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Changes of Astringent Sensation of Soy Milk during Tofu Curd Formation

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The effect of isoflavone on soy milk and tofu astringency was investigated, and no consistency was found between an undesirable astringent taste and isoflavone contents. Isoflavone-enriched extract (~39% isoflavones) showed no astringency. Soybean foods having high amounts of isoflavones showed less astringency. About 80% of isoflavones exist freely in both soy milk and tofu, but 55% of phytates (which play an important role in the formation of the tofu curd network) exist freely in the soy milk, and 6−13%, on the basis of coagulation, existed freely in the tofu curds. A 1% potassium phytate solution at pH 7 showed the very same astringency as soy milk; however, calcium phytate at the same concentration and pH showed no undesirable sensation. Thus, it is assumed that the astringent characteristics caused by phytic ions in soy milk are lost upon conversion of phytic ions to their insoluble salt forms during soy milk coagulation.

KEYWORDS: Isoflavone; phytic acid; astringency; soy milk; tofu curd network

INTRODUCTION

Soy foods such as soy milk and tofu have been consumed as a traditional food in East Asian countries such as China, Korea, and Japan. Soy milk has become popular as a beverage in the Far East and is gradually spreading to the Western world. Large-scale production has evolved, along with commercial marketing of soy milk in a worldwide market.

Unfortunately, soy milk is not well accepted for its beanlike (beany) flavor and astringent taste. Many attempts to improve the unfavorable characteristics of soybean seeds have been made. Heating during disruption of the beans (*1*) or before disruption (*2*) can control and the development of the odorless soybean variety have made possible the control of the objectionable beanlike (beany) flavor. The elimination of lipoxygenase isozymes by mutagenesis and breeding was successfully achieved (*3*) and contributes to the improvement of the bean flavor of soybean products. Nevertheless, factors that impart bitter and astringent flavors have remained, and the control of astringency in soy milk has proved to be a fairly difficult job for the manufacturers.

There have been several investigations on the objectionable aftertaste of soybeans. Arai et al. (*4*) identified a number of phenolic acids from defatted soy flour and showed that these phenolic acids had sour, bitter, and astringent tastes. Moreover, many studies have revealed that soybean isoflavones (soybean seed containing 0.2% approximately on dry basis) are responsible for the astringent taste in soy foods (*5*−*8*). Tsukamoto et al. (*9*) stated that different glycosides in soybeans may cause an undesirable taste.

Tofu curd, which is prepared from soy milk by the addition of coagulants, generally does not have an astringent taste even though the soy milk itself does. In these cases both the soy milk and tofu contain the same amounts of isoflavones. Matsuura et al. (*7*) pointed out that aglycons are much more undesirable than glycosides and that aglycon formation is inhibited by the addition of glucono-δ-lactone (GDL) during soaking. The soybean hypocotyl axis has higher concentrations of isoflavones compared to cotyledons (*6*). One approach to reduce the objectionable aftertaste of soy products is to remove the axis with seed coat before soy food preparation. Chien and Snyder (*10*) assumed that phenolic substances in soy milk contribute to the objectionable astringent taste. After that, they attempted to decrease the astringency of soy milk, adding skimmed cow's milk, calcium sulfate, or citric acid in the soy milk's preparation. In the same study they noticed that warm temperatures (65 °C) of the soy milk resulted in a loss of astringency compared to sensory evaluations at room temperature or 4 °C.

Although many efforts to decrease the astringency of soy foods have been performed, changes of taste sensation in tofu curd prepared from the same soy milk are still unknown when the physical characteristics of soy milk changed to form a curd after the addition of coagulant at the desired temperature.

Even though it is obvious that tofu curd shows fewer or no objectionable tastes when compared with soy milk, there is no report indicating the cause of less objectionable sensation in

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tofu curd. Taste characteristics of tofu are not explained by the quantitative chemical analysis of components in material soybeans. Soy milk and tofu curd do not show the same sensation of astringency, even though they are prepared from same the soybean.

