FOOD COMPOSITION AND ADDITIVES

Determination of the Levels of Isoflavonoids in Soybeans and Soy-Derived Foods and Estimation of Isoflavonoids in the Japanese Daily Intake

YUMIKO NAKAMURA, SUMIKO TSUJI, and YASUHIDE TONOGAI
National Institute of Health Sciences, Division of Food Chemistry, Osaka Branch, 1-1-43, Hoenzaka, Chuo-ku, Osaka, 540-00 06, Japan

The levels of 6 kinds of isoflavonoids found in 11 domestic and imported soybeans, and 12 kinds of soybean-based processed foods in Japan were systematically analyzed, and the Japanese daily intake of isoflavonoids from those foods was estimated. The total isoflavonoids (daidzein, glycitein, and genistein) were analyzed with acid hydrolysis and the intact isoflavonoids (daidzein, glycitein, genistein, daidzin, glycitin, and genistin) were analyzed without hydrolysis. This was followed by cleanup with an ODS cartridge column and determined by liquid chromatography with a diode array detector. The highest content of isoflavonoids was found in kinako (a roasted soybean powder) and the lowest was found in soy sauce. The contents and composition of the isoflavonoids in the 11 soybeans varied by species and country of origin. The level of isoflavonoids found in the processed foods varied by manufacturing method or ingredients. The percentage of aglycone tended to be higher in miso (fermented soybean paste) and soy sauce, which are heated and fermented during the manufacturing process. Japanese daily intake of isoflavonoids from soybeans and soybean-based processed foods was estimated as 27.80 mg per day (daidzein 12.02 mg, glycitein 2.30 mg, and genistein 13.48 mg).

Isoflavonoids are a category of phytoestrogens which have sterile or estrogenic activities (1), and are mainly found in legumes such as soybeans (1–3). Japanese typically ingest phytoestrogens mainly from soybean and its derived processed foods (2).

The metabolic fate of isoflavonoids differs by species, and sensitivity to phytoestrogens is dependent on the concentration of free phytoestrogens (4). Isoflavonoids have a protective effect against cancer, cardiovascular diseases, and osteoporosis (5–10). People in Eastern Asia have consumed soybean and its derived processed foods since ancient times. The adverse effects of isoflavonoids to humans are obscure in spite of the potential risk to children who are exposed to isoflavonoids in high concentration at an early stage of development.

Studies of the levels of phytoestrogens in soybean and its processed foods (2, 11–23) do not sufficiently describe the Japanese intake of isoflavonoids. Therefore, we analyzed the levels of isoflavonoids in domestic and imported soybeans and their processed foods thoroughly, and calculated the Japanese daily intake of isoflavonoids from soybean and its processed foods based on the report *Japanese Nutrition Research in 1996 (the 8th year of Heisei)* (24).

Experimental

Apparatus and Materials

Trade names and sources are for user information only.

(**a**) *Homogenizer*.—National MX-X52 (Osaka, Japan) or equivalent.

(**b**) *Water bath*.—Boiling.

(**c**) *Liquid chromatographic (LC) system*.—Equipped with binary gradient pump, 100 μL injection loop, autosampler set at 8°C, column oven set at 35°C, diode array detector (DAD), and data-handling system (Hewlett-Packard, Co., Palo Alto, CA). Chromatogram at DAD was monitored at 260 and 280 nm.

(**d**) *LC column*.—*Separator column*.—250 × 4.6 mm id, stainless steel, packed with STR ODS-II (5 μm particle size). Ready-to-use columns from Shinwa-Kako, Inc., Kyoto, Japan, are suitable.

(**e**) *Mini cartridge column*.—Sep-pak plus C_{18} cartridges from Waters Corp. (Milford, MA) are suitable.

(**f**) *Centrifuge*.—Adjustable to 1000 × *g* and 5°C.

(**g**) *Centrifugal tube*.—Glass tube with screw cap, 50 mL.

(**h**) *Rotary evaporator*.—Water bath set at 35°C.

Reagents

All reagents are of analytical purity unless otherwise stated. The water used was Milli-Q grade or equivalent. Trade names and sources are for user information only.

Received June 18, 1999. Accepted by JL November 11, 1999.

(**a**) *Solvents and reagents*.—Acetonitrile (LC grade), ethanol (LC grade), methanol (LC grade), *n*-hexane, hydrochloric acid (36%), phosphoric acid, 2,6-di-*t*-butyl-4-methylphenol (BHT), and dimethyl sulfoxide (DMSO).

(**b**) *Standard (isoflavonoids and internal standard)*.—Genistein (MW270.24) from Extrasynthèse (Genay, France). Daidzein (MW254.24), daidzin (MW416.38), genistin (MW432.38), glycitein (MW284.27), glycitin (MW446.41) from Fujicco Co., Ltd. (Kobe, Japan). Flavone (MW222.24) as internal standard from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The chemical structures of these isoflavonoids and flavone are shown in Figure 1.

(**c**) *Standard stock solution*.—Dissolve the preweighed amount of each isoflavonoid standard with a small amount of DMSO, then dilute with methanol to 10 mL (547–1319 µg/mL).

(**d**) *Standard working solution (isoflavonoid mixture)*.—Mix isoflavonoid stock solutions of daidzin, glycitin, and genistin (A) or daidzein, glycitein, and genistein (B) and dilute with methanol 5–50 times. The concentrations of the standard working solutions are 38.48–159.5 µg/mL (1A and 1B) and 54.7–263.8 µg/mL (2A and 2B), respectively.

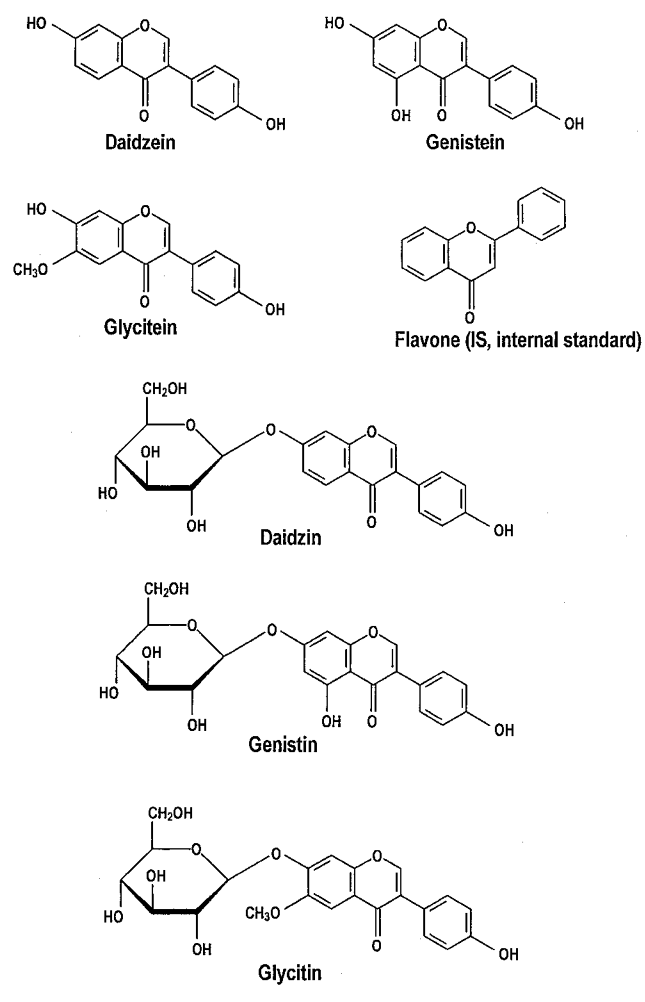

Figure 1. Chemical structures of 6 isoflavonoids and flavone.

(**e**) *Standard solution*.—Mix adequate amounts of each isoflavonoid stock solution and internal standard (IS) solution and dilute with methanol.

(**f**) *Internal standard (IS) solution*.—Dilute flavone stock solution with methanol 5 times; prepare 210 µg/mL IS solution.

(**g**) *80% Methanol*.—Add 20 mL water to 80 mL methanol, and mix well.

(**h**) *10N HCl solution*.—Mix 200 mL hydrochloric acid and 34 mL water.

(**i**) *0.05% BHT solution*.—Dissolve 0.25 g BHT with ethanol and adjust to 500 mL. Prepare solution before use and store in the dark.

(**j**) *20% Methanol*.—Add 20 mL methanol to 80 mL water, and mix well.

(**k**) *Mobile phase*.—(*1*) A: Water–phosphoric acid, 1000 + 1 (v/v). (*2*) B: Acetonitrile–water–phosphoric acid, 800 + 200 + 1 (v/v/v).—Degas solutions.

