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Content and Composition of Isoflavonoids in Mature or Immature Beans and Bean Sprouts Consumed in Japan

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The content of 9 types of isoflavonoids (daidzein, glycitein, genistein, formononetin, biochanin A, coumestrol, daidzin, glycitin and genistin) in 34 domestic or imported raw beans including soybeans, 7 immature beans and 5 bean sprouts consumed in Japan were systematically analyzed. Each isoflavonoid was analyzed in total after acid hydrolysis to the aglycone, and intact individual isoflavonoids were also analyzed without hydrolysis. After the sample clean up, daidzein, glycitein, genistein, formononetin, biochanin A, daidzin, glycitin and genistin were determined by HPLC with a diode array detector and coumestrol was determined by spectrofluorimetry. The content and composition of isoflavonoids varied greatly between soybean sprouts, immature soybeans and mature beans of the same type but of different source. Isoflavonoid content was highest in mature soybeans. The composition of isoflavonoids differed in each growth stage of soybeans. In other beans, the largest content of isoflavonoids was found in mature chickpeas, but this value was less than 1/27 of the isoflavonoid content in mature soybeans. Thus, the contribution of beans other than soybeans to the daily intake of isoflavonoids in a Japanese diet is negligible.

Key words —— isoflavonoid, bean, acid hydrolysis, high performance liquid chromatography, diode array detection, spectrofluorometric detection

INTRODUCTION

Isoflavonoids, which are found in legumes such as soybeans,[1,2] have both sterile and estrogenic activities[1] and reported to be protective against cancer, cardiovascular diseases and osteoporosis.[3–9] Much research has been reported about the content of isoflavonoids in soybeans and soybean-derived processed foods.[10–23] In contrast, there are few reports about the isoflavonoid content in beans other than soybeans.[11,12,18,23]

Japanese people are reported to ingest isoflavonoids mainly through the consumption of soybeans and its derived processed foods.[20] Recently, we estimated that the Japanese daily intake of isoflavonoids from soybeans and soybean-based processed foods is 27.80 mg per day (daidzein 12.02 mg, glycitein 2.30 mg and genistein 13.48 mg).[24] However, isoflavonoid intake from the consumption of immature beans, sprouts and beans other than soybeans has not been elucidated. Here we have measured the content of isoflavonoids in mature and immature beans and bean sprouts consumed in Japan, and have compared the content and composition variation between different types and different growth stages of the beans.

MATERIALS AND METHODS

Materials —— Genistein, formononetin and biochanin A were purchased from Extrasynthèse (Genay, France). Daidzein, daidzin, genistin, glycitein and glycitin were from Fujicco Co., Ltd. (Kobe, Japan). Coumestrol was obtained from Fluka Chemie AG (Bucks, Switzerland). Flavone, 2,6-di-*t*-butyl-4-methylphenol (BHT) and dimethyl sulfoxide (DMSO) were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The chemical structures of these isoflavonoids and flavone are shown in Fig. 1. Acetonitrile, ethanol, *n*-hexane and methanol were analytical grade for high performance liquid chromatography (HPLC). All other reagents were analytical grade. The water used was Milli-Q grade or equivalent.

A YMC-pack ODS-AM-303 column (4.6 mm i.d. × 250 mm, 5 μm particle size) was purchased from YMC Co., Ltd. (Kyoto, Japan). Mini-cartridge column Sep-pak^R plus C_{18} cartridges were obtained from Waters Corporation (Milford, MA, U.S.A.).

The domestic and imported mature beans, immature beans and sprouts that we analyzed are foods commonly consumed in Japan. The imported mature beans were obtained at a port in Hyogo Prefecture and others were purchased in retail stores in Osaka Prefecture.

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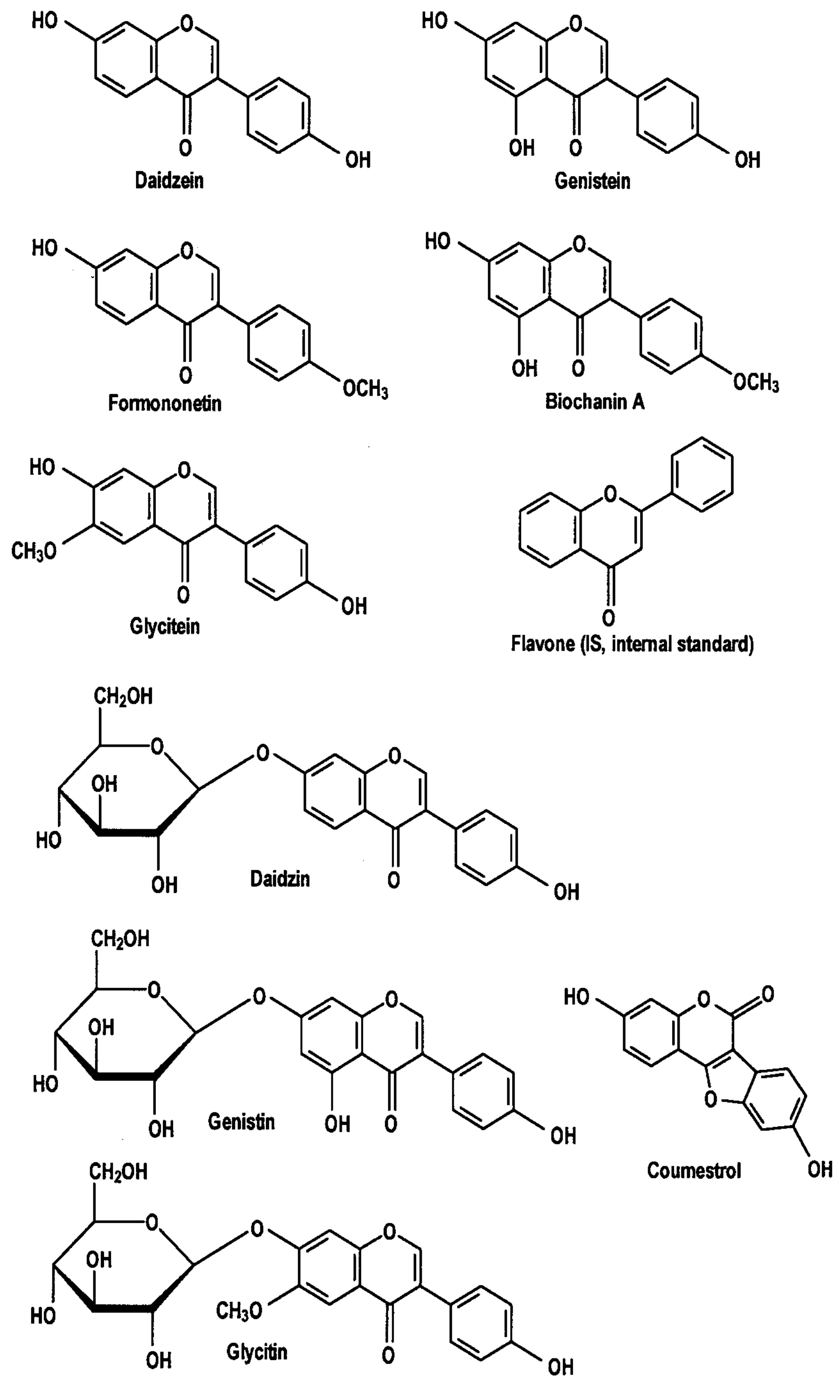

Fig. 1. Chemical Structures of 9 Isoflavonoids and Flavone (Internal Standard)

Instruments —— A Shimadu LC-10 series equipped with binary gradient pump LC-10AD, 100 μl injection loop, autosampler SIL-10A, column oven CTO-10A set at 40°C, diode array detector (DAD) SPD-M10A*VP*, fluorospecrometric detector RF-10A and system controller SCL-10A (Kyoto, Japan) was used for isoflavonoid analysis.

