Soybean Isoflavones: Effect of Environment and Variety on Composition

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The effects of environment and variety on the isoflavones and isoflavone glucosides of soybeans were studied. Extracting the oil from soybeans did not remove the isoflavones or the isoflavone glucosides since they are not soluble in hexane. The total isoflavone of soybeans varied from 116 to 309 mg/g within variety and varied from 46 to 195 mg/g with the same variety grown in different locations. The isoflavones in soybeans also varied from year to year when soybeans were grown in the same location. On an equal weight basis most of the isoflavones are concentrated in the hypocotyl and the isoflavone content of soybean hulls is quite low.

Soybeans contain several biologically active components (Anderson et al., 1979). The isoflavones of soybean products are of interest because of their estrogenic (Drane et al., 1980; Kitts et al., 1980), antifungal (Wyman and Van Etten, 1978), and antibacterial (Naim et al., 1974) activities. The estrogenic activity of soybeans is of particular interest because soybean protein products are being used in food products such as infant formulas, health foods, and mass feeding programs.

Recently, Murphy (1981) reported the separation of two soybean isoflavone glucosides and their aglucons by gradient high-performance liquid chromatography with a methanol–water gradient. Earlier reports from this laboratory described a method for the quantitative determination of six isoflavones (daidzin, glycitin, genistin, daidzein, glycitein, and genistein) in soybeans (Eldridge, 1982a) and analysis of several commercial soybean protein products which are used for food products (Eldridge, 1982b). In the latter publication, variation was noted in the isoflavone content of several defatted soybean flours. To better understand the observed variation, we conducted the current study on the effects of variety, location, and crop year on the amount of isoflavone and isoflavone glucosides in soybeans. Distribution of the isoflavones in various seed components was also examined.

MATERIALS AND METHODS

Sources of Soybeans. All soybeans used in this study were certified seed grade. All samples except Tiger variety were supplied by Dr. Richard L. Bernard, U.S. Department of Agriculture, U.S. Regional Soybean Laboratory, University of Illinois, Urbana, IL 61801. The soybeans from the U.S. Regional Soybean Laboratory were used because as part of the soybean breeding program the varieties are grown several years at different locations. The parentage of each variety from the U.S. Regional Soybean Laboratory are included as footnotes in the tables. Tiger soybeans (TS-280) were purchased from Sommer Bros. Seed Co., Pekin, IL 61554.

Sample Preparation. For initial studies, soybeans were cracked, dehulled, flaked, and extracted with pentane–hexane (Eldridge et al., 1971). The air-dried defatted soybean flakes were analyzed and compared to full-fat (unextracted) soybean flakes.

Two varieties of soybeans were cracked mechanically between rollers and hand separated into various seed parts. The hull, hypocotyl, and cotyledon were analyzed for isoflavones and isoflavone glucosides to determine the within-seed distribution.

After these initial studies, whole soybeans which were stored under identical conditions and contained 9.2–12.2% moisture were ground for 1 min in a Varco electric grinder, Model 228.1.00. The full-fat powder from this grinder gave particles, 90% of which passed a 40-mesh screen. The larger particles consisted primarily of hull fragments. At least two 1-g subsamples were taken from each soybean sample preparation.

Preparation of Extracts. A 1-g subsample in 25 mL of 80% aqueous methanol containing an internal standard (*n*-butyrophenone) was heated (boiling) on a steam bath 4 h, cooled, filtered through a type AP prefilter followed by a type HA, 0.45-μm filter (Millipore Corp., Bedford, MA).

Chromatography. The previously published high-performance liquid chromatographic procedure (Eldridge, 1982a) was followed, using a linear gradient from 25 to 50% methanol in 20 min followed by an isocratic hold period of at least 30 min. Response factors which were used for quantification of the individual isolated glucosides and aglucons were determined relative to the internal standard. These response factors were used to calculate the isoflavone and isoflavone glucoside composition of various soybean preparations.

Statistical Analysis. Variation among sample means and interactions were examined by use of analyses of variance (Snedecor and Cochran, 1980). The between-subsample variance was used to determine an LSD (least significant difference, 0.05 level) for comparing sample

Table I. Effect of Defatting Soybeans on Isoflavone Content of Soybean Meal[a] (mg/100 g)

isoflavone	full-fat flakes (fat-free basis)	defatted flakes	LSD[b]
daidzin	118.5	114.0	5.2
glycitin 7-β-glucoside	0.9	0.8	2.8
genistin	204.1	188.5	9.6
daidzein	2.0	2.5	1.8
glycitein	1.0	1.2	1.6
genistein	4.4	4.4	0.9
total	330.9	311.4	17.2

[a] Tiger variety with 21.4% oil. [b] Least significant difference at 0.05 probability level.

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Table II. Anatomical Distribution[a] of Soybean Isoflavones in Two Varieties of Soybeans (mg/100 g)

isoflavone	hull		hypocotyl		cotyledon		
	Amsoy[b]	Tiger	Amsoy	Tiger	Amsoy	Tiger	LSD[c]
daidzin	6.6	8.6	1031.5	759.9	37.5	102.8	539.2
glycitin 7-β-glucoside			664.1	588.8	1.7	1.6	127.0
genistin	2.8	7.4	5.3	9.1	113.9	205.8	167.4
daidzein	0.7	1.0	19.0	14.0	1.4	2.8	10.6
glycitein		1.5	11.8	9.3			5.8
genistein	0.5	1.5	24.7	24.2	2.8	5.9	5.8
total	10.6	20.0	1756.5	1405.2	158.5	319.2	808.1

[a] Soybeans contain about 8% hull, 2% hypocotyl, and 90% cotyledon (Bailey et al., 1935). [b] Amsoy variety = Adams × Harosoy. [c] Least significant difference at 0.05 probability level, based on part-variety interaction.

Table III. Isoflavone Content of Different Varieties of Soybeans Grown in Urbana, IL, in 1980 (mg/100 g)

isoflavone	variety[a]				
	Hardin	Amcor	Sprite	Century	LSD[b]
daidzin	30.5	38.8	83.7	84.9	4.3
glycitin 7-β-glucoside	9.5	9.9	15.2	15.1	2.3
genistin	68.0	96.3	200.5	140.5	7.0
daidzein	2.0	0.8	3.2	3.0	1.5
glycitein	2.2	1.3	3.0	2.2	1.3
genistein	3.2	2.5	3.7	4.6	0.7
total	115.9	149.8	309.3	250.2	14.7

[a] Parentage of varieties: Hardin = Corsoy[3] × Cutlass-71; Amcor = Amsoy-71 × Corsoy; Sprite = Williams × Ransom; Century = Catlin × Bonus. [b] Least significant difference at 0.05 probability level, based on sample variