Isoflavones in Retail and Institutional Soy Foods

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A national sampling plan was developed to select the most widely used isoflavone-containing foods in the United States. Foods were selected based on their retail volume and sampled in five geographical areas representing seven metropolitan areas. Isoflavones were analyzed from composite samples, raw and cooked, and reported by brand. Quality control measures were evaluated throughout the study. Isoflavone levels ranged from 1 μg/g in soy sauces to 540 μg/g in tempeh. Soymilk and tofu represented the major portion of soy foods evaluated. These data will appear in the electronic version of USDA Handbook No. 8 of Food Composition Data in 1999.

Keywords: *Phytoestrogens; genistein; glycitein; daidzein; biochanin A; formononetin*

INTRODUCTION

Phytochemicals are reported to have a number of health protective effects. Isoflavones from soybeans and several other legumes are classified as phytoestrogens in many literature citations due to their weak estrogenic activity in mammalian systems. A number of epidemiological studies have suggested that consumption of soybeans and soy foods is associated with lowered risks for several cancers, including breast, prostate, and colon (Messina et al., 1997; Messina, 1995), cardiovascular diseases (Schultz, 1998; Anderson et al., 1995; Anthony et al., 1996, 1997), and bone health (Barham et al., 1996). The mechanisms for these effects remains to be delineated. The beneficial effects of isoflavones may be due, but only in part, to the weak estrogenic activity of the isoflavones. Genistein, daidzein, glycitein, and their glucoside forms are 100 000−500 000 times less potent estrogens than estradiol in vivo (Farmakalidis et al., 1985; Song et al., 1999). The interest in soybean components has been so strong that two international conferences have been convened to report the state of knowledge in the field (Messina, 1995; Setchell, 1998). Therefore, there is major interest in studying the biological effects of phytoestrogens. In contrast, our knowledge of the levels of these components in foods is limited.

Fortunately, the isoflavones are confined to a few plant foods consumed by humans (Harborne, 1994). The major source of phytoestrogens in human diets are isoflavones in soybeans and soy foods. Isoflavones are found in alfalfa and clover seeds, usually consumed as sprouts, chick peas, or garbanzo beans, and some pulses (Reinli and Block, 1996). The estrogenic isoflavones found in soy are genistein, daidzein, and glycitein predominately as their glucosides and malonylglucosides but also as acetylglucosides and aglycons (Wang and Murphy, 1994a,b). The phytoestrogens found in the other legumes are biochanin A, formononetin, and coumesterol. Recently, Reinli and Block (1996) summarized 22 reviewed studies to create a phytoestrogen database, although glycitein data was excluded. Mazur et al. (1998) reported isoflavone concentrations from a variety of legume seeds. Of those consumed as human foods, only soybeans had significant isoflavone levels. Coward et al. (1998) recently reported the effect of baking and frying on isoflavone distribution in several soyfoods. Krishnan (1998) has reported significant levels of genistein in the American groundnut (*Apios americana* Medikus), which was a food source for native Americans and early European settlers in the northeastern United States. Our development of a database on isoflavone levels in human foods has been simplified by segregation of isoflavones into a few food plant species. To provide a functional database for clinicians, dietitians, food scientists, and consumers, we attempted to analyze the major retail soy foods in the U.S. food supply for isoflavones.

The three isoflavones in soybeans and soy products (genistein, daidzein, and glycitein) occur in four possible forms, the aglycon, the glucoside, the malonylglucoside, and the acetylglucoside (Wang and Murphy, 1994a,b). The bioavailability of the isoflavones is apparently affected by the gut microflora of the consumer (Xu et al., 1994). There does not appear to be a difference between the bioavailability of the glucosides or aglycon (Farmakalidis et al., 1985; Xu, 1995). The effects of processing alter the distribution of the forms and can result in the loss of some isoflavones through leaching and removal of undesirable fractions. The distribution of the isoflavone yields a picture of the processing history of a particular soy product (Wang and Murphy, 1996). We have developed a routine HPLC method to evaluate isoflavones in soy products (Murphy et al., 1997) and have employed it to examine systematically the isoflavone levels in retail and institutional isoflavone-containing foods sampled nationally based on retail sales volume.

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MATERIALS AND METHODS

Samples. Isoflavone-containing foods were selected based on market share volume and expected isoflavone content. Food samples included soymilk, tofu, miso, soy sauce, tempeh, soybean (vegetable) oil, alfalfa sprouts, and chicken and beef analogues. Isoflavone analysis was conducted on composites and reported by brand. Products were composited one of three ways: on a nationwide basis; by city; or by region. Nationwide composites were formed by combining all units of a food from all cities to yield one sample. An aliquot was removed and analyzed for isoflavones to represent the entire nation. City composites were formed by combining all units of a given food purchased in one particular city to yield one sample from which an aliquot was analyzed. Several products were composited by region to yield more information than a nationwide composite and yet reduce the number of analytical samples required for city composites. Cities were grouped into regions (east, central, and west). By region, each brand had three analytical values representing each region of the United States.

Lot to lot variation was examined in soymilk. Four containers of Eden Soy milk, two representing one lot and two representing a different lot, were purchased in the Baltimore/Washington area, composited by lot, and analyzed for isoflavones.