Standard solutions —— A pre-weighed amount of each isoflavonoid standard was dissolved in a small amount of DMSO and diluted with methanol to form 10 ml of stock solutions (547–1319 μg/ml). An appropriate amount of each isoflavonoid stock solution was then diluted with methanol to make isoflavonoid standard solutions. Flavone stock solution was diluted with methanol 5 times to prepare a 210 μg/ml internal standard (IS) solution.

Extraction —— Edible portions of the bean samples were analyzed. Intact individual isoflavonoids were analyzed without hydrolysis, and the total content of each isoflavonoid was analyzed as the aglycone after acid hydrolysis by the method of Franke *et al.*[11] with a previously described modification.[24] Each analysis was performed in triplicate. For the analysis of peanuts, lipids were removed before isoflavonoid analysis by extraction with 50 ml of *n*-hexane overnight at an ambient temperature.

(A) Intact isoflavonoids (sample solution A): Ground mature bean powder (1.0 g), homogenized immature beans (2.0 g) or homogenized bean sprouts (3.0 g) were placed in centrifuge tubes. One ml of IS solution (flavone 210 μg) and 50 ml of 80% methanol were added to the centrifugal tubes, which were then sonicated for 30 min. Isoflavonoids were extracted for 24 hours at ambient temperature, the tube was centrifuged at $1000 \times g$ for 20 min at 5°C and the volume was adjusted to 50 ml with methanol to form sample solution A.

(B) Total isoflavonoids (sample solution B): Ground mature bean powder (1.0 g), homogenized immature beans (2.0 g) or homogenized bean sprouts (3.0 g) were placed in centrifuge tubes. One ml of IS solution (flavone 210 μg/ml), 10 ml of 10 M HCl solution and 40 ml of ethanol containing 0.05% BHT were added to the centrifugal tube, which was then sonicated for 30 min. Hydrolysis was carried out by reflux in a boiling water bath for 3 hr.[11] The tube was cooled and centrifuged at $1000 \times g$ for 15 min at 5°C, and the volume was adjusted to 50 ml with methanol to form sample solution B.

Clean Up (Test Solution) —— Sample solutions A and B prepared above were cleaned up using Sep-pak^R plus C_{18} cartridges.[17] One ml of each sample solution was diluted with 10 ml of water and applied to a Sep-pak^R plus C_{18} cartridge column preconditioned with methanol and water. The column was washed with 20 ml of water followed by 2 ml of 20% methanol, and then isoflavonoids and flavone were eluted with exactly 2 ml of methanol to form the test solution.

HPLC Analysis —— The content of isoflavonoids in each test solution was determined by HPLC using flavone as an internal standard.[11] Coumestrol was determined by fluorospectrometry, and other isoflavonoids and flavone were determined by DAD. HPLC conditions were as follows. Apparatus, Shimadzu LC-10 series; column, YMC-Pack ODS-AM-303; column oven temperature, 40°C; Mobile phase, (solvent A) water : phosphoric acid 1000 : 1 (v/v), (solvent B) water : acetonitrile : phosphoric acid 200 : 800 : 1 (v/v/v), (linear gradient program) B: 0% (0 min) → 20% (5 min) → 100% (50–60 min) → 0% (61 min); flow rate, 1.0 ml/min; DAD monitoring wavelength, 260 nm for daidzein, daidzin, genistein, genistin, glycitein, glycitin, biochanin A, formononetin, 280 nm for flavone, 343 nm for coumestrol; spectrofluorometric detector for coumestrol, excitation and emission wavelength, 344 nm and 413 nm; injection volume, 10 μl.

Recovery Tests —— A recovery test was performed for mature soybean #1, mature kidney bean #2, immature green peas #4 and soybean sprout (moyashi) #1 by adding 1 ml of the appropriate concentration of the isoflavonoid standard solution and 1 ml of IS solution to each sample.

RESULTS AND DISCUSSION

HPLC Analysis of Standard Solution

The HPLC chromatogram of the standard solution is shown in Fig. 2. All nine phytoestrogens and flavone could be determined individually. Previously, we used STR ODS-II (4.6 mm i.d. × 250 mm, 5 μm particle size) column to determine isoflavonoids in soybeans and soybean-derived processed foods.[24] However, there is not good separation of coumestrol from genistein or biochanin A from flavone with the STR ODS-II column. Therefore, as some beans contain biochanin A,[11] we used the YMC-pack ODS-AM-303 column in this study.

The coefficients of variations of the relative retention time and peak area of the nine isoflavonoids and flavone (10.4–26.1 μg/ml) using DAD were 0.01–0.04% and 0.41–1.15%, respectively; those of coumestrol using fluorospectrometric detection were 0.07% and 0.66%, respectively (data not shown). The relative retention time (flavone set as 1.000), detection limits and linear dynamic range are shown in Table 1. The detection limits (S/N = 5) of the nine isoflavonoids and coumestrol were 44.8–260.8 ng/ml (0.108–0.973 nmol/ml) and 26.1 ng/ml (0.097 nmol/ml) by DAD and fluorospectrometric detection, respectively. The linear dynamic ranges of the standard solution were more than 1,000-fold as shown in Table 1. The sensitivity of coumestrol detection by fluorospectrometry was 10 times higher than by DAD, therefore further determinations of coumestrol were carried out by fluorospectrometry in this study.

