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Phytoestrogen Enriched Tofu from Soybean Meal

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ABSTRACT

Isoflavone, a group of phytoestrogen, reduces postmenopausal symptoms and the risk of osteoporosis of women. Glycosidic forms of isoflavones are presented in non-fermented soyfoods such as tofu and they are less bioavailable than the aglycone isoflavones. Aglycone forms of isoflavones or more bioavailable forms can be increased by acid hydrolysis during tofu processing. The present study investigated the possibility of increasing the aglycone forms of isoflavones by acid hydrolysis. We used five types of tofu in this study: soybean tofu with hydrolysis, soybean meal tofu with hydrolysis, soybean tofu in general process, soybean meal tofu in general process, and commercial tofu. Defatted soybean meal was used as the major ingredient in the tofu which was made by using the new method—acid hydrolysis. To identify the isoflavone quantities in all five types of tofu, high performance liquid chromatography with diode array detection (HPLC-DAD) analysis was employed. The genistein ratio between hydrolyzed tofu and standard tofu was 1:1-8, and the daidzein ratio between hydrolyzed tofu and standard tofu was 1:6-12. The five types of tofu were analyzed for the crude protein and micronutrients such as calcium (Ca), magnesium (Mg), potassium (K), iron (Fe), zinc (Zn), and selenium (Se) by the modified Kjeldahl method and inductively coupled plasma emission spectroscopy (ICP-ES), respectively. The mean crude protein concentration of hydrolyzed tofu from soybean meal was 40.8%. In addition, especially the hydrolyzed soybean meal tofu showed the higher concentration of Ca (27,307 mg/kg) and K (25,553 mg/kg). By and large, soybean meal tofu with acid hydrolysis is a rich source of isoflavone aglycone compared with other types of tofu.

KEYWORDS

Tofu; Soybean Meal; Isoflavone; Aglycone; Daidzein; Genistein

1. Introduction

Soybean products are the source of phytochemicals with the range of nutritional benefits. Among many soybean products, tofu is the most popular soy food product in Korea, Japan, and China. According to US soyfood directory, raw regular tofu contains 84.6% water, 1.9% carbohydrates, 8.1% protein, 4.8% fat, and a range of micronutrients, and phytoestrogen, especially isoflavone. Tofu is, not only rich in proteins and unsaturated fatty acids, but also, it is rich in range of isoflavones. Isoflavone, a class of plant-derived phytoestrogen, compounds with estrogenic activity. These phytoestrongens have similar molecular structures as of human, animal, or synthetic estrogens. Thus, it can easily bind to an estrogen receptor. Isoflavones show beneficial pharmacological and physiological effects on human health [1]. It has been hypothesized that isoflavones may lower the risk of cancers in breast, prostate, urinary tract, and colon. Moreover, increased isoflavone consumption reduced coronary heart disease and osteoporosis [2].

Isoflavone is defined as a class of isoflavonoids that characteristically has apolyphenolic structure [3]. They are found in legumes, and grains such as wheat, corn, and oats. Among these crops, soybean has the highest isoflavone concentration. According to USDA, the total isoflavone (genistein, daidzein, and glycitein) concentration

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of raw soybean mature seeds is 155 mg/100g. To date, 19 soybean isoflavones have been reported, and 12 types of isoflavones are regarded as the major isoflavones. It can be classified into four types depending on the chemical structure. Each type has three compounds [*i.e.*, malonyl glycoside (malonyl genetin, malonyl daidzin, malonyl glycitin) and acetyl-beta glycosides, beta glycosides, and aglycones (genestein, daidzein, glycitein)] [4].