Sample Handling. Most foods were shipped to the Food Composition Laboratory, ARS, USDA, at Beltsville, MD. Equal weights of individuals foods were composited and homogenized in a Robot Coupe R-2 for 2−3 min depending on food properties. Selected foods were analyzed in raw and in cooked (according to package directions) state. Soy/beef hamburger patties had no cooking instructions and were cooked on open skillet to an internal temperature 140 °F. All tofus, except for a small subsample of the leading brand, were analyzed raw. The leading brand of tofu was analyzed cooked (steamed) and raw. Homogeneous aliquots of each homogenized composite were placed in 125-mL sample cups and stored at −60 °C. Frozen samples were shipped on dry ice to Iowa State University for isoflavone analysis. For each food, two reserve samples were kept (at −60 °C) at the Food Composition Laboratory. All samples were given a unique letter and numeric code.

Standards. All chemicals were reagent grade from Fisher Scientific unless noted otherwise. Milli-Q system (Millipore Co., Bedford, MA) HPLC-grade water was used. Nine isoflavone and one coumestan standards were used in the calibration curves. Genistein, daidzein, genistin, daidzin, acetylgenistin, glycitin, and glycitein were isolated or synthesized and purified as described previously (Murphy et al., 1997). Biochanin A and formononetin were from Sigma Chemical Co. (St. Louis, MO). Coumesterol was from ACROS Organics (a division of Fisher Scientific, Pittsburgh, PA). The internal standard, 2,4,4′-trihydroxydeoxybenzoin (THB), was synthesized as described previously (Murphy et al., 1997; Song et al., 1998).

Isoflavone standard solutions were prepared based on UV maximum absorbance and molar extinction coefficients (Ollis, 1962). The purity of the standards was based on the percentage peak area according to the Beckman System Gold software, and final concentrations were adjusted on the basis on these purities. For isoflavones without pure standards, the malonyl forms and acetyldaidzin and acetylglycitin, the appropriate standard curves were adjusted on the basis of molecular weight differences as confirmed by our previous work with most of these standards (Wang and Murphy, 1994a,b). Stock solutions were stored at −15 °C and thoroughly warmed and redissolved at room temperature for 2 h to ensure complete solubility.

Quality Control. Quality control measures included the following: analysis of authentic standards each day food samples were analyzed; recoveries of genistin, genistein, daidzein, and THB on five different soyfood matrices were conducted monthly; and analysis of four reference materials weekly over the duration of this project (24 months). Internal and external standards were added to the dry matrix as 200-μL aliquots of a 400 μg/mL THB solution and/or 50−700 μL of the appropriate isoflavone, depending on isoflavone concentration in the food matrix, from stock solutions in methanol of daidzein (800 μg/mL), genistein (1600 μg/mL), or genistin (600 μg/mL). The samples were mixed thoroughly and allowed to air-dry at room temperature to evaporate the methanol. Recovery samples were then processed as described below for samples. Precision and accuracy estimates were made based on these quality control measures. Any significant deviations (>5% for standards, >8% for reference materials) from baseline of these reference materials would lead to system inspection and correction before any further samples would be analyzed. All data were corrected for recovery based on internal standard.

Isoflavone Analysis. The moisture content of all samples was determined by vacuum oven-drying (AOAC, 1997). Foods were extracted as 2-g samples, either as is, if food could be ground in a coffee mill to a free-flowing powder, or as a freeze-dried sample followed by grinding in a coffee mill, in 10 mL of acetonitrile, 2 mL of 0.1 N HCl, and an amount of water that was optimized depending on the food matrix in a 125-mL screw-top Erlenmeyer flask with stirring for 2 h at room temperature. The extracts were filtered and taken to dryness on a rotary evaporator at <30 °C. The residues were dissolved in 80% HPLC-grade methanol. An aliquot was filtered through a 0.45-μm PTFE filter (Alltech Associates, Deerfield, IL) and analyzed by HPLC within 10 h of extraction to minimize malonylglucoside to glucoside interconversion. We observed that 0.2% of total moles of daidzein and 0.3% of total moles of genistein convert from malonylglucoside to glucoside per hour at room temperature. Soybean isoflavone HPLC analysis was performed as reported previously (Murphy et al., 1997). All isoflavone peaks were rescanned from 200 to 350 nm with Beckman System Gold software to confirm UV spectra match with isoflavone identity. Alfalfa isoflavone and coumsterol HPLC analysis was a modification of Murphy et al. (1997). The HPLC gradient was the same as Murphy et al. (1997) through 35% acetonitrile. Then, instead of recycling to zero time, the gradient increased to 50% acetonitrile for 8 min prior to recycling back to 15% acetonitrile at zero time. Formononetin, biochanin A, and coumesterol were monitored at 262 nm and scanned from 200 to 350 nm. Alfalfa isoflavone and coumesterol recoveries were corrected with internal standard, THB.

Statistical Analysis. Statistical evaluation was conducted using the General Linear Models procedure and

Student's *t* test with SAS package (version 6.03, 1995, Cary, NC). Differences were considered significant if $p \leq 0.05$.

RESULTS AND DISCUSSION

Food Sampling. Foods representing different categories of soy products were selected for isoflavone analysis based on the use in the human diet, particularly vegetarian and ethnic populations, and the availability at the retail level. Unique soy products such as tofu, miso, and tempeh were included in this study. Alfalfa sprouts were included as the only predominant non-soy food that contains isoflavones. The majority of the products were purchased from cities representing five geographic regions of the United States (northeast, southeast, north central, southwest, and west). The seven cities selected for sample pickup were identified either as major population centers having the highest grocery sales for their representative region (Toth et al., 1995a) or as cities with high densities of vegetarians. The seven cities selected were New York; Baltimore/Washington, DC; Tampa; Kansas City; Houston; Los Angeles; and Denver.

