Isoflavone Content in Commercial Soybean Foods

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The concentration and distribution of isoflavones in 29 commercial soybean foods, categorized into soy ingredients, traditional and second-generation, were evaluated by high-performance liquid chromatography and photodiode array detection. Twelve isomers were quantified, three aglycons (daidzein, genistein, glycitein) and nine glucosides (daidzin, genistin, glycitin; 6″-*O*-acetyldaidzin, -genistin, -glycitin; 6″-*O*-malonyldaidzin, -genistin, -glycitin). Compared with unprocessed soybeans, high-protein soy ingredients contained similar concentrations, except alcohol-leached soy concentrate. Traditional soybean foods showed differences between nonfermented and fermented foods. Nonfermented foods had greater levels of glucosides, while in contrast, greater levels of aglycons were found in fermented foods. Second-generation soy foods contained only 6–20% of the isoflavones of whole soybeans. The variety of soybean, method of processing, and addition of other components affect the retention and distribution of isoflavone isomers in soy foods.

Keywords: *Isoflavones; daidzein; genistein; glycitein; daidzin; genistin; glycitin; soy foods; HPLC*

INTRODUCTION

Certain phytochemicals in fruits, vegetables, and grains possess possible cancer-preventive properties that may inhibit tumor initiation, prevent oxidative damage, or affect steroid hormones or prostaglandin metabolism to block tumor promotion (Caragay, 1992). Isoflavones are one class of these compounds and are found in soybeans in large amounts. The major soybean isoflavone aglycons, genistein, and daidzein (Figure 1), have been identified for decades (Walter, 1941). Because these compounds seem to act as anticarcinogens by exerting a biological antioxidant effect, their content and bioavailability in foods have been topics of recent interest (Messina and Barnes, 1991).

Isoflavones differ from flavones in that the benzyl ring B is linked to position 3 instead of position 2 (Figure 1). Soybeans contain three types of isoflavones, as four chemical forms: the aglycons daidzein, genistein, and glycitein; the glucosides daidzin, genistin, and glycitin; the acetylglucosides 6″-*O*-acetyldaidzin, 6″-*O*-acetylgenistin, and 6″-*O*-acetylglycitin; and the malonylglucosides 6″-*O*-malonyldaidzin, 6″-*O*-malonylgenistin, and 6″-*O*-malonylglycitin (Kudou *et al.*, 1991).

The flavones quercetin and kaempherol have been found to be *in vitro* mutagens when examined by the *Salmonella typhimurium* short-term mutagenesis Ames test system (Brown *et al.*, 1977; Brown and Dietrich, 1979). In contrast, Bartholomew and Ryan (1980) found that isoflavones yielded negative results in the Ames test. Messina and Barnes (1991) showed that soybean chips, soy protein isolate, and soy molasses, all of which were rich in isoflavones, inhibited mammary tumorigenesis in animals induced by 7,12-dimethylbenz[*a*]-anthracence or methylnitrosourea. The decrease of mammary tumor estrogen receptors by feeding soy chips paralleled the inhibition of tumorigenesis. The binding property of isoflavones to estrogen receptors may reduce breast cancer, which is estrogen dependent (Henderson *et al.*, 1982; Verdeal *et al.*, 1980).

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Isoflavones possess antioxidant and antifungal activity. Naim *et al.* (1976) reported that the isoflavones inhibited lipoxygenase action and prevented peroxidative hemolysis of sheep erythrocytes *in vitro*. The extent depends on the structures of the isoflavones. Pratt and Birac (1979) found that soybeans, defatted soy flour, soy protein concentrates, and soy isolates have appreciable antioxidant activity detected by the rate of β-carotene bleaching in a lipid–aqueous system, which was due to phenolic compounds. Fleury *et al.* (1992) showed that malonyl isoflavones are good antioxidants in the storage test carried out at 37 °C and in UV light-induced oxidation of a β-carotene/linoleic acid system. Isoflavones have no antioxidant properties in the Rancimat test performed at 100 °C or in the heat-induced oxidation of a β-carotene/linoleic acid system in comparison with BHA and BHT. The rapid decomposition of malonyl isoflavones at 100 °C renders them inactive. Pratt and Birac (1979) suggested that isoflavones have a protective effect but do not act as radical scavengers like BHA and BHT.

In vitro, genistein specifically inhibits epidermal growth factor receptor tyrosine kinase activity (Akiyama *et al.*, 1987) and protein histidine kinase from yeast cell extracts (Huang *et al.*, 1992). Genistein is a unique topoisomerase inhibitor in selectively suppressing the growth of oncogene *ras*-transformed NIH 3T3 cells but not normal NIH 3T3 cells (Okura *et al.*, 1988). In addition, Sariaslani and Kunz (1986) found that soybean flour and genistein induced cytochrome P-450 in *Streptomyces griseus*. Peterson and Barnes (1991) found that genistein was a potent inhibitor of the growth of human breast carcinoma cell lines and that daidzein and biochanin A were weaker inhibitors. In a short-term rat study, hepatic cumene hydroperoxidase activity was increased by feeding a soy isoflavone extract (Hendrich *et al.*, 1994). These findings may be alternative mechanisms of inhibition of non-hormone-related tumorigenesis by soy isoflavone.

There have been reports on the content of genistein and daidzein and their glycosides in a few soybean varieties and in soy foods (Murphy, 1982; Farmakalidis and Murphy, 1985) as well as discussion of the effects

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of processing on these compounds. More recently, the variations in glucoside substitution have been recognized (Farmakalidis and Murphy, 1985; Kudou *et al.*, 1991). Glycitein, a 5-methoxy form, has been reported by some researchers (Eldridge, 1982; Naim *et al.*, 1973), but not all soybeans seem to contain this form. The isoflavones seem to be concentrated in the soybean hypocotyl with low to moderate amounts in the cotyledon. Because traditional processing of soybeans into food products does not separate these seed parts, we have evaluated soybean seeds without fractionation.