Although all isoflavones are efficiently absorbed from the intestinal tract, there are differences in the fate of aglycones and beta-glycosides in terms of absorption [5]. Aglycones of isoflavones have higher bioavailability than corresponding glycoside forms, as glycosidicisoflavones are not directly transported across the gastro intestinal tract [5]. Most soyfood products have a complex mixture of glycoside conjugates (genistin, daidzin, and glicytin). These isoflavone glycosides are hydrolyzed by intestinal, mucosal, and bacterial beta glucosidase. Probiotics such as *Latobacillus* and *Bifidobacterium* have been known to possess endogenous beta glucosidase. Thus, depending on the hydrolysis, the profile of isoflavone can be changed. This hydrolytic action leads to increase of bioactive isoflavone aglycones, which are then either absorbed directly or further metabolized by intestinal microflora in the large intestine into other metabolites [6].

In the 1960s, Japan started using defatted soybean meal in tofu preparation [7]. However, today, instead of soybean meal, whole soybeans are widely used in tofu preparation to obtain richer tofu flavor and nutritional value. The mealy texture deprives tofu of some of its cohesiveness, making it more breakable [7]. So far, there are no data available on the soybean meal tofu nutritional profiles. The evaluation of soybean meal tofu nutritional profiles is still important, especially, for developing countries as soybean meal based tofu might have potential to provide more nutritious, high protein based food source with less cost.

In comparison with the whole soybean, the soybean meal has higher composition of protein and amino acids, and lower fat concentration. For example, soybeans contain approximately 37% crude protein and 18% crude fat, and soybean meal contains 44% of crude protein and 3% of crude fat [8]. Therefore, soybean meal could be a good source for tofu preparation due to high isoflavone concentrations and low fat contents. This study was carried out to 1) determine the feasibility of tofu preparation using soybean meal from North Dakota, USA; 2) assess nutrient profiles and isoflavone concentrations in both soybean meal and soybean meal tofu prepared from the newest hydrolyzed method. As isoflavone bioavailability is critical for better human nutrition, in our study, we suggest a new tofu preparation procedure for improved isoflavone bioavailability.

2. Materials and Methods

2.1. Materials

Defatted soybean meal samples were collected from Northern Crops Institute, North Dakota State University, Fargo, ND and Hubbard Feeds Inc., Moorhead, MN, USA. Three types of commercially grown soybean (*Glycine max* L.) samples were obtained from the Department of Plant Sciences, North Dakota State University, Fargo, ND, USA. Seven commercially sold pre-packed tofu samples products were purchased from four local supermarkets in Fargo, ND, USA. All study samples were stored in a 4˚C refrigerator until further processing and sample analysis. All samples were analyzed in triplicates.

2.2. Preparation Soybean Meal and Soybean Tofu with Acid Hydrolysis

Tofu was made on a laboratory scale. Before tofu preparation, approximately 200 g of the two types of defatted soybean meals were soaked in 1600 mL of water at room temperature (25˚C) for 3 hours, and the three types of whole soybeans were soaked for 12 hours in the same conditions. Then both the soybean meals and whole soybeans were ground in a household coffee grinder (Hamilton Beach Inc., NC, USA) to make a slurry. This resultant slurry was cooked to 100˚C for 10 minutes. After cooking, the insoluble soy residue was filtered using a cloth. Approximately, 200 mL of the filtrate, (*i.e.*, soy milk) was collected. For making general tofu, 20 mL of 10% calcium sulfate ($CaSO_4$) was added as a coagulant to the soymilk. On the other hand, for making hydrolyzed tofu, 200 mL of soymilk from soy bean and soybean milk were boiled with 20 mL of 4 M hydrochloric acid (HCl) to 100˚C for 5 minutes. Then 20 mL of 10% $CaSO_4$ was added to coagulate, and 20 mL of 4 M potassium hydroxide (KOH) was added to neutralize. The precipitate was filtrated using acloth, and the supernatant was discarded.

