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Effect of Water-to-Bean Ratio on the Contents and Compositions of Isoflavones in Tofu

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The present study investigated the changes of the bioavailable isoflavones, including daidzin, genistin, daidzein, and genistein, during the making of tofu. The amount of extracted daidzin and genistin in soy milk increased with increasing water-to-bean ratios from 5 to 9 and reached the maximum level at the ratios of 9−11. On the other hand, the amount of extracted free isoflavones (daidzein and genistein) was not affected by the water-to-bean ratio at the range of 5−11, and their extracted amounts in soy milk were 2−4-fold those in raw soybean. It is suggested that these free isoflavones are mainly derived from daidzin, genistin, malonyldaidzin, and malonylgenistin through enzymatic hydrolysis during the making of soy milk. Tofu made with water-to-bean ratios of 9:1 and 10:1 had the maximal retentions of daidzin and genistin, which were due to the fine homogeneous network microstructure that is supposed to be more effectively retained through hydrophilic interaction with protein. On the contrary, the retained amount of free isoflavones decreased significantly as the water-to-bean ratio increased from 7 to 11, due to their weakening hydrophobic interaction with protein. In this study it was found that the homogeneous microstructure of tofu improved the retention of hydrophilic daidzin and genistin and that the increased amount of drained water does not significantly reduce their retention in the final tofu products as generally imagined.

KEYWORDS: Daidzin; genistin; daidzein; genistein; isoflavones; soy milk; tofu

INTRODUCTION

Recently, increasing evidence has indicated that the consumption of soy-containing foods is associated with lower blood cholesterol and the rate of osteoporosis, protection against cardiovascular disease, and reduced incidence of certain cancers (prostate and breast) (*1*−*3*). Results from these studies have suggested the isoflavones in soybeans might be the contributing factors.

Twelve isoflavone components have been isolated from soybeans; three free isoflavones (genistein, daidzein, and glycitein), and their respective nine glucosidic conjugates. The glucosides include three β-glucosides forms (genistin, daidzin, and glycitin), three malonylglucosides (6′′-*O*-malonylgenistin, 6′′-*O*-malonyldaidzin, and 6′′-*O*-malonylglycitin), and three acetylglucosides (6′′-*O*-acetylgenistin, 6′′-*O*-acetyldaidzin, and 6′′-*O*-acetylglycitin). Isoflavones when ingested are metabolized extensively in the intestinal tracts, absorbed, and transported to the liver and undergo enterohepatic recycling (*4*). Intestinal bacterial β-glucosidase cleaves the sugar moieties and releases the biologically active isoflavones, daidzein and genistein, and in the adult these can be further biotransformed by bacteria to the specific metabolites, including equol and desmethylangolensin metabolites of daidzein and *p*-ethyl phenol metabolites of genistein before absorption (*5*). All of these phytoestrogens can share the physiological features and behavior of endogenous estrogens (*4*). However, it was noted that daidzin and genistin were readily hydrolyzed by the intestinal bacterial β-glucosidase, whereas 6′′-*O*-malonylglucosides and 6′′-*O*-acetylglucosides were not (*6*, *7*). When daidzin and genistin are ingested, daidzein or genistein appears rapidly in plasma, and thereafter the plasma profiles were similar to those for the ingestion of the corresponding free isoflavones (*8*). This evidence in the literature suggests that the bioavailability of daidzin, genistin, daidzein, and genistein predominates over the other forms of isoflavones (*6*−*8*).

The processing of soybeans affects the nutritional content of the soy food products significantly (*9*, *10*). There have been reports on the isoflavone content of some soybean varieties and soy foods as well as the effects of processing on these compounds (*11*, *12*). It was suggested that enzymatic hydrolysis, heat processing, and fermentation significantly alter the free and bound isoflavones distribution (*10*).

Hui et al. (*13*) found a 2−3-fold difference in isoflavone content between different brands or varieties of tofu. These variations in isoflavone content may result from different processing techniques and soybean varieties. According to Wang and Murphy (*10*) and Jackson et al. (*14*), some isoflavones were lost in the processing steps, which involved soaking, heating, filtration, and coagulation. The loss of isoflavones was significant when the soy milk was separated from okara and coagulated

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to form tofu. Because the extraction and retention of protein and other water-soluble components of soybean were affected by the water-to-bean ratios during soy milk and tofu manufacturing as reported in our previous research (*15*), the content and composition of isoflavones might be influenced as well. Therefore, the objectives of the present study are to investigate the effect of water-to-bean ratios on the extraction and retention of the bioavailable isoflavones (daidzin, genistin, daidzein, and genistein) in soy milk and to develop the optimal processing condition for making tofu products with higher amounts of bioavailable isoflavones.

