyutaka that was harvested in Asakura, Fukuoka in 2000. \square denotes the plot of the breaking stress of tofu made from Sachiyutaka that was harvested at the Agricultural Experiment Station of Okayama prefecture in Akaiwa in 2000. Vertical bars indicate the standard deviation for two experiments.

stress (Fig. 3). When the plots for Sachiyutaka in Fig. 3 were excluded, a significant correlation appeared between the protein content and the breaking stress at a concentration of 0.25% $MgCl_2$ ($P < 0.001$, $r=0.52$). Sachiyutaka showed the highest protein content of soybean seeds but the breaking stress of tofu prepared with 0.25% $MgCl_2$ was the lowest among the six varieties (Table 2). For these reasons, there was no significant correlation between the protein content and the breaking stress of tofu prepared with 0.25% $MgCl_2$

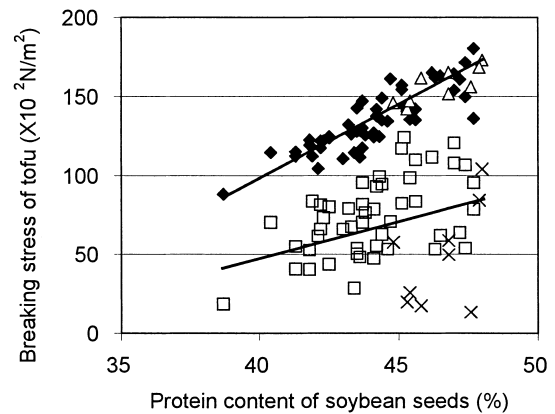


Fig. 3. Correlation between the protein content of soybean seeds and the maximum breaking stress of tofu (\blacklozenge and \triangle) and the breaking stress of tofu prepared with 0.25% $MgCl_2$ (\square and \times). \blacklozenge and \square denote the plots for five varieties, Fukuyutaka, Enrei, Aya-kogane, Hatayutaka and Tachinagaha. \triangle and \times denote the plots for Sachiyutaka. Two lines denote the approximate linear equations for all six varieties.

when the plots of Sachiyutaka were added to those of the other varieties.

Varietal differences in the correlation between seed protein content and tofu breaking stress, and coagulation reactivity with coagulant

We examined the correlation between the protein content and the breaking stress of tofu prepared with 0.25% $MgCl_2$ and the maximum breaking stress using six varieties (Fig. 4 and Table 2). For all the varieties, the protein content showed a stronger correlation with the maximum breaking stress than the breaking stress of tofu prepared with 0.25% $MgCl_2$. The six varieties differed in the relationship between the maximum breaking stress and seed protein content, that is, the correlation between the maximum breaking stress and the seed protein content produced different approximate linear equations with variable slopes among the six varieties. The lowest value of the slope of the approximate linear equation was 384 for Tachinagaha and the highest value was 1059 for Enrei. $MgCl_2$ concentration for the maximum breaking stress differed among the six varieties (Table 2). Fukuyutaka required the lowest concentration while Sachiyutaka required the highest concentration. As for tofu prepared with 0.25% $MgCl_2$, tofu made from Sachiyutaka showed the lowest breaking stress, although its protein content and its maximum breaking stress were high, while tofu made from Fukuyutaka showed the highest breaking stress.

Discussion

In this study, we obtained soymilk by squeezing the slurry of ground soybeans and water, followed by heating of raw soymilk. This step is named 'Namashibori' and used

Table 2. Seed protein content, tofu breaking stress and magnesium chloride concentrations for the maximum breaking stress for six soybean varieties

	Enrei	Fukuyutaka	Sachiyutaka	Ayakogane	Hatayutaka	Tachinagaha
Protein content of soybean seeds (%)	45.0±1.5	44.9±1.6	46.4±1.2	44.3±2.7	43.6±1.2	42.3±1.2
Maximum breaking stress (X 10 ² N/m ²)	153±17	135±13	156±11	132±27	131±12	121±7
Breaking stress of tofu at 0.25% MgCl ₂ (X10 ² N/m ²)	72±22	97±21	45±31	72±31	74±13	58±19
Magnesium chloride concentrations for the maximum breaking stress (%)	0.42±0.04	0.34±0.03	0.45±0.04	0.38±0.04	0.41±0.04	0.42±0.04

The values express means ± S.D.

mainly in Okinawa and other Asian countries than Japan. Many tofu manufacturers in Japan use the 'Kanetsushibori' step, in which soymilk is obtained by squeezing the slurry after heating. The yield of protein, oil and saccharides was not affected by the temperature used in squeezing the slurry, while the amount of glycosides increased and the texture of the tofu gel changed when the squeezing temperature increased (Asano *et al.* 1987, 1989). The yield of protein from soybeans to soymilk recorded in our method (Fig. 1) was similar to the value estimated by Nakayama *et al.* (1960), in which soymilk was obtained by the 'Kanetsushibori' step.