In this study we tried to make clear through sensory evaluation the phenomenon of an undesirable astringent sensation in soy milk disappearing after tofu curd formation even though the same variety of soybean seeds was used.

MATERIALS AND METHODS

Soybean Seeds. A main sample of soybeans (*Glycine max* var. Suzuyutaka) was harvested in 2000 at the Iwate University Experimental Farm located in Morioka, Iwate, Japan, and another variety, Fukyutaka, harvested in 2000 in Saga prefecture, Japan, were stored at 4 °C until use.

Commercial Soy Foods. The commercial soy milks and Kinu tofu (Taishi Food Co. Ltd.), Long Life tofu (Morinaga Co. Ltd.), and soy yogurt (Soyfarm Co. Ltd.) were bought at a local supermarket in Morioka, Iwate, Japan.

Chemicals. Phytic acid was purchased from Nacalai Tesque Inc. The other chemicals from Wako and Kanto Chemical Co. were of the highest purity available and used without further purification.

Preparation of Soy Milk and Tofu Curd. The soy milk was prepared as follows: washed clean soybean seeds were soaked in deionized water for 18 h at 4 °C. The swollen beans were ground into a homogenate with water 8 times the weight of those soybeans without soaking using an Oster blender (Oster Co., Milwaukee, WI) for 4 min, and the homogenate was then filtered through a defatted cotton sheet to separate okara (soybean residues). The filtrate was heated at ~100 °C for 5 min using steam and then cooled quickly to 5 °C with ice to obtain soy milk.

Soy milk (25 mL) was placed in a beaker, and then air was removed from the soy milk using a vacuum pump for 30 min. After that, the soy milk was kept in ice for 30 min. GDL (0.3%), $CaCl_2$ (0.15%), or $MgCl_2$ (0.15%) was added and well stirred. Then the soy milk was poured into a syringe to make tofu curd according to the method of Wada et al. (*11*) and then placed in hot water of 90 °C for GDL or 70 °C for $CaCl_2$ or $MgCl_2$ for 1 h. The tofu curds were allowed to cool for several minutes at room temperature and then kept in a refrigerator at 4 °C for further experimental purpose.

Dialysis. Soy milk and tofu homogenate were dialyzed by cellulose membrane (molecular weight cutoff of 10000) against at least a 50-fold volume of deionized water at 4 °C for 24 h with stirring. Water was changed three times at 8 h intervals.

HPLC Analysis of Isoflavone Components. *Sample Drying.* Soybean powder and okara were dried in a vacuum oven at around 50 °C for 24 h. Soy milks were dried in a vacuum chamber. Tofu and soy yogurt were first homogenized using a Potter-Elvehjem homogenizer (Takashima Co.) and dried in the same way.

Extraction of Glycoside Components. Dried samples were weighed in a sample tube, and a 3−10-fold volume of 70% aqueous ethanol containing 0.1% acetic acid was added. This stood at 4 °C for 24 h and was centrifuged at 8000*g* for 3 min. The supernatant was directly used as the sample solution for HPLC analysis.

HPLC Analysis. The analysis of soybean isoflavones was carried out according to the method of Kudou et al. (*6*) with some modification. A model LC-9A instrument (Shimadzu Co. Ltd.), composed of a microprocessor-controlled dual-pump system and a variable-wavelength detector (model SPD-6A, Shimadzu Co. Ltd.) connected to a computing integrator (Chromatopac C-R6A, Shimadzu Co. Ltd.), was used for the HPLC analysis. The Develosil 250 × 4.6 mm ODS-HG-5 (C_{18}) reverse phase column was used throughout the experiments.

The linear gradient elution by acetonitrile/water solutions containing 0.1% acetic acid was applied for the separation. The concentration of the acetonitrile was changed from 15 to 40% linearly for 62.5 min. The solvent flow rate was 1.0 mL/min. The samples were injected in varied amounts between 5 and 20 μL with a microsyringe. Eluted isoflavones were monitored at 260 nm, and the areas of the resultant eluted peaks were integrated by the above-described integrator.