MATERIALS AND METHODS

Materials. Soybeans of the Ohio FG1 cultivar were obtained from a local agency. Food grade calcium sulfate dihydrate (Ako Kasei Co., Ltd., Hyogo, Japan) was used as the coagulant in the tofu production and was obtained from Gemfont Corp. (Taipei, Taiwan).

Soy Milk and Tofu Making. Soy milk and tofu were produced according to the traditional method as follows. The procedure used for making soy milk and tofu were similar to that published by Cai and Chang (*16*) with some modifications. Five aliquots of 300 g of soybeans were soaked in tap water (3 times bean weight) at room temperature for 9 h to bring the weight of the soaked beans to ∼2.2 times their initial weight. After the initial soak water had been drained away, tap water was added to the hydrated beans to a final weight of 1800, 2400, 3000, 3300, or 3600 g to give water-to-bean ratios of 5:1, 7:1, 9:1, 10:1, and 11:1 (w/w), respectively. The mixtures were then ground with a soy milk grinder (pineapple grinder, Great Yen Electric Food Grinder Co., Ltd., Taipei, Taiwan) equipped with an automatic centrifugal filter to separate raw soy milk from the okara. After grinding, the raw soy milk was further filtered through a 100-mesh sieve to remove the remaining okara. After the addition of 1 g of antifoaming agent (containing 90% glycerin fatty acid ester, 5% calcium carbonate, 4.3% soybean phospholipids, and 0.7% silicone resin), the raw soy milk was heated to 96 °C in 20 min and held at this temperature for 5 min. After the soy milk had cooled to room temperature, the weight of the soy milk was measured. To produce tofu, the boiled soy milk was cooled to ∼73 °C, 50 mL of calcium sulfate solution was added to the soy milk to give a final $CaSO_4 \cdot 2H_2O$ concentration used in the soy milk of 0.4% (w/v), and the solution was then stirred at a speed of 250 rpm for 10 s and then incubated for 20 min to form bean curds. The soybean curd was then broken slightly and transferred into a muslin cloth-lined stainless steel mold (13 × 13 × 5.5 cm^3) and pressed at 22 g/cm^2 for 10 min, at 44 g/cm^2 for 10 min, and at 65 g/cm^2 for 30 min. At the end of pressing, the cloth was removed, and the weight of tofu was recorded. The tofu yield was expressed as grams of fresh tofu per 100 g of soybeans. The tofu was transferred into a plastic bag and stored at 4 °C overnight for subsequent measurements.

Determination of the Protein and Solid Contents and Their Recoveries. The protein contents in soybean and tofu were determined in triplicate according to the micro-Kjeldahl method (*17*). The solid contents of soybean and tofu were determined by drying a 5 g homogenized sample in an oven at 105 °C until constant weight was obtained. Protein recovery in tofu was expressed as the amount of protein in tofu divided by the amount of protein in raw soybean multiplied by 100% on a dry weight basis. The same calculation was applied to solid recovery.

Determination of Water Retention Ability (WRA) of Tofu. WRA was determined according to a modification of the water-holding capacity (WHC) method of Puppo and Añón (*18*). About 5 g (w_1) tofu was placed on a cotton cloth membrane maintained in the middle position of a 250 mL centrifuge tube (62 mm × 120 mm). The sample weight was recorded after centrifugation at 120*g* for 5 min at 15 °C (w_2) and subsequent heating to a constant weight (w_3) at 105 °C. The WRA of tofu was calculated as follows:

$$\mathrm{WRA} = [(w_2 - w_3)/(w_1 - w_3)] \times 100\%$$

Scanning Electron Microscopy (SEM). A JEOL JSM-6300 scanning electron microscope (JEOL USA, Inc., Peabody, MA) was used to examine the fine structure of tofu made with different water-to-bean ratios. The procedure used for sample preparation was that of deMan et al. (*19*) with some modifications. A small piece of each tofu sample was fixed at room temperature with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.1) for 2 h. After five washings with 0.1 M phosphate buffer (pH 7.1) at 10 min intervals, it was then postfixed with 1% osmium tetraoxide in the same buffer for 90 min at room temperature. The fixed sample was rinsed five times with phosphate buffer at 10 min intervals. The sample was frozen in liquid nitrogen and freeze-dried. The dried sample was finally mounted on an aluminum stub and sputter-coated with gold/palladium (60:40). The observations were carried out at 20 kV.