2.3. Isoflavone Extraction and HPLC Analysis

All samples (two types of soybean meal tofu, three types of soybean tofu, and seven types of processed tofu) were dried in a vacuum oven (10 mmHg) (Napco Inc., VA, USA) at 60˚C for 48 hours. Dried samples were ground using a household coffee blender (Hamilton Beach Inc., NC, USA). Two grams of dried, finely ground samples were placed in a 50 mL conical plastic tube to which 10 mL of acetonitrile, 2 mL of 0.1 M HCl, and 5 mL of distilled water were added. The mixture was shaken for 2 hours using a mini incubating shaker (VWR International, IL, USA) for the complete isoflavone extraction. Extracted sample was filtered (No. 41 filter paper, What man, Hillsboro, OR, USA), and again through a 0.45 μm

membrane (VWR International, IL, USA).

Isoflavone profiles of dry ground tofu were performed on an Agilent 1200 series separation module attached to a Agilent 1200 series photodiode array detector (PDA) (Agilent Technologies Inc., Torrance, CA, USA) and a C18 column (250 × 4.60 mm) (Prodigy 5u Phenomnex Inc, CA, USA). The photo diode array detector monitored absorbance from 200 nm to 350 nm. The following standards were used for quantification purposes: daidzin, genistin, daidzein, and genistein (Sigma-Aldrich, St. Louis, USA). The mobile phase consisted of glacial acetic acid (0.1% v/v; eluent A) and acetonitrile/glacial acetic acid (99.9:0.1, v/v; eluent B). All elutions were carried out at a flow rate of 1.0 mL/min at 23˚C. The solvent gradient was as follows: After the injection of 20 µL of sample, solvent B was increased from 15% to 29% over 36 min, then increased to 35% within the next 8 min. From 44 min to 45 min, solvent B was increased to 40%, and finally to 45% within the following 5 min. After that, solvent B was re-equilibrated back to 15%. Solvent A was manipulated depending on solvent B gradient. The total run time was 55 minutes.

2.4. Protein Analysis

To calculate crude protein concentrations of tofu, modified AOAC official method (988.05) was used. Approximately, 0.2 g of dry ground tofu samples was transferred into the digestion tube in duplicates. Two catalyst tablets composed of 3.5 g of potassium sulfate (K_2SO_4) and 0.4 g of copper (II) sulfate ($CuSO_4$) and 15 mL of 98% concentrated sulfuric acid (H_2SO_4) were added into the tube. Tubes were placed in a preheated digestion block with heat shields at 420˚C, and then the exhaust manifold was covered. Water vacuum was run at high flow for 5 minutes, and the flow was reduced gradually with the closed fume hood. The samples were digested for 1 hour until the samples became a clear green color. After digestion, the samples were removed from the digestion block and the heat shields, and then cooled for 5 minutes with the exhaust system running. The exhaust manifold was removed and the samples were cooled for additional 5 minutes. To prevent solidification of samples, 10 mL distilled deionized water was added to each tube. Each sample was diluted with 90 mL distilled deionized water. The digestion tube filled with one third of water was seton the Kjeltec 1030 autoanalyzer (Tecator, Höganäs, Sweden), and the distillation was begun. To reduce the error, chemical blanks were run first. These served as the correction factor for calculations. After finishing the distillation cycle completely, the recorded values indicated the milliliters of 1 M H_2SO_4 which were titrated for each sample.

2.5. Micronutrient Analysis

Micronutrients, calcium (Ca), magnesium (Mg), potassium (K), iron (Fe), zinc (Zn), and selenium (Se), concentrations were determined using the modified HNO_3-H_2O_2 digestion method [9]. Approximately, 0.5 g of ground tofu samples was digested with 6 mL of concentrated 70% nitric acid (HNO_3) for 1 hour at 90˚C. After hand shaking the digestion tubes, 3 mL of 30% hydrogen peroxide (H_2O_2) were added to the samples and kept for 15 min at 90˚C. Afterwards, 3 mL of 6 M HCl were added and kept for 5 min at 90˚C, and then cooled to room temperature. Completely digested samples were diluted with millipore water until the final volume became 10 mL. Mineral concentrations were determined by inductively couple plasma-emission spectrometry (ICP-ES; ICP-6500 Duo, Thermo Fisher Scientific, Pittsburg, PA, USA). Measurements of total potassium (K), magnesium (Mg), iron (Fe), zinc (Zn) and selenium (Se) using this modified method were validated using National Institute of Standards and Technology (NIST) standard reference material 1515a (apple leaves) and inter-laboratory standards.