We have estimated the amount of soy isoflavones that humans would need to consume to provide an anticarcinogenic dose at 1.5–2.0 mg (kg of body weight)$^{-1}$ day^{-1} (Hendrich *et al.*, 1994). To evaluate the potential of the isoflavones as a dietary anticarcinogen, the amounts available in typical soy foods and in soybeans must be quantified. In the present study, we investigated the isoflavone content in a number of locally purchased soy foods including soy ingredients (soybeans, soy flour, soy granule, texturized vegetable protein, isolates, concentrates), traditional soy foods (roasted soybeans, instant soy beverage powder, tofu, tempeh, bean paste, fermented bean curd, miso), and soy-added second-generation soy foods (soy hot dog, soy bacon, tempeh burger, tofu yogurt, soy Parmesan cheese, soy Cheddar and mozzarella cheese, soy noodles).

MATERIALS AND METHODS

Instrumentation. The high-performance liquid chromatography (HPLC) apparatus consisted of a Beckman-Altex Model 420 microprocessor system controller, two Beckman-Altex Model 110A liquid chromatograph pumps, a Beckman mixer (Beckman Instruments Inc., Fullerton, CA), a Waters 991 photodiode array detector (PDA) (Waters, Marlborough, MA) monitoring 200–350 nm, and a NEC computer with Waters PDA data processing software. A YMC-pack ODS-AM-303 column (5 μm, 25 cm × 4.6 mm i.d.) (YMC Inc., Wilmington, NC) was used for quantitative HPLC analyses. A YMC-pack ODS-AM-323 column (10 μm, 25 cm × 10 mm i.d.) was used for semipreparative HPLC. All HPLC analyses were performed at ambient temperatures.

Materials. All soy ingredients and processed soybean products were purchased locally except Vinton 81 soybeans, which were from our collection. Soy protein isolates and soy concentrates were generously provided by Protein Technologies International (St. Louis, MO) and Grain Processing Corp. (Muscatine, IA). Milli-Q system (Millipore Co., Bedford, MA) HPLC grade water was used. Other HPLC grade organic solvents were from Fisher Scientific Co. (Pittsburgh, PA).

Isoflavone Standards. Ten isoflavone standards were used for quantifying the amounts of isoflavones in the food samples. Authentic standards of daidzein and genistein were obtained from commercial sources (ICN Pharmaceuticals, Plainview, NY, and Calbiochem Corp., San Diego, CA). Daidzin and genistin were from previous work in the laboratory (Murphy, 1981). 6″-*O*-Acetyldaidzin and 6″-*O*-acetylgenistin were isolated according to the method of Farmakalidis and Murphy (1985), except that semipreparative HPLC, instead of crystallization, was used for the final purification. A linear gradient of 0–60% acetonitrile (ACN) in 30 min at a flow rate of 2 mL/min was used to separate 6″-*O*-acetyldaidzin from daidzin and 6″-*O*-acetylgenistin from genistin, respectively. 6″-*O*-Malonyldaidzin, 6″-*O*-malonylgenistin, and 6″-*O*-malonylglycitin were purified as follows: ground soybeans were defatted with hexane for 1 h at room temperature. Defatted flour was extracted with ACN and 0.1 N HCl (1:5:1 w/v/v) according to the procedure of Murphy (1981) for at least 2 h at room temperature and then filtered through Whatman No. 42 filter paper (Micron Separation Inc., Westborough, MA). The filtrate was concentrated by using a rotary evaporator at 30 °C. An aliquot was applied to a 2.5 × 75 cm Sephadex LH-20 (Pharmacia LKB Biotech Inc., Piscataway, NJ) column with eluent of 50% methanol (MeOH) to separate 6″-*O*-malonylgenistin from 6″-*O*-malonyldaidzin and 6″-*O*-malonylglycitin. Further purification to 6″-*O*-malonylgenistin was achieved by a semipreparative column and isocratic mobile phase of 25% ACN with 0.1% glacial acetic acid. 6″-*O*-Malonyldaidzin and 6″-*O*-malonylglycitin were separated from each other with 18% ACN with 0.1% glacial acetic acid by using semipreparative HPLC and compared with the results of Kuduo *et al.* (1991) for confirmation. The malonyl isoflavones in crude extract degraded into glucosides after 5 days, even stored at 5 °C. During extraction, acidic conditions and temperatures lower than 30 °C were necessary to prevent malonyl isoflavones from degrading. Glycitin was purified according to the method of Naim *et al.* (1974) with a slight modification. Pure 6″-*O*-acetylglycitin and glycitein were not obtained because of their minute amounts in soybeans from Iowa. The quantitation of these two compounds in the soy foods was estimated by using the same standard curve of glycitin and by adjusting for the molecular weight differences.

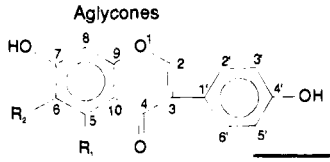
Isoflavone Extraction. All wet samples were freeze-dried except the samples of soy ingredients, instant beverage, and noodle. Two grams of dried, finely ground samples was placed in a 125-mL screw-top Erlenmeyer flask containing 10 mL of ACN and 2 mL of 0.1 N HCl (Murphy, 1981) and stirred for 2 h at room temperature. Extractants were filtered through Whatman No. 42 filter paper. The filtrate was taken to dryness on a rotatory evaporator at ≤30 °C. The dried material was redissolved in 10 mL of 80% HPLC grade MeOH in water. A aliquot of sample was filtered through a 0.45-μm PTFE filter unit [poly(tetrafluoroethylene), Alltech Associates Inc., Deerfield, IL] and analyzed by HPLC. The moisture content of the sample was determined according to AOAC Method 14.003 (AOAC, 1984) in order to calculate the concentrations of isoflavones on an as-is basis.

HPLC Quantitation of Isoflavones. A linear HPLC gradient was composed of (A) 0.1% glacial acetic acid in H_2O and (B) 0.1% glacial acetic acid in ACN. Following injection of 20 μL of sample, solvent B was increased from 15% to 35% over 50 min, and then held at 35% for 10 min. The solvent flow rate was 1 mL/min. A Waters 991 series photodiode array detector monitored from 200 to 350 nm. The minimum detectable concentrations for daidzein and genistein are 185 and 100 ng/mL, respectively. UV spectra were recorded and area responses were integrated by Waters PDA software.

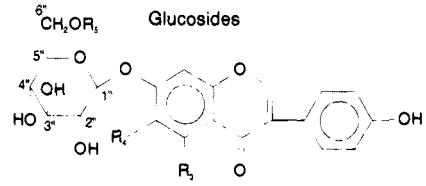
Statistical Analysis. All samples were run in triplicate. Statistical analysis was done by using the SAS package developed by the SAS Institute, Inc. (Box 8000, Cary, NC). Analyses of variance using the general linear models (GLM) were conducted, and differences between the sample means were analyzed by Fisher's least significant difference (lsd) test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

It is now recognized that the three soy isoflavone aglycons, genistein, daidzein, and glycitein, are each found in four isomeric forms in soybeans and soy foods (Figure 1). The distribution of these 12 forms has not been quantified in soy foods produced in or imported to the United States. Our results show the amounts and scope of variation in the distribution of these isoflavonoids in typical soy foods. Because the same extraction protocol was used for all soybeans and soy foods, the relative differences in the distribution of the 12 isomers cannot be due to changes that occurred during sample workup. The conditions used for sample preparation have been designed to minimize changes in the distribution of the four classes of isomers, malonylglucoside, acetylglucoside, glucoside, or aglycon. The HCl–ACN extraction procedure followed the method described in Murphy (1981), which was superior to all other solvent systems in terms of maximizing extraction efficiency and minimizing coextractives. Limited by the



R_1	R_2	Compounds
H	H	daidzein
OH	H	genistein
H	OCH_3	glycitein



R_3	R_4	R_5	Compounds
H	H	H	daidzin
OH	H	H	genistin
H	OCH_3	H	glycitin
H	H	$COCH_3$	6"-*O*-Acetyldaidzin
OH	H	$COCH_3$	6"-*O*-Acetylgenistin
H	OCH_3	$COCH_3$	6"-*O*-Acetylglycitin
H	H	$COCH_2COOH$	6"-*O*-Malonyldaidzin
OH	H	$COCH_2COOH$	6"-*O*-Malonylgenistin
H	OCH_3	$COCH_2COOH$	6"-*O*-Malonylglycitin

Figure 1. Chemical structures of 12 isoflavone isomers.

expense of standards and numbers of samples, we chose five soy foods to examine the recovery efficiency. Recovery estimation was performed by adding genistein standard to dry samples and extracting with solvents. Recovery data of five soy foods were as follows: textured vegetable protein, 70% ± 16%; soy beverage, 79% ± 13%; tofu, 95% ± 5%; soy bacon, 90% ± 11%; and tempeh, 86% ± 7%. Values in the tables are uncorrected.

Figure 2 shows the HPLC chromatograms of Vinton 81 soybeans (a), textured vegetable protein (TVP) (b), and soy hot dog (c) monitored at 254 nm. All 12 isoflavone compounds eluted within 60 min with baseline resolution. The spectrum from 200 to 350 nm of each isoflavone was evaluated by using the PDA. Both the retention time and spectrum of each compound in samples were used for identification compared to those of the standards daily. The elution order and retention times (minutes) of isoflavones were as follows: daidzin, 14.33 ± 0.52; glycitin, 15.64 ± 0.60; genistin, 23.52 ± 0.64; 6″-*O*-malonyldaidzin, 25.37 ± 0.54; 6″-*O*-malonylglycitin, 26.18 ± 0.66; 6″-*O*-acetyldaidzin, 30.76 ± 0.89; 6″-*O*-acetylglycitin, 32.00 ± 1.06; 6″-*O*-malonylgenistin, 34.67 ± 0.88; daidzein, 39.23 ± 0.80; glycitein, 41.29 ± 0.93; 6″-*O*-acetylgenistin, 41.91 ± 0.76; genistein, 53.78 ± 0.85.

The soy foods used in this study were classified into three groups: soy ingredients, traditional soy foods, and second-generation soy foods. The data for the individual isoflavone contents in all soy products are presented in Tables 1–3. Total isoflavone contents for each food, normalized for molecular weight differences of the isoflavone isomers, are presented in Table 4 on a wet-weight or as-is basis.

With the exception of soy concentrate, all 10 soy ingredients contained total isoflavone amounts ranging from 2003 to 2404 μg/g (Table 1) or from 466 to 1636 μg/g (Table 4) when normalized for molecular weight differences. Vinton 81 soybeans, green soybeans, soy granules, soy flour, and TVP had significantly higher ($p < 0.05$) isoflavone amounts than the other soybean products examined in this study. Murphy (1982) reported that significant amounts of isoflavones were carried over into processed soy protein products such as TVP, soy protein isolate, and soy flour. Isoflavones were not found in soybean oil (Eldridge and Kwolek, 1983). These soy ingredients were not diluted with other food components that could reduce isoflavone concentration.

The concentrations of the 12 isoflavone isomers in each soy ingredient are given in Table 1. These data indicate that 97–98% of total isoflavone in whole soybeans or other high-soy-protein products was in the esterified forms, glucoside, malonylglucoside, or acetylglucoside. However, the distribution of these nine glucosides varied according to the different types of soy ingredients. The major isomers in whole Vinton 81 and green soybeans and soy flour, which were minimally processed, were 6″-*O*-malonylgenistin and 6″-*O*-malonyldaidzin. Glycitin forms were approximately equally distributed between glucosides and malonylglucosides isomers. Vinton 81 soybeans are a tofu variety of bean characterized by a large seed size, yellow seed coat and hilum, and high protein content. Green soybeans were a commercial product for retail sale to produce soybean sprouts and have green seed coats. Almost all soybeans in commerce today have yellow seed coats; thus, these green soybeans are quite unusual. Whole soybeans have an extremely variable isoflavone content, depending on variety and environmental conditions (Table 1; Eldridge and Kwolek, 1983; Farmakalidis and Murphy, 1985; Wang and Murphy, 1994). The isoflavone distributions for Vinton 81 soybeans grown in two locations in Iowa in two crop years have significantly different isoflavone amounts and distributions. Soy flour was prepared by defatting dehulled soy flakes and grinding so that 97% of the flour passes a 100-mesh screen. Accordingly, minimal processing for soy flour yielded an isoflavone profile similar to that of intact soybeans.

In contrast, in soy granules, TVP, and soy isolates, the principal isoflavone isomers were genistin and daidzin (Table 1). In addition, soy granules and TVP have an appreciable amount of 6″-*O*-acetylgenistin and 6″-*O*-acetyldaidzin, probably reflecting their heat treatment during extrusion processing. Different processing seemingly affects the distribution. In heat treatment, some malonyl isoflavones were transformed into acetyl forms (Farmakalidis and Murphy, 1985). The malonyl isoflavones themselves were heat-labile and very unstable (Kudou *et al.*, 1991). The amounts of the glycitin group were lower as compared with daidzin and genistin groups but distributed in the same manner as the two predominant isoflavonoids.

The total isoflavone contents of soy protein isolates were more than 2 times smaller than in Vinton 81 soybeans or soy flour (Table 4). Soy protein isolate was prepared by dilute alkali extraction of soluble protein from defatted soy flakes, precipitation at the isoelectric point, neutralization, and drying of the protein fraction. The ratio of malonyl isomers to glucosides was significantly lower as compared with intact soybeans, whereas the ratio of acetyl isomers to glucosides was considerably higher. The higher ratio of acetylglucosides to glucoside isomers and lower ratio of malonylglucoside to glucoside

Table 1. Isoflavone Contents (Micrograms per Gram, As Is) of Soy Ingredients[a,b]

	glucoside			malonyl			acetyl			aglycon		
product	Din	Gin	Glin	Din	Gin	Glin	Din	Gin	Glin	Dein	Gein	Glein
Vinton 81 90H[c]	690 b	852 a	56 d	300 b	743 b	50 b	1 e	9 e	nd	26 b	29 c	20 f
Vinton 81 91I[c]	180 e	394 de	53 def	241 c	738 b	61 a	tr	2 e	35 de	7 f	17 f	20 ef
green soybean	451 d	430 d	48 f	515 a	851 b	57 ab	tr	2 e	nd	10 de	16 f	18 g
soy flour	147 f	407 de	41 g	261 bc	1023 a	57 a	tr	1 e	32 e	4 g	22 e	19 fg
soy granule	727 a	870 a	132 b	106 d	193 cd	60 a	72 c	135 d	48 c	12 c	27 d	22 d
TVP A[d]	507 c	634 b	146 a	93 d	192 cd	60 a	187 b	320 b	90 a	12 c	29 cd	25 c
TVP B[d]	463 d	552 c	93 c	129 d	256 c	58 a	231 a	355 a	68 b	8 ef	22 e	26 b
soy isolate A[d]	tr	137 g	34 h	20 e	100 d	39 c	6 e	nd	33 e	63 a	136 a	53 a
soy isolate B[d]	88 g	301 f	49 ef	18 e	88 d	36 c	74 c	215 c	46 c	11 cd	36 b	25 bc
soy isolate C[d]	133 f	382 e	55 de	19 e	95 d	37 c	36 d	122 d	40 d	12 c	36 b	22 de
soy concentrate	tr	18 h	31 h	nd	tr	nd	tr	1 e	nd	nd	nd	23 d
lsd[e]	31	47	6	41	117	7	14	34	2	2	2	1

[a] Data from three replications. Values in column with different letters were significantly different at $\alpha = 0.05$; dry samples. [b] Abbreviations: Din, daidzin; Gln, genistin; Glin, glycitin; Dein, daidzein; Gein, genistein; Glein, Glycitein; tr, trace; nd, not detected. [c] 90 and 91, 1990 and 1991 crop years; H and I, different locations. [d] Different commercial sources. [e] Least significant difference.

Table 2. Isoflavone Contents (Micrograms per Gram, As Is) of Traditional Soy Foods[a,b]

	glucoside			malonyl			acetyl			aglycon		
product	Din	Gin	Glin	Din	Gin	Glin	Din	Gin	Glin	Dein	Gein	Glein
roast soybeans	460 b	551 d	68 b	45 b	63 f	72 a	397 a	743 a	102 a	39 e	69 e	52 a
instant beverage A[c]	444 b	775 a	76 a	39 b	144 d	40 c	5 b	24 b	33 b	18 g	44 g	20 d
instant beverage B[c]	404 c	718 b	77 a	58 b	202 b	43 b	8 b	27 b	33 b	15 g	38 h	20 d
instant beverage C[c]	468 b	674 c	68 b	61 b	179 c	42 bc	9 b	22 b	33 b	15 g	32 i	20 d
instant beverage D[c]	525 a	745 ab	75 a	98 b	259 a	44 b	12 b	26 b	33 b	30 f	50 f	21 cd
tofu	25 e	84 f	8 e	159 ab	108 e	nd	8 b	1 b	29 b	46 d	52 f	12 f
tempeh	2 e	65 f	14 d	255 a	164 c	nd	11 b	nd	nd	137 c	193 b	24 b
bean paste	nd	96 ef	21 c	nd	nd	19 e	1 b	2 b	nd	271 a	183 c	54 a
honzukuri miso[d]	72 d	123 e	18 c	nd	nd	22 d	1 b	11 b	nd	34 f	93 d	15 e
fermented bean curd	nd	tr	nd	nd	nd	nd	nd	nd	nd	143 b	223 a	23 bc
lsd[e]	35	32	3	127	16	3	23	38	8	4	5	2

[a] See Table 1. [b] See Table 1. [c] Different commercial sources. [d] Made of rice and soybeans. [e] Least significant difference.

Table 3. Isoflavone Contents (Micrograms per Gram, As Is) of Second-Generation Soy Foods[a,b]

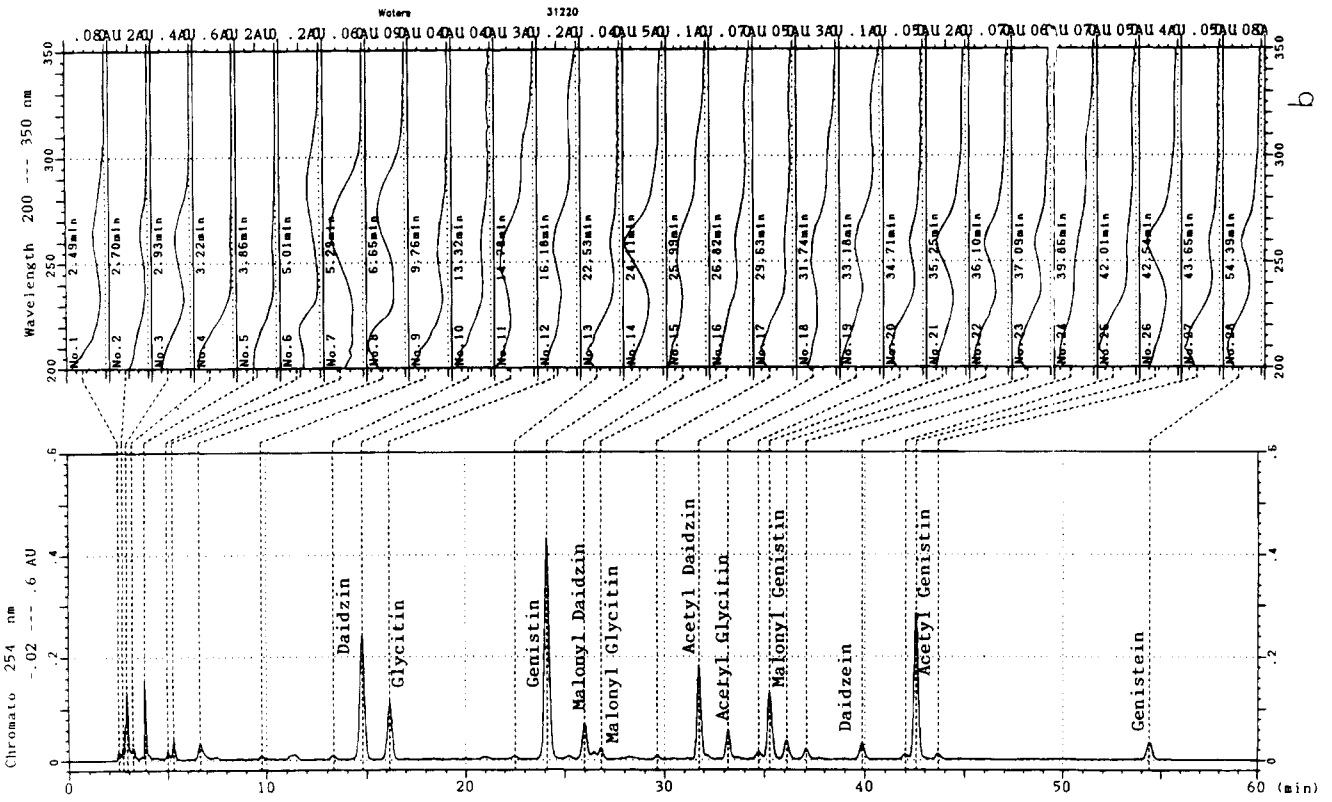
	glucoside			malonyl			acetyl			aglycon		
product	Din	Gin	Glin	Din	Gin	Glin	Din	Gin	Glin	Dein	Gein	Glein
soy hot dog	35 b	67 b	15 cd	12 b	42 b	15 b	tr	4 ab	14 c	8 c	16 c	8 d
soy bacon	tr	27 d	14 cd	tr	5 c	12 c	tr	3 ab	nd	26 b	48 b	9 c
tempeh burger	36 ab	158 a	18 a	25 b	nd	nd	nd	1 b	nd	34 a	96 a	18 b
tofu yogurt	42 a	80 b	12 d	61 a	79 a	nd	nd	tr	nd	tr	3 e	5 e
soy Parmesan	tr	tr	nd	26 b	tr	nd	tr	tr	36 a	tr	6 de	20 a
Cheddar cheese A[c]	tr	nd	12 d	nd	tr	nd	tr	tr	19 c	tr	4 e	8 d
Cheddar cheese B[c]	16 c	46 c	17 ab	67 a	7 c	nd	nd	tr	27 b	tr	9 d	8 cd
mozzarella cheese	7 d	33 cd	15 bc	17 b	6 c	nd	nd	9 a	19 c	tr	8 d	9 cd
flat noodle	tr	6 e	nd	15 b	37 b	37 a	nd	tr	nd	tr	13 c	19 ab
lsd[d]	8	17	3	27	11	2	1	4	7	2	3	1

[a] See Table 1. [b] See Table 1. [c] Different commercial sources. [d] Least significant difference.

in isolate may be due to the toasting of defatted soy flakes after hexane extraction or heating during the drying of the soy isolate.

Soy concentrate can be produced by a water or alcohol wash of soy flakes to remove soluble carbohydrates and improve functionality. Alcohol washing should remove most of the isoflavones. The concentrate analyzed here was obviously an alcohol-washed concentrate as evidenced by the extremely low concentration of isoflavones.

Traditional soy foods, such as soy beverage, tofu, tempeh, and miso, are consumed frequently in Asian countries and increasingly in Western countries. The amounts of individual isoflavones in these soy products are presented in Table 2. The total isoflavone amounts, adjusted for molecular weight differences, are given in Table 4. Traditional nonfermented soy foods, roasted soybeans (1625 μg/g), and instant soy beverage powder (1001–1183 μg/g), have 2–3 times the total amount of isoflavone as compared with fermented soy foods, tempeh (625 μg/g), bean paste (593 μg/g), miso (294 μg/g), and fermented bean curd (390 μg/g). Instant soy beverages analyzed in this study were in a dry powder form. Therefore, the amounts of isoflavone in these soy beverages would be diluted by 4 when the powder was reconstituted with water for consumption. In this nonfermented group, tofu contained lesser isoflavone totals (337 μg/g) than roasted soybeans or soy beverage powders ($p < 0.05$). However, reconstituted soy beverage would be very comparable to this tofu. The distribution of isoflavone isomers in nonfermented foods was greater in the glucosides, genistin and daidzin, whereas the fermented soyfoods retained low amounts of glucosides. The aglycon isomers were the major forms in these fermented soy foods. Roasted soybeans were produced by frying whole, hydrated soybeans. As expected from heat processing, roasted soybeans had significantly larger amounts of acetylisoflavones and


b
Daidzin
Glycitin
Genistin
Malonyl Daidzin
Malonyl Glycitin
Acetyl Daidzin
Acetyl Glycitin
Malonyl Genistin
Daidzein
Acetyl Genistin
Genistein
Chromato 254 nm
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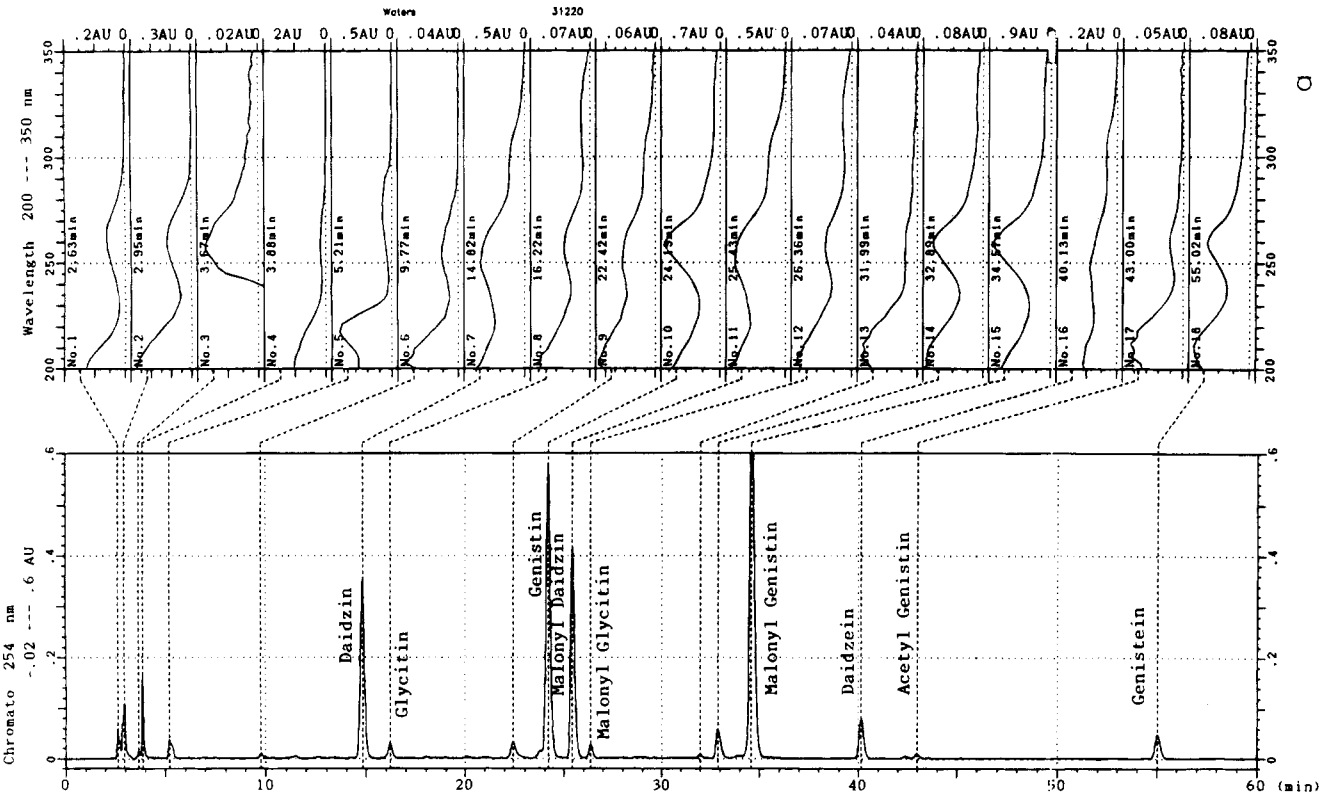
a
Daidzin
Glycitin
Genistin
Malonyl Daidzin
Malonyl Glycitin
Malonyl Genistin
Daidzein
Acetyl Genistin
Genistein
Chromato 254 nm
Wavelength 200 --- 350 nm
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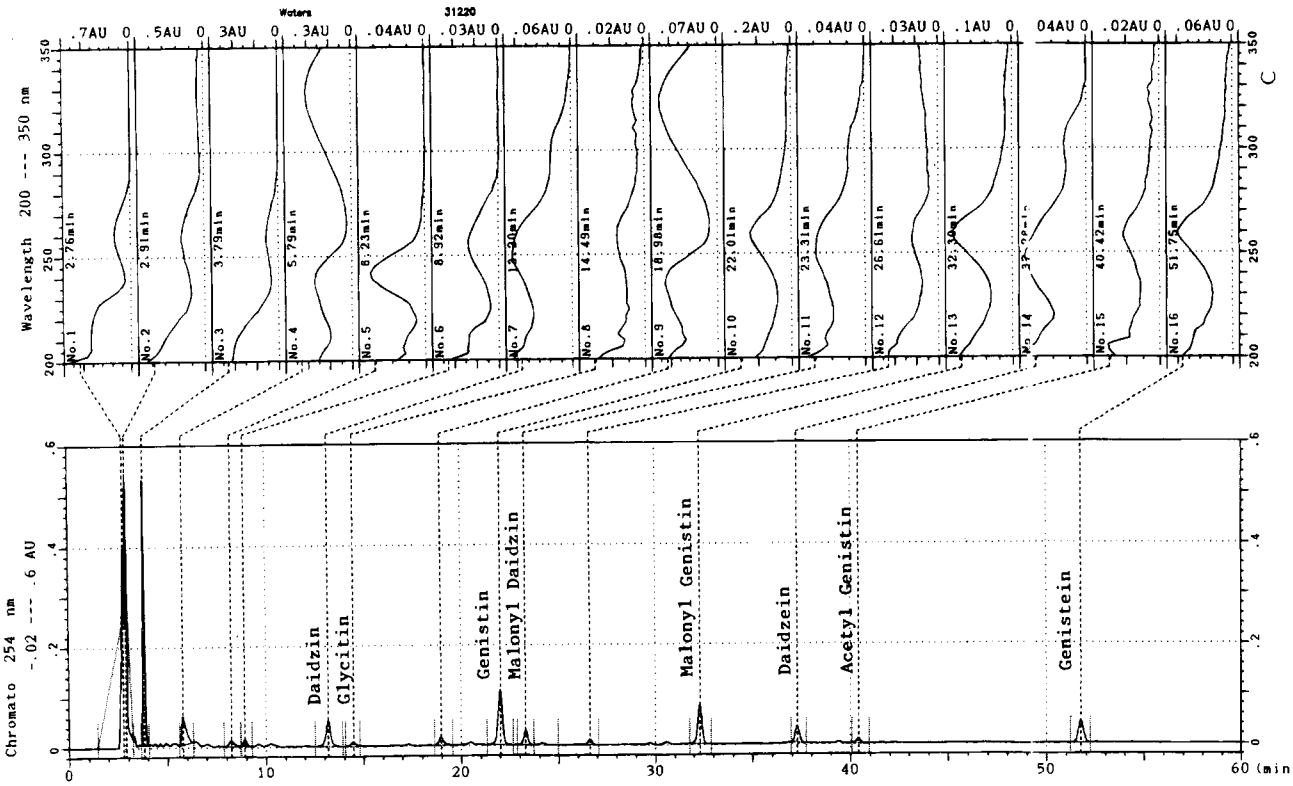

Figure 2. HPLC retention profiles and UV absorbance spectra of Vinton 81 soybean (a), texturized vegetable protein (b), and soy hot dog (c).

Table 4. Total Contents[a] (Micrograms per Gram, As Is) of Daidzein, Genistein, and Glycitein in Soy Food Products

product	daidzein	genistein	glycitein	total
soy ingredients[b]				
Vinton 81 90H	600 a	954 a	82 gh	1636 a
Vinton 81 91I	240 gh	648 fg	107 def	995 e
green soybean	546 b	729 d	79 gh	1354 c
soy flour	226 h	810 c	88 g	1124 d
soy granule	549 b	748 d	167 b	1464 b
TVP A[c]	473 c	707 de	202 a	1382 bc
TVP B[c]	484 c	702 de	156 c	1342 c
soy isolate A[c]	77 jkl	273 jk	115 d	466 g
soy isolate B[c]	115 ij	392 i	102 ef	610 f
soy isolate C[c]	122 i	393 i	99 f	615 f
soy concentrate	tr o	13 q	42 i	56 lm
traditional soy foods				
roasted soybeans	563 ab	869 b	193 a	1625 a
instant beverage A[b,c]	311 ef	617 g	109 de	1037 e
instant beverage B[b,c]	295 ef	607 g	111 de	1014 e
instant beverage C[b,c]	336 e	560 h	105 ef	1001 e
instant beverage D[b,c]	407 d	665 ef	111 de	1183 d
tofu	146 i	162 n	29 lmn	337 hi
tempeh	273 fg	320 j	32 jklmn	625 f
bean paste	272 fg	245 kl	77 h	593 f
fermented bean curd	143 i	224 lm	23 n	390 gh
honzukuri miso (rice and soybeans)	79 jk	177 n	38 ijkl	294 i
second-generation soy foods				
soy hot dog	34 lmno	82 op	34 ijklm	150 jk
soy bacon	28 mno	69 op	24 n	122 jkl
tempeh burger	64 klm	196 mn	30 klmn	289 i
tofu yogurt	57 klmn	94 o	12 o	164 j
soy Parmesan[b]	15 no	8 q	41 ij	65 lm
Cheddar cheese A[c]	2 o	5 q	27 mn	34 m
Cheddar cheese B[c]	34 mno	40 pq	35 ijklm	109 jklm
mozzarella cheese	11 o	36 pq	30 klmn	76 klm
flat noodle[b]	9 o	37 pq	39 ijk	85 jklm

[a] With normalization of molecular weight differences. Data from three replications. Values in column with different letters were significantly different at $\alpha = 0.05$. [b] Dry samples. [c] Different sources.

lesser malonylglucosides compared with unprocessed soybeans (Table 2).

Commercial soy milk powder was made by grinding hydrated soybeans in water, heating, filtering, spray-drying, and adding other flavoring ingredients. Addition of other ingredients diluted the isoflavone contents. The distribution of isoflavones in soy beverages was similar to that of TVP and soy flour analyzed in this study, with large amounts of genistin and daidzin. Different commercial sources of soy milk powder were different in isomer distribution as well. These soy milk powders would usually be reconstituted with water in a 1:3 ratio.

Tofu, or soy curd, was produced by precipitating a curd from soy milk by using calcium salts and removal of fluid by pressing. The total content of isoflavone in tofu was similar to that of soy beverage powder if the former was expressed on a dry-matter basis (Table 4). The moisture of this tofu was 73%. In Table 2, the aglycons in tofu were greater than in reconstituted soy milk, suggesting action by native glucosidases during tofu production (Matsuura and Obata, 1993). Because we do not know the specific variety of soybean used to produce this tofu, it is difficult to make mass-balance comparisons.

The four fermented soy foods have a common pattern for isoflavone distribution (Table 2). The aglycons were the major forms, probably due to the hydrolysis during fermentation by *Rhizopus oligosporus* for tempeh, *Aspergillus oryzae*, *Pediacoccus halophylus*, and *Saccharomyces rouxii* for bean paste (ko chu jang in Korea) and miso, and *Actinomycor elegans* for fermented bean curd (su-fu in China). The different inocula provided different extents of hydrolysis. Some malonyl isoflavones might be changed into glucosides, and then into aglycons, because of hydrolysis. This hydrolysis may be caused by glucosidases in soybeans (Matsuura and Obata, 1993) before the heat treatment.

The third group of soy foods, second-generation soy foods, were made by adding soy ingredients to a wide variety of foods to replace animal protein and/or to reduce fat while simulating these American foods. To maintain the similarity to original foods, the addition of soy ingredients was in limited amounts. The total isoflavone contents in nine second-generation products are presented in Table 4. The second-generation soy foods contained isoflavones ranging from 34 to 289 μg/g. Compared with whole soybeans, there was only 6–20% of the total isoflavone contents because most of the food matrices in these foods were nonsoybean constituents. The distribution of the isomers was not similar to that of the soy ingredients or traditional soy foods used in their manufacture. During formulation of these second-generation foods, modification such as hydrolysis of the malonyl group and glucose must occur.

The results show that the contents of commercial soybean food products were affected by the variety of soybean, the processing, and the dilution with nonsoy ingredients. However, inasmuch as these commercial soy foods were produced from unknown varieties of soybeans or unknown sources of ingredients, it is impossible to follow the mass balance of the isoflavones during soy food production. Given the major variation in isoflavone content of soybeans from environmental conditions (location and/or crop year), further analysis of soy foods and ingredients is necessary to obtain an accurate estimation of soy isoflavones available for consumers. Additionally, it seems that heat processing, enzymatic hydrolysis, and fermentation significantly alter the isomer distribution of the three isoflavonoids. There are no data regarding the bioavailability of the different isomers of genistein and daidzein and no data on the biological activity of glycitein and its isomers.