Test Materials

Domestic and imported soybeans (cropped at 1997) were obtained in Hyogo Prefecture and Osaka Prefecture in Japan from August 1998 to January 1999. All of the processed foods derived from soybeans and analyzed here are commonly used in Japan, and were purchased from retail stores in Osaka Pref. Details were as follows: Boiled soybeans, roasted soybeans, kinako (powdered roasted soybeans), tofu (traditional food used in Eastern Asia, including Japan), kori-dofu (freeze-dried tofu), okara (soybean residue as a by-product of tofu), tofu-derived processed foods (cooked and fried tofu), natto (fermented soybeans, a Japanese traditional food), miso (bean paste, traditional food used in Eastern Asia, including Japan), soy sauce, soy milk, and yuba (processed food derived from soy milk, a Japanese traditional food).

Domestic and imported soybeans, roasted soybeans, kori-dofu, and dried yuba were ground into powders. Tofu-derived foods were defatted with 20 volumes of *n*-hexane for 24 h, dried at ambient temperature, and homogenized. Boiled soybeans, tofu, natto, kinzanji-miso, and raw yuba were homogenized. Kinako, okara, miso (except kinzanji-miso), soy sauce, and soy milk were mixed homogeneously before use.

Extraction

Intact isoflavonoids were analyzed without hydrolysis, and total isoflavonoids were analyzed as aglycone with acid hydrolysis by the method of Franke et al. (15), with some modification. Flavone was used as an internal standard (15). Analyses were performed in triplicate throughout the experiment.

(**a**) *Intact isoflavonoids*.—One gram each of ground, homogenized, or mixed soybeans and the processed foods (except soy milk and soy sauce), or 5 mL soy milk was placed in a centrifuge. One milliliter IS solution (flavone 210 µg) and 50 mL 80% methanol were added to the centrifuge tubes, and

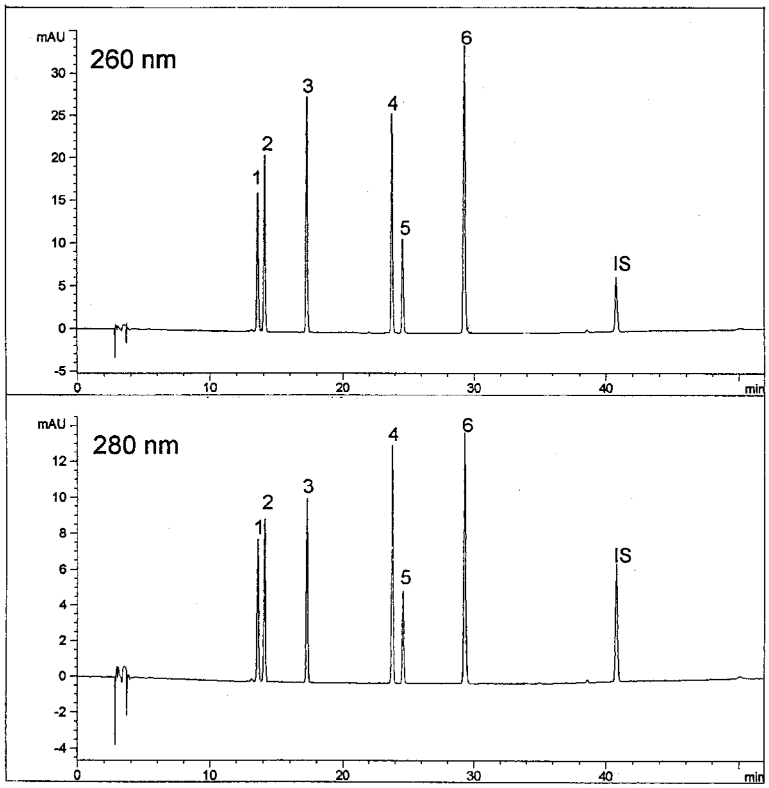

Figure 2. Liquid chromatograms of the standard solution. Concentrations of 6 isoflavonoids and flavone are as follows: 1) daidzin 3.619 μg/mL; 2) glycitin 5.277 μg/mL; 3) genistin 4.528 μg/mL; 4) daidzein 4.056 μg/mL; 5) glycitein 2.188 μg/mL; 6) genistein 4.400 μg/mL; and IS) flavone 2.096 μg/mL.

sonicated for 30 min. Isoflavonoids were extracted for 24 h at an ambient temperature, the tube was centrifuged at 1000 × *g* for 20 min at 5°C, and the volume was adjusted to 50 mL with methanol (sample solution A1).

Table 1. Variations of 6 isoflavonoids and flavone in retention times and peak areas in LC[a]

Flavonoids	Concentration, μg/mL	Retention time, min	Peak area, mAU
Daidzin	9.048	13.80 ± 0.06	353.9 ± 3.2
Glycitin	13.19	14.29 ± 0.06	346.9 ± 11.4
Genistin	11.32	17.48 ± 0.06	493.6 ± 7.4
Daidzein	10.14	23.83 ± 0.08	516.8 ± 5.0
Glycitein	5.469	24.65 ± 0.08	219.5 ± 2.2
Genistein	11.00	29.34 ± 0.09	757.1 ± 13.4
Flavone	10.50	40.83 ± 0.09	394.9 ± 6.1

[a] Data are expressed as means ± SD (n = 10).
LC conditions are as follows. Apparatus, HP 1100 series; column, STR ODS-II (4.6 mm id × 250 mm); column oven temperature, 35°C; mobile phase, (solvent A) water–phosphoric acid, 100 + 1 (v/v), (solvent B) water–acetonitrile–phosphoric acid, 200 + 800 + 1 (v/v/v), (linear gradient program) B%: 10 (0 min) → 80 (50–52 min) → 10 (53 min); flow rate, 1.0 mL/min; detector, DAD; monitoring wavelength, 260 nm for daidzein, daidzin, genistein, genistin, glycitein, and glycitin; 280 nm for flavone; injection volume, 10 μL.

One milliliter soy sauce was diluted to 10 mL with water and 0.1 mL IS solution (flavone 210 μg) was added (sample solution A2).

(**b**) *Total isoflavonoids*.—One gram each of ground, homogenized, or mixed soybeans or their processed foods (except soy milk and soy sauce), or 5 mL soy milk was placed in a centrifuge. One milliliter IS solution (flavone 210 μg), 10 mL 10N HCl solution, and 40 mL 0.05% BHT solution were added to the centrifuge tubes, and sonicated for 30 min. Hy-

Table 2. Detection limits of 6 isoflavonoids

	Detection limit[a] (ng/g fresh weight or ng/mL)		
Flavonoids	ng/g solid foods[b]	ng/mL soy milk	ng/mL soy sauce
Daidzin	2263	452.6	45.26
Glycitin	3299	659.8	65.98
Genistin	1133	226.6	22.66
Daidzein	1014	202.9	20.29
Glycitein	547.2	109.4	10.94
Genistein	1100	220.0	22.00

[a] Detection limits were expressed for the sample base. Analytical methods are described in the text.
[b] Solid foods are soybean and the soybean-derived processed foods, except soy milk and soy sauce.

Table 3. Recoveries of 6 isoflavonoids from Sep-pak plus C_{18} cartridge[a]

Flavonoids	Spiked amounts, µg	Recoveries, %
Daidzin	3.618	102.4 ± 1.2
Glycitin	5.277	106.3 ± 1.5
Genistin	4.527	102.2 ± 1.9
Daidzein	4.055	102.7 ± 2.1
Glycitein	2.189	99.6 ± 1.8
Genistein	4.628	104.2 ± 0.1

[a] Data are represented as means ± SD ($n = 3$).
Nine isoflavonoids and flavone (2.100 µg, internal standard) were added onto a Sep-Pak plus C_{18} cartridge column preconditioned by 10 mL methanol followed by 10 mL distilled water. The column was washed with 10 mL water followed by 2 mL 20% methanol, and the flavonoid was eluted by 2 mL methanol. Eluate was evaporated and redissolved with 2 mL methanol. Nine isoflavonoids were determined using flavone as internal standard by LC.
LC conditions are described in the text.

drolysis was performed in a boiling water bath by reflux for 3 h (15). The tube was cooled and centrifuged at 1000 × *g* for 20 min at 5°C, and the volume was adjusted to 50 mL with ethanol (sample solution B1).

Five mL soy sauce was placed into a centrifuge tube. One milliliter IS solution (flavone 210 µg), 10 mL 10N HCl solution, and 40 mL 0.05% BHT solution were added to the centrifuge tube and sonicated for 30 min. Hydrolysis was performed in a boiling water bath by reflux for 3 h. The tube was cooled and centrifuged at 1000 × *g* for 20 min at 5°C, and the volume was adjusted to 50 mL with ethanol. Ten mL of this extractant was evaporated to dryness, and dissolved with 10 mL water (sample solution B2).