Isoflavone Extraction. The procedure used for isoflavone extraction was that of Wang and Murphy (*10*) with some modifications. All wet samples were freeze-dried except raw soybean. Two grams of dried, finely ground samples was mixed with 9 mL of double-distilled water and 10 mL of acetonitrile (ACN), stirred for 2 h at room temperature, and filtered through Whatman no. 42 filter paper. The filtrate was taken to dryness under vacuum at a temperature below 30 °C. The dried material was redissolved in 80% methanol to a final volume of 10 mL and filtered through a 0.45 μm nylon syringe filter (Micron Separation Inc., Westborough, MA) prior to HPLC analysis. A methanolic solution of 5000 ppm of benzoic acid was used as the internal standard solution. One milliliter of internal standard solution was mixed with 9 mL of sample solution for the HPLC analysis.

HPLC Analysis of Isoflavones. Analysis of isoflavones was performed by a modification of a method described by Wang and Murphy (*10*). A linear HPLC gradient was composed of (A) 0.1% glacial acetic acid in H_2O and (B) 0.1% glacial acetic acid in acetonitrile (ACN). Following injection of 20 μL of sample, solvent B was increased from 10 to 30% over 60 min, then increased to 90% within the next 3 min and finally to 10% within 2 min, and held at that percentage for the next 12 min. The solvent flow rate was 1 mL/min. The HPLC system included Thermo Separation Products ConstaMeric 3200 and 3500 gradient pumps equipped with a Thermo Separation Products SpectroMonitor 3200 digital UV−vis detector. Peak areas were integrated using the SISC Chromatography Data Station version 3.0. A reversed-phase analytical column [Phenomenex Luna C18 (2), 250 × 4 mm, 5 μm] was used to carry out the separation. The eluted components were detected at 254 nm.

Data Analysis. All results were analyzed by analysis of variance (ANOVA) using the general linear model (*20*). Duncan's multiple-range test was used to determine differences among the samples. Significant levels were defined as probabilities of 0.05 or less. All processing treatments were in triplicate.

RESULTS AND DISCUSSION

Isoflavone Content in Tested Soybeans. The amounts of daidzin, genistin, daidzein, and genistein in the raw soybean used in this study were determined by RP-HPLC, and the contents were found to be 339.9, 363.2, 65.1, and 28.2 μg/g of soybeans (on a dry basis), respectively. These corresponded to 73.0, 75.2, 22.9, and 9.3 μmol/100 g of soybeans in that order. These results were in agreement with the findings of Wang and Murphy (*21*), who showed that the daidzin, genistin, daidzein, and genistein contents in the tested soybean varieties ranged from 37 to 780 μg/g, from 128 to 806 μg/g, from 4 to 59 μg/g, and from 7 to 45 μg/g of soybeans (on a dry basis), respectively. Tsukamoto et al. (*22*) found that not only the variety but also the crop year, growing location, and growing temperature would affect the contents of isoflavones in soybean seeds.

Content of Extracted Isoflavones in Soy Milk Prepared with Different Water-to-Bean Ratios. For basic traditional soy milk production, soybeans were first washed, soaked, ground with tap water, and filtered to obtain the soy milk. Most of the water-soluble nutrients and solids were present in the soy milk, and the insoluble residue, the okara, contained most of the fiber. In this study we found that the amount of the extracted daidzin

Table 1. Contents of the Main Isoflavones in Raw Soybean, Soy Milk, and Tofu through Processing[a]