Market share and sales volume data were utilized to identify the highest volume supermarket chains within each city (Toth et al., 1995a). Whenever possible, foods were purchased in the supermarket chain having the highest grocery sales for a particular city. Top chains for the seven cities were Baltimore/Washington, Giant Foods; New York, Pathmark/Waldbaum; Tampa, Publix; Kansas City, Price Chopper; Denver, King Sooper; Houston, Kroger; and Los Angeles, Vons (Toth et al., 1995a). If a food item was not available in the supermarkets, the product was purchased from the leading health food chains or ethnic markets in each city. National chains, such as Whole Foods, Wild Oats, and Fresh Fields, were used whenever possible. Soy/beef patties used in the school lunch program and the military were purchased directly from the manufacturer.

National brands representing the different categories of soy products were chosen using sales volume data obtained from A. C. Nielsen Co. Only store brands were purchased for vegetable cooking oil. Kroger and American Stores Co. were selected to represent store brands based on highest overall grocery sales (Toth et al., 1995b). Samples representing two different lot numbers were purchased at Kroger in Houston and Jewel (division of American Stores Co.) in Chicago.

The number of samples for any given food purchased in each store varied by product. In most instances, two units, each representing a different lot number, were purchased in the designated store in each city. Only one unit was purchased in each store for foods thought to contain little or no isoflavones. Due to the uncertainty of levels in alfalfa sprouts, three units were purchased in each store in each city. All products were purchased over an 18-month period beginning March 1995.

Extraction Optimization. Extraction conditions were optimized for each soy matrix. The amount of water included in the acetonitrile/0.1 N HCl/water extraction mixture had a significant effect of the amount of isoflavone extracted and varied with food extracted. Figure 1 presents optimization for total genistein and total daidzein extraction in soybeans, soy isolate, tofu, and miso. The reproducibility of the extraction

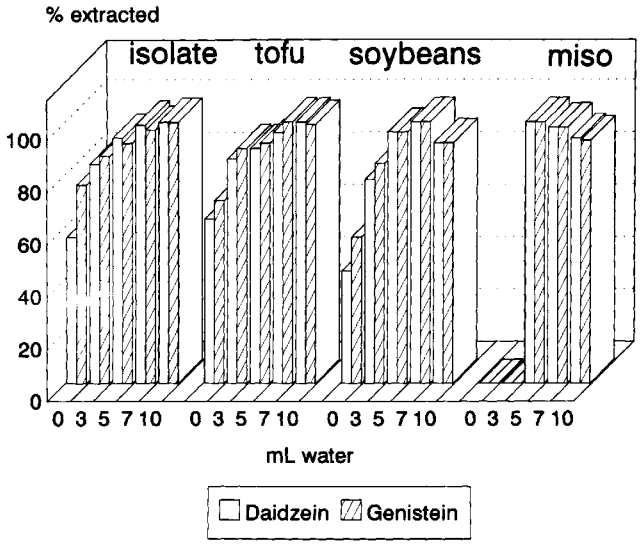

Figure 1. Soy isoflavone extraction optimization for soy isolate, soy flour, tofu, and miso. All extractions were replicated 2−8 times.

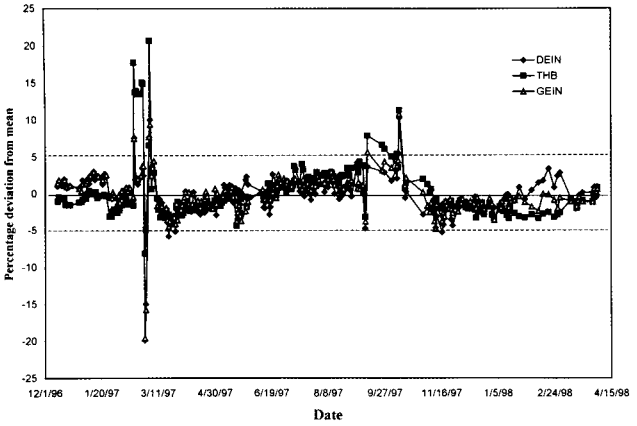

Figure 2. Quality control analysis of standards in routine HPLC analysis of soybean isoflavones. DEIN = daidzein; THB = 2,4,4′-trihydroxydeoxybenzoin; GEIN = genistein.

increased as the amount of individual isoflavone extracted reached a maximum level. The standard deviation of the maximum extraction percentage ranged from 0.6 to 4.8% and from 0.2 to 3.5% for total daidzein and total genistein, respectively, for the four food matrices evaluated. Glycitein followed a similar pattern. Optimum water varied between 5 and 10 mL depending on the matrix. Miso yielded no isoflavone extraction until the water level reached 5 mL because high salt content of the miso matrix resulted in a miso gum-like ball upon acetonitrile addition at lower water concentrations.