Cleanup

Cleanup of sample solutions A1, A2, B1, and B2 was performed using Sep-pak plus C_{18} cartridges (12) before LC.

One milliliter of each sample solution A1 and B1 was diluted with 10 milliliter water and applied to a Sep-pak C_{18} plus cartridge column preconditioned with methanol and water. The remaining volume of sample solutions A2 and B2 was applied to a Sep-pak plus C_{18} cartridge column preconditioned with methanol and water. The column was washed with 20 mL water followed by 2 mL 20% methanol; isoflavonoids and flavone were eluted with exactly 2 mL methanol (test solution).

Table 4. Recoveries of 9 isoflavonoids with or without hydrolysis from soybean, tofu, and miso[a]

		Recovery, %					
		Soybean		Tofu		Miso	
Flavonoids	Spiked amounts, µg/g	Intact[b]	Hydrolysis[c]	Intact	Hydrolysis	Intact	Hydrolysis
Daidzin	181.0	97.3 ± 4.3	95.0 ± 9.2	102.8 ± 0.6	83.6 ± 4.5	101.0 ± 5.9	99.0 ± 9.1
Glycitin	131.7	94.3 ± 8.0	90.4 ± 4.3	102.1 ± 0.9	88.6 ± 0.8	110.4 ± 1.7	96.9 ± 2.8
Genistin	226.4	97.3 ± 6.6	96.1 ± 9.0	100.6 ± 0.3	85.8 ± 3.5	105.2 ± 0.1	93.7 ± 6.3
Daidzein	202.8	106.8 ± 9.8	98.3 ± 8.2	105.8 ± 1.7	89.4 ± 4.3	94.9 ± 2.5	94.9 ± 0.0
Glycitein	54.69	94.6 ± 1.6	90.5 ± 6.4	106.5 ± 3.0	82.2 ± 8.8	98.3 ± 4.3	100.6 ± 0.3
Genistein	220.0	112.6 ± 6.0	103.6 ± 6.0	102.7 ± 1.2	88.2 ± 4.0	93.5 ± 2.7	81.9 ± 1.7

		Recovery, %			
		Soy milk		Soy sauce	
Flavonoids	Spiked amounts, µg/mL	Intact[b]	Hydrolysis[c]	Intact	Hydrolysis
Daidzin	36.19 (3.619)[d]	100.7 ± 6.8	100.4 ± 4.2	90.6 ± 6.9	100.5 ± 8.8
Glycitin	26.39 (5.277)	93.7 ± 1.6	88.2 ± 2.2	94.2 ± 6.1	90.8 ± 7.4
Genistin	45.37 (4.527)	93.9 ± 1.7	97.4 ± 5.4	108.2 ± 3.7	92.3 ± 9.4
Daidzein	40.55 (4.055)	88.7 ± 1.4	105.5 ± 5.3	81.2 ± 8.0	80.9 ± 7.6
Glycitein	10.94 (2.189)	103.6 ± 1.3	110.6 ± 6.1	105.2 ± 4.1	66.4 ± 6.1
Genistein	44.00 (4.400)	86.0 ± 1.1	98.7 ± 8.6	108.6 ± 2.5	82.7 ± 7.9

[a] Data are represented as means ± SD ($n = 3$).
[b] Intact (without hydrolysis): *See* text for analytical method.
[c] Hydrolysis (with hydrolysis): *See* text for analytical method.
[d] Values in parentheses are spiked amounts to soy sauce (nmol/mL).

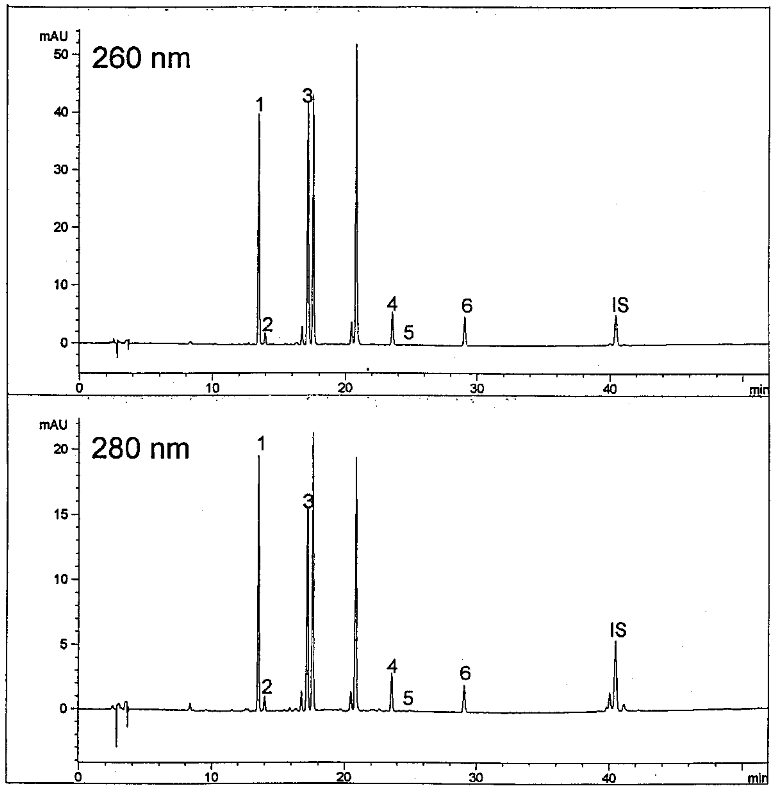

Figure 3-1. Liquid chromatograms of test solution of soybean #2 (without hydrolysis). Numbers of peaks are as follows: 1) daidzin; 2) glycitin; 3) genistin; 4) daidzein; 5) glycitein; 6) genistein; and IS) flavone (internal standard).

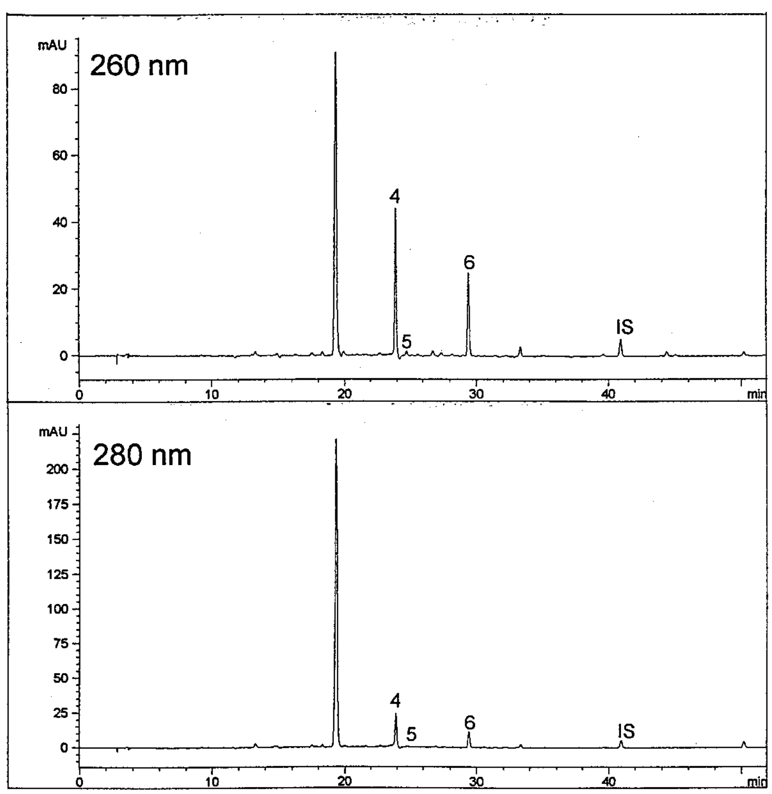

Figure 3-2. Liquid chromatograms of test solution of soybean #2 (with hydrolysis). Numbers of peaks are as follows: 4) daidzein; 5) glycitein; 6) genistein; and IS) flavone (internal standard).