	μmol/100 g of soybean					
	daidzin	genistin	malonyldaidzin	malonylgenistin	daidzein	genistein
raw soybean	73.0 ± 0.8 a	75.2 ± 1.1 a	233.0 ± 4.6 a	158.3 ± 4.4 a	22.9 ± 0.9 c	9.3 ± 0.7 c
soy milk[b]	61.0 ± 0.1 b	61.2 ± 0.2 b	97.3 ± 2.8 b	72.6 ± 3.9 b	49.2 ± 0.6 a	38.7 ± 0.2 a
tofu[b]	44.6 ± 1.7 c	58.9 ± 0.2 c	18.1 ± 0.7 c	17.6 ± 0.2 c	30.6 ± 0.9 b	25.7 ± 1.0 b

[a] Means (± standard deviation) bearing the same letters within the same column are not significantly different (P < 0.05). [b] Soy milk and tofu were made at the water-to-bean ratio of 10:1.

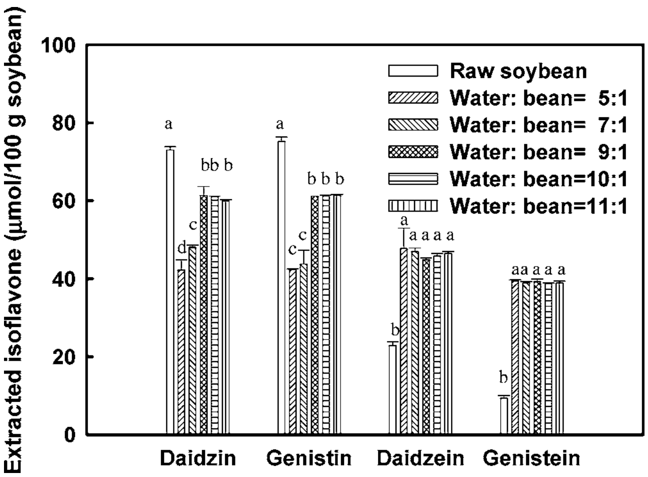

Figure 1. Effect of water-to-bean ratio on the extracted amounts of daidzin, genistin, daidzein, and genistein in soy milk. Columns bearing the same letters are not significantly different (P < 0.05).

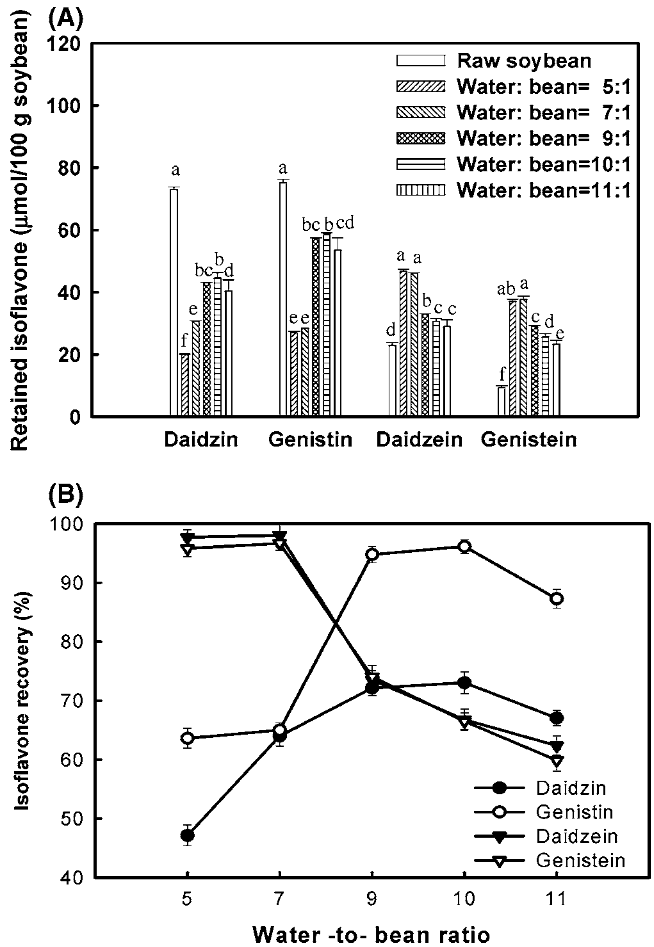

Figure 2. Effects of water-to-bean ratio on the retained amount of isoflavones from soybean (**A**) and the recovery of isoflavones from soy milk in tofu (**B**). Columns bearing the same letters are not significantly different (P < 0.05).

and genistin in soy milk increased with increasing water-to-bean ratios from 5 to 9 and reached the maximum level at the ratios of 9 to 11, whereas the amount of extracted free isoflavones (daidzein and genistein) was not affected by the water-to-bean ratio (**Figure 1**). This is similar to the extraction efficiencies of protein and other water-soluble components as reported in our previous research (*15*). Our previous study found that the soy milk prepared with water-to-bean ratios of 9:1 to 11:1 had the maximum protein and maximum solid recoveries.