Recovery Tests

The recovery of the standard solutions from the Sep-pak^R plus C_{18} cartridge is shown in Table 2. The

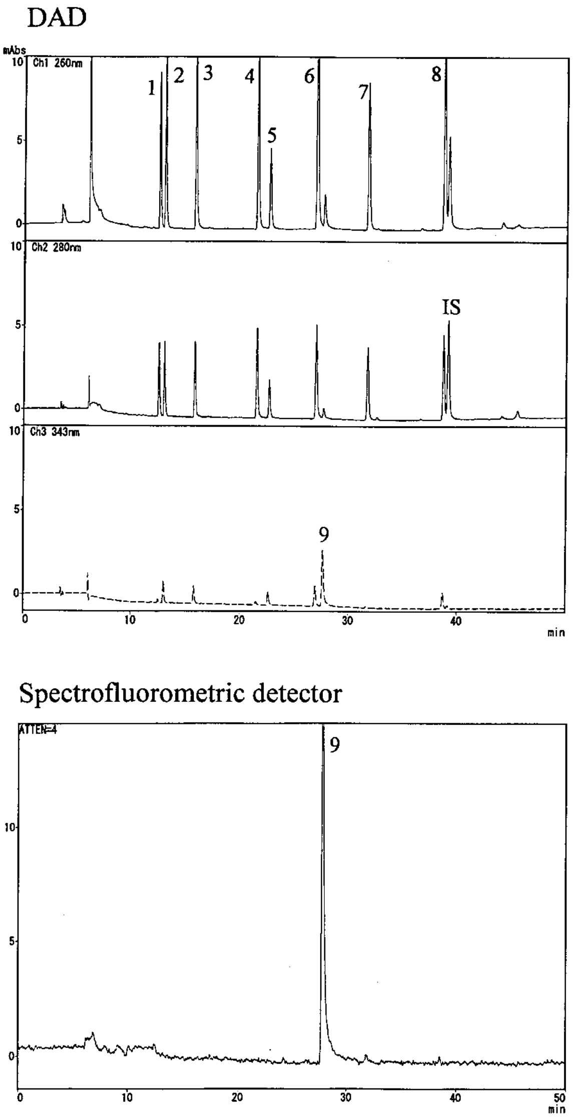

Fig. 2. HPLC Chromatograms of 9 Isoflavonoids and Flavone (Standard Solution)

Conditions of HPLC are as follows: Apparatus, Shimadzu LC-10 series; column, YMC-Pack ODS-AM-303 (4.6 mm i.d. × 250 mm); column oven temperature, 40°C; Mobile phase, (solvent A) water:phosphoric acid 1000 : 1 (v/v), (solvent B) water : acetonitrile : phosphoric acid 200 : 800 : 1 (v/v/v), (linear gradient program) B: 0% (0 min) → 20% (5 min) → 100% (50–60 min) → 0 (61 min); flow rate, 1.0 ml/min; DAD monitoring wavelength, 260 nm for daidzein, daidzin, genistein, genistin, glycitein, glycitin, biochanin A, formononetin, 280 nm for flavone, 343 nm for coumestrol; spectrofluorometric detector for coumestrol, excitation wavelength, 344 nm; emission wavelength, 413 nm; injection volume, 10 μl. Peaks are 1, daidzin 17.9 μg/ml; 2, glycitin 26.1 μg/ml; 3, genistin 22.4 μg/ml; 4, daidzein 20.1 μg/ml; 5, glycitein 10.8 μg/ml; 6, genistein 43.6 μg/ml; 7, formononetin 20.5 μg/ml; 8, biochanin A, 21.5 μg/ml 9, coumestrol 10.4 μg/ml and IS, flavone 21.0 μg/ml.

recovery of the nine isoflavonoids was 84.0–102.6% and the coefficient of variation was 0.21–2.14% ($n = 5$), *i.e.*, all nine isoflavonoids were recovered constantly and efficiently from the Sep-pak^R plus C_{18} cartridge.

Recovery tests were performed both with and without hydrolysis for soybean #1, kidney bean #2, premature green peas #4 and soybean sprout #1 (Table 3). Recoveries of the nine isoflavonoids were 84.8–94.6% without hydrolysis and 72.0–100.8% with hydrolysis from mature soybean, 79.4–91.5% without hydrolysis and 65.0–105.6% with hydrolysis from mature kidney bean, 71.0–93.1% without hydrolysis and 74.8–105.9% with hydrolysis from immature green peas, and 79.8–102.6% without hydrolysis and 73.0–96.4% with hydrolysis from soybean sprout. The coefficient of variance was below 10% for the triplicate analysis, except for coumestrol in immature green peas without hydrolysis. A lower recovery was obtained for coumestrol in kidney bean with hydrolysis, and for glycitin and coumestrol in soybean sprout with hydrolysis. A larger coefficient of variance (11.4%) was obtained for coumestrol in premature green peas without hydrolysis. These results might be caused by interference from other material derived from the sample.

Content of Isoflavonoids in Mature Beans, Immature Beans and Sprouts

Twelve 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, have been reported to be found in soybean.[22] Toda *et al.*[21] reported that natto (fermented soybeans, a traditional Japanese food) contained 6″-*O*-succinyldaidzin, 6″-*O*-succinylgenistin and 6″-*O*-succinylglycitin. Because we could not obtain the 6″-*O*-acetyl or 6″-*O*-malonyl derivatives of daidzin, genistin and glycitin, nor the glycosides of formononetin, biochanin A and coumestrol, we analyzed each isoflavonoid in total as the aglycones (daidzein, glycitein and genistein) by acid hydrolysis and also the intact individual isoflavonoids as aglycones and glucosides (daidzin, glycitin and genistin).

As the lipid content in peanuts is very high (47.4% w/w),[25] we removed lipids by *n*-hexane extraction before isoflavonoid analysis. Some of the B sample solutions were colored purple-red owing to acidification of anthocyanidin-related compounds present in some mature beans (possibly in the skin) such as black soybean, some kidney beans, azuki beans, black mappe, green gram, broad bean, Saltapia bean, green or red peas and peanuts; in some immature beans such as black soybean and broad bean, green pea pod; and in the sprouts of black and

Table 1. Relative Retention Time, Detection Limit and Linear Range of 9 Isoflavonoids by HPLC

Isoflavonoids	Relative retention time[a)] (Flavone = 1.000)[b)]	Detection limit [ng/ml]	Detection limit [μg/g][c)]	Linear range [μg/ml]	(R^2)
Daidzin	0.305	44.8	2.24	0.045– 89.6	(0.999)
Glycitin	0.314	65.3	3.26	0.065–130.6	(0.999)
Genistin	0.386	56.1	2.80	0.056–112.1	(1.000)
Daidzein	0.542	50.2	2.51	0.050–100.4	(1.000)
Glycitein	0.568	108.3	5.41	0.108–108.3	(1.000)
Genistein	0.682	54.5	2.73	0.054–108.9	(0.999)
Coumestrol	0.691	260.8	13.04	0.261– 52.2	(1.000)
Coumestrol (fluorospectrometry)		26.1	1.30	0.026– 52.2	(0.993)
Formononetin	0.807	51.2	2.56	0.051– 95.4	(1.000)
Biochanin A	0.985	53.7	2.68	0.054– 94.5	(1.000)

Conditions of HPLC are as follows. Apparatus, Shimadzu LC-10 series; column, YMC-Pack ODS-AM-303 (4.6 mm i.d. × 250 mm); column oven temperature, 40°C; Mobile phase, (solvent A) water : phosphoric acid 1000 : 1 (v/v), (solvent B) water : acetonitrile : phosphoric acid 200 : 800 : 1 (v/v/v), (linear gradient program) B→ 20% (5 min) → 100% (50–60 min) → 0% (61 min); flow rate, 1.0 ml/min; DAD monitoring wavelength, 260 nm for daidzein, daidzin, genistein, genistin, glycitein, glycitin, biochanin A and formononetin, 280 nm for flavone, 343 nm for coumestrol; spectrofluorometric detector for coumestrol, excitation wavelength, 344 nm; emission wavelength, 413 nm; injection volume, 10 μl. *a*) Values are means of 10 trials. *b*) Retention time of flavone was 39.78 ± 0.01 min (mean ± S.D. for 10 trials). *c*) Values are expressed as μg/g fresh weight of sample (S/N=5).