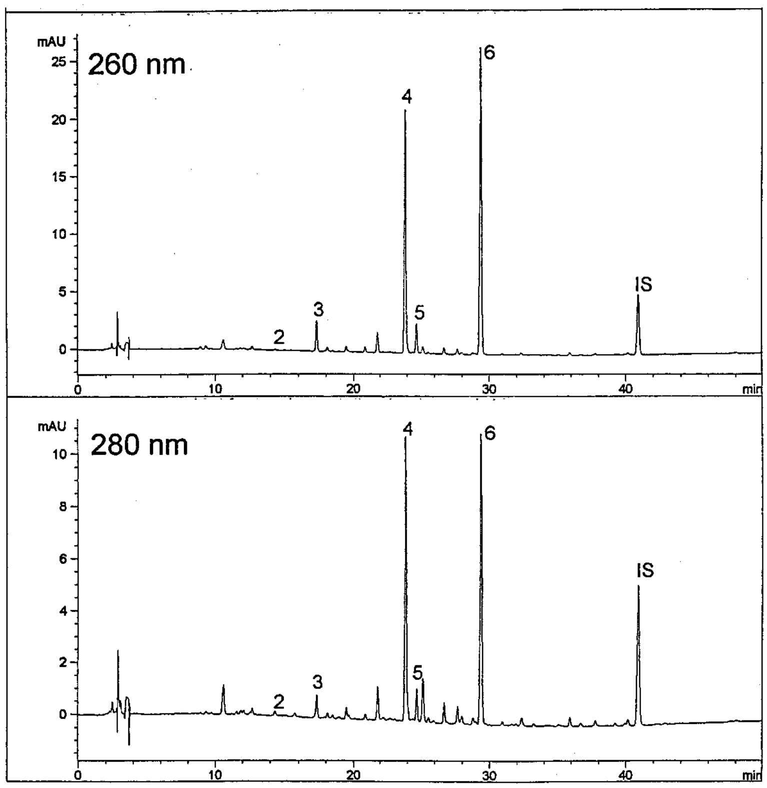

Figure 4-1. Liquid chromatograms of test solution of miso #8 (without hydrolysis). Numbers of peaks are as follows: 2) glycitin; 3) genistin; 4) daidzein; 5) glycitein; 6) genistein; and IS) flavone (internal standard).

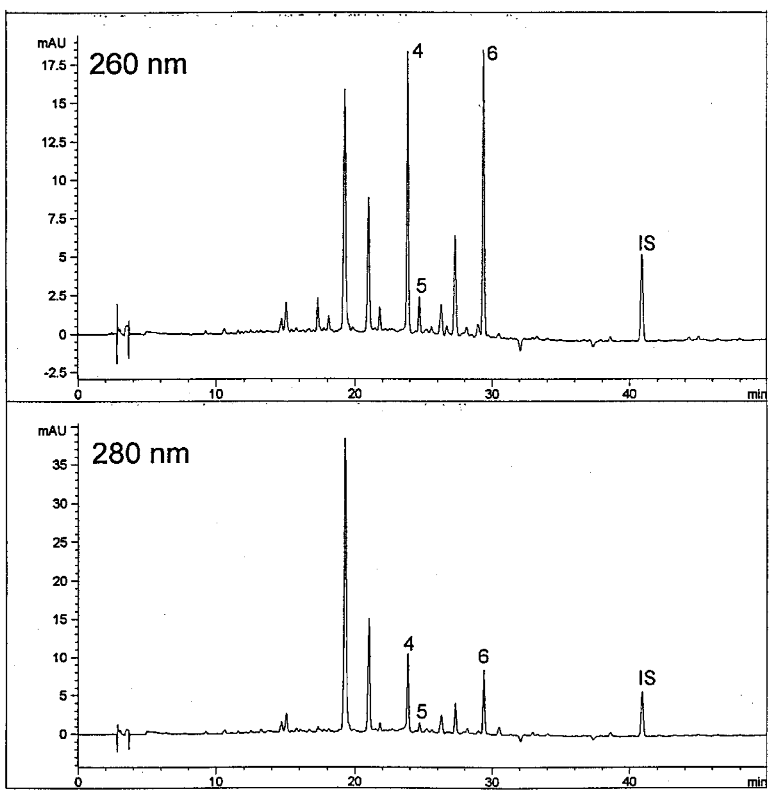

Figure 4-2. Liquid chromatograms of test solution of miso #8 (with hydrolysis). Numbers of peaks are as follows: 4) daidzein; 5) glycitein; 6) genistein; and IS) flavone (internal standard).

Table 5. Contents of isoflavonoids in soy beans and their processed foods

Sample No.	Total isoflavonoids[a] (mg/g or mg/mL)			Composition of isoflavonoids (%, molar ratio)[b]									
				Total isoflavonoids			Glucoside			Aglycone			
	Daidzein	Glycitein	Genistein	Daidzein	Glycitein	Genistein	Daidzin	Glycitin	Genistin	Daidzein	Glycitein	Genistein	Total
(1) Soybean	[μg/g fresh weight]												
1 Soybean (Tsurunoko, domestic)	1138 ± 126	44.3 ± 6.3	894 ± 95	56.4	2.0	41.7	29.0	1.8	21.1	5.1	0.2	3.5	8.8
2 Soybean (harvested in U.S.A.)	424 ± 18	85.6 ± 0.6	373 ± 93	39.8	7.2	32.9	6.4	2.9	6.9	1.6	0.7	2.1	4.4
3 Soybean (harvested in U.S.A.)	723 ± 72	91.8 ± 14.8	686 ± 50	49.8	5.7	44.5	10.2	1.8	10.2	2.0	0.6	1.8	4.3
4 Soybean (harvested in Canada)	716 ± 59	60.8 ± 7.1	405 ± 56	62.2	4.7	33.1	11.8	2.9	11.8	2.2	0.5	1.8	4.5
5 Soybean (harvested in Australia)	784 ± 97	124 ± 24	745 ± 52	49.1	7.0	43.9	7.8	2.5	10.3	1.9	0.4	1.9	4.2
6 Soybean (harvested in China)	960 ± 63	145 ± 7	723 ± 68	54.2	7.3	38.4	12.9	2.3	14.5	1.5	0.2	1.0	2.7
7 Soybean (harvested in China)	625 ± 74	102 ± 13	654 ± 81	46.9	6.9	46.2	12.7	3.5	16.1	4.9	0.8	3.7	9.4
8 Black soybean (domestic)	452 ± 58	36.7 ± 11.9	831 ± 193	35.7	2.6	61.7	10.6	1.3	28.0	2.2	0.2	4.1	6.6
9 Black soybean (harvested in Korea)	599 ± 60	40.9 ± 6.3	579 ± 165	50.7	3.1	46.2	28.8	2.5	29.9	12.2	0.6	9.5	22.4
10 Green soybean (domestic)	650 ± 55	38.7 ± 9.7	701 ± 46	48.4	2.6	49.1	14.6	1.6	26.7	5.5	0.4	7.5	13.4
11 Green soybean (harvested in Canada)	630 ± 37	44.3 ± 3.4	335 ± 81	64.0	4.0	32.0	11.6	2.3	11.6	2.6	0.8	2.0	5.4
(2) Boiled soybean	[μg/g fresh weight]												
1 Soybean	270 ± 8	8.53 ± 1.08	411 ± 19	40.7	1.2	58.2	28.2	0.9	42.4	9.1	2.2	1.9	13.1
2 Soybean (domestic soybean)	278 ± 9	22.2 ± 1.0	428 ± 31	39.7	2.8	57.5	26.8	2.4	41.4	10.6	0.6	8.5	19.7
3 Black soybean (domestic soybean)	369 ± 9	23.5 ± 0.4	354 ± 19	51.0	2.9	46.1	39.3	2.4	31.9	6.3	0.2	1.1	7.7
(3) Roasted soybeans	[μg/g fresh weight]												
1 Soybean	970 ± 91	144 ± 19	892 ± 59	50.1	6.7	43.3	15.1	3.0	18.3	35.0	0.7	1.4	37.1
(4) Kinako	[μg/g fresh weight]												
1 Kinako (soybean)	1844 ± 69	80.2 ± 5.4	1289 ± 55	59.0	2.3	38.8	29.9	1.5	32.1	29.0	0.5	2.8	32.3
2 Kinako (domestic black soybean)	1181 ± 37	99.5 ± 5.1	830 ± 61	57.6	4.3	38.1	22.0	2.6	27.5	35.6	1.2	5.0	41.7
(5) Tofu	[μg/g fresh weight]												
1 Kinukoshi-tofu	74.7 ± 7.1	26.4 ± 2.0	80.3 ± 1.9	43.0	13.6	43.4	15.0	9.4	17.8	5.2	3.4	4.3	12.8
2 Momen-tofu	63.3 ± 2.8	23.5 ± 0.9	83.8 ± 3.8	38.9	12.9	48.3	13.6	9.0	18.7	7.5	3.9	6.5	17.9
3 Yaki-tofu	83.1 ± 2.8	28.7 ± 1.1	103 ± 4	40.4	12.5	47.1	14.3	8.5	18.5	6.3	3.1	5.2	14.6
4 Packed tofu	106 ± 2.5	25.7 ± 1.1	112 ± 5	45.1	9.8	45.1	19.0	7.3	21.2	4.9	2.5	3.8	11.2
(6) Freeze-dried tofu (kori-dofu)	[μg/g fresh weight]												
1 Kori-dofu	310 ± 17	34.4 ± 2.3	540 ± 20	36.5	3.6	59.8	5.6	1.2	19.0	29.2	2.5	35.0	66.6