Thin-Layer Chromatography (TLC). TLC was carried out as follows: A precoated silica gel plate (Kieselgel 60; Merck) was used. The solvent system used for developing was a lower layer of chloroform/methanol/water (13:7:2), separated by a liquid separator. After developing, spots on the plate were visualized for isoflavones by UV irradiation (254 nm) and for saponins by spraying with 10% sulfuric acid and heating at 115 °C for 13 min in an oven.

Determination of Phytic Acid by Chemical Method. The phytate content of soy milk and tofu was measured according to the method followed by Ishiguro (*12*). Soy milk or homogenized tofu was mixed with trichloroacetic acid (TCA) containing a sodium sulfate solution. The mixture was settled at room temperature for 20 min and centrifuged at 8000*g* for 3 min. Supernatant was mixed with $FeCl_3$ solution (0.2% Fe^{3+} in 0.5 M HCl). The mixture was incubated in boiling water for 90 min, cooled at room temperature, and then centrifuged at 1000*g* for 6 min. The precipitate was collected and dispersed in washing solution (0.6% HCl plus 2.5% Na_2SO_4) and then centrifuged again at 1000*g* for 6 min. The precipitate was further dispersed in 0.6 N NaOH and centrifuged at 1000*g* for 6 min. The precipitate was dissolved into 0.9 N HCl, and the desired volume was adjusted with deionized water. This sample solution was used for the determination of iron content according to an *o*-phenanthroline method. The sample solution was placed in a flask and then mixed with acetate buffer (pH 4.6), 10% hydroxylamine hydrochloride, and 0.12% *o*-phenantholine and then filled to the desired volume with deionized water. Samples stood for >30 min at room temperature, and the absorbance was measured at 510 nm by a spectrophotometer (Shimadzu UV−vis spectrophotometer, Mini 1240 model). The calibration curve of iron was made by iron standard solution for atomic absorbance analysis. One phytate molecule links with four iron molecules, so that the phytate content is equal to a quarter of the iron's.

Preparation of Isoflavone-Enriched Extract. Soybean seeds were dried at room temperature for a few days and milled by using a food mill for 2 min to obtain soybean powder.

The soybean powder (20 g) was extracted with 200 mL of 70% aqueous ethanol at room temperature for one night. The extract was evaporated to dryness under reduced pressure using a rotary evaporator at 35 °C. Dried extracts were dispersed in butanol/water = 1:1 (v/v) and allowed to stand overnight. The upper layer (butanol layer) was evaporated to dryness under reduced pressure. After lyophilization, dry matter was used as the isoflavone-containing dry extract fraction.

Preparation of Potassium Phytate (1%) Solution. One gram of phytic acid was taken in a beaker to which was added 1 N potassium hydroxide solution under a pH-meter and well stirred at pH up to 7. The volume was finally adjusted to 100 mL.

Preparation of Potassium Phytate Added Soy Milk. Soy milk with 0.2% or more potassium phytate was prepared by adding 20 mL of a 1% potassium phytate solution (pH 7) directly into 100 mL of soy milk and mixing well.

Preparation of Isoflavone-Enriched Extract (Dry) Added Soy Milk. The extract contains 39.4 mg of isoflavone in 100 mg of dry matter. However, soy milk (100 mL) contains on average 41.0 mg of isoflavones. Therefore, ~104 mg of dry extract, which contains 41.0 mg of isoflavones, was added into soy milk (100 mL) as a one-time increase of isoflavone concentration.

Sensory Evaluation. Sensory evaluation was done by 10 panel members who can recognize the difference between 0.04% tannic acid and water at a 5% level of significance. A triangle presentation of randomized combinations was done to evaluate the differences of the taste of sample solutions. The samples (2 mL each per test tube) were directly put into the mouth. Panel members were supplied with water in each trial for overcoming the astringent sensation, but each panel member was asked to use the water according to his/her own judgment for the best procedure for overcoming the remaining astringent sensation. When panel members answered correctly three times with three combinations, then the probability of a correct answer was expected at a <5% level of significance.

Isoflavone-enriched extract (isoflavone contents of 39.4 mg in 100 mg of dry extract), 1% potassium phytate, and 1% calcium phytate

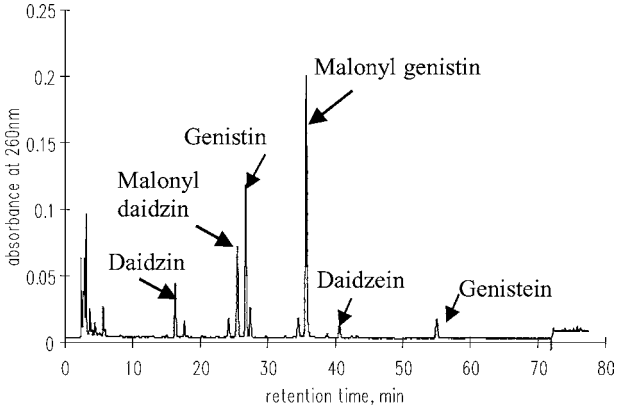

Figure 1. Typical HPLC chromatogram of isoflavone components of soy milk: column, Develosil 250 × 4.6 mm ODS–HG-5 (C_{18}); solvent, CH_3CN/H_2O containing 0.1% CH_3COOH (HPLC grade); elution, CH_3CN gradient linearly 15–40% in 62.5 min; flow rate, 1.0 mL/min; detection, UV at 260 nm.

solutions were evaluated by 10 trained panel members by descriptive analysis. Four milligrams of isoflavone-enriched extract (dry sample) was offered to the panels. Five milliliters of 1% potassium and calcium phytate was offered to taste in similar way. Isoflavone-enriched extract and potassium phytate added soy milks were offered to the panelists to investigate in the same above-mentioned way. Five panels were considered to control the sample condition of the evaluation.

Panelists were asked to taste each sample at about 1 h intervals. Samples were presented in triplicate, and panelists were supplied some blank sheets to write down their taste perception and comments. Moreover, the physical condition of the panel members and the environment of the place were also considered.

RESULTS AND DISCUSSION

Relationship of Taste Characteristics and Isoflavone Contents. According to some studies cited in the Introduction, soybean isoflavones are the main cause of an astringent sensation in soy milk. If isoflavones are responsible for this astringent sensation, its intensity will obviously increase with elevated concentrations of isoflavones. To investigate the relationship between the taste characteristics and the isoflavone content, different kinds of soybean foods (including pure soy milk products) were bought at the market, and different preparations of soy milk and tofu curd were used in our laboratory. Isoflavone contents in the different soybean foods were determined by HPLC. A typical chromatogram of isoflavone components in soy milk analyzed by HPLC is shown in **Figure 1**. Taste characteristics and isoflavone concentrations of different kinds of soy foods, such as soy milk, tofu, and soy yogurt, are shown in **Table 1**. Soy milk prepared in the laboratory contained nearly 41 mg of isoflavones in 100 mL of soy milk and showed a strong astringent taste. Tofu curd coagulated with GDL demonstrated a bitter and astringent taste, whereas tofu curd made with calcium chloride showed less astringency, as evaluated by the panel members. Another commercial tofu (Kinu tofu) contains a high isoflavone concentration (88 mg of isoflavones in 100 g of tofu curd) but almost no undesirable taste. Thus, it is observed that there might be no consistency between isoflavone concentration and astringency. From these results, an idea is assumed: isoflavones exist freely in soy milk but are attached to some large components after curd formation or are buried by protein aggregates; for this reason we may not feel the same sensation in tofu.

Moreover, it is known that phytic acid (soybean generally contains 0.2−0.3%) combines with protein and calcium (or magnesium) during tofu curd formation (*13*, *14*). Therefore, phytates may have the affect of trapping the astringent components to prevent them from interacting with taste cells of the mouth and throat. In the section we try to make clear whether isoflavones exist freely or not in soy milk and tofu or if other components are responsible for that sensation.

Table 1. Taste Characteristics and Their Isoflavone Concentration of Some Soyfoods, Prepared in the Laboratory and Purchased at the Market

sample (coagulant)	isoflavone concn[a] (mg/100 g of fresh wt)	taste characteristics[b]
Prepared in the Laboratory		
soymilk	41.4	strong, bitter, and astringent
tofu (GDL)	41.0	bitter, astringent
tofu ($CaCl_2$)	41.0	more desirable
Purchased at the Market		
soy milk[c]	75.0	limited undesirable
soy milk[d]	49.0	astringent, undesirable
soy milk[e]	28.0	smooth taste
soy milk[f]	23.0	slightly undesirable
soy milk[g]	30.0	astringent
Kinu tofu ($MgCl_2$)[c]	88.0	no undesirable
Morinaga tofu (Mg + GDL)[h]	30.9	astringent
soybean yogurt[i]	15.0	astringent

[a] Average values of three replicate analysis. [b] Taste evaluation was performed by 10 panel members. [c] Taishi Food Co. Ltd. [d] Kibun Food Co. Ltd. [e] Marusan Food In. Ltd. [f] Akokasei Food In. Ltd. [g] Meiraku Food Co. Ltd. [h] Morinaga Co. Ltd. [i] Soyfarm Co. Ltd.

Screening of Factors Affecting the Undesirable Taste in Soy Milk. *Location of Isoflavones and Phytic Acid in Soy Milk and Tofu.* It was assumed that isoflavones exist freely in soy milk and that they bind with some large components during tofu curd formation. Phytates are known to be bound with soy proteins during tofu curd formation, so phytates will be a good marker of this study. Therefore, first, the location of isoflavones and phytates by dialysis, if they exist freely or not in both soy milk and tofu, was investigated. Isoflavone and phytate contents before dialysis were 41.4 mg% and 220 mg%, respectively. After dialysis, >83% (34.36 mg) of the isoflavones were diffused into the outer solution from the dialysis tube. With regard to phytates, ∼45% (99 mg) diffused out, but 55% (121 mg) remained inside the dialysis tube. This result suggests that isoflavones are almost free and that about half of the phyates existed freely; however, the rest of them were attached to large components in soy milk.

Next, dialysis of tofu curds was performed to make clear the location of isoflavones and phytates in tofu curd at different coagulations. Three kinds of tofu curd were prepared by using different coagulants: GDL, $CaCl_2$, and $MgCl_2$. With regard to isoflavones, 38, 40.8, and 47.2 mg (per 100 g of tofu curd) of isoflavones were detected in tofu curds before dialysis, respectively. After dialysis, about 65% (13.4 mg) of isoflavones of GDL-coagulated curd, 70% (12.1 mg) of Ca-coagulated curd, and 72% (13.2 mg) of Mg-coagulated curd were diffused from the dialysis tube. For phytates, only 13, 6, and 9% from GDL-, Ca-, and Mg-coagulated curds, respectively, were diffused out (**Figure 2**). Therefore, it is possible to say that isoflavones are almost free even in tofu curd, whereas most phytates are attached to large components in the tofu curds. If isoflavones are the components that effect the astringent taste in soy milk, we cannot explain why tofu has less or no astringent taste because tofu