This study investigated a wide variety of soy products and provides basic information for future human and animal feeding studies. Proposed anticarcinogenic doses of soybean isoflavones range from 1.5 to 2.0 mg (kg of body weight)$^{-1}$ day^{-1} (Hendrich *et al.*, 1994). There are a number of soy food choices that will fit this dose requirement without the need to consume unusual amounts of these soy foods.

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LITERATURE CITED

Akiyama, T.; Ishida, J.; Nakagawa, S.; Ogawara, H.; Watanabe, S.-I; Itoh, N.; Shibuya, M.; Fukami, Y. Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J. Biol. Chem.* **1987**, *262*, 5592–5595.

AOAC. *Official Methods of Analysis*, 14th ed.; Association of Official Analytical Chemists: Washington, DC, 1984; p 249.

Bartholomew, R. M.; Ryan, D. S. Lack of mutagenicity of some phytoestrogens in the Salmonella/mammalian microsome assay. *Mutat. Res.* **1980**, *78*, 317–320.

Brown, J. P.; Dietrich, P. S. Mutagenicity of plant flavonols in the Salmonella/Mammalian microsome test: activation of flavonol glycosides by mixed glycosidases from rat cecal bacteria and other sources. *Mutat. Res.* **1979**, *66*, 223–224.

Brown, J. P.; Dietrich, P. S.; Brown, R. J. Frameshift mutagenicity of certain naturally occurring phenolic compounds in the *Salmonella*/Microsome test: activation of anthraquinone and flavonol glycosides by gut bacterial enzymes. *Biochem. Soc. Trans.* **1977**, *5*, 1489–1492.

Caragay, A. B. Cancer-preventive foods and ingredients. *Food Technol.* **1992**, *46*, 65–68.

Eldridge, A. C. Determination of isoflavones in soybean flours, protein concentrates and isolates. *J. Agric. Food Chem.* **1982**, *30*, 353–355.

Eldridge, A. C.; Kwolek, W. F. Soybean isoflavones: effect of environment and variety on composition. *J. Agric. Food Chem.* **1983**, *31*, 394–396.

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

Fleury, Y.; Welti, D. H.; Philippossian, G.; Magnolato, D. Soybean (malonyl) isoflavones characterization and antioxidant properties. In *Phenolic compounds in food and their effects on health*; Huang, M.-T., Ho, C.-T., Lee, C. Y., Eds.; American Chemical Society: Washington, DC, 1992; Vol. II, pp 98–113.

Henderson, B. E.; Ross, R. K.; Pike, M. C.; Casagrande, J. T. Endogenous hormones as a major factor in human cancer. *Cancer Res.* **1982**, *42*, 3232–3239.

Hendrich, S.; Lee, K-W.; Xu, X.; Wang, H-J.; Murphy, P. A. Defining food components as new nutrients. *J. Nutr.* **1994**, in press.

Huang, J.; Nasr, M.; Kim, Y.; Matthews, R. Genistein inhibits protein histidine kinase. *J. Biol. Chem.* **1992**, *267*, 15511–15515.

Kudou, S.; Fleury, Y.; Welt, D.; Magnolato, D.; Uchida, T.; Kitamura, K. Malonyl isoflavone glycosides in soybean seeds (*Glycine max* Merrill). *Agric. Biol. Chem.* **1991**, *55*, 2227–2233.

Matsuura, M.; Obata, A. β-Glucosidases from soybeans hydrolyze daidzin and genistin. *J. Food Sci.* **1993**, *58*, 144–147.

Messina, M.; Barnes, S. The role of soy products in reducing cancer risk. *J. Natl. Cancer Inst.* **1991**, *83*, 541–546.

Murphy, P. A. Separation of genistin, daidzin and their aglucones and coumesterol by gradient high-performance liquid chromatography. *J. Chromatogr.* **1981**, *211*, 166–169.

Murphy, P. A. Phytoestrogen content of processed soybean products. *Food Technol.* **1982**, *36*, 60–64.

Naim, M.; Gestetner, B.; Kirson, I.; Birk, Y.; Bondi, A. A new isoflavone from soya bean. *Phytochemistry* **1973**, *12*, 169–170.

Naim, M.; Gestetner, B.; Zilkah, S.; Birk, Y.; Bondi, A. Soybean isoflavones. Characterization, determination and antifungal activity. *J. Agric. Food Chem.* **1974**, *22*, 806–810.

Naim, M.; Gestetner, B.; Bondi, A.; Birk, Y. Antioxidative and antihemolytic activities of soybean isoflavones. *J. Agric. Food Chem.* **1976**, *24*, 1174–1177.

Okura, A.; Arakawa, H.; Oka, H.; Yoshinari, T.; Monden, Y. Effect of genistein on topoisomerase activity and on the growth of [Val12]Ha-*ras*-transformed NIH 3T3 cells. *Biochem. Biophys. Res. Commun.* **1988**, *157*, 183–189.

Peterson, G.; Barnes, S. Genistein inhibition of the growth of human breast cancer cells: independence from estrogen receptors and the multi-drug resistance gene. *Biochem. Biophys. Res. Commun.* **1991**, *179*, 661–667.

Pratt, D. E.; Birac, P. M. Source of antioxidant activity of soybeans and soy products. *J. Food Sci.* **1979**, *44*, 1720–1722.

Sariaslani, F. S.; Kunz, D. A. Induction of cytochrome P-450 in *Streptomyces griseus* by soybean flour. *Biochem. Biophys. Res. Commun.* **1986**, *141*, 405–410.

Verdeal, K.; Brown, R. R.; Richardson, T.; Ryan, D. S. Affinity of phytoestrogens for estradiol binding proteins and effect of coumesterol on growth of 7,12-dimethylbenz(a)anthracene-induced rat mammary tumors. *J. Natl. Cancer Inst.* **1980**, *64*, 285–290.

Walter, E. D. Genistin (an isoflavone glucoside) and its aglycone, genistein from soybean. *J. Am. Chem. Soc.* **1941**, *63*, 3273–3276.

Wang, H.; Murphy, P. A. Isoflavone composition of American and Japanese soybeans in Iowa: Effects of variety, crop year, and location. *J. Agric. Food Chem.* **1994**, following paper in this issue.

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