3. Results and Discussion

In this study, to make nutritional tofu with increased concentrations of bioavailable aglycone, a modified standard tofu making processing was used. Therefore, the effect of applying acid hydrolysis to the standard tofu procedure was determined. By using the HPLC-DAD analysis, four isoflavones in tofu were quantified including aglycones (daidzein, genestein) and ß-glycosides (daidzin, genistin). In our preliminary experiment, the isoflavone profile of defatted soybean meal was confirmed to have a higher content of isoflavone ß-glycosides than general soybeans. We observed that the concentrations of genistin and daidzin in soybean meal were 17 and 9 times higher than in whole soybean. In addition, the ratio of isoflavone glycoside to aglycone is much higher in soybean meal than in whole soybean. In other words, as an ingredient source soybean meal was the optimal raw material for acid-hydrolyzed tofu. As a result, the concentration of isoflavone aglycones had a significant increase in the modified tofu made with the acid-hydrolysis than tofu made with the standard procedure. Hydrolyzed tofu and standard tofu were made by two ingredients: soybean and defatted soybean meal. As shown in **Table 1**, in the hydrolyzed soybean tofu and defatted soybean meal tofu, the mean total isoflavone aglycone concentrations were found to be 203 µg/mg and 556 µg/mg, respectively. Without hydrolysis, in the normal soybean tofu and defatted soybean meal tofu, the mean total isoflavone aglycone concentrations were 140 µg/mg and 138 µg/mg, respectively, and the average total aglycone concentration in commercial tofu was 253 µg/mg. On average, the aglycone concentration of tofu made by soybean meal was increased 4 fold during acid

Table 1. Isoflavone concentrations in different types of Tofu.

Tofu sample type		ß-Glucoside		Aglycone	
		Daidzin (µg/mg)	Genestin (µg/mg)	Daidzein (µg/mg)	Genestein (µg/mg)
Soybean Tofu with Hydrolysis	Prosoy	627.34	ND	609.74	ND
	ND 1005T	332.21	ND	ND	ND
	P.91M10	ND	ND	ND	ND
	Mean ± SD	319.85 ± 256.26	-	203.25 ± 287.44	-
Soybean Meal Tofu with Hydrolysis	Northern Crops Institute	671.58	402.75	324.79	382.19
	Hubbard Feeds Inc.	1923.97	794.56	183.79	221.10
	Mean ± SD	1297.77 ± 626.20	598.65 ± 195.91	254.29 ± 70.50	301.64 ± 80.55
Soybean Tofu in General Process	Prosoy	1200.74	770.93	53.59	75.18
	ND 1005T	873.49	566.46	27.78	50.84
	P.91M10	1086.72	531.04	87.65	125.54
	Mean ± SD	1053.65 ± 135.63	622.81 ± 105.73	56.34 ± 24.52	83.85 ± 31.10
Soybean Meal Tofu in General Process	Northern Crops Institute	1173.55	869.80	27.48	45.03
	Hubbard Feeds Inc.	975.07	1021.03	0.00	202.84
	Mean ± SD	1074.31 ± 99.24	945.41 ± 75.61	13.74 ± 13.74	123.94 ± 78.90
Commercial Tofu	Azumaya firm tofu	341.03	788.35	64.98	154.73
	Nasoya extra firm tofu	475.42	740.34	94.45	198.48
	Pulmuone soft tofu	556.38	621.45	96.71	135.90
	Melissa's extra firm tofu	1032.63	593.24	55.58	91.91
	Wildwood firm sprou tofu	974.83	540.19	55.44	90.56
	Mori-nu silken extra firm tofu	288.17	457.15	212.85	369.98
	House feeds premium extra firm tofu	806.69	595.70	63.00	89.93
	Mean ± SD	639.31 ± 278.08	619.49 ± 104.90	91.86 ± 51.92	161.64 ± 92.99