Table 5. (*continued*)

Sample No.	Total isoflavonoids[a] (mg/g or mg/mL)			Composition of isoflavonoids (%, molar ratio)[b]									
				Total isoflavonoids			Glucoside			Aglycone			
	Daidzein	Glycitein	Genistein	Daidzein	Glycitein	Genistein	Daidzin	Glycitin	Genistin	Daidzein	Glycitein	Genistein	Total
(7) Okara		[μg/g fresh weight]											
1 Okara	44.7 ± 3.3	12.2 ± 0.3	48.1 ± 1.4	44.3	10.8	44.9	13.6	6.0	13.6	12.9	4.9	17.4	35.2
(8) Tofu-derived processed foods		[μg/g fresh weight]											
1 Atsu-age	108 ± 4	35.5 ± 1.1	144 ± 3	39.1	11.6	49.4	10.7	5.3	14.4	9.8	2.3	4.2	16.3
2 Usu-age	191 ± 8	39.2 ± 7.4	304 ± 15	37.4	6.9	55.8	16.0	3.4	24.0	21.3	1.4	10.1	32.8
3 Ganmodoki	128 ± 0.8	38.9 ± 0.6	191 ± 2	36.4	10.3	53.3	13.3	6.3	23.8	14.4	2.8	7.5	24.7
(9) Natto (fermented soybean)		[μg/g fresh weight]											
1 Natto (soybean)	342 ± 10	67.7 ± 0.9	404 ± 31	43.7	7.7	48.6	35.7	6.8	35.9	7.8	1.0	5.9	14.7
2 Natto (black soybean)	323 ± 28	36.7 ± 0.6	296 ± 18	51.0	5.2	43.9	31.9	3.9	29.5	9.6	1.1	9.6	20.3
(10) Miso (bean paste)		[μg/g fresh weight]											
1 Rice-koji miso #1	261 ± 2	40.7 ± 0.3	350 ± 9	41.7	5.8	52.5	17.4	1.9	22.0	18.4	3.7	18.9	40.9
2 Rice-koji miso #2	346 ± 10	31.0 ± 0.6	355 ± 4	48.8	3.9	47.3	8.9	0.9	5.2	39.0	3.0	40.8	82.8
3 Shiro-miso (rice-koji miso)	46.9 ± 1.8	12.0 ± 0.2	84.6 ± 8.9	34.2	7.8	58.0	16.3	4.2	33.1	17.9	3.6	20.4	41.9
4 Akadashi-miso (mixed miso)	281 ± 0.5	48.6 ± 0.6	319 ± 29.7	45.0	7.0	48.1	2.4	0.8	5.2	42.6	6.2	42.8	91.6
5 Koji-miso (rice-koji miso)	108 ± 5	23.0 ± 0.7	166 ± 25	37.9	7.3	54.9	17.4	3.6	28.1	20.5	3.7	26.8	51.0
6 Salt-reduced miso (rice-koji miso)	185 ± 5	32.7 ± 1.1	271 ± 6	39.4	6.2	54.4	7.0	1.8	11.7	25.4	4.3	29.4	59.1
7 Barley-koji miso	80.1 ± 3.1	16.6 ± 0.9	101 ± 5	42.3	7.8	49.9	15.0	4.3	13.8	24.9	3.4	35.0	63.3
8 Soybean-koji miso	363 ± 3	53.4 ± 0.6	398 ± 6	46.3	6.1	47.7	0	0.3	3.3	46.3	5.8	44.4	96.4
9 Kinzanji-miso	49.8 ± 8.9	16.2 ± 1.4	62.4 ± 9.2	40.5	11.8	47.7	18.2	6.2	25.9	21.2	5.6	11.5	38.3
(11) Soy sauce (shoyu)		[μg/mL]											
1 Koikuchi shoyu #1	6.25 ± 0.48	1.90 ± 0.19	1.29 ± 0.00	68.2	18.5	13.2	18.5	0	0	42.5	18.5	13.2	74.2
2 Koikuchi shoyu #2	9.13 ± 0.46	2.02 ± 0.18	2.84 ± 1.32	67.1	13.3	19.6	0	0	0	53.1	13.3	18.2	84.6
3 Koikuchi shoyu #3	7.14 ± 1.30	2.32 ± 0.06	3.13 ± 0.27	58.7	17.0	24.3	0	0	0	49.6	17.0	23.7	90.3
4 Usukuchi shoyu	5.26 ± 0.33	1.37 ± 0.03	3.32 ± 0.35	54.7	12.8	32.5	0	0	0	54.7	12.8	32.5	100.0
5 Tosa shoyu	5.62 ± 0.28	1.06 ± 0.06	2.29 ± 2.43	64.4	10.9	24.8	0	0	0	64.4	10.9	21.6	96.9
6 Tamari shoyu	6.13 ± 0.53	0.82 ± 0.51	1.00 ± 0.05	78.5	9.4	12.1	12.4	0	0	66.1	6.1	12.1	84.3
7 Sashimi shoyu	8.54 ± 0.41	2.62 ± 0.10	3.32 ± 0.08	61.0	16.8	22.3	11.5	0	0	49.7	16.8	22.3	88.7
8 Salt-reduced shoyu	7.81 ± 0.69	1.76 ± 0.22	2.44 ± 0.31	66.9	13.5	19.7	0	0	0	34.2	13.5	15.1	62.7

Table 5. (continued)

Sample No.	Total isoflavonoids[a] (mg/g or mg/mL)			Composition of isoflavonoids (%, molar ratio)[b]									
				Total isoflavonoids			Glucoside			Aglycone			
	Daidzein	Glycitein	Genistein	Daidzein	Glycitein	Genistein	Daidzin	Glycitin	Genistin	Daidzein	Glycitein	Genistein	Total
(12) Soy milk		[µg/mL]											
1 Soy milk	126 ± 7	16.3 ± 0.7	168 ± 9	42.3	4.9	52.9	14.9	2.5	19.1	4.2	0.3	1.5	6.0
2 Soft drink #1	42.5 ± 0.2	1.64 ± 0.01	78.6 ± 1.1	36.0	1.2	62.7	25.1	1.1	44.4	2.1	0	0.7	2.8
3 Soft drink #2	26.4 ± 1.8	1.51 ± 0.18	52.4 ± 3.5	34.4	1.8	63.9	26.0	1.8	48.1	1.0	0	0.8	1.8
(13) Yuba		[µg/g fresh weight]											
1 Dried yuba	759 ± 28	176 ± 9	1158 ± 71	37.8	7.8	54.3	26.4	6.0	38.3	7.2	1.0	5.5	13.7
2 Raw yuba	161 ± 10	40.9 ± 1.7	236 ± 16	38.5	8.7	52.8	18.9	5.6	25.8	11.3	2.3	11.2	24.9

[a] Isoflavonoids were analyzed with hydrolysis. Values are means ± SD for 3 replications.
[b] Values are the means of 3 replications. Malonyl glucosides, acetyl glucosides, and succinyl glucosides were not quantitated because of the lack of standard.

LC Analysis

Isoflavonoids in the test solution were analyzed by LC using flavone as the internal standard (15).

The LC conditions for isoflavonoid analysis were as follows: column, STR ODS-II; mobile phases, (A) water–phosphoric acid, 1000 + 1 (v/v), (B) acetonitrile–water–phosphoric acid, 800 + 200 + 1 (v/v/v); a gradient program, (B) 10% (0 min) → 80% (50 min), linear gradient; column oven temperature, 35°C; detector, DAD; injection volume, 10 µL.

The λmax of 6 isoflavonoids and flavone in methanol solution at DAD was as follows: 250 nm for daidzin; 258 and 320 nm for glycitin; 258 nm for genistin; 248 and 302 nm for daidzein; 256 and 320 nm for glycitein; 260 nm for genistein; and 251 and 295 nm for flavone. The selected wavelengths for the quantitation of 6 isoflavonoids and flavone were 260 nm for daidzein, daidzin, genistein, genistin, glycitein, and glycitin; 280 nm for flavone because of the vicinity of λmax for each isoflavonoids and the avoidance of interferences by colored sample solutions.

Recovery Test

A recovery test was performed for soybeans, tofu, miso, soy milk, and soy sauce with or without hydrolysis in triplicate. One milliliter standard working solution (1A or 1B) and 1 mL IS solution were added to 1 g ground soybean powder, homogenized tofu, and miso, or 5 mL soy milk. For soy sauce analysis, 1 milliliter standard working solution (2A or 2B) and 0.1 mL IS solution were added to 1 mL soy sauce and analyzed by the above-mentioned method. The standard working solutions 1A (daidzin, glycitin, and genistin) and 1B (daidzein, glycitein, and genistein) or 2A (daidzin, glycitin, and genistin) and 2B (daidzein, glycitein, and genistein) were added separately.

