Isoflavone Composition of American and Japanese Soybeans in Iowa: Effects of Variety, Crop Year, and Location

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The amounts of 12 isoflavones were measured in 8 American and 3 Japanese soybean varieties by using C_{18} reversed-phase high-performance liquid chromatography. In Vinton 81 soybeans of 1989–1991, variation in total isoflavone ranged from 1176 to 3309 μg/g. Isoflavone amounts of soybeans grown in different locations in 1991 ranged from 1176 to 1749 μg/g. Crop year seemed to have a much greater influence on isoflavone content in Vinton 81 than did location. Isoflavones in the other seven American varieties (Pioneer 9111, Pioneer 9202, Prize, HP204, LS301, XL72, Strayer 2233) ranged from 2053 to 4216 μg/g. The major isoflavone constituents were 6″-*O*-malonylgenistin, genistin, 6″-*O*-malonyldaidzin, and daidzin. Isoflavone contents in three Japanese varieties (Keburi, Kuro diazu, Raiden) ranged from 2041 to 2343 μg/g and from 1261 to 1417 μg/g for soybeans grown in 1991 and 1992, respectively. Compared with American varieties, Japanese varieties had higher 6″-*O*-malonylglycitin contents and higher ratios of 6″-*O*-malonyldaidzin to daidzin and 6″-*O*-malonylgenistin and genistin.

Keywords: *Isoflavones; daidzin; genistin; glycitin; soybeans*

INTRODUCTION

Soybeans have been consumed by Asians for a significant amount of history. Recently, to decrease the risk of diseases by changing dietary patterns, the use of soybeans has increased in the United States. Soybeans contain a variety of biologically active compounds (Messina and Messina, 1991). From epidemiological data, consumption of soybeans might contribute to the low incidence of breast cancer in Japanese women and men (Adlercreutz *et al.*, 1991). A similar result was found in the investigation on dietary soy consuming people with lower breast cancer risk in Singapore (Lee *et al.*, 1991). Isoflavones are one group of these potential anticarcinogenic soy compounds that have been intensively studied. Evidence has shown that isoflavones possess anticarcinogenic properties by acting as antiestrogens (Adlercreutz *et al.*, 1986), antioxidants (Naim *et al.*, 1976), and tyrosine protein kinase inhibitors (Akiyama *et al.*, 1987). The major isoflavones, daidzin and genistin, and their corresponding aglycons, daidzein and genistein, have been isolated and identified from soybean meal (Walter, 1941). The third kind of isoflavone, glycitein (6-methoxydaidzein), and its 7-*O*-β-glucoside, glycitin, were first isolated from soybeans by Naim *et al.* (1973). More recently, researchers discovered acetylated and malonylated isoflavones. 6″-*O*-Acetyldaidzin (Ohta *et al.*, 1979), 6″-*O*-acetylgenistin (Ohta *et al.*, 1980), and 6″-*O*-acetylglycitin (Kudou *et al.*, 1991a) have been isolated from soybean seeds, separately. These compounds were found at greater concentrations in the hypocotyl of soybeans than in the cotyledon. Farmakalidis and Murphy (1985) isolated 6″-*O*-acetyldaidzin and 6″-*O*-acetylgenistin from toasted defatted soy flakes and quantified their contents in four varieties of soybeans. 6″-*O*-Malonyldaidzin, 6″-*O*-malonylgenistin, and 6″-*O*-malonylglycitin were found in soybean seeds by Kudou *et al.* (1991b) and were shown to be the thermally unstable constituents.

Soybeans and soy foods are consumed in significant amounts in Asian countries because of their inexpensive, high-quality protein (Koury and Hodges, 1968). Isolated soy proteins have been used in a wide range of products in food industry. In the accompanying paper (Wang and Murphy, 1994), we reported that isoflavone contents in 29 commercial soy food products were remarkably different, due in part to the variety of soybeans used as starting material. Eldridge and Kwolek (1983) investigated the isoflavone contents in Illinois soybeans; however, they did not report acetylated or malonylated isoflavone compounds. In the present study, we measured all 12 isoflavones in 8 varieties of Iowa soybeans, usually used for tofu production, and in 3 varieties of Japanese soybeans and compared the effects of different varieties, crop years, and locations on the isoflavone content in these soybeans.

MATERIALS AND METHODS

Soybean and Isoflavone Sources. All American soybeans analyzed in this study were of food grade and generously provided by Dr. L. A. Wilson and Dr. C. Hurburgh of the Department of Food Science and Human Nutrition, Iowa State University. Three Japanese soybeans were grown by Dr. R. G. Palmer of the Department of Agronomy, Iowa State University. Authentic standards of daidzein and genistein were obtained from commercial sources (ICN Pharmaceuticals, Plainview, NY, and Calbiochem Corp., La Jolla, CA). Other isoflavone standards were purified as described in the accompanying study (Wang and Murphy, 1994). Milli-Q system (Millipore Co.) HPLC grade water was used. Other HPLC grade organic solvents were from Fisher Scientific Co.