SD = Standard deviation, ND = Not detected.

hydrolysis, and the defatted soybean meal provided by Northern Crops Institute contained the highest value of aglycone (707 µg/mg). In detail, in the hydrolyzed soybean meal tofu, isoflavone aglycone concentrations of genistein increased 1- to 8-fold, and daidzein, 6- to 12-fold in all the samples. Although the total aglycone concentrations ranged widely, according to the report of tofu-type soy varieties, ratios of genistein to daidzein were stable around 1.4 [10]. However, in the hydrolyzed soybean tofu, only sample prosoy had 610 µg/mg of daidzein, and none of the isoflavone aglycones were detected in other types of soybean such as ND1005T and P.91M10. This may occur, because the tofu may get overboiled, and the over-boiling may cause severe acid hydrolysis leading aglycone to be changed into a different form.

The crude protein concentrations of all types of tofu are presented in **Table 2**. Notably, acid hydrolysis had a significant effect on crude protein concentration. The raw ingredient—soybean meal from Hubbard Feeds Inc.—had 46% of crude protein; however, during standard tofu making 13.6% protein was lost, and when acid hydrolysis occurred 16% of crude protein was lost. The mean crude protein concentration of commercial tofu was recorded at 56% by the modified Kjeldahl method. The protein concentration in commercial tofu was superior than lab-scale tofu. One reason for this can be the external protein sources added during commercial tofu preparation.

During tofu preparation in the laboratory, $CaSO_4$ was used as a coagulant. This is because, $CaSO_4$ was found to be the most suitable coagulant for tofu making in terms of its high yield, retention of maximum amount of isoflavones, and in obtaining a firm, but not as hard as the texture of tofu [11]. **Table 3** shows the concentrations of Ca, K, Mg, Zn, Fe, and P for all tofu samples. In the soybean meal tofu with hydrolysis, the mean total Ca concentration was 27,306 mg/kg. This value was lower when compared with the Ca value for normal tofu (47,493 mg/kg). This is because the crude protein concentration was decreased during acid hydrolysis; therefore, the ca-protein binding was reduced. Comparing commercial tofu, for basic tofu production, soymilk is coagulated with one of the several coagulants used in the

Table 2. Crude protein percentage in different types of Tofu.

Tofu sample type		Crude protein (%)
Soybean Tofu with Hydrolysis	Prosoy	36.57
	ND 1005T	38.02
	P.91M10	41.81
	Mean ± SD	38.80 ± 2.21
Soybean Meal Tofu with Hydrolysis	Northern Crops Institute	42.80
	Hubbard Feeds Inc.	38.75
	Mean ± SD	40.78 ± 2.03
Soybean Tofu in General Process	Prosoy	45.47
	ND 1005T	46.70
	P.91M10	37.97
	Mean ± SD	43.38 ± 3.86
Soybean Meal Tofu in General Process	Northern Crops Institute	51.51
	Hubbard Feeds Inc.	40.22
	Mean ± SD	45.87 ± 5.65
Commercial Tofu	Azumaya firm tofu	55.78
	Nasoya extra firm tofu	55.23
	Pulmuone soft tofu	54.60
	Melissa's extra firm tofu	53.82
	Wildwood firm sprou tofu	53.64
	Mori-nu silken extra firm tofu	63.09
	House feeds premium extra firm tofu	57.77
	Mean ± SD	56.28 ± 3.07

SD = Standard deviation.

Table 3. Micronutrient concentrations in different types of Tofu.