Table 2. Recovery of Standard Solutions of 9 Isoflavonoids from Sep-pak plus C_{18}^R Cartridge Column

Isoflavonoids	Spiked amounts [μg]	Recovery [%]
Daidzin	3.584	102.6 ± 1.4
Glycitin	5.224	102.1 ± 0.8
Genistin	4.482	99.0 ± 0.5
Daidzein	4.014	100.2 ± 0.5
Glycitein	4.332	97.0 ± 0.5
Genistein	4.356	95.5 ± 0.2
Coumestrol	2.086	88.8 ± 1.9
Formononetin	4.094	100.2 ± 0.4
Biochanin A	4.296	84.0 ± 0.9

Data are represented as means ± S.D. (n = 5). Each isoflavonoid and 4.2 μg flavone (internal standard) were applied to a Sep-pak plus C_{18}^R cartridge column preconditioned by 20 ml of methanol followed by 10 ml of distilled water. The column was washed with 10 ml of water followed by 2 ml of 20% methanol and flavonoid was eluted by exactly 2 ml of methanol. Each isoflavonoid was determined by HPLC using flavone as an internal standard. Conditions for HPLC are described in Table 1.

green mappe (Table 4). We removed these purple-red impurities with the Sep-pak^R plus C_{18} cartridge before HPLC analysis. Figures 3-1 to 4-2 show the HPLC chromatograms of the test solutions of chick pea (#21) and soybean sprout (#1) with and without hydrolysis.

Table 4 summarizes the isoflavonoid content in 11 mature soybeans, 23 mature beans (but not soybean), 7 immature beans and 5 sprouts. Each isoflavonoid was identified by comparing its relative retention time (flavone = 1) and DAD spectra with those of the standard solutions. Pseudo-peaks near glycitin, glycitein and formononetin were observed, in particular near glycitin and glycitein in Azuki-bean (#17–18) and near formononetin in kidney beans (#1–5), scarlet runner bean (#6), peas (#14–16) and peanut (#23). These were shown to be artifacts by comparison with the DAD spectra.

The isoflavonoid total content was determined as the aglycone obtained by acid hydrolysis of each isoflavonoid (Table 4). The content of total individual isoflavonoids was as follows. In 11 mature soybeans: daidzein 382.0–910.2 μg/g, glycitein 27.0–150.8 μg/g and genistein 433.5–1118 μg/g. In 23 other mature beans: daidzein ND (not detected) to 6.50 μg/g, glycitein ND, genistein ND to 3.55 μg/g, formononetin ND to 9.59 μg/g, biochanin A ND to 25.18 μg/g, glycitein and coumestrol ND. In 7 immature beans: daidzein ND to 173.8 μg/g, glycitein ND to 67.16 μg/g, genistein ND to 62.27 μg/g, formononetin, biochanin A and coumestrol, ND. In 5 sprouts, daidzein ND to 67.80 μg/g, glycitein ND to 10.47 μg/g, genistein ND to 94.04 μg/g, coumestrol ND to 21.04 μg/g, formononetin and biochanin A, ND. None of the isoflavonoids that we tested was detected in kidney bean (#1–4), scarlet runner bean (#6), peas (#14–16), black mappe (#19), green gram (#20), peanut (#23), immature broad bean (#3), immature green pea (#4–5), green pea sprout (#6) and kidney bean pod (#7). The content of daidzein, glycitein and

Table 3. Recovery of Isoflavonoids with or without Hydrolysis

	Isoflavonoids	Spiked amounts [μg/g]	Recovery [%] Without hydrolysis	With hydrolysis
Soybean #1				
	Daidzin	1315	88.6 ± 5.6	100.8 ± 7.9
	Glycitin	1368	87.8 ± 4.6	74.0 ± 7.4
	Genistin	1832	88.2 ± 8.2	76.7 ± 6.0
	Daidzein	1054	89.7 ± 1.5	90.4 ± 1.0
	Glycitein	650	88.5 ± 5.3	72.0 ± 3.1
	Genistein	1943	86.3 ± 5.7	86.8 ± 9.9
	Coumestrol	805	93.7 ± 5.4	96.6 ± 2.0
	Formononetin	1068	94.6 ± 3.1	96.4 ± 3.1
	Biochanin A	1075	84.8 ± 2.5	92.1 ± 1.3
Kidney bean #2				
	Daidzin	263	83.3 ± 0.5	99.4 ± 9.9
	Glycitin	684	86.6 ± 2.6	87.6 ± 7.5
	Genistin	916	80.0 ± 2.1	80.0 ± 0.7
	Daidzein	527	86.1 ± 1.7	104.8 ± 5.7
	Glycitein	130	89.0 ± 0.3	105.6 ± 0.9
	Genistein	389	85.1 ± 1.9	86.9 ± 3.0
	Coumestrol	403	87.0 ± 6.8	65.0 ± 7.5
	Formononetin	214	91.5 ± 0.8	95.4 ± 3.1
	Biochanin A	215	79.4 ± 2.5	84.8 ± 0.3
Immature green peas #4				
	Daidzin	132	89.5 ± 2.6	78.8 ± 9.1
	Glycitin	342	93.1 ± 8.4	105.1 ± 10.0
	Genistin	458	82.3 ± 3.2	78.7 ± 1.3
	Daidzein	164	85.7 ± 0.7	94.1 ± 9.1
	Glycitein	65.0	71.0 ± 3.2	99.1 ± 9.2
	Genistein	199	85.5 ± 1.5	84.3 ± 8.0
	Coumestrol	201	91.7 ± 10.5	74.8 ± 3.4
	Formononetin	107	90.8 ± 3.7	105.9 ± 8.7
	Biochanin A	108	72.3 ± 1.6	91.4 ± 7.5
Soybean sprout #1				
	Daidzin	87.7	83.1 ± 1.0	84.0 ± 1.6
	Glycitin	91.2	81.0 ± 0.4	68.6 ± 1.5
	Genistin	122	92.0 ± 0.1	85.3 ± 1.4
	Daidzein	70.3	95.4 ± 0.3	73.0 ± 7.0
	Glycitein	43.3	87.0 ± 6.0	73.4 ± 4.9
	Genistein	130	102.6 ± 2.5	85.4 ± 7.8
	Coumestrol	134	79.8 ± 7.7	65.9 ± 3.1
	Formononetin	71.2	99.4 ± 3.0	96.4 ± 1.4
	Biochanin A	71.7	83.8 ± 2.4	85.6 ± 7.1

Data are represented as means ± S.D. ($n = 3$).

genistein was high in mature and immature soybeans, and in soybean sprouts, as compared with the other beans. The content of isoflavonoids varied within the same type of mature bean, including soybeans, immature soybean and soybean sprouts, and the values that we obtained for both soybean and other types of bean were in good agreement with other studies.[10–24]

Composition of Isoflavonoids in Mature Beans, Immature Beans and Sprouts

The composition of isoflavonoids (% of total, molar ratio) in the samples is summarized in Table 5. Data below detection limits (Table 1) were omitted. Glucosides of daidzein, glycitein and genistein (daidzin, glycitin and genistin, respectively) were detected in 11 mature and 2 immature soybeans, and

Table 4. Contents of Isoflavonoids

Agricultural products	Scientific name	Coloured red-purple by hydrolysis	Contents of lipids[a)] [%]	Moisture[a)] [%]
Mature beans (soybean)				
1 Soybean (domestic)	*Glycine max*		19	12.5
2 Soybean (domestic)	*Glycine max*		19	12.5
3 Soybean (China)	*Glycine max*		19.5	12.5
4 Soybean (Australia)	*Glycine max*			
5 Soybean (U.S.A.)	*Glycine max*		21.7	11.7
6 Soybean (Canada)	*Glycine max*			
7 Soybean (China), black and small	*Glycine max*	○		
8 Black soybean (domestic)	*Glycine max*	○	19	12.5
9 Black soybean (Korea)	*Glycine max*	○		
10 Green soybean (domestic)	*Glycine max*		19	12.5
11 Green soybean (Canada)	*Glycine max*			
Mature beans (except soybean)				
1 Kidney bean (U.S.A.)	*Phaseolus vulgaris*		2.2	16.5
2 Kidney bean (domestic), daifuku-mame	*Phaseolus vulgaris*		2.2	16.5
3 Kidney bean (domestic), uzura-mame	*Phaseolus vulgaris*	○	2.2	16.5
4 Kidney bean (domestic), taisho-kintoki	*Phaseolus vulgaris*	○	2.2	16.5
5 Kidney bean (Canada), kintoki-mame	*Phaseolus vulgaris*	○		
6 Scarlet runner bean (domestic), shirohana-mame	*Phaseolus coccineus*		1.7	17.2
7 Lima bean (U.S.A.)	*Phaseous lunatus*		1.8	11.9
8 Lima bean (U.S.A.)	*Phaseous lunatus*		1.8	11.9
9 Lima bean (U.S.A.)	*Phaseous lunatus*		1.8	11.9
10 Butter bean (Myanmar)	*Phaseous lunatus*		1.8	11.9
11 Butter bean (Myanmar)	*Phaseous lunatus*		1.8	11.9
12 Butter bean (Myanmar)	*Phaseous lunatus*		1.8	11.9
13 Saltapia bean (Myanmar)	*Phaseous lunatus*	○		
14 Peas (Canada), yellow	*Pisum sativum*		2.3	13.4
15 Peas (China), green	*Pisum sativum*	○	2.3	13.4
16 Peas (Newzealand), red	*Pisum sativum*	○	2.3	13.4
17 Azuki bean (domestic), dainagon	*Vigna angularis*	○	2.2	15.5
18 Azuki bean (domestic)	*Vigna angularis*	○	2.2	15.5
19 Black mappe (Thailand)	*Vigna mungo*	○		
20 Green gram (China)	*Vigna radiata*	○	1.5	10.8
21 Chick pea (U.S.A.)	*Cices arietinum*		5.2	10.4
22 Broad bean (China)	*Vicia faba*	○	2	13.3
23 Peanuts (South Afica) (defatted with *n*-hexane)	*Arachis hypogaea*	○	47.4	6.2
Immature beans				
1 Soybean (domestic)	*Glycine max*		6.6	69.8
2 Black soybean (domestic)	*Glycine max*	○	6.6	69.8
3 Broad bean (domestic)	*Vicia faba*	○	0.2	68.1
4 Green pea (domestic)	*Pisum sativum*		0.4	76.5
5 Green pea (domestic)	*Pisum sativum*		0.4	76.5
6 Green pea pod (domestic)	*Pisum sativum*	○	0.1	89.8
7 Kidney bean pod (domestic)	*Phaseous vulgaris*		0.1	93.1
Sprouts				
1 Soybean sprout	*Glycine max*		2.2	88.3
2 Soybean sprout	*Glycine max*		2.2	88.3
3 Green pea sprout	*Pisum sativum*			
4 Black mappe sprout	*Vigna mungo*	○		
5 Green gram sprout	*Vigna radiata*	○	0.1	91.6

a) Data are cited from "Standard Tables of Food Composition in Japan." *b*) Data are means ± S.D. for 3 trials. *c*) $n = 1$. *d*) ND, not detected.