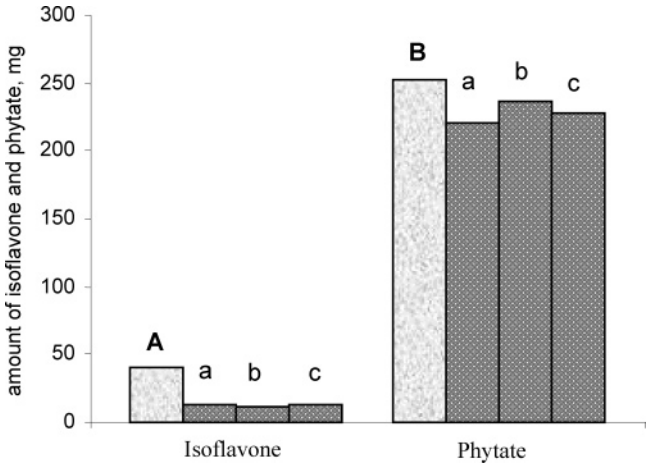

Figure 2. Amount of isoflavone and phytate in GDL-, Ca-, and Mg-coagulated tofu curds before (lightly shaded bars) and after (heavily shaded bars) dialysis: (**A**) isoflavone content; (**B**) phytate content; (**a**) GDL-, (**b**) Ca-, and (**c**) Mg-coagulated tofu curds.

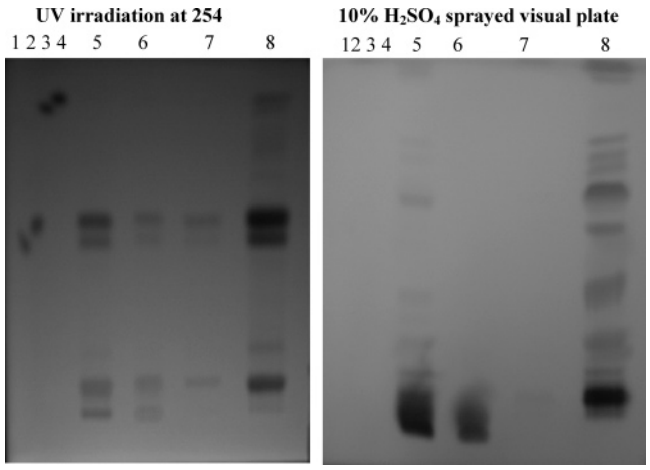

Figure 3. TLC pattern of isoflavone-enriched extract of the soybean seeds: (lane 1) daidzin; (lane 2) daidzein; (lane 3) genistin; (lane 4) genistein; (lane 5) 70% ethanol extract; (lane 6) butanol/H_2O (1:1), lower layer (water layer); (lane 7) butanol/H_2O (1:1), upper layer (butanol layer), partitioned by liquid separator; (lane 8) methanol-extracted isoflavones contain dry extract. Conditions: silica gel plate (Kieselgel 60; Merck); solvent, $CHCl_3/CH_3OH/H_2O$ (13:7:2 lower phase); detection, spraying with 10% H_2SO_4, heating at 115 °C for 13 min.

itself contains isoflavones as a free form in the very same amount as in soy milk.

Taste Characteristics of Isoflavone-Enriched Extract and Phytate Salts. Dry extract was prepared from soybean seeds in which isoflavone contents (purity) were 39.4 mg/100 mg of dry matter. By HPLC determination, the contents of isoflavone components were daidzin (8.3 mg), malonyl daidzin (1.9 mg), genistin (14.6 mg), malonyl genistin (0.52 mg), daidzein (5.3 mg), and genistein (8.8 mg). TLC analysis revealed that this fraction also contained group B and DDMP conjugated saponins (*15*, *16*), confirmed by other TLC and HPLC procedures (*15*, *16*), and phospholipids, but not sugar **(Figure 3)**. Taste characteristics of the isoflavone-containing extract were evaluated by 10 panelists. None of the 10 perceived astringency, but 8 of the10 panelists perceived bitterness (**Table 2**), which may be caused by saponins. At the same time the taste characteristics of potassium phytate and calcium phytate solutions (1%) were evaluated. Eight of 10 panel members expressed that a 1% potassium phytate solution had a very strong astringency but

Table 2. Taste Characteristics of the Isoflavone-Enriched Extract (Dry), Potassium Phytate (1% Solution), and Calcium Phytate (1% Solution)

panel	dry extract[a]	potassium phytate	calcium phytate
1	bitter, chalky	astringent	smooth
2	bitter	astringent	not bad taste
3	bad taste and not smooth	undesirable	smooth
4	undesirable and bitter	astringent	no undesirable
5	bitter	not smooth	not bad taste
6	no taste, chalky	astringent	no taste
7	bitter	astringent	smooth
8	bitter	astringent	not undesirable
9	bitter	astringent	not bad taste
10	bitter, chalky	astringent	smooth

[a] Dry extract (4 mg of each panel) was applied to sensory test.