Database Development and Quality Control. Analysis of isoflavones was performed by HPLC separation and quantification by photodiode array detection of the 12 isoflavones found in soy products and of biochanin A, formononetin, and coumesterol in alfalfa and clover sprouts. Routine quality control and documentation is critical to developing a valid database (Mangels et al., 1993). Quality control measures were routinely performed throughout the isoflavone analysis period. These included analysis of isoflavone standards every day samples were run (Figure 2); accuracy estimations by recovery of external and internal standards in five soy food matrices monthly (Table 1); and precision estimation by evaluation of coefficient of variation

Table 1. Recovery of Genistin, Genistein, Daidzein, and 2,4,4′-Trihydroxydeoxybenzoin (THB) in Soy Foods

	recovery analyte							
	daidzein		genistein		genistin		THB	
food	X + SD[a]	CV[b]	X + SD	CV	X + SD	CV	X + SD	CV
n	25		25		8		21	
TVP	90 ± 9	10	91 ± 6	7	98 ± 6	6	94 ± 5	5
soymilk	88 ± 8	9	90 ± 11	13	99 ± 7	7	95 ± 8	8
soybean	81 ± 16	20	95 ± 10	10	99 ± 4	4	98 ± 5	5
tofu	92 ± 8	8	91 ± 9	10	94 ± 4	4	95 ± 5	5
tempeh	90 ± 10	11	87 ± 17	19	96 ± 6	6	95 ± 4	4

[a] X = average; SD = standard deviation. [b] CV = coefficient of variation.

Table 2. Within-Day and Between-Day Precision of Isoflavone Analysis[a]

		coefficient of variation (%)	
isoflavone	mean (μg/g)	within day[b]	between day[c]
daidzin	188 ± 6	4.3 ± 3.7	9.6 ± 4.9
glycitin	52 ± 5	3.8 ± 1.9	6.9 ± 2.5
genistin	223 ± 10	4.8 ± 3.4	11.0 ± 6.0
malonyldaidzin	1430 ± 29	3.2 ± 1.9	4.5 ± 2.9
malonylglycitin	131 ± 3	3.8 ± 2.3	5.1 ± 2.8
malonylgenistin	1379 ± 26	3.0 ± 1.8	5.2 ± 4.4
acetylgenistin[d]	28 ± 1	2.0 ± 1.9	3.7 ± 3.0
daidzein	21 ± 1	3.2 ± 2.0	7.0 ± 3.5
genistein	19 ± 1	2.7 ± 1.7	5.3 ± 2.5
total daidzein	860 ± 17	2.9 ± 1.6	3.8 ± 2.5
total glycitein	103 ± 4	2.7 ± 1.6	4.6 ± 3.6
total genistein	893 ± 18	3.3 ± 1.5	4.6 ± 2.1

[a] Soybean flour stored −29 °C over 24 months. [b] n = 47. [c] n = 24. [d] Acetyldaidzin, acetylglycitin, and glycitein not detected.

for four food matrices for within-day and between-day variance bimonthly (Table 2).

In Figure 2, the importance of this quality control check is evident for running daily standards every time samples are analyzed to confirm that the HPLC system is operating correctly. The first major positive deviation from the mean in mid-February 1997 represented autosampler failure. Any samples run on these days were rerun after the instrumentation problem was corrected. The second deviation from the mean, both negative and positive, was on days when laboratory room heating failed and then exceeded column heater temperature. The third major deviation occurred in mid-October 1997 resulting from HPLC pump failure. Although the second deviation from our standard means would be evident to anyone working in the laboratory, the first and third were not as obvious without this internal quality control measure.

The recovery of external standards (genistin, genistein and daidzein) were reasonable (Table 1). Recoveries were always higher and more consistent (lower coefficients of variation) for the glucoside, genistin, than for the aglycones, genistein and daidzein. These recovery differences were anticipated since our extraction scheme is optimized to extract the predominate forms of isoflavones in most soy matrices, the glucosides. The glucoside forms (malonylglucoside, acetylglucoside, and underivatized glucoside) account for >95% of the isoflavanoid forms in soy-based foods except for foods that are highly fermented such as miso and soy sauce. Recovery of our internal standard, THB, was almost as consistent as genistin. Multiple recovery levels of both internal and external standards were linear over the range of concentrations found in soybeans and soy foods (data not shown).

Table 2 presents an example of a bimonthly precision estimate for isoflavone analysis of one soy matrix stored over the duration of the project. No differences were seen in precision between the two soy matrices, full fat soy flour and freeze-dried soymilk, stored at room temperature (data not shown) and at −29 °C over the duration of the project. Additionally, no changes were observed in isoflavone form distribution during storage at room temperature nor at −29 °C (data not shown). Excellent precision was observed for all within-day estimates. Genistin and daidzin but not malonylgenistin and malonyldaidzin deviated outside of our 8% CV limits for between-day % CV. Genistin and daidzin represented 14 and 11% of each total in this matrix. However, total genistein and total daidzein deviated by less than 5% CV. The total individual isoflavone amounts are the more important dietary component values in the database. These four soy foods represent our standard reference materials. Additionally, all four matrices show that total isoflavone extraction was constant over 24 months at room temperature and at −29 °C.

Data Compilation. Data compilation was presented two ways. Individual isoflavone contents were measured for all 12 forms found in soy. Since isoflavones are absorbed as the aglycon, the total concentration of isoflavones in food products should not be expressed as the arithmetic sum of the individual forms. The molecular weight of the glucosides is 1.6−1.9 greater than the aglycon. Ideally, the molar concentration of isoflavones could be used. However, since soy products are used by consumers not familiar with scientific units, mass concentrations units (mg/g and μg/g) are already used on some retail soy products. Total isoflavone contents were adjusted for the molecular weight differences. Total isoflavones are the sum of the adjusted sums of total genistein + total daidzein + total glycitein.

Soyfoods. Soybean oil was evaluated for isoflavone levels to demonstrate that it is not a source of isoflavones in U.S. diets. Two national brands, Wesson vegetable oil and Crisco all natural pure vegetable oil, were sampled nationally in seven cities and composited. Two bottles of different lot numbers for two store brands, Kroger vegetable oil and Jewel vegetable oil, were purchased in Chicago and composited by brand. Samples were extracted directly with ACN−water−acid mixture. Internal and external standards were extracted within acceptable limits. No isoflavones were detected in any of the soybean oils. Commercial processing of soybean oil does not allow for any carryover of isoflavones from the protein meal fraction. Vegetable oils are not a source of isoflavones.