Table 4. Continued

Total isoflavonoids (with acid hydrolysis)[b]					
Daidzein MW254.24	Glycitein MW284.27	Genistein MW270.24	Formononetin MW268.27	Biochanin A MW284.27	Coumestrol MW268.23
[μg/g fresh weight]					
725.3 ± 30.3	49.4 ± 5.7	986.7 ± 70.2	ND	ND	ND
904.1[c]	59.8[b]	1567[b]	ND	ND	ND
889.9 ± 39.8	150.8 ± 15.3	677.5 ± 89.7	ND	ND	ND
492.6 ± 6.3	72.9 ± 6.1	495.4 ± 18.3	ND	ND	ND
528.4 ± 58.0	84.1 ± 15.6	454.1 ± 14.6	ND	ND	ND
701.0 ± 30.6	81.8 ± 11.9	719.6 ± 79.0	ND	ND	ND
550.3 ± 75.6	90.9 ± 10.5	433.5 ± 81.9	ND	ND	ND
382.0 ± 75.1	27.0 ± 2.9	570.3 ± 109.5	ND	ND	ND
910.2 ± 14.0	48.3 ± 4.6	931.9 ± 22.6	ND	ND	ND
460.0 ± 21.2	37.1 ± 6.5	1118 ± 162.2	ND	ND	ND
536.9 ± 73.9	72.6 ± 13.6	514.7 ± 64.2	ND	ND	ND
[μg/g fresh weight]					
ND[d]	ND	ND	ND	ND	ND
ND	ND	ND	ND	ND	ND
ND	ND	ND	ND	ND	ND
ND	ND	ND	ND	ND	ND
ND	ND	ND	ND	trace	ND
ND	ND	ND	ND	ND	ND
ND	ND	ND	9.59 ± 0.24	trace	ND
ND	ND	ND	2.91 ± 0.89	trace	ND
ND	ND	ND	4.21 ± 0.10	trace	ND
ND	ND	ND	4.12 ± 0.73	trace	ND
ND	ND	ND	3.47 ± 0.18	trace	ND
ND	ND	ND	3.76 ± 0.77	trace	ND
ND	ND	ND	6.84 ± 0.09	ND	ND
ND	ND	ND	ND	ND	ND
ND	ND	ND	ND	ND	ND
ND	ND	ND	ND	ND	ND
4.97 ± 0.11	ND	3.55 ± 0.45	ND	ND	ND
5.70 ± 0.59	ND	3.44 ± 0.85	ND	ND	ND
ND	ND	ND	ND	ND	ND
ND	ND	ND	ND	ND	ND
6.50 ± 0.56	ND	ND	3.60 ± 0.24	25.18 ± 0.40	ND
ND	ND	ND	ND	4.62 ± 0.76	ND
ND	ND	ND	ND	ND	ND
[μg/g fresh weight]					
64.14 ± 0.87	23.30 ± 1.05	36.37 ± 7.96	ND	ND	ND
173.8 ± 6.9	67.16 ± 0.31	62.27 ± 3.13	ND	ND	ND
ND	ND	ND	ND	ND	ND
ND	ND	ND	ND	ND	ND
ND	ND	ND	ND	ND	ND
ND	ND	ND	ND	ND	ND
ND	ND	ND	ND	ND	ND
[μg/g fresh weight]					
67.80 ± 4.23	6.09 ± 0.46	94.04 ± 3.84	ND	ND	4.36 ± 0.15
31.14 ± 1.52	10.47 ± 1.07	49.89 ± 2.62	ND	ND	ND
ND	ND	ND	ND	ND	ND
ND	ND	ND	ND	ND	21.04 ± 0.20
ND	ND	ND	ND	ND	15.91 ± 1.17

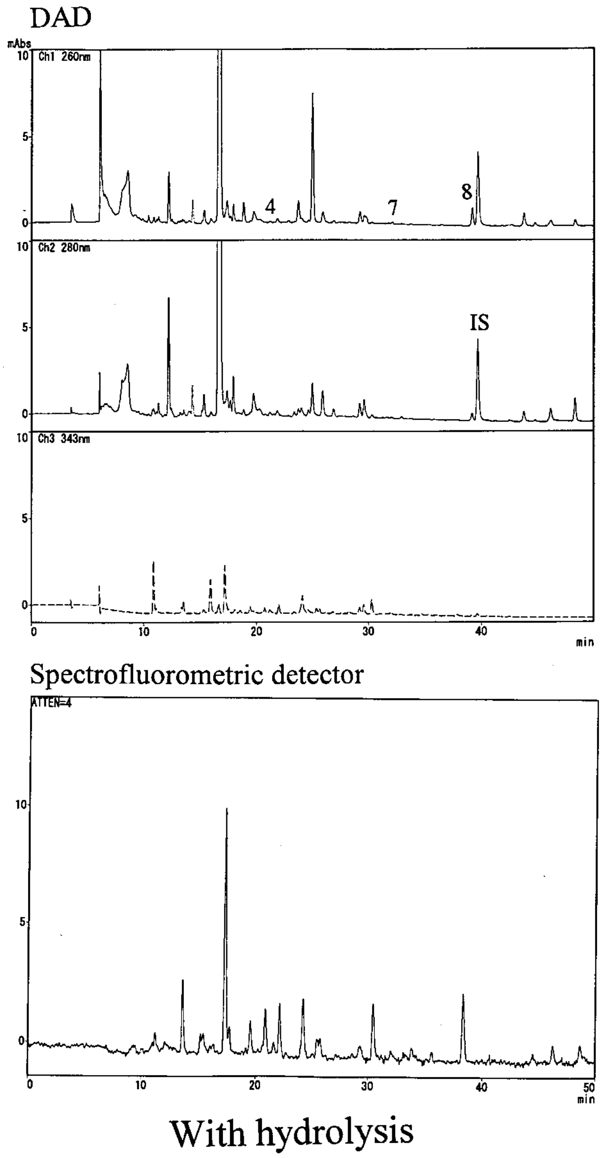

Fig. 3-1. HPLC Chromatograms of the Test Solutions of Mature Chick Pea (#21) with Hydrolysis

Analysis method and conditions of HPLC are described in the text. Peaks are 4, daidzein; 7, formononetin; 8, biochanin A, and IS, flavone.

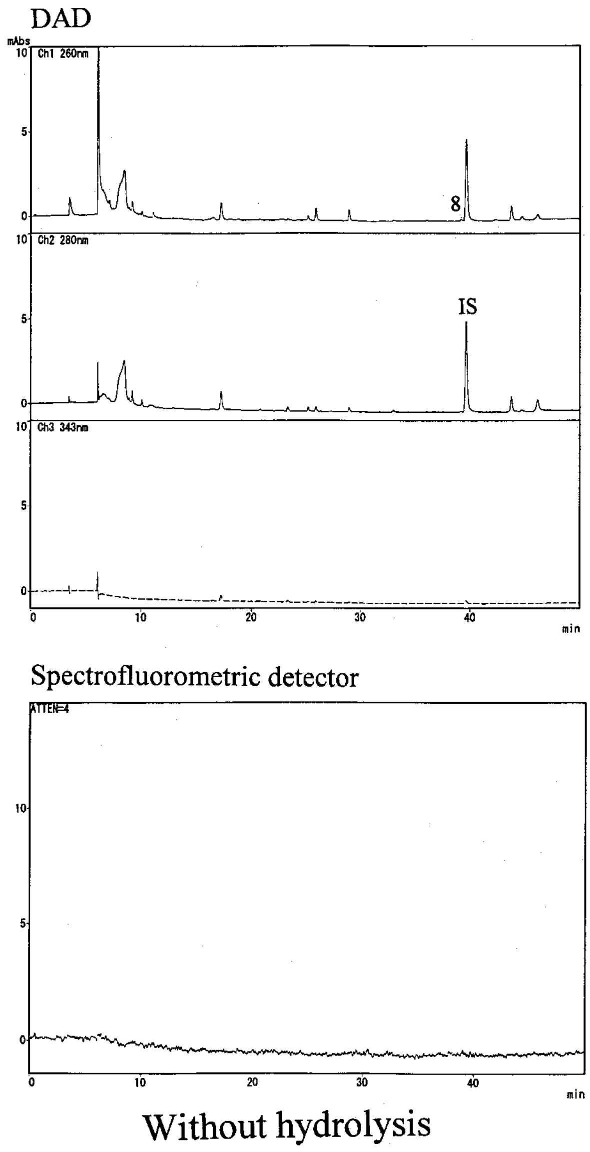

Fig. 3-2. HPLC Chromatograms of the Test Solutions of Mature Chick Pea (#21) without Hydrolysis

Analysis method and conditions of HPLC are described in the text. Peaks are 8, biochanin A, and IS, flavone.