Generally, soybean varieties with a high protein content are suitable for tofu manufacturing. Soybeans with a high protein content, however, do not always produce tofu with sufficient toughness. When MgCl₂ is used as a coagulant, the manufacturers prefer to use a concentration of 0.25% (personal communication from a tofu manufacturer). Breaking stress decreased by the addition of more MgCl₂ than the concentration required for the maximum breaking stress (Fig. 2), therefore excess concentration of a coagulant may result in the production of softer tofu. Manufacturers selected the concentration of 0.25% because the use of MgCl₂ at a concentration of above 0.25% may result in poor quality of tofu for some soybean varieties and production areas. In this study, we observed that the breaking stress of tofu prepared with 0.25% MgCl₂ showed a low significant correlation, while the maximum breaking stress of tofu exhibited a high significant correlation with the protein content of soybean seeds (Fig. 3), which is related to the different MgCl₂ concentrations used for the maximum breaking stress (Table 2). There was a significant correlation between the protein content and the breaking stress at 0.25% MgCl₂ when the plots of Sachiyutaka, which required the highest MgCl₂ concentration for the maximum breaking stress of tofu, were excluded. The difference in the MgCl₂ concentration for the maximum breaking stress may be due to a difference in the coagulation reactivity of soymilk. In general, soymilk prepared by using seeds of soybeans which have just been harvested shows a high pH and requires more coagulants (Ohta *et al.* 1979, Ohara *et al.* 1992). Since phytic acid is capable of binding with calcium and magnesium ions, soymilk containing more phytic acid needs more coagulants (Saio *et al.* 1969b). It was suggested that nonstarchy polysaccharides may exhibit cross-linkages with calcium which affect the texture of tofu (Shen *et al.* 1991, Wang and Chang 1995). Those factors and others may prevent a coag-

ulant, such as MgCl₂, from reacting with the proteins in soymilk and differences in the reactivity may result from the difference in such components. It is important to identify the factors that act as buffers between soy proteins and coagulants. At the maximum breaking stress, the buffer reactivity of soymilk may disappear and the protein content of soybeans mainly affects tofu consistency. The breaking stress of tofu at a concentration of 0.25% MgCl₂ showed larger standard deviations than the maximum breaking stress (Table 2). It is possible that the variation in the components related to the coagulation reactivity may affect the quality of tofu more than the variation in the protein contents.

The six soybean varieties investigated in this study were adapted to different regions in Japan and we collected soybean samples grown at different locations. A part of the samples included different soybean varieties grown at the same locations in the same year (Table 1). Therefore both variety and environment factors affected the results shown in Table 2. However, the average values of the varieties listed in Table 2 reflected the properties of the respective varieties to some extent because similar trends were observed when different soybean varieties grown at the same locations in the same year (data not shown) were compared. Among the six varieties, tofu made from Fukuyutaka showed the highest breaking stress when prepared with 0.25% MgCl₂ and required the lowest MgCl₂ concentration for the maximum breaking stress (Table 2). Therefore, Fukuyutaka is the most suitable variety for obtaining a firm tofu in using 0.25% MgCl₂ and is one of the soybean varieties that tofu manufacturers prefer to use. The tofu made from Sachiyutaka showed a low breaking stress at a concentration of 0.25% MgCl₂ at several production locations in spite of its high content of seed protein (Fig. 4). In contrast to tofu made from Fukuyutaka, the consistency of tofu made from Sachiyutaka could be easily reduced at a concentration of 0.25% MgCl₂ under certain conditions of cultivation, which may be related to the largest requirement of MgCl₂ for the maximum breaking stress (Table 2).

Besides the buffer reactivity of soymilk, slopes of the linear equations between the protein content and the maximum breaking stress differed among the six varieties (Fig. 4). The slopes of the approximate linear equations for Tachinagaha and Enrei were 384 and 1059, respectively. The value of the latter variety was about 2.8 times as large as that of the former variety. The slopes reflected the production of tofu with a steady consistency. If the consistency varies