Soy milk isoflavone levels are presented in Table 3. Two brands of soymilk were selected for analysis based on market share. The aseptically processed product by Eden Soy and the pasteurized soy milk by White Wave represented the two types of commercially available soymilks. The aseptic product was purchased in the seven cities in regional chain stores. The pasteurized product was available in health food store chains. Each sample was the result of a composite of two different lot numbers, and two replicate samples were extracted and analyzed by HPLC. A composite of two lots of Washington, DC, Eden soymilk was used for comparison with intercity samples.

The Eden soymilk had significant differences by lot (city) in total isoflavones, individual totals, and indi-

Table 3. Isoflavone Content of Soymilk (μg/g Wet Weight)

		glucosides			malonylglucoside			acetylglucoside			aglycon			total[e]			
soymilk	% M[a]	D[b]	G	Gl	D	G	Gl	D	G	Gl	Dein	Gein	Glein	Dein	Gein	Glein	total
Aseptically Processed																	
DC[c]	86	50^{D}	78^{C}	6^{D}	11^{CD}	17^{ABC}	0^{D}	0	0^{E}	0	2^{AB}	1^{B}	0	38^{B}	59^{BC}	4^{C}	101^{C}
HO	90	49^{D}	71^{C}	8^{D}	8^{DE}	12^{CD}	2^{C}	0	4^{D}	0	1^{B}	1^{B}	0	36^{B}	54^{C}	6^{C}	96^{C}
TA	86	54^{CD}	70^{C}	14^{ABC}	18^{B}	22^{A}	4^{A}	0	7^{BC}	0	2^{A}	2^{A}	0	44^{B}	61^{BC}	11^{A}	116^{BC}
KC	86	82^{AB}	105^{B}	15^{AB}	10^{D}	14^{BCD}	3^{B}	0	6^{BC}	0	2^{A}	2^{A}	0	58^{A}	78^{AB}	11^{AB}	147^{AB}
DN	86	65^{CD}	73^{C}	11^{C}	14^{C}	19^{AB}	3^{BC}	0	7^{BC}	0	2^{A}	2^{AB}	0	45^{B}	63^{BC}	9^{B}	117^{BC}
NY	89	94^{A}	130^{A}	16^{A}	6^{E}	10^{D}	0^{D}	0	8^{B}	0	2^{A}	2^{A}	0	63^{A}	92^{A}	10^{AB}	165^{A}
DC composite	89	68^{BC}	88^{BC}	13^{BC}	29^{A}	20^{A}	3^{B}	0	10^{A}	0	2^{A}	2^{A}	0	59^{A}	74^{B}	10^{AB}	143^{AB}
LSD[f]		18	23	3	3	5	1		2		1	1		13	18	2	33
Pasteurized																	
DN[d]	89	26^{A}	34^{A}	6^{A}	42^{A}	45^{A}	5	0	5	0	2	3	1	40^{A}	50^{A}	7	97^{A}
HO	91	23^{B}	33^{A}	5^{A}	39^{AB}	44^{A}	5	0	5	0	3	4	1	37^{B}	50^{A}	7	94^{A}
KC	91	23^{B}	34^{A}	5^{A}	39^{AB}	44^{A}	5	0	5	0	3	4	1	37^{AB}	51^{A}	7	95^{A}
DC	90	24^{B}	34^{A}	5^{A}	39^{B}	44^{A}	5	0	5	0	3	4	1	37^{B}	51^{A}	7	95^{A}
TA	92	20^{C}	25^{B}	4^{B}	38^{B}	39^{B}	5	0	4	0	2	2	0	34^{C}	41^{B}	5	80^{B}
LSD		2	4	1	3	4								3	5	0	7

[a] Moisture. [b] D = daidzin; G = genistin; Gl = glycitin; Dein = daidzein; Gein = genistein; Glein = glycitein. [c] Aseptically processed soy milk, Original Eden Soy Beverage; DC = Giant Foods, Washington, DC; HO = Kroger, Houston; TA = Publix, Tampa; KC = Price Chopper; DN = Albertsons, Denver; NY = Walbaum. [d] Pasteurized soy milk, White Wave Silk Dairyless Soy Beverage, TA = Nature's Food Patch, Tampa; KC= Wild Oats, Kansas City; HO = Whole Foods, Houston; DC = Fresh Fields, Washington, DC; DN = Vitamin Cottage. [e] Total = moles of isoflavone × molecular weight of isoflavone. [f] Values in a column with different superscripts are different at $\alpha \leq 0.05$; Lsd = least significant difference; each sample analyzed in duplicate.