Isoflavone Extraction. Raw soybean seeds (2 g) with seed coat were ground, mixed with 2 mL of 0.1 N HCl and 10 mL of acetonitrile, stirred for 2 h at room temperature, and filtered through Whatman No. 42 filter paper. The filtrate was taken to dryness under vacuum a temperature below 30 °C. The dried material was redissolved in 10 mL of 80% methanol and then filtered through a 0.45-μm filter unit (Alltech Associates,

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Table 1. Isoflavone Contents (Micrograms per Gram) of Vinton 81 Soybeans in Different Crop Years in Iowa[a,b]

isoflavone	1989	1990	1991A[c]	1991B[c]	1991C[c]
daidzein	59 a	26 b	10 cd	17 c	7 d
genistein	56 a	29 b	19 c	20 c	17 c
glycitein	20 bc	20 c	22 b	24 a	20 bc
diadzin	779 a	690 b	234 c	231 c	180 c
genistin	850 a	852 a	326 c	325 c	394 b
glycitin	69 a	56 a	66 a	65 a	53 a
6″-*O*-malonyldaidzin	410 a	300 b	121 d	237 c	241 c
6″-*O*-malonylgenistin	958 a	743 b	290 b	545 c	738 b
6″-*O*-malonylglycitin	69 a	50 b	58 ab	72 a	61 ab
6″-*O*-acetyldaidzin	tr[d]	tr	tr	tr	tr
6″-*O*-acetylgenistin	2 c	9 a	5 b	4 b	2 c
6″-*O*-acetylglycitin	36 a	nd[d]	25 ab	23 ab	35 a
total	3309 a	2776 b	1176 d	1563 c	1749 c

[a] Samples measured in triplicate. [b] Means followed by the same letter in the same row are not significantly different ($p < 0.05$). [c] Different crop-growing locations. [d] tr, trace; nd, not detected.

Deerfield, IL). Twenty microliters of filtrate was applied in the HPLC analysis.

HPLC Analysis. The instrumentations for high-performance liquid chromatography (HPLC) analysis used were described in the accompanying paper (Wang and Murphy, 1994). A linear HPLC gradient was employed: solvent A was 0.1% glacial acetic acid in H_2O, and solvent B was 0.1% glacial acetic acid in acetonitrile; 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. UV spectra were recorded, and area responses were integrated by Waters 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 for variety, crop year, and variety−crop year interaction. Differences between the sample means were analyzed by Fisher's least significant difference (lsd) test.

RESULTS AND DISCUSSION

The eight American varieties of soybeans analyzed in this study are commonly used in tofu production and characterized as large uniform seed size, light-colored hilum, thin seed coat, and high protein content. Because the proportion of seed coat is as little as 8%, the step of dehulling the soybeans was eliminated from the extraction procedure of isoflavone. Typically, soybeans processed for soy milk and tofu are not dehulled. There have been reports that only small quantities of isoflavones existed in the seed coat of soybeans (Eldridge and Kwolek, 1983). Most of the isoflavones were concentrated in the hypocotyl and are 4 times higher than in cotyledons (Kudou *et al.*, 1991b). Glycitein and its three derivatives exclusively occurred in the hypocotyl (Kudou *et al.*, 1991b). The extraction method used in this study followed that of Murphy (1981). Acetonitrile with dilute acid increased extraction efficiency. During the extraction, low temperature (<30 °C) was employed because storage of the crude extracts at higher temperature resulted in hydrolysis of malonyl esters (data not shown; Köster *et al.*, 1983). This might be due to the heat sensitivity of malonyl esters themselves (Kudou *et al.*, 1991b) or to β-glucosidases from soybeans (Matsuura and Obata, 1993).