2 soybean sprouts. Aglycones were detected in 11 mature soybeans, chick pea (#21), 2 immature soybeans, sprouts of 2 soybean (#1–2), black mappe (#4) and green gram (#5). Composition varied within the same type of bean, as was found for the total content (Table 4). In 11 mature soybeans (#1–11), the glucosides and aglycones of daidzein, glycitein and genistein accounted for 19.99–47.71% and 1.3–12.99%, respectively. Small amounts of formononetin were detected in 3 mature lima beans (#7–9), butter beans (#10–12) and Saltapia bean (#13), but no aglycones were detected. Small amounts of daidzein and genistein were detected in mature Azuki-beans (#17–18), but no aglycones were detected. In mature chick pea (#21), biochanin A and small amounts of daidzein and formononetin were detected, and 12.48% of biochanin A was present as the aglycone. A small amount of biochanin A was detected in broad bean (#22), but the aglycone was not detected. In immature soybeans (#1 and 2), the glucosides and aglycones of daidzein, glycitein and genistein accounted for 16.79–31.37% and 10.31–12.03%, respectively. In soybean sprouts (#1 and 2), the glucosides and aglycones of daidzein, glycitein and genistein accounted for 11.61–17.46% and 16.24–34.32%, respectively. For the bean

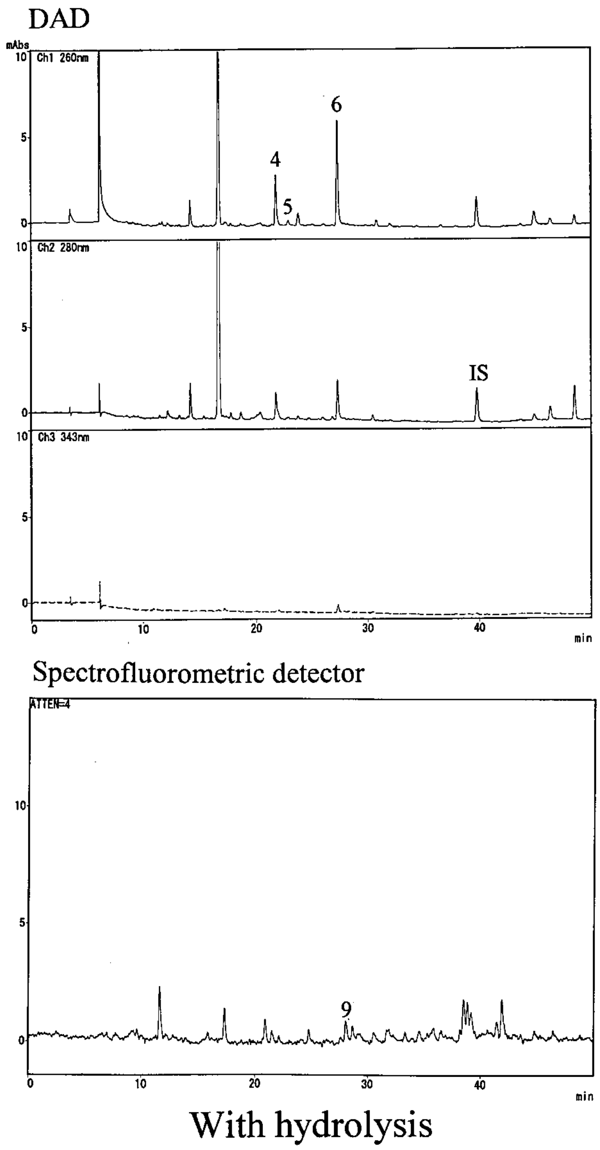

Fig. 4-1. HPLC Chromatograms of the Test Solutions of Soybean Sprout (#1) with Hydrolysis

Analysis method and conditions of HPLC are described in the text. Peaks are 4, daidzein; 5, glycitein; 6, genistein; 9, coumestrol, and IS, flavone.

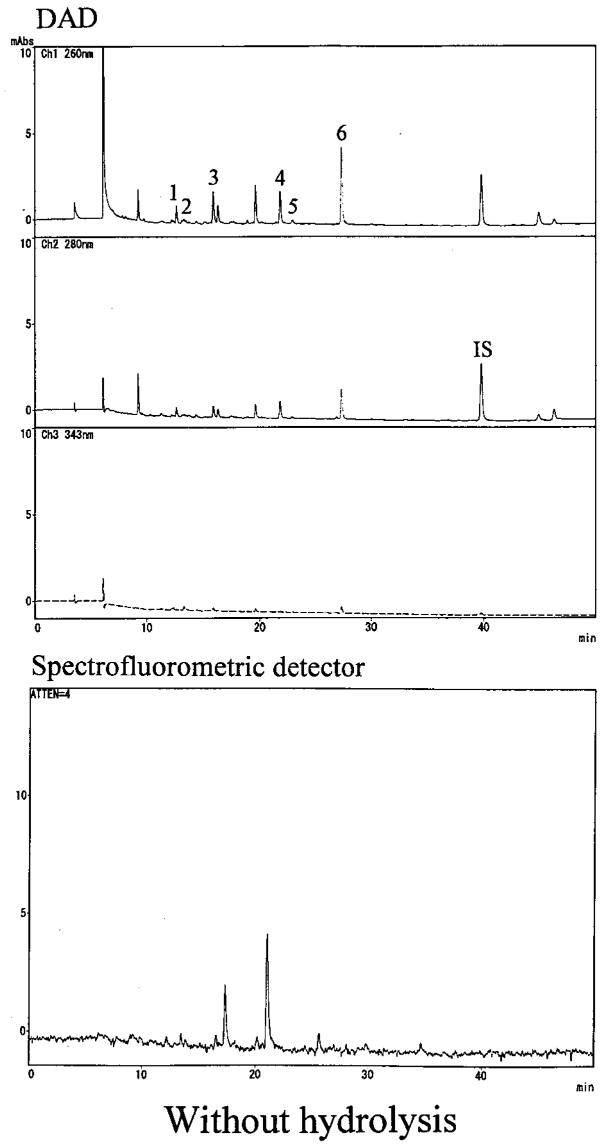

Fig. 4-2. HPLC Chromatograms of the Test Solutions of Soybean Sprout (#1) without Hydrolysis

Analysis method and conditions of HPLC are described in the text. Peaks are 1, daidzin; 2, glycitin; 3, genistin; 4, daidzein; 5, glycitein; 6, and IS, flavone.

sprouts, coumestrol was detected only in soybean sprout (#1), black mappe sprout (#4) and green gram sprout (#5), and coumestrol was not detected in the corresponding mature beans. In black mappe sprout (#4) and green gram sprout (#5), 27.58–27.7% of coumestrol was present as the aglycone.