Table 4. Isoflavone Content of Tofu (μg/g Wet Weight)

		glucosides			malonylglucoside			acetylglucoside			aglycon			total[d]			
tofu	% M[a]	D[b]	G	Gl	D	G	Gl	D	G	Gl	Dein	Gein	Glein	Dein	Gein	Glein	total
tofu A[c]	83	59^{F}	99^{DE}	18^{C}	86^{G}	113^{F}	16^{C}	8^{D}	12^{BC}	0	8^{C}	11^{C}	2^{C}	91^{G}	138^{F}	22^{C}	251^{G}
tofu B	86	50^{G}	83^{G}	15^{F}	64^{J}	85^{H}	11^{E}	8^{C}	10^{D}	0	6^{E}	9^{D}	2^{C}	74^{J}	111^{I}	17^{G}	202^{J}
tofu C	84	58^{F}	98^{DE}	17^{D}	69^{I}	95^{G}	13^{E}	5^{F}	11^{CD}	0	8^{C}	11^{C}	2^{C}	80^{I}	128^{G}	20^{E}	228^{I}
tofu D	86	66^{D}	97^{E}	16^{E}	129^{D}	144^{C}	17^{C}	7^{E}	11^{CD}	0	3^{H}	4^{H}	2^{D}	113^{F}	146^{DE}	21^{D}	280^{E}
tofu E	82	74^{C}	113^{C}	18^{C}	140^{C}	158^{A}	19^{A}	18^{A}	13^{B}	0	4^{G}	5^{G}	2^{C}	130^{C}	165^{B}	24^{B}	319^{B}
tofu F	86	68^{D}	90^{F}	14^{G}	149^{B}	149^{B}	16^{C}	0^{G}	12^{BC}	0	5^{F}	7^{F}	2^{C}	122^{D}	148^{DE}	20^{E}	290^{D}
tofu G	84	78^{B}	100^{DE}	21^{B}	144^{BC}	151^{B}	15^{C}	0^{G}	17^{A}	0	6^{E}	8^{EF}	3^{B}	127^{C}	158^{C}	25^{A}	310^{CD}
tofu H	84	73^{C}	98^{E}	14^{G}	156^{A}	144^{C}	19^{A}	17^{B}	12^{BC}	0	6^{E}	8^{EF}	2^{C}	138^{B}	150^{D}	21^{D}	309^{CD}
tofu I	85	64^{E}	102^{D}	15^{F}	105^{F}	129^{D}	13^{D}	0^{G}	11^{CD}	0	6^{E}	8^{E}	2^{C}	98^{F}	145^{E}	18^{F}	261^{F}
tofu J	85	40^{H}	64^{H}	11^{H}	75^{H}	90^{H}	11^{E}	0^{G}	8^{E}	0	22^{A}	30^{A}	4^{A}	84^{HE}	121^{H}	17^{G}	222^{I}
tofu K	82	105^{A}	135^{B}	19^{C}	119^{E}	119^{E}	13^{D}	0^{G}	13^{B}	0	22^{B}	25^{B}	3^{B}	146^{A}	178^{A}	22^{C}	347^{A}
tofu L	86	105^{A}	143^{A}	29^{A}	143^{BC}	40^{I}	7^{F}	0^{G}	17^{A}	0	7^{D}	9^{D}	2^{C}	86^{H}	129^{G}	24^{AB}	239^{H}
LSD[e]		2	4	1	5	5	2	0	1		0	1	0	4	5	1	9

[a] Moisture. [b] See footnote *b* in Table 3. [c] Tofu A = Azumaya, extrafirm, composite; B = Azumaya, extrafirm, raw; C = Azumaya, extrafirm, cooked; D = Azumaya, Albertsons, firm, raw; E = Azumaya, Albertsons, firm, cooked; F = Azumaya, Bell Market, firm, raw; G = Azumaya, Bell Market, firm, cooked; H = Azumaya, composite, firm; I = Hinoichi regular; J = Hinoichi firm, 2-city composite; K = Naysoya, firm, 4-city composite; L = Mori-nu, firm, silken, 6-city composite. According to package labels, all tofus were coagulated with nigari except Naysoya (calcium sulfate) and Mori-nu (δ-gluconolactone). [d] See footnote *e* in Table 3. [e] See footnote *f* in Table 1.

vidual isoflavones on either a wet or dry (data not shown) weight basis. The total isoflavone concentrations ranged from 96 to 165 μg/g (wet weight). In these soymilks, the differences among lots could be principally attributed to differences in total genistein concentration. The different lots of Eden soymilk showed differences in processing time as reflected in differences in malonylglucoside forms versus glucoside forms. The New York and Houston lots had lower levels of the malonyl forms but higher glucosides, which indicates longer thermal processing treatment than other lots (Wang and Murphy, 1996). Thermal processing was not extreme for any of the soymilk lots since little acetylglucosides were observed. Soymilks appear to be thermally processed rapidly from the soybeans because minimal concentrations of aglycons, produced by soybean β-glucosidases, are present in these soymilks.

The White Wave soymilks had smaller differences among lots. The isoflavone concentrations were only different for the Tampa lot as compared to the other city lots on a wet weight basis. On a dry weight basis, there were significant differences between total isoflavones among lots. These observations suggest that the percent solids were different in production of these soymilks. However, these products will be used on an "as is" or wet basis. Since a pasteurized soymilk would be expected to have a shorter shelf life than the aseptically processed soymilk, the smaller variance in the former's isoflavone levels may reflect soymilks made from a single batch of soybeans. The aseptically processed milks, with much longer shelf life, could have been produced over a much longer period and reflect a wider array of soybeans used in production. The lower variability among isoflavone forms in White Wave's soymilks also suggests very consistent processing operations.