The effect of different crop years on the isoflavone distribution and content in Vinton 81 soybeans is presented in Table 1. Vinton 81 soybeans are used as tofu beans in Iowa. Similar distribution patterns of isoflavones were observed among the soybeans within three crop years (1989−1991). 6″-*O*-Malonylgenistin was the major isoflavone constituent, representing 25−42% of isoflavones, followed by genistin, 6″-*O*-malonyldaidzin, and daidzin, respectively. These four components composed from 83% to 93% of total isoflavone contents. The other eight isoflavones were in very low amounts, 7−17%. The forms of 7-*O*-glucoside 6″-*O*-acetylates were especially low. According to Farmakalidis and Murphy (1985) and Kudou *et al.* (1991b), 6″-*O*-acetylated isoflavones were the products of heat treatment. Therefore, it was expected that these compounds existed in trace amounts in the intact, minimally processed soybeans. This is in agreement with results reported by other researchers (Graham *et al.*, 1990; Kudou *et al.*, 1991b) that isoflavones predominantly exist as the 7-*O*-glucoside 6″-*O*-malonylated conjugates in soybean tissues. This phenomenon also occurs in the isoflavones in chickpea, formononetin, and biochanin, the 4′-*O*-methylated derivatives of daidzein and genistein, respectively (Kessmann and Barz, 1987). Because of their polar, hydrophilic properties, the isoflavone conjugates are located in vacuoles (Barz and Welle, 1992). Malonylation may facilitate the transport of flavonoid glycosides through the tonoplast into the vacuole (Teusch and Forkmann, 1987). The functions of isoflavones in soybeans are as inducing compounds of *nod* genes in *Bradyrhizobium japonicum* (Kosslak *et al.*, 1990) and as precursors for inducible pterocarpan phytoalexins, glyceollin I−III, in soybean (*Glycine max*) (Ebel and Grisebach, 1988).

There was a significant difference ($p < 0.05$) in the concentrations of isoflavones among different crop years in terms of the total isoflavone and the individual isoflavones. Soybeans grown in 1989 contained the highest total isoflavone amounts, which were 1.2 times and 1.9−2.8 times higher than those in 1990 and 1991 soybeans, respectively. This dramatic change of total isoflavones from 1989 to 1991 was also reflected in the amounts of 6″-*O*-malonylgenistin, 6″-*O*-malonyldaidzin, genistin, and daidzin. In contrast, 6″-*O*-malonylglycitin and glycitin contents remained constant across crop years and were at lesser concentrations than the genistein and daizein forms. The data in Table 1 present the effect of different growth locations on the isoflavone content in 1991. All three 1991 soybeans had significantly greater 6″-*O*-malonylgenistin and genistin contents in the soybeans at location C than did those at locations A and B. However, the total or the individual amounts were not greatly influenced by the growth locations. It seemed that effect of crop year was more influential than that of growth location on the isoflavone concentrations.

Seven varieties of soybeans developed for tofu production, Pioneer 9111, Pioneer 9202, Prize, HP204, LS301, XL72, and Strayer 2233, from the same crop year of 1989 were analyzed. The total and individual isoflavone contents are given in Table 2. Total isoflavone concentrations were in the range of 2053−4216 μg/g and varied from variety to variety. Pioneer 9111, Pioneer 9202, Prize, and LS301 contained more than 3500 μg/g of total isoflavones, which was higher than that of Vinton 81 of 1989 (Table 1). The other three varieties, HP204, XL72, and Strayer 2233, had much lower total isoflavones. The greater amounts of 6″-*O*-malonylgenistin and 6″-*O*-malonyldaidzin resulted in the larger amounts of total isoflavones in the former four varieties of soybeans. XL72 had significantly more glycitin and 6″-*O*-malo-

Table 2. Isoflavone Contents (Micrograms per Gram) in Different Varieties of Iowa Soybeans in 1989[a,b]

isoflavone	Pioneer 9111	Pioneer 9202	Prize	HP204	LS301	XL72	Strayer 2233
daidzein	28 b	23 c	38 a	4 e	10 d	12 d	25 bc
genistein	30 c	34 b	33 b	15 e	16 d	45 a	30 c
glycitein	19 ab	20 ab	20 ab	19 b	19 b	21 a	20 ab
daidzin	637 b	531 c	780 a	196 f	442 d	148 f	367 e
genistin	888 a	668 c	806 b	330 f	562 d	481 e	444 e
glycitin	60 b	70 b	68 b	63 b	64 b	97 a	74 b
6″-*O*-malonyldaidzin	690 ab	630 b	709 a	349 c	752 a	198 d	385 c
6″-*O*-malonylgenistin	1756 a	1705 a	1342 c	945 e	1558 b	1042 d	883 e
6″-*O*-malonylglycitin	72 b	88 b	87 b	94 b	92 b	118 a	81 b
6″-*O*-acetyldaizdin	tr[c]	tr	tr	tr	tr	tr	tr
6″-*O*-acetylgenistin	2 a	1 ab	1 ab	1 ab	1 ab	2 ab	tr
6″-*O*-acetylglycitin	33 c	35 b	tr	36 a	33 bc	37 a	34 bc
total	4216 a	3806 b	3886 b	2053 e	3551 c	2201 de	2344 d

[a] Samples measured in triplicate. [b] Means followed by the same letter in the same row are not significantly different ($p < 0.05$). [c] tr, trace.