Calculation of Japanese Daily Intake of Isoflavonoids

The total Japanese daily intake of soybean and its processed foods was 70.4 g in 1996; 13.9 g came from miso (rice-koji miso, barley-koji miso, soybean-koji miso, kinzanji-miso, mixed miso, etc.), 40.0 g from tofu (kinukoshi-tofu, momen-tofu, yaki-tofu, and packed tofu), 7.8 g from tofu-derived processed foods (atsu-age, usu-age, and ganmodoki), and 8.8 g from soybeans and other soybean-derived processed foods (kori-dofu, yuba, okara, soy milk, kinako, boiled soybean, natto, etc.; soy sauce was not included) (25). The Japanese daily intake of isoflavonoids from soybeans and soybean-derived foods was calculated using the results of this study.

Table 6. Comparison of isoflavonoid contents in several foods cited from several references[a]

Sample		Present study (as aglycone), µg/g fresh weight	Takamatsu (2) (aglycone + glucoside), µg/g	Toda et al. (11) (aglycone + glycosides), µg/g	Pettersson and Kiessling (12) (as aglycone), µg/g	Dwyer et al. (13) (as aglycone), µg/g	Wang and Murphy (14) (as aglycone), µg/g	Franke et al. (15, 17) (as aglycone), µg/g	Nguyenle et al. (16) (aglycone + glucoside), µg/g	Franke et al. (18) (as aglycone), µg/g
Soybeans	*n*	11	?		1		3	4		4
	Daidzein	700 (424–1138)	983		706		462 (240–600)	846 (676–1007)		913
	Glycitein	74.2 (36.7–145)	not determined		not determined		89.3 (79–107)	not determined		114
	Genistein	630 (335–894)	885		1000		777 (648–954)	1107 (940–1382)		763
Boiled beans	*n*	3		18				1		
	Daidzein	306 (270–369)		199 (160–247)				68.5		
	Glycitein	18.2 (8.5–23.5)		43 (29–60)				not determined		
	Genistein	398 (354–428)		395 (356–445)				69.4		
Roasted beans	*n*	1					1	1		1
	Daidzein	970					563	848		786
	Glycitein	144					193	not determined		168
	Genistein	892					869	1106		889
Kinako	*n*	2	?	3						
	Daidzein	1513 (1181–1844)	1140	1097						
	Glycitein	89.8 (80.2–99.5)	not determined	0						
	Genistein	1060 (830–1289)	682	1492						
Tofu	*n*	4	2?	11		9	1	1	1	4
	Daidzein	81.6 (63.4–106)	113 (80–145)	180 (151–220)		82.3 (57.3–117)	146	113	224	139
	Glycitein	26.2 (23.5–28.7)	not determined	73 (66–80)		not determined	29	not determined	not determined	17
	Genistein	94.9 (80.3–112)	130 (105–154)	257 (225–294)		206 (159–306)	162	166	334	141
Kori-dofu	*n*	1	?					1		
	Daidzein	310	105					225		
	Glycitein	34.5	not determined					not determined		
	Genistein	540	190					227		
Okara	*n*	1								
	Daidzein	44.7								
	Glycitein	12.2								
	Genistein	48.1								

Table 6. (*continued*)

Sample		Present study (as aglycone), µg/g fresh weight	Takamatsu (2) (aglycone + glucoside), µg/g	Toda et al. (11) (aglycone + glycosides), µg/g	Pettersson and Kiessling (12) (as aglycone), µg/g	Dwyer et al. (13) (as aglycone), µg/g	Wang and Murphy (14) (as aglycone), µg/g	Franke et al. (15, 17) (as aglycone), µg/g	Nguyenle et al. (16) (aglycone + glucoside), µg/g	Franke et al. (18) (as aglycone), µg/g
Tofu-derived	*n*	3		6						
processed	Daidzein	141 (108–191)		262 (118–336)						
foods	Glycitein	37.9 (35.5–39.2)		56 (0–168)						
	Genistein	213 (144–304)		377 (317–412)						
Natto	*n*	2	?	25						
	Daidzein	333 (323–342)	466	441 (319–587)						
	Glycitein	52.2 (36.7–67.7)	not determined	192 (128–319)						
	Genistein	350 (296–404)	432	642 (569–810)						
Miso	*n*	9	?	14			1			
	Daidzein	191 (46.9–363)	235	140 (88–235)			79			
	Glycitein	30.5 (12.0–53.4)	not determined	16 (0–27)			38			
	Genistein	234 (62.4–398)	221	217 (166–278)			177			
Soy sauce	*n*	8 [µg/mL]	?	4						
	Daidzein	7.00 (5.26–9.13)	13	8 (7–8)						
	Glycitein	1.73 (0.82–2.62)	not determined	1						
	Genistein	2.45 (1.00–3.32)	3	7 (4–11)						
Soy milk	*n*	3 [µg/mL]		3			2 (soy drink)		2	3
	Daidzein	54.0 (26.4–126)		140			7.0 (6.8–7.2)		18 (16–20)	81
	Glycitein	6.46 (1.51–16.3)		17			not determined		not determined	not determined
	Genistein	99.6 (52.4–168)		200			21.0 (20.2–21.7)		32.5 (28–37)	78
Yuba	*n*	2 (dried and raw)	? (dried)							
	Daidzein	460 (161–759)	863							
	Glycitein	108 (40.9–176)	not determined							
	Genistein	697 (236–1158)	906							

Table 6. (*continued*)

Sample		Present study (as aglycone), µg/g fresh weight	Song et al. (22) (as aglycone), µg/g	Franke et al. (20) Singapore (as aglycone), µg/g	Franke et al. (20) Hawaii (as aglycone), µg/g	Murphy et al. (23) (as aglycone), µg/g
Soybeans	*n*	11		4, raw soybean		
	Daidzein	700 (424–1138)		913 (797–1163)		
	Glycitein	74.2 (36.7–145)		92 (61–152)		
	Genistein	630 (335–894)		541 (436–720)		
Boiled beans	*n*	3		4, cooked soybean	3, cooked soybean	
	Daidzein	306 (270–369)		649 (577–803)	418 (396–438)	
	Glycitein	18.2 (8.5–23.5)		43 (29–60)	61 (56–64)	
	Genistein	398 (354–438)		395 (356–445)	425 (364–536)	
Roasted beans	*n*	1	2			
	Daidzen	970	379			
	Glycitein	144	174			
	Genistein	892	506			
Kinako	*n*	2	2, roasted soyflour			
	Daidzein	1513 (1181–1844)	1696			
	Glycitein	89.8 (80.2–99.5)	109			
	Genistein	1060 (830–1289)	1626			
Tofu	*n*	4	3	4, raw tofu	3, soft tofu	12
	Daidzein	81.6 (63.4–106)	806	139 (107–117)	137 (43–185)	107 (74–146)
	Glycitein	26.2 (23.5–28.7)	179	17 (15–19)	30 (16–47)	20.9 (17–25)
	Genistein	94.9 (80.3–112)	939	141 (124–164)	129 (61–164)	143 (111–165)
Kori-dofu	*n*	1		2, fermented tofu	3, firm tofu	
	Daidzein	310		250 (232–269)	169 (72–256)	
	Glycitein	34.5		49	31 (14–54)	
	Genistein	540		288 (262–313)	155 (67–217)	
Okara	*n*	1				
	Daidzein	44.7				
	Glycitein	12.2				
	Genistein	48.1				

Table 6. (*continued*)

Sample		Present study (as aglycone), µg/g fresh weight	Song et al. (22) (as aglycone), µg/g	Franke et al. (20) Singapore (as aglycone), µg/g	Franke et al. (20) Hawaii (as aglycone), µg/g	Murphy et al. (23) (as aglycone), µg/g
Tofu-derived processed foods	*n*	3	2,fried tofu	4, cooked tofu		3, cooked tofu, dry weight bases
	Daidzein	141 (108–191)	490	124 (103–150)		679 (490–827)
	Glycitein	37.9 (35.5–39.2)	106	15 (36–145)		134 (119–147)
	Genistein	213 (144–304)	693	119 (92–149)		910 (782–1071)
Natto	*n*	2			1	
	Daidzein	333 (323–342)			553	
	Glycitein	52.2 (36.7–67.7)			198	
	Genistein	350 (296–404)			490	
Miso	*n*	9	3		3	2
	Daidzein	191 (46.9–363)	759		98 (35–171)	88.5 (88–89)
	Glycitein	30.5 (12.0–53.4)	150		16 (4–36)	24 (23–25)
	Genistein	234 (62.4–398)	625		115 (51–210)	119 (117–121)
Soy sauce	*n*	8 [µg/mL]				2
	Daidzein	7.00 (5.26–9.13)				3.5 (1–6)
	Glycitein	1.73 (0.82–2.62)				2.5 (0–5)
	Genistein	2.45 (1.00–3.32)				1.5 (0–3)
Soy milk	*n*	3 [µg/mL]	3		3	12
	Daidzein	65.0 (26.4–126)	632		58 (40–75)	44 (34–63)
	Glycitein	6.46 (1.51–16.3)	124		15 (5–23)	7.83 (4–11)
	Genistein	99.6 (52.4–168)	1384		52 (22–58)	60.3 (41–92)
Yuba	*n*	2 (dried and raw)				
	Daidzein	460 (161–759)				
	Glycitein	108 (40.9–176)				
	Genistein	697 (236–1158)				

[a] Values are the average, and values in parentheses are the range.