Tofu isoflavone levels are presented in Table 4. The tofu brands were selected on national market share. There were significant differences by lot (city) in total isoflavones, individual totals, and individual isoflavones on a wet weight basis. Tofus A, H, J, K, and L represent multiple city composites among brands. Tofus K and L are aseptically produced with curd formation and whey retention in the package. The other tofus are produced by coagulation of soymilk, separation of whey, followed by packaging in water. Different firmness styles of tofu

Table 5. Isoflavone Content of Raw and Cooked Tofu (μg/g Dry Weight Basis)

	total							
	raw				cooked			
tofu[a]	Dein	Gein	Glein	total	Dein	Gein	Glein	total
Azumaya extrafirm	531	806	123	1460	490	782	119	1391
Azumayu firm A	787	1018	146	1951	736	937	134	1807
Azumayu firm B	845	1024	134	2003	827	1071	147	1985
average	721	949	137	1804	679	910	134	1727
LSD	192	140	19	347				

[a] Extrafirm tofu = tofus C and B; firm A = tofus E and D; firm B = tofus G and F in Table 4, respectively. $n = 2$. [b] See footnote *e* in Table 3.

Table 6. Isoflavone Content of Fermented Soyfoods (μg/g Wet Weight)

		glucosides			malonylglucoside			acetylglucoside			aglycon			total[e]			
food[a]	% M[b]	D[c]	G	Gl	D	G	Gl	D	G	Gl	Dein	Gein	Glein	Dein	Gein	Glein	total
soy saucea[d]		0	0	0	0	0	0	0	0	3	6	4	3	6	3	5	14
soy sauce B		0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1
miso A	53	62	89	10	32	37	8	14	23	0	25	29	12	88	117	23	228
miso B	53	66	92	13	14	17	0	8	20	9	38	43	12	89	121	25	235
tempeh A	61	93	206	14	64	111	8	49	46	0	85	103	9	201	316	22	538
tempeh B	61	105	226	14	66	111	8	35	50	0	77	89	8	193	316	22	531

[a] $n = 2$. [b] Moisture. [c] See footnote *b* in Table 3. [d] Soy sauce A = Kikkoman, fermented; soy sauce B = La Soy; both 7-city composite; miso A = shiro (white) and miso B = aka (red), each 6-bag composite from supplier; tempeh A = raw and tempeh B = cooked, both 5-city composite, White Wave. [e] See footnote *e* in Table 3.

Table 7. Isoflavone Content of Retail Soy Meat Analogues (μg/g Wet Basis)

		glucosides			malonylglucoside			acetylglucoside			aglycon			total[e]			
food[a]	% M[b]	D[c]	G	Gl	D	G	Gl	D	G	Gl	Dein	Gein	Glein	Dein	Gein	Glein	total
chicken[d]																	
raw	74	30	78	8	5	6	0	17	35	0	5	7	3	35	79	9	123
cooked	70	35	92	9	13	7	0	19	43	0	6	8	4	44	94	9	147
frank																	
raw	59	11	17	5	0	0	0	7	13	0	0	3	0	10	21	3	34
cooked	58	11	16	6	0	0	0	7	0	13	3	3	0	14	20	4	38
burger A																	
raw		12	25	6	24	42	7	18	29	0	8	13	4	37	66	12	115
cooked	57	10	22	6	13	25	3	15	27	0	6	8	4	25	47	10	82
burger B																	
raw	63	10	20	5	15	29	6	19	28	0	5	7	4	29	51	10	90
cooked	60	10	23	6	14	27	6	20	31	0	5	8	4	30	54	11	95
burger C																	
raw	64	8	17	6	11	21	5	16	24	0	5	7	4	24	42	10	76
cooked	58	8	16	5	10	19	0	14	24	0	5	8	4	23	40	8	71
link A																	
raw	65	7	12	5	6	12	0	5	11	0	3	3	0	13	23	3	39
cooked	63	7	15	5	7	12	0	0	14	0	0	4	0	7	27	3	37
link B																	
raw	66	7	12	4	7	13	0	7	13	0	3	3	0	15	24	3	42
cooked	64	7	16	5	7	13	0	0	14	0	0	4	0	7	28	3	38
link C																	
raw	65	7	15	5	7	13	0	0	13	0	0	3	0	8	27	3	38
cooked	62	7	14	5	7	13	0	0	14	0	0	4	0	8	27	3	38

[a] $n = 2$. [b] Moisture. [c] See footnote *b* in Table 3. [d] Chicken = Worthington Foods FriChik, 5-city, 2-can/city composite; frank = Loma Linda meatless franks, 5-city, 2-can/city composite; burger A = Harvest burger, west; burger B = Harvest burger, midwest composite; burger C = Harvest burger, east composite; link A = Morningstar Farm, west; link B = Morningstar Farm, midwest; link C = Morningstar Farm, east. [e] See footnote *e* in Table 3.

were evaluated. City composites ranged from 222 to 347 μg/g total isoflavones. Tofus produced by different processing schemes had different individual isoflavone profiles. Tofus K and L, which undergo significantly greater heat processing, had greater concentrations of glucosides than malonylglucosides. The less intensively heat processed tofus, A−J, had proportionately more malonylglucosides to glucosides. The longer heat processing time required to produce tofu generated more acetylglucosides as compared to soymilks that are produced with shorter heat processing times. The β-glucosidases of soybeans had longer times to remain active in tofus as compared to soymilks and resulted in higher levels of free forms in tofus as compared to soymilk. The effect of cooking, as in steaming for 3 min and cooling for 5 min, was compared in three tofus, evaluated, and presented in Table 5. No significant difference was observed between raw and cooked tofus in individual and total isoflavones.