Table 3. Isoflavone Contents (Micrograms per Gram) in Three Japanese Varieties of Soybean[a,b]

isoflavone	Keburi 1991	Keburi 1992	Kuro diazu 1991	Kuro diazu 1992	Raiden 1991	Raiden 1992
daidzin	1 b	4 a	tr[c]	tr	tr	tr
genistein	9 b	8 c	8 c	7 d	11 a	8 c
glycitein	21 a	19 ab	tr	12 b	22 a	20 ab
daidzin	91 bc	96 b	80 c	37 e	115 a	53 d
genistin	179 b	136 cd	174 b	128 d	237 a	148 c
glycitin	68 c	50 d	66 c	42 e	96 a	73 b
6″-*O*-malonyldaidzin	562 a	322 d	375 c	222 e	407 b	242 e
6″-*O*-malonylgenistin	1232 a	670 b	1187 a	717 b	1191 a	723 b
6″-*O*-malonylglycitin	127 b	70 d	111 c	60 e	183 a	111 c
6″-*O*-acetyldaidzin	12 a	tr	1 bc	tr	2 b	tr
6″-*O*-acetylgenistin	tr	2 b	tr	tr	tr	4
6″-*O*-acetylglycitin	41 a	33 c	37 b	35 c	40 a	34 c
total	2343 a	1411 c	2041 b	1261 d	2305 a	1417 c

[a] Samples measured in triplicate. [b] Means followed by the same letter in the same row are not significantly different ($p < 0.05$). [c] tr, trace.

Table 4. Ratios of Malonyl Isoflavones to Glucosides in Various Soybeans

soybean (year)	6″-*O*-malonyl-daidzin/daidzin	6″-*O*-malonyl-genistin/genistin
American varieties		
Vinton 81 (1989)	0.5	1.1
Vinton 81 (1990)	0.4	0.9
Vinton 81 (1991)	1.0	1.7
Pioneer 9111 (1989)	1.1	2.0
Pioneer 9202 (1989)	1.2	2.6
Prize (1989)	0.9	1.7
HP204 (1989)	1.8	2.9
LS301 (1989)	1.7	2.8
XL72 (1989)	1.3	2.2
Strayer 2233 (1989)	1.0	2.0
Japanese varieties		
Keburi (1991)	6.2	6.9
Keburi (1992)	3.4	4.9
Kuro diazu (1991)	4.7	6.8
Kuro diazu (1992)	6.0	5.6
Raiden (1991)	3.5	5.0
Raiden (1992)	4.6	4.9

nylglycitin than did all others. The trends for other isoflavones were similar to that in Vinton 81.

Isoflavone contents were determined in three Japanese varieties of soybeans, Keburi, Kuro diazu, and Raiden, grown in a single experimental unit for 1991 and 1992 (Table 3). These data indicated that there was variation among Japanese varieties. In 1991 and 1992, Kuro diazu soybeans were significantly lesser than the other two varieties in total and individual isoflavone contents. The 1992 group constituted only 60% of the isoflavone amounts in the 1991 group. Crop year seemed to affect isoflavone concentration to a greater extent than genetics in these Japanese varieties. We have examined the effect of the interaction of variety and crop year. From the results of statistical analysis of variety−year interactions, a significant effect ($p < 0.05$) was observed for the concentrations of daidzein, genistein, daidzin, genistin, 6″-*O*-malonyldaidzin, 6″-*O*-malonylglycitin, 6″-*O*-acetyldaidzin, 6″-*O*-acetylgenistin, and 6″-*O*-acetylglycitin. In comparison with the eight American varieties, these three Japanese varieties soybeans contained greater 6″-*O*-malonylglycitin concentration levels. The ratios of 6″-*O*-malonyldaidzin to daidzin and of 6″-*O*-malonylgenistin to genistin were evaluated (Table 4). The American varieties, with ratios of 1−3, were in marked contrast to Japanese varieties, with ratios of 4−6. This clearly suggests that genetics played a significant role in soy isoflavone distribution.

In this study, the content and distribution of isoflavone were measured in eight American and three Japanese varieties of soybeans. Isoflavone contents were influenced by genetics, crop years, and growth location. From the results, the effect of crop year had a greater impact on the isoflavone contents than did location. The climate condition might be the attributing factor to variation in isoflavone contents. The isoflavone distribution patterns were different between American and Japanese soybeans. Japanese soybeans had higher ratios of 6″-*O*-malonyl isoflavones to glucosides than did American soybeans. The compounds 6″-*O*-malonylgenistin, genistin, 6″-*O*-malonyldaidzin, and daidzin were the dominant isoflavones in the soybeans. Further studies of the extent of bioavailability of different isoflavone isomers are required.

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