The total isoflavonoid content seemed to be different for the three growth stages of soybeans (Table 4); however, after correction for moisture content, the difference was not found to be significant [1-way analysis of variance (ANOVA), Scheff s multiple comparison test]. The composition of isoflavonoids differed significantly in the three growth stages of soybeans (Table 5). The percentage of aglycones decreased from the sprout to the immature bean to the mature bean ($p < 0.001$), whereas the percentage of glucosides increased in the reverse order ($p < 0.05$; 1-way ANOVA). The biological activity of isoflavonoids is stronger in the aglycone form than in the glycoside form. It may be that isoflavonoids such as daidzein, glycitein and genistein have a specific role during the growth stages of soybeans.

The Japanese daily intake of pulses during the past 9 years has varied only between 65.9 and 72.5 g, of which 64.1–70.4 g is derived from soybeans and

Table 5. Compositions of Isoflavonoids (% of Total)

Agricultural products	Composition of isoflavonoids [%], molar ratio Total isoflavonoids (analyzed with acid hydrolysis)					
	Daidzein	Glycitein	Genistein	Formononetin	Biochanin A	Coumestrol
Mature beans (soybean)						
1 Soybean (domestic)	42.7	2.6	54.7	—[a]	—	—
2 Soybean (domestic)	37.2	2.2	60.6	—	—	—
3 Soybean (China)	53.5	8.1	38.4	—	—	—
4 Soybean (Australia)	48.1	6.4	45.5	—	—	—
5 Soybean (U.S.A.)	51.3	7.3	41.4	—	—	—
6 Soybean (Canada)	48.3	5.0	46.7	—	—	—
7 Soybean (China), black, small	52.9	7.8	39.2	—	—	—
8 Black soybean (domestic)	40.5	2.6	56.9	—	—	—
9 Black soybean (Korea)	49.7	2.4	47.9	—	—	—
10 Green soybean (domestic)	29.8	2.2	68.1	—	—	—
11 Green soybean (Canada)	50.7	3.6	45.7	—	—	—
Mature beans (except soybean)						
1 Kidney bean (U.S.A.)	—	—	—	—	—	—
2 Kidney bean (domestic), daifuku-mame	—	—	—	—	—	—
3 Kidney bean (domestic), uzura-mame	—	—	—	—	—	—
4 Kidney bean (domestic), taisho-kintoki	—	—	—	—	—	—
5 Kidney bean (Canada), kintoki-mame	—	—	—	—	—	—
6 Scarlet runner bean (domestic), shirohana-mame	—	—	—	—	—	—
7 Lima bean (U.S.A.)	—	—	—	100	—	—
8 Lima bean (U.S.A.)	—	—	—	100	—	—
9 Lima bean (U.S.A.)	—	—	—	100	—	—
10 Butter bean (Myanmar)	—	—	—	100	—	—
11 Butter bean (Myanmar)	—	—	—	100	—	—
12 Butter bean (Myanmar)	—	—	—	100	—	—
13 Saltapia bean (Myanmar)	—	—	—	100	—	—
14 Peas (Canada), yellow	—	—	—	—	—	—
15 Peas (China), green	—	—	—	—	—	—
16 Peas (Newzealand), red	—	—	—	—	—	—
17 Azuki bean (domestic), dainagon	58.3	—	41.7	—	—	—
18 Azuki bean (domestic)	62.4	—	37.6	—	—	—
19 Black mappe (Thailand)	—	—	—	—	—	—
20 Green gram (China)	—	—	—	—	—	—
21 Chick pea (U.S.A.)	18.4	—	—	10.2	71.4	—
22 Broad bean (China)	—	—	—	—	100	—
23 Peanuts (South Afica) (defatted with *n*-hexane)	—	—	—	—	—	—
Immature beans						
1 Soybean (domestic)	53.8	17.5	28.7	—	—	—
2 Black soybean (domestic)	57.3	22.2	20.5	—	—	—
3 Broad bean (domestic)	—	—	—	—	—	—
4 Green pea (domestic)	—	—	—	—	—	—
5 Green pea (domestic)	—	—	—	—	—	—
6 Green pea pod (domestic)	—	—	—	—	—	—
7 Kidney bean pod (domestic)	—	—	—	—	—	—
Sprouts						
1 Soybean sprout	40.1	3.6	55.6	—	—	0.8
2 Soybean sprout	34.0	11.4	54.5	—	—	—
3 Green pea sprout	—	—	—	—	—	—
4 Black mappe sprout	—	—	—	—	—	100
5 Green gram sprout	—	—	—	—	—	100

a) Not detected or trace amounts was detected (below detection limit).

Table 5. Continued

Glucosides of daidzein, glycitein and genistein				Aglycone						
Daidzin	Glycitin	Genistin	Total	Daidzein	Glycitein	Genistein	Formononetin	Biochanin A	Coumestrol	Total
19.3	1.2	27.3	47.7	6.5	0.2	6.3	—	—	—	13.0
7.1	1.1	11.9	20.0	0.6	—	0.8	—	—	—	1.4
9.9	0.7	11.6	22.2	1.6	—	1.3	—	—	—	2.9
14.4	3.1	21.5	39.0	1.4	0.2	1.5	—	—	—	3.1
14.8	3.3	19.2	37.2	1.3	0.2	1.2	—	—	—	2.7
11.1	1.6	13.5	26.2	0.6	0.1	0.6	—	—	—	1.4
17.9	3.8	25.4	47.1	2.2	0.3	2.1	—	—	—	4.6
13.2	1.6	27.9	42.7	3.5	—	4.9	—	—	—	8.4
15.8	1.1	17.9	34.8	4.1	0.2	3.4	—	—	—	7.8
7.5	0.4	22.7	30.7	0.3	—	1.1	—	—	—	1.3
12.5	1.5	14.5	28.5	0.9	—	0.8	—	—	—	1.7
—	—	—	—	—	—	—	—	—	—	—
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—	—	—	—	—	—	—	—	12.5	—	12.5
—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—
12.2	6.7	12.5	31.4	5.3	1.5	5.3	—	—	—	12.0
7.1	6.3	3.4	16.8	6.0	2.2	2.1	—	—	—	10.3
—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—
4.2	1.1	6.3	11.6	11.4	1.6	21.4	—	—	—	34.3
6.7	0.7	10.1	17.5	5.0	5.6	5.6	—	—	—	16.2
—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	27.6	27.6
—	—	—	—	—	—	—	—	—	27.7	27.7

soybean-derived products, and 1.8–2.6 g is derived from other beans.[26] Here we found that the largest content of isoflavonoids in pulses other than soybeans was in the mature chick pea (#21), but this value was less than 1/27 of that in mature soybeans (Table 4). We conclude that the Japanese daily intake of isoflavonoids from beans other than soybeans is less than 0.5% of the total isoflavonoid intake from pulses, and thus could be neglected when evaluating phytoestrogen intake in the Japanese diet.

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