Three fermented soy foods were sampled based on national market share (Table 6). Two types of soy sauce were evaluated as 7-city composites. A fermented product (soy sauce A) and a nonfermented one (soy sauce B) were evaluated. Soy sauce is a very poor source of

Table 8. Isoflavone Content of Alfalfa Sprouts (μg/g Wet Weight Basis)

location[a]	% moisture	formononetin	biochanin A	coumestrol
DC	96	3	0	0
HO	95	3	0	0
NY	95	13	58	5
KC	95	3	0	0
DN	95	22	1	5
TA	95	4	0	3
LA	95	1	0	0

[a] DC = Giant Foods, Washington, DC; HO = Kroger, Houston; TA = Publix, Tampa; KC = Price Chopper; DN = Albertsons, Denver; NY = Walbaum.

isoflavones as the fermentation organisms have probably degraded them. Two types of miso, shiro or white and aka or red, were sampled as 6-bag composite from supplier. The isoflavone distribution in these products was very uniform. Miso represents a food containing high concentrations of isoflavones. Tempeh, as a 5-city composite, was evaluated raw and after cooking according to package directions (nonstick skillet cooking, 3 min per side). No difference was observed between raw and cooked tempehs (data not shown). Tempeh contained the highest concentration of isoflavones of products surveyed in this study. Tempeh and miso contained the largest concentration of aglycons resulting from the action of the β-glucosidases of the fermentation organisms. The effects of multiple heat processing steps in tempeh and in miso production are evident based on the significant concentrations of the acetylglucosides.

Four types of retail soy/meat analogues were evaluated for isoflavone content: soy chicken, meatless franks, soy burgers, and soy links (Table 7). These soy foods provide modest amounts of isoflavones. One chicken analogue was evaluated as a 5-city composite, and the effects of cooking according to package direction were examined. Total isoflavones were lower in raw product as compared to cooked. The moisture loss in cooking may account for this difference since a much smaller difference was observed on a dry basis. The meatless frank yielded minimal moisture loss and no difference in isoflavone levels between cooked and raw product. The soy burgers were sampled as regional composites (east, midwest, and west) and evaluated for raw versus cooked products. There appears to be a regional variation in this product as sampled. Cooking appeared to have little effect on isoflavone concentration. The distribution of isoflavone forms changed due to cooking with malonylglucosides decreasing and glucosides increasing in most products. Soy links were evaluated as regional composites as well. No variation in isoflavone content was observed between regional composites nor due to cooking.

Alfalfa sprouts were the only non-soy food evaluated in this isoflavone survey (Table 8). This food is the only other isoflavone-containing food that had a large enough market share to be considered for sampling. The products were collected from seven cities. The isoflavones found in alfalfa (and clover) seeds and sprouts are different than soy. None of the soy isoflavones were detected in alfalfa sprouts. Formononetin was detected in all alfalfa-labeled products. The product collected in New York and in Denver had labels that indicated these were mixtures of clover and alfalfa sprouts. The other five cities' product labels indicated that only alfalfa seeds were used to produce the sprouts. The two products with mixed seeds (sprouts) contained biochanin A and coumestrol. The alfalfa product contained coumesterol as well. None of the products containing only alfalfa had detectable biochanin A. On a wet weight basis, the sprouts appear to be a minor source of isoflavones for most U.S. diets.

Institutional soy-extended hamburgers were evaluated as raw and cooked composites from the supplier (Table 9). These foods are a very minimal source of isoflavones ranging from 6 to 18 μg/g raw and 9−30 μg/g cooked. Cooked burgers appear to have higher concentrations of isoflavones, but moisture and fat loss probably account for the apparent increase in concentration. Given that the overall levels are so low for these products, the differences among products are not very significant.

In summary, 63 isoflavone-containing foods were evaluated for isoflavone levels based on market sales and isoflavone abundance. These data will be available electronically as an appendix to USDA Handbook No. 8 at http://www.nal.fnic/foodcom/Data/index.html.

Table 9. Isoflavone Content of Institutional Soy/Beef Hamburgers (μg/g Wet Weight Basis)

		glucosides			malonylglucoside			acetylglucoside			aglycon			total[e]			
food[a]	% M[b]	D[c]	G	Gl	D	G	Gl	D	G	Gl	Dein	Gein	Glein	Dein	Gein	Glein	total
burger A[d]																	
raw	63	2	4	0	3	4	0	0	5	0	0	1	0	3	8	0	11
cook	50	5	8	2	4	6	0	5	7	0	5	0	0	10	12	1	23
burger B																	
raw	65	3	5	0	2	3	0	4	7	0	0	0	0	5	9	0	14
cook	58	4	7	2	4	5	0	5	8	0	0	0	0	7	12	1	20
burger C																	
raw	61	3	6	1	3	4	0	4	7	0	0	1	0	6	11	1	18
cook	51	6	10	3	5	7	0	7	10	0	1	1	0	11	17	2	30
burger D																	
raw	62	2	3	0	2	3	0	0	0	0	0	0	0	2	4	0	6
cook	52	3	5	2	4	5	0	0	0	0	0	0	0	3	5	1	9
burger E																	
raw	58	2	4	0	0	3	0	0	6	0	0	0	0	2	8	0	10
cook	48	3	5	0	0	4	0	5	8	0	0	0	0	4	10	0	14

[a] $n = 2$. [b] Moisture. [c] See footnote *b* in Table 3. [d] Burger A = school lunch USDA Commodity patty, 6-patty composite; burger B = school lunch precooked patty, Hudson Foods, 5-patty composite; burger C = Excel (Cargill) soy/beef patty, 6-patty composite; burger D = Aksarben soy/beef patty (U.S. Army Military Command), 6-patty composite; burger E = Tyson soy/beef patty, 6-patty composite. [e] See footnote *e* in Table 3.

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