Table 3. Sensory Evaluation (Triangle Randomized Test) of the Difference between Isoflavone-Enriched Dry Extract or Potassium Phytate Added Soy Milks and Normal Soy Milk

	level[a]			
	A	B	C	D[b]
added isoflavone-enriched extract (one time increased[c]) into soy milk	3	2	0	0
added 0.2% potassium phytate solution in soy milk[d]	0	0	1	4

[a] Number of persons who gave correct answer: A, no correct answer; B, 1/3; C, 2/3; and D, 3/3. [b] Probability of difference at 5% level of significance. [c] 104 mg of dry extract (isoflavone contents 39.4 mg/100 mg of dry matter) added into 100 mL of soy milk (100 mL of soy milk contains an average of 41 mg of isoflavone). [d] 20 mL of 1% potassuim phytate (pH 7) added into 100 mL of soy milk.

that calcium phytate had no astringent taste. Next, soy milks mixed with isoflavone-enriched extract or potassium phytate were prepared to compare the astringency with normal soy milk. Five panelists tested did not identify the difference between dry extract added and normal soy milk at a 5% level of significance. On the other hand, when the soy milk increased with potassium phytate (0.2% more) was tasted, panel members identified the difference (increased astringency) at a 5% level of significance **(Table 3)**. All previous results suggest that isoflavones perhaps show no astringent taste in soy milk or tofu but that phytates with a potassium ion may cause an astringent sensation.

Ono (*13*, *14*) has provided one attractive mechanism on how soy milk coagulates into tofu. According to this mechanism, when Ca and/or Mg ions are added as a coagulant, Ca and/or Mg ions bind with phytic acid to release hydrogen ions and form salts. Proteins, oil bodies, and phytates coagulate together to form a network structure of tofu curds. The network structure matures to hold water inside. On the basis of this theory, a hypothesis has been assumed as to why tofu has less or no astringency than soy milk. Isoflavones and some phytates exist freely in soy milk. Isoflavones do not show astringency, but potassium phytate (soluble) ions show astringent sensation, whereas calcium phytate (insoluble) shows no astringency. After curd formation, soluble potassium phytate becomes an insoluble form. This is why we do not feel the same sensation in tofu curd.

Some reports have shown that some isoflavone components produced an undesirable astringent sensation in soy milk. Kudou et al. (*6*) reported that malonyl isoflavones comprised 66% of the total beans showing astringency. However, the report considered that the conversion of malonyl isoflavone glycosides into isoflavone glycosides during processing may produce soy

milk with a very low degree of objectionable aftertaste. Matsuura et al. (*7*) have demonstrated that an increase in daidzein and genistein by the action of β-glucosidase in soybeans during the soy milk manufacturing process resulted in an increase in the objectionable aftertaste. However, Kudou et al. (*6*) reported that isoflavone compounds are thermally stable, but they convert into a conjugated compound under certain conditions. Some other research has been performed to control the astringency in soy milk considering the processing method or the incorporation of other chemicals, but the astringency of the soy milk usually disappears after tofu curd formation. There is no report at the present time considering why tofu curd is less astringent. However, we found some soy milk that has high amounts of isoflavones but is less astringent. Therefore, there is no consistency within the above information regarding the astringency of isoflavone compounds. The astringent sensation is very different among other basic tastes; therefore, the evaluation of an astringent sample should be compared to a known substance, which should depend on panel behavior. In this study we assume that either conjugated compounds or components incorporated with other components probably cause astringency. During tofu curd formation, this kind of incorporation occurs according to the report of Ono (*13*, *14*). The phenomena of this study derived from the concept and report of Ono (*13*, *14*). However, other factors such as soybean variety, other components (instead of phytates), physical properties, and pH of tofu curds should remain under investigation.

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