Table 7. Japanses daily intake of isoflavonoids from soybean and its processed foods[a]

	Daily intake of foods, g	Daily intake of isoflavonoids, mg/day			
		Daidzein	Glycitein	Genistein	Total
Miso (bean paste)	13.9	2.66	0.42	3.25	6.33
Tofu	40.0	3.27	1.04	3.79	8.10
Tofu-derived processed foods	7.8	1.10	0.30	1.66	3.06
Soybean and other soybean-derived processed foods	8.8	4.99	0.54	4.78	10.31
Total	70.4	12.01	2.30	13.48	27.80

[a] Soybean-derived processed foods contain boiled soybeans, roasted soybeans, kinako (powdered roasted soybeans), kori-dofu (freeze-dried tofu), okara (soybean residue as a by-product of tofu), natto (fermented soybeans), soy milk, and yuba (processed food derived from soy milk).

Results and Discussion

LC Analysis of Standard Solution

The liquid chromatogram of the standard solution is shown in Figure 2. All of the 6 isoflavonoids and flavone were determined individually.

Variations of 6 isoflavonoids and flavone in retention times and peak areas in LC ($n = 10$) are shown in Table 1. The coefficients of variation (CVs) of retention time and peak area were 0.22–0.43% and 0.90–3.28%, respectively.

Linear dynamic ranges of the standard solution for LC determination were 0.0090–181.0 μg/mL for daidzin (R^2 0.999), 0.0132–131.9 μg/mL for glycitin (R^2 0.999), 0.0227–226.4 μg/mL for genistin (R^2 1.000), 0.0020–202.8 μg/mL for daidzein (R^2 0.999), 0.0011–54.69 μg/mL for glycitein (R^2 0.999), and 0.0022–220.0 μg/mL for genistein (R^2 1.000) (data not shown). Thus, determination of isoflavonoids of variable amounts in the sample solutions was possible in this study.

The detection limits for a sample base are shown in Table 2. The detection limit (S/N = 3) was 0.547–3.299 μg/g fresh weight for solid foods (soybeans, boiled soybeans, roasted soybeans, kinako, tofu, freeze-dried tofu, okara, tofu-derived processed foods, natto, miso, and yuba); 0.109–0.660 μg/mL for soy milk; and 0.011–0.066 μg/mL for soy sauce. It was sensitive enough for quantitaion of these samples.

Recovery Test

Recoveries of the standard solution from the Sep-pak plus C_{18} cartridge are shown in Table 3. The recovery of 6 isoflavonoids was 99.6–106.3% and the CV was 0.10–2.04% (n = 3). These results indicate that all 6 isoflavonoids were recovered constantly and absolutely from the Sep-pak plus C_{18} cartridge. Therefore, cleanup using the Sep-pak plus C_{18} cartridge was applied to the analysis of soybean and its processed foods before LC to reduce impurities such as lipids and pigments.

The recovery tests were performed with or without hydrolysis for soybean #1, tofu #1, miso #1, soy milk #1, and soy sauce #1. Spiked amounts of isoflavonoids were set at 3 levels as the intrinsic content of isoflavonoids in each sample (Table 4). Several large peaks were detected in the test solution of soy sauce, but there was no interfering peak to determine the 6 isoflavonoids in each test solution (data not shown). The recoveries of 6 isoflavonoids were 94.3–112.6% without hydrolysis, and 90.4–103.6% with hydrolysis from soybeans; 102.1–106.5% without hydrolysis and 82.2–89.4% with hydrolysis from tofu; 93.5–110.4% without hydrolysis and 81.9–100.6% with hydrolysis from miso; 86.0–103.6% without hydrolysis and 88.2–110.6% with hydrolysis from soy milk; and 81.2–108.6% without hydrolysis and 66.4–100.5% with hydrolysis from soy sauce. The CV was below 10% for the 3 trials. Good recoveries were obtained for 6 isoflavonoids, except glycitein in soy sauce with hydrolysis. The relatively low recovery of glycitein in soy sauce by acid hydrolysis method might be due to interference material derived from the sample. Murphy et al. (23) reported recoveries of daidzein, genistein, and genistin in soybean, tofu, and soymilk to be 81–99%; our results (Table 4) coincide with this data.

Levels and Composition of Isoflavonoids in Soybeans and Soy-Derived Processed Foods

Previously, isoflavonoids were extracted with or without hydrolysis and measured by LC after centrifugation or filtration (25). In this study, we measured isoflavonoids both with and without hydrolysis and followed with cleanup using a Sep-pak plus C_{18} cartridge (13). Anthocyanidins (black or dark purple pigments) contained in the rind of black soybean and its processed foods and some impurities including lipids were removed by cleanup using a Sep-pak plus C_{18} cartridge.

Fifteen isoflavonoids, daidzein, daidzin, glycitein, glycitin, genistein, genistin, 6″-O-acetyldaidzin, 6″-O-acetylgenistin, 6″-O-acetylglycitin, 6″-O-malonyldaidzin, 6″-O-malonylgenistin, 6″-O-malonylglycitin, 6″-Osuccinyldaidzin, 6″-O-succinylgenistin, and 6″-O-succinylglycitin have been detected in soybean and soy-derived processed foods (11, 14). We could not obtain 6″-O-acetyl or 6″-O-malonyl or 6″-O-succinyl compounds of daidzin, genistin, and glycitin. Therefore, we analyzed the total isoflavonoids as aglycones (daidzein, glycitein, and genistein) with hydrolysis and the intact isoflavonoids as aglycones and glucosides (daidzin, glycitin, and genistin). Acetyl-, malonyl-, or succinyl-glucosides could not be quantitated in this study.

Several large peaks were detected in the test solution of soy sauce but there was no interfering peak to determine isoflavonoids in each test solution. The liquid chromatograms of soybean #2 and miso #8 with and without hydrolysis are shown in Figures 3 and 4. Compared with the liquid chromatogram of Wang and Murphy (14), the large peaks not determined at t_R 17.7 and 20.8 min in Figure 3-1 might be 6″-O-malonyldaidzin and 6″-O-malonylgenistin, respectively, which are abundant in soybeans, soy milk, and tofu (14, 20, 22).

Contents of isoflavonoids in soybean and its processed foods analyzed by the described method are shown in Table 5. Total isoflavonoids contain aglycone, glucosides, and malonyl-, succinyl- and acetyl- daidzin, glycitin, and genistin. Contents are expressed as μg/g fresh weight (soybean and its processed foods except soy milk and soy sauce) or μg/mL (soy milk and soy sauce).

The contents of total isoflavonoids indicated as aglycone were as follows: daidzein 452–1138 μg/g, glycitein 36.7–145 μg/g, and genistein 335–894 μg/g in 11 soybeans; daidzein 270–369 μg/g, glycitein 8.53–23.5 μg/g, and genistein 354–428 μg/g in 3 boiled soybeans; daidzein 970 μg/g, glycitein 144 μg/g, and genistein 892 μg/g in 1 roasted soybean; daidzein 1181–1844 μg/g, glycitein 80.2–99.5 μg/g, and genistein 830–1289 μg/g in 2 kinako; daidzein 63.3–106 μg/g, glycitein 23.5–28.7 μg/g, and genistein 80.3–112 μg/g in 4 tofu; daidzein 310 μg/g, glycitein 34.4 μg/g, and genistein 540 μg/g in 1 kori-dofu; daidzein 44.7 μg/g, glycitein 12.2 μg/g, and genistein 48.1 μg/g in 1 okara; daidzein 108–191 μg/g, glycitein 35.5–39.2 μg/g, and genistein 144–304 μg/g in 3 tofu-derived processed foods; daidzein 323–342 μg/g, glycitein 36.7–67.7 μg/g, and genistein 296–404 μg/g in 2 natto; daidzein 49.8–363 μg/g, glycitein 12.0–53.4 μg/g, and genistein 62.4–398 μg/g in 9 miso; daidzein 5.26–8.54 μg/mL, glycitein 0.82–2.62 μg/mL, and genistein 1.00–3.32 μg/mL in 8 soy sauce; daidzein 26.4–126 μg/mL, glycitein 1.51–16.3 μg/mL, and 52.4–168 μg/mL in 3 soy milk; daidzein 161–769 μg/g, glycitein 40.9–176 μg/g, and genistein 236–1158 μg/g in 2 yuba. The highest content of isoflavonoids was in kinako #1 and the lowest was in soy sauce #6 (Table 5).