As compared with the raw soybean material, the amounts of daidzein and genistein in soy milk increased to 2- and 4-fold, respectively. The results of **Table 1** indicated that this might be due to the conversion of isoflavone-β-glucosides, such as daidzin, genistin, malonyldaidzin, and malonylgenistin, to the free isoflavones by enzymatic hydrolysis. It was well documented that the amount of daidzein and genistein increased at the expense of malonyldaidzin and malonylgenistin during heating (*10*, *23*−*26*).

Content of Retained Isoflavones in Tofu Prepared with Different Water-to-Bean Ratios. The coagulation step was also an important reason for the loss of isoflavones in the traditional style of tofu making. In this study we found that the retention of isoflavones in tofu was significantly affected by the water-to-bean ratios used during tofu making. The tofus made with water-to-bean ratios of 9:1 and 10:1 were found to give the maximal retentions of daidzin and genistin (**Figure 2A**). The amount of retained daidzin and genistin in tofu increased with the increasing water-to-bean ratios from 5:1 to 9:1 and reached the maximum at the water-to-bean ratios of 9:1 and 10:1 and then decreased as the water-to-bean ratio further increased to 11:1. However, the tofus made with the water-to-bean ratios of 5 and 7 were found to have the maximal retention of the free isoflavones, daidzein and genistein. The amount of retained daidzein and genistein in tofu decreased with increasing water-to-bean ratios from 7:1 to 11:1. This might be due to the bigger holes of the porous microstructure of the tofu made with higher water-to-bean ratios (**Figure 3**). The bigger holes could not prevent the hydrophobic daidzein and genistein from being drained. **Figure 2B** shows the isoflavone recoveries in tofu from soy milk. It indicated that the recoveries of daidzin and genistin increased from 47 and 64% at a water-to-bean ratio of 5:1 to the maximum levels of recoveries of about 73 and 96%, respectively. On the contrary, the recoveries of daidzein and genistein in tofu from soy milk both decreased significantly as the water-to-bean ratio increased from 7:1 to 11:1.

Correlations between the Retention of Isoflavones and Microstructure of Tofu Prepared with Different Water-to-Bean Ratios. The tofu made with water-to-bean ratios of 9:1 and 10:1 had the maximal retention of daidzin and genistin as well as the maximal tofu yield, maximal solid and protein recoveries, and the best WRA (**Table 2**). In our previously study

Table 2. Effect of Water-to-Bean Ratio on the Properties of Tofu[a]

water/bean ratio	moisture content (%)	WRA[b] (%)	protein recovery (%)	solid recovery (%)	tofu yield (g of tofu/100 g of soybeans)
5:1	79.6 ± 0.4 b	72.9 ± 1.9 c	56.2 ± 0.6 d	45.1 ± 0.2 c	195 ± 3 d
7:1	80.2 ± 0.4 ab	75.0 ± 0.2 b	66.2 ± 0.9 c	51.5 ± 0.6 b	230 ± 2 c
9:1	80.4 ± 1.0 ab	76.5 ± 0.1 ab	70.4 ± 0.8 ab	55.5 ± 2.1 a	250 ± 3 a
10:1	80.5 ± 0.5 ab	77.2 ± 0.1 a	70.9 ± 0.7 a	55.9 ± 0.8 a	253 ± 3 a
11:1	81.2 ± 0.3 a	75.5 ± 0.7 b	69.5 ± 0.4 b	53.8 ± 0.4 b	254 ± 2 a

[a] Mean (± standard deviation) bearing the same letters among the same column are not significantly different ($P < 0.05$). [b] Water retention ability of tofu.