Tofu sample type		Element concentrations (mg/kg)					
		Ca	K	Mg	Zn	Fe	P
Soybean Tofu with Hydrolysis	Prosoy	20,401	29,956	2714	29	53	6329
	ND 1005T	25,500	28,215	3634	9	2	5821
	P.91M10	19,421	24,682	2150	0.3	7	7243
	Mean ± SD	21,774 ± 2665	27,618 ± 2194	2832 ± 612	13 ± 12	21 ± 23	6465 ± 588
Soybean Meal Tofu with Hydrolysis	Northern Crops Institute	25,473	25,828	3113	0.1	1	3939
	Hubbard Feeds Inc.	29,140	25,277	3354	0.03	0.2	1287
	Mean ± SD	27,307 ± 1834	25,553 ± 275	3233 ± 120	0.1 ± 0.03	0.7 ± 0.5	2613 ± 1326
Soybean Tofu in General Process	Prosoy	42,577	6472	3440	42	88	8994
	ND 1005T	49,383	5440	3393	39	75	10,030
	P.91M10	56,706	8725	3814	34	66	8059
	Mean ± SD	49,555 ± 5769	6879 ± 1312	3549 ± 188	38 ± 3	76 ± 9	9028 ± 805
Soybean Meal Tofu in General Process	Northern Crops Institute	70,935	9813	3865	33	38	7064
	Hubbard Feeds Inc.	24,051	18,875	2173	33	40	3404
	Mean ± SD	47,493 ± 23442	14,344 ± 4531	3019 ± 846	33 ± 0.01	39 ± 1.1	5234 ± 1830
Commercial Tofu	Azumaya firm tofu	14,744	6206	3577	8	56	8958
	Nasoya extra firm tofu	6238	2980	6994	44	45	9134
	Pulmuone soft tofu	6797	6145	3985	62	68	9594
	Melissa's extra firm tofu	10,914	4090	3687	59	71	12,630
	Wildwood firm sprou tofu	6644	4172	1184	46	89	5590
	Mori-nu silken extra firm tofu	2306	7844	1269	30	86	4585
	House feeds premium extra firm tofu	5341	4651	1583	40	69	5150
	Mean ± SD	7569 ± 3752	5156 ± 1531	3183 ± 1920	41 ± 17	69 ± 14.3	7949 ± 2719

SD = Standard deviation.

US, such as $CaSO_4$, calcium chloride ($CaCl_2$), magnesium sulfate ($MgSO_4$), nigari, or delta gluconolactone, which is a non dairy coagulant derived from corn starch. Accordingly, Ca and Mg concentrations were influenced depending on the types of coagulants. In comparison with commercial tofu and generally handmade tofu, hydrolyzed tofu showed higher concentrations of K. The highest concentration of K was observed in Prosoy (29,956 mg/kg). The mean K concentration of defatted soybean meal tofu was 25,553 mg/kg, whereas the mean K concentrations of the standard soybean tofu and soybean meal tofu with the same type of raw materials were 6819 mg/kg and 14,344 mg/kg, respectively. This is because in the process of hydrolyzed tofu making, KOH was added at the final step to neutralize the acid (*i.e.*, HCl). Soybeansareknown as a relatively rich source of P, and are important for growth and bone integrity of livestock and poultry. However, through the results presented in **Table 3**, the concentration of P did not have any apparent properties. The micronutrients such as Zn and Fe were low in all types of tofu. Unfortunately, in the acid-hydrolyzed soybean meal tofu, the concentrations of Zn and Fe were severely reduced to 0.06 mg/kg and 0.63 mg/kg, respectively.

4. Conclusion

By using soybean meal as a major ingredient, the hydrolyzed soybean meal tofu was devised not only as the cost-effective alternative of protein source to developing countries, but also richer source of phytoestrogen. Furthermore, newly applied tofu preparation which was used in the acid hydrolysis before coagulation of tofu, recorded maximum 8-fold increase in genistein and 12-fold increase in daidzein concentrations, respectively. Overall, the crude protein concentration and the micronutrient profile were ideal in the hydrolyzed soybean meal tofu. Even though this study showed the limitation of making tofu in a laboratory scale in terms of finding the optimal conditions, this modified tofu procedure enhanced the value of tofu as functional food.

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