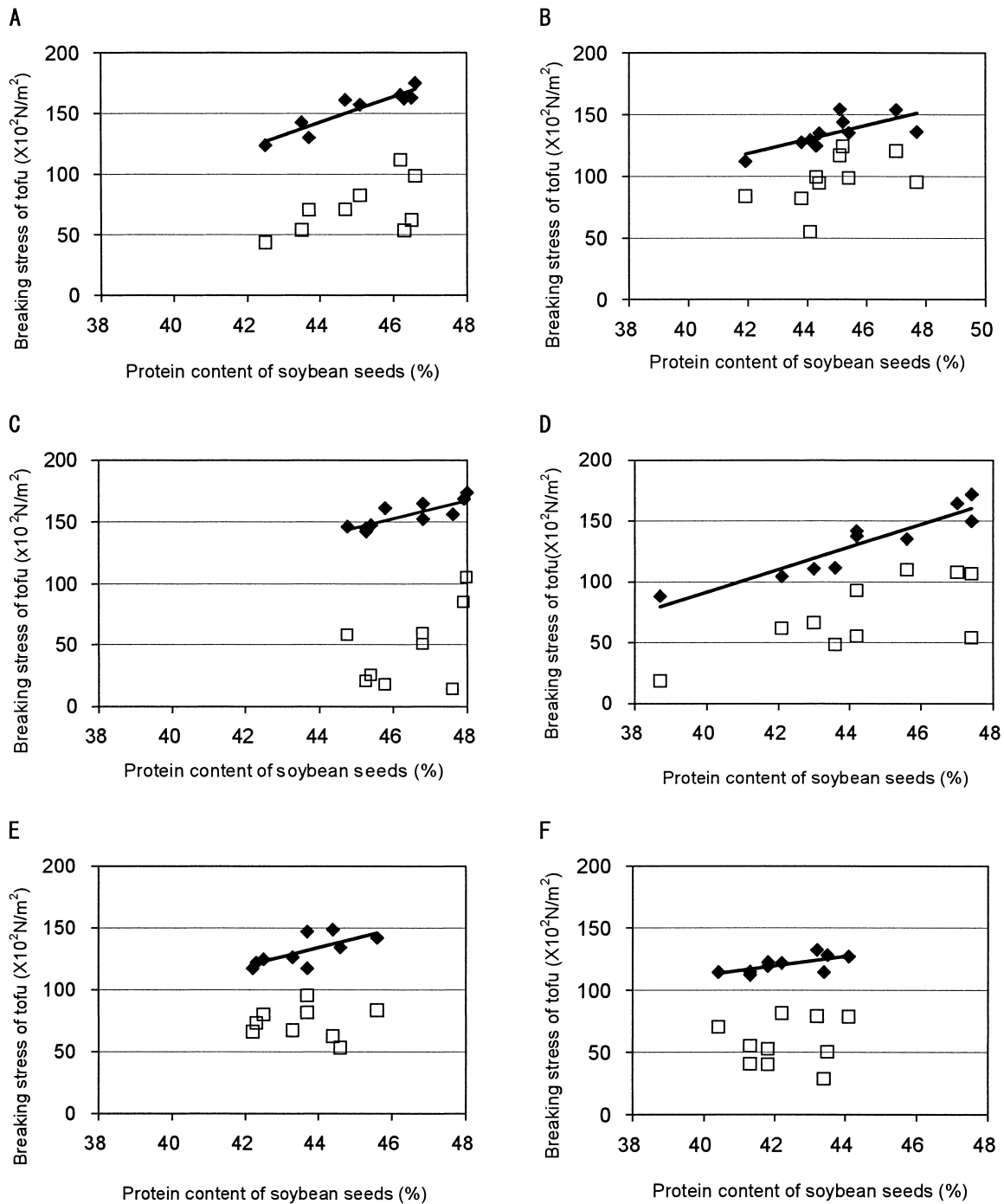


Fig. 4. Correlation between the protein content of soybean seeds and the breaking stress of tofu made from six varieties, Enrei (A), Fukuyutaka (B), Sachiutaka (C), Ayakogane (D), Hatayutaka (E) and Tachinagaha (F). \blacklozenge denotes the plot of the maximum breaking stress of tofu. \square denotes the plot of the breaking stress of tofu prepared with 0.25% MgCl₂. Lines for approximate linear equations are drawn for the maximum breaking stress.

within a limited range although the protein content varies largely, manufacturers can expect a stable consistency, which is required for large-scale manufacturing. Therefore, suitable characteristics for tofu making may include a high protein content, proper coagulation reactivity like that of soymilk made from Fukuyutaka, and steady consistency like that of tofu made from Tachinagaha.

Our results suggested that the consistency of tofu could be estimated not only by the protein content but also by the buffer reactivity of soymilk to a coagulant. Protein content could determine the consistency when the buffer reactivity of soymilk disappears by the addition of sufficient amount of coagulants, while the buffer reactivity is likely to largely affect the consistency of tofu made with a coagulant below

the concentration required for the maximum breaking stress. Our next objective is to reveal the buffer reactivity of soymilk, which may be associated with some components other than protein.

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