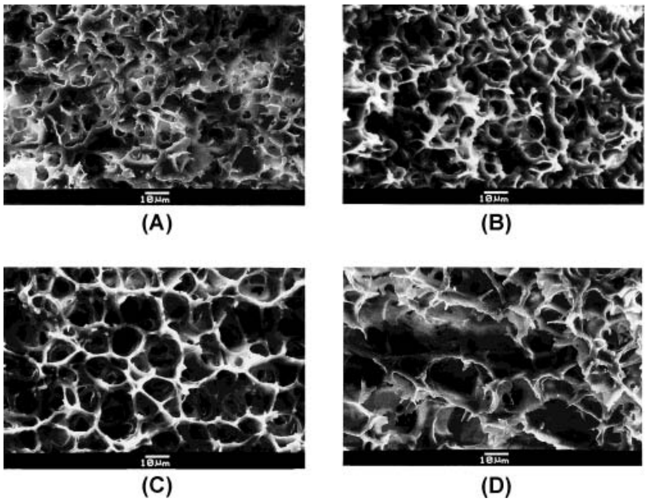

Figure 3. SEM images of freeze-dehydrated tofu prepared with various water-to-bean ratios: (**A**) water/bean = 5:1; (**B**) water/bean = 7:1; (**C**) water/bean = 9:1; (**D**) water/bean = 11:1.

(*27*) we suggested that the tofu had the better uniform and homogeneous microstructure, retained more water and water-soluble components in the tofu matrix, and, consequently, resulted in the maximal tofu yield and maximal protein and solid recoveries, as well as the better WRA. In **Figure 3**, the SEM images of tofu prepared with different water-to-bean ratios clearly show the different microstructures that were closely related to their water retention abilities (**Table 2**). The network of tofu obtained with a water-to-bean ratio of 5:1 revealed a dense and compact structure with many small pores (**Figure 3A**). When the water-to-bean ratio was increased to 7:1, there was a trend to increase the pore size, regularity, and uniformity (**Figure 3B**). The microstructure of tofu made with a water-to-bean ratio of 9:1 showed the most continuous and uniform honeycomb-like structure (**Figure 3C**). However, the network structure of the tofu prepared with a water-to-bean ratio of 11:1 was discontinuous and had many large broken fragments and holes (**Figure 3D**). These events explained why the tofu prepared with water-to-bean ratios of 9:1 and 10:1 (the SEM image of tofu prepared at 10:1 is not shown) had a uniform and continuous tofu network and, consequently, could effectively trap the maximal amount of hydrophilic daidzin and genistin, maximal soluble solids, maximal proteins, and much water in the tofu matrix, therefore resulting in the maximal retention of daidzin and genistin, as well as the maximal tofu yield and maximal protein and solid recoveries.

However, the maximal contents of free isoflavones, daidzein and genistein, were found to be in the tofu made with water-to-bean ratios of 5 and 7. This result might be due to the hydrophobic interaction of the free isoflavones with the hydrophobic area of the protein. We suggest that when the water-to-bean ratio increased to more than 7:1, the holes of the protein network would become too big to retain the free isoflavones by hydrophobic interaction with the hydrophobic area of the protein molecules. Therefore, daidzein and genistein were drained away with tofu whey. Consequently, the retained daidzein and genistein decreased as the water-to-bean ratio increased. Kohyama et al. (*28*) reported that hydrophobic interaction and charge−charge interaction both contributed to the network formation in Ca^{2+}-induced tofu gel. The protein aggregation was induced by the hydrophobic interaction of neutralized protein molecules (*28*). The strength of the hydrophobic interaction between neutralized protein molecules decreased with the decreasing compactness and denseness of the network structure of tofu. We, therefore, speculated that the association between free isoflavones and protein decreased with the increasing water-to-bean ratio used in tofu making. Therefore, we concluded that the tofu prepared with water-to-bean ratios at 5:1 and 7:1 gave the maximum contents of daidzein and genistein, as a result of the relatively stronger hydrophobic interaction between protein molecules and the free isoflavones.

Effects of Water-to-Bean Ratios on the Total Bioavailable Isoflavones Contents in Soy Milk and Tofu. The amounts of total bioavailable isoflavones (daidzin, genistin, daidzein, and genistein) (*6*−*8*) in raw soybean material and in the soy milks prepared with water-to-bean ratios of 5, 7, 9, 10, and 11 were 180.4, 171.9, 177.9, 206.7, 206.8, and 206.6 μmol/100 g of soybeans, respectively. In view of the total bioavailable isoflavones content, the water-to-bean ratios of 9, 10, and 11 were considered to be optimal for soy milk preparation. In addition, the amounts of total bioavailable isoflavones in the final tofu products prepared with the water-to-bean ratios of 5, 7, 9, 10, and 11 were 130.6, 143.1, 160.5, 159.8, and 146.2 μmol/100 g of soybeans, respectively. Accordingly, the water-to-bean ratios of 9 and 10 were considered to be optimal for tofu making (**Figure 2A**).