The composition of isoflavonoids (% of total, molar ratio) are also shown in Table 5 for comparison of abundance of each isoflavonoids. The percentage of aglycon was as follows: soybeans, 2.7–22.4%; boiled soybeans, 7.7–13.1%; roasted soybeans 37.1%; kinako 32.3–41.7%; tofu, 11.2–17.9%; kori-dofu, 66.6%; okara, 35.2%; tofu-derived processed foods, 16.3–32.8%; natto, 14.7–20.3%; miso, 38.3–96.4%; soy sauce, 62.7–100%; soy milk, 1.8–6.0%; yuba, 13.7–24.9%.

The level of isoflavonoids in 11 soybeans varied by species and country of origin (Table 5). The CV in 3 trials was relatively high in soybeans, probably because of the heterogeneous localization of isoflavonoids inside the soybeans (2), which are most abundant in soygerm (20). The level of isoflavonoids in processed foods varied by the manufacturing method or ingredients (Table 5). With hydrolysis, the most abundant isoflavonoids were daidzein and genistein. The composition of isoflavonoids in soybeans varied by species and origin, and those in processed foods varied by the manufacturing method or ingredients (Table 5). The percentage of aglycone tended to be higher in the processed foods such as soy sauce and miso, which were heated and fermented in the manufacturing process (Table 5).

The variances in content and composition of isoflavone in soybeans and soybean-derived foods have been elucidated by several researchers (19, 20). Murphy et al. (19) assayed 6 of the major soy-based infant formulas marketed in the United States for their isoflavone levels and showed that isoflavone levels were variable across brands probably because of the different amounts of soy isolate used in product formulation. Franke et al. (20) determined the concentrations and glucosidic conjugation patterns of isoflavones by multiethnic populations in Singapore and Hawaii and showed that total isoflavone levels and conjugation patterns varied as a function of soybean variety, storage conditions, and food processing. They also indicated that a large contribution to the differences in total isoflavone content between food groups was due to the water content in foods and to leaching of polar analytes into the water phase during boiling (20).

Wang and Murphy (21) investigated the effects of processing techniques on the distribution of isoflavones by manufacturing tempeh, soymilk, tofu, and protein isolate. The manufacturing steps caused significant losses ($p < 0.05$) of isoflavones by soaking (12%) and heat processing (49%) in tempeh production; coagulation (44%) in tofu processing; and alkaline extraction (53%) in soy protein isolate production (21). In the production of tempeh, soymilk, and tofu, malonyldaidzin and malonylgenistin decreased after soaking and cooking (21). After fermentation, daidzein and genistein concentrations increased in tempeh, apparently as a result of fungal enzymatic hydrolysis. Acetyldaidzin and acetylgenistin were generated during heat processing (21). In protein isolate processing, alkaline extraction caused the generation of daidzein and genistein, probably through alkaline hydrolysis (21).

Our results are compatible with previous data (2, 11–18, 20, 22, 23) in Table 6. Murphy et al. (23) developed a national sampling plan to select the greatest variety of isoflavone-containing foods in the United States. These data have appeared on the Internet version of USDA Handbook No. 8 of Food Composition Data in 1999 (26). Contents of isoflavonoids in soybeans and soybean-derived foods analyzed in this study (Table 5) are compatible with these data. Moreover, our study is comprehensive, with a large amount of data, both in terms of variety and quantity of data.

Japanese Daily Intake of Isoflavonoids from Soybeans and Soy-Derived Processed Foods

Table 7 shows Japanese daily intake of isoflavonoids from soybeans and its processed foods, calculated from the results of this study (Table 5), and based on the *Japanese Nutrition Research in 1996 (the 8th year of Heisei)* (24). The total daily intake was 27.80 mg, which was classified into 12.02 mg daidzein, 2.30 mg glycitein, and 13.48 mg genistein.

Toda et al. (11) reported the Japanese daily intake of isoflavonoids from processed foods of soybean as 17.96 mg. Kimira et al. (27) calculated the Japanese daily intake of isoflavonoids as 16.2 mg daidzein, 23.27 mg genistein, and a total of 39.46 mg. Our results, which say that Japanese total isoflavonoid intake is 27.80 mg, is higher than the data of Toda et al. (11) and lower than that of Kimira et al. (27). These variations are probably due to the difference in the methods of isoflavonoid analysis and calculation of Japanese daily intake.

Acknowledgments

This study was supported by grants from the Japanese Ministry of Health in 1998.

References

(1) Price, K.R., & Fenwick, G.R. (1985) *Food Addit. Contam.* **2**, 73–106
(2) Takamatsu, K. (1997) *Proceeding of the Symposium on Food and Health Science Symposium '97*, pp 10–20 (in Japanese)
(3) Kurzer, M.S., & Xu, X. (1997) *Annu. Rev. Nutr.* **17**, 353–381
(4) Lundh, T. (1995) *Proc. Soc. Exp. Biol. Med.* **208**, 33–39
(5) Uesugi, T., Toda, T., Ishida, H., & Tsuji, K. (1997) *Shokuhin Kogyo,* 11/30/97, pp 24–30 (in Japanese)
(6) Ishimi, Y., & Ikegami, S. (1998) *J. Jpn. Soc. Nutr. Food Sci.* **51**, 294–298 (in Japanese)
(7) Messina, M., & Messina, V. (1991) *Perspect. Prac.* **91**, 836–840
(8) Adlercreuz, H., Goldin, B.R., & Gorbach, S.L. (1995) *J. Nutr.* **125**, 757S–770S
(9) Knight, D.C., & Eden, J.A. (1995) *Maturitas* **22**, 167–175
(10) Setchell, K.D.R., Borriello, S.P., Hulme, P., Kirk, D.N., & Axelson, M. (1984) *Am. J. Clin. Nutr.* **40**, 569–578
(11) Toda, T., Tamura, J., & Okuhira, T. (1997) *FFI Journal* **172**, 83–88 (in Japanese)
(12) Pettersson, H., & Kiessling, K.-H. (1984) *J. Assoc. Off. Anal. Chem.* **67**, 503–509
(13) Dwyer, J.T., Goldin, B.R., Saul, N., Gualtieri, L., Barakat, S., & Adlercreutz, H. (1994) *J. Am. Diet. Assoc.* **94**, 739–743
(14) Wang, H.-J., & Murphy, P.A. (1994) *J. Agric. Food Chem.* **42**, 1666–1673
(15) Franke, A.A., Custer, L.J., Cerna, C.M., & Narala, K.K. (1994) *J. Agric. Food Chem.* **42**, 1905–1913
(16) Nguyenle, T., Wang, E., & Cheung, A.P. (1995) *J. Pharm. Biomed. Anal.* **14**, 221–232
(17) Franke, A.A., Custer, L.J., Cerna, C.M., & Narala, K. (1995) *Proc. Soc. Exp. Biol. Med.* **208**, 18–26
(18) Franke, A.A., Custer, L.J., Wang, W., & Shi, C.Y. (1998) *Proc. Soc. Exp. Biol. Med.* **217**, 263–273
(19) Murphy, P.A., Song, T., Buseman, G., & Barua, K. (1997) *J. Agric. Food Chem.* **45**, 4635–4638
(20) Franke, A.A., Hankin, J.H., Yu, M.C., Maskarinec, G., Low, S.-H., & Custer, L.J. (1999) *J. Agric. Food Chem.* **47**, 977–986
(21) Wang, H.-J., & Murphy, P.A. (1996) *J. Agric. Food Chem.* **44**, 2377–2383
(22) Song, T., Barua, K., Buseman, G., & Murphy P.A. (1998) *Am. J. Clin. Nutr.* **68** (suppl. 1) 1474S–1479S
(23) Murphy, P.A., Song, T., Buseman, G., Barua K., Beecher, G.R., Trainer, D., & Holden, J. (1999) *J. Agric. Food Chem.* **47**, 2697–2704
(24) Health Insurance Bureau, Ministry of Health and Welfare of Japan (1998) *Japanese Nutrition Research in 1996 (the 8th year of Heisei)*, Dai-ichi Shuppan, Tokyo, Japan (In Japanese)
(25) Reinli, K., & Block, G. (1996) *Nutr. Cancer* **26**, 123–148
(26) http://www.nal.usda.gov/fnic/foodcomp/Data/isoflav/isoflav.html
(27) Kimira, M., Arai, Y., Shimoi, K., & Watanabe, S. (1998) *J. Epidemiol.* **8**, 168–175