Furthermore, Matsuura and Obata (*29*) found that genistein and daidzein had much stronger aftertastes than their respective glucosidic conjugates. Consequently, higher amounts of daidzein and genistein in soy food seemed to have a negative effect on food quality. Therefore, the tofus made with water-to-bean-ratios of 5:1 and 7:1 might be unacceptable due to the objectionable flavor of genistein and daidzein. Consequently, the tofus made with water-to-bean ratios of 9:1 and 10:1 should be better because of the weaker aftertastes and the maximal amounts of total bioavailable isoflavones.

In the studies of Wang and Murphy (*10*) and Jackson et al. (*14*), the water-to-bean ratios employed for tofu making were 11 and 5, respectively. The estimated losses of total bioavailable isoflavones (daidzin, genistin, daidzein, and genistein) in okara in the studies of Wang and Murphy (*10*) and Jackson et al. (*14*) were 11 and 54%, respectively. In our study, we concluded that the optimum water-to-bean ratios for soy milk preparation and tofu making were in the range of 9−10, and the estimated loss of total bioavailable isoflavones in okara was 21%. In the study of Wang and Murphy (*10*), the much lower loss of isoflavones in soy milk preparation was due to the satisfactory extraction of isoflavones with sufficient of water, namely, with an amount of water 11 times the bean weight. However, the free isoflavones were greatly lost with the drained tofu whey due to the weakening of their hydrophobic interaction with protein. On the other hand, the results of the study of Jackson et al. (*14*) suggested that the high percentage of total bioavailable isoflavones lost in okara was due to the poor extraction efficiency by using such a low water-to-bean ratio of 5:1.

In this study, the recovery of total bioavailable isoflavones in tofu was 82% (relative to the amount in raw soybean). This was significantly higher than those reported by Wang and Murphy (*10*) and Jackson et al. (*14*). Their recoveries were 44 and 49%, respectively.

LITERATURE CITED

(1) Anderson, J. J. B.; Garner, S. C. Phytoestrogens and human function. *Nutr. Today* **1997**, *32*, 232−239.

(2) Caragay, A. B. Cancer preventive foods and ingredients. *Food Technol.* **1992**, *46*, 65−68.

(3) Messina, M. Soy, soy phytoestrogens (isoflavones), and breast cancer. *Am. J. Clin. Nutr.* **1999**, *70*, 574−575.

(4) Setchell, K. D. R.; Zimmer-Nechemias, L.; Cai, J.; Heubi, J. E. Exposure of infants to phyto-oestrogens from soy-based infant formula. *Lancet* **1997**, *350*, 23−27.

(5) Zubik, Z.; Meydani, M. Bioavailability of soybean isoflavones from aglycone and glucoside forms in American women. *Am. J. Clin. Nutr*. **2003**, *77*, 1459−1465.

(6) Andlauer, W.; Kolb, J.; Fürst, P. Isoflavones from tofu are absorbed and metabolized in the isolated rat small intestine. *J. Nutr.* **2000**, *130*, 3021−3027.

(7) Coward, L.; Kirk, M.; Albin, N.; Barnes, S. Analysis of plasma isoflavones by reversed-phase HPLC-multiple reaction ion monitoring-mass spectrometry. *Clin. Chim. Acta* **1996**, *247*, 121−142.

(8) Piskula, M. K. Soy isoflavone conjugation differs in fed and food-deprived rats. *J. Nutr.* **2002**, *130*, 1766−1771.

(9) Wang, H.; Murphy, P. A. Isoflavone content in commercial soybean foods. *J. Agric. Food Chem.* **1994**, *42*, 1666−1673.

(10) Wang, H.; Murphy, P. A. Mass balance study of isoflavones during soybean processing. *J. Agric. Food Chem.* **1996**, *44*, 2377−2383.

(11) Farmakalidis, E.; Murphy, P. A. Isolation of 6′′-*O*-acetylgenistin and 6′′-*O*-acetyldaidzin from toasted defatted soyflakes. *J. Agric. Food Chem.* **1985**, *33*, 385−389.

(12) Murphy, P. A. Phytoestrogen content of processed soybean products. *Food Technol.* **1982**, *36*, 60−64.

(13) Hui, E.; Henning, S. M.; Park, N.; Heber, O.; Go, V. L. W. Genistein and daidzein/glycitein content in tofu. *J. Food Compos. Anal.* **2001**, *14*, 199−206.

(14) Jackson, C. J. C.; Dini, J. P.; Lavandier, C.; Rupasinghe, H. P. V.; Faulkner, H.; Poysa, V.; Buzzell, D.; DeGrandis, S. Effects of processing on the content and composition of isoflavones during manufacturing of soy beverage and tofu. *Process Biochem.* **2002**, *37*, 1117−1123.

(15) Kao, F. J.; Su, N. W.; Lee, M. H. The characteristics of firm tofu as affected by the water-to-bean ratio and calcium sulfate concentration of soymilk. *Food Sci. Agric. Chem.* **2002**, *4*, 128−135.

(16) Cai, T. D.; Chang, K. C. Dry tofu characteristics affected by soymilk solid content and coagulation time. *J. Food Qual.* **1997**, *20*, 391−401.

(17) AOAC. *Official Methods of Analysis*, 13th ed.; Association of Official Analytical Chemists: Washington, DC, 1980.

(18) Puppo, M. C.; Añón, M. C. Structural properties of heated-induced soy protein gels as affected by ionic strength and pH. *J. Agric. Food Chem.* **1998**, *46*, 3583−3589.

(19) deMan, J. M.; deMan, L.; Gupta, S. Texture and microstructure of soybean curd (tofu) as affected by different coagulants. *Food Microstruct.* **1986**, *5*, 83−89.

(20) SAS Institute. *SAS/STAT User's Guide*, version 6.03; Cary, NC, 1990.

(21) Wang, H.; Murphy, P. A. Isoflavone composition of American and Japanese soybeans in Iowa: effects of variety, crop year and location. *J. Agric. Food Chem.* **1994**, *42*, 1674−1677.

(22) Tsukamoto, C.; Shimada, S.; Igita, K.; Kudou, S.; Kokubun, M.; Okubo, K.; Kitamura, K. Factors affecting isoflavone content in content in soybean seeds: changes in isoflavones, saponins, and composition of fatty acids at different temperatures during seed development. *J. Agric. Food Chem.* **1995**, *43*, 1184−1192.

(23) Barnes, S.; Kirk, M.; Coward, L. Isoflavones and their conjugates in soy foods: extraction conditions and analysis by HPLC-mass spectrometry. *J. Agric. Food Chem.* **1994**, *42*, 2466−2474.

(24) Mahungu, S. M.; Diaz-Mercado, S.; Li, J.; Schwenk, M.; Singletary, K.; Faller, J. Stability of isoflavones during extrusion processing of corn/soy mixture. *J. Agric. Food Chem*. **1999**, *47*, 279−284.

(25) Murphy, P. A.; Barua, K.; Hauck, C. C. Solvent extraction selection in the determination of isoflavones in soy foods. *J. Chromatogr. B* **2002**, *777*, 129−138.

(26) Ha, E. Y. W.; Morr, C. V.; Seo, A. Isoflavone aglycones and volatile organic compounds in soybeans; effects of soaking treatments. *J. Food Sci.* **1992**, *57*, 414−417.

(27) Kao, F. J.; Su, N. W.; Lee, M. H. Effect of calcium sulfate concentration in soymilk on the microstructure of firm tofu and the protein constitutions in tofu whey. *J. Agric. Food Chem.* **2003**, *51*, 6211−6216.

(28) Kohyama, K.; Sano, Y.; Doi, E. Rheological characteristics and gelation mechanism of tofu (soybean curd). *J. Agric. Food Chem.* **1995**, *43*, 1808−1812.

(29) Matsuura, M.; Obata, A.; Fukushima, D. Objectionable flavor of soy milk developed during the soaking of soybean and its control. *J. Food Sci.* **1989**, *54*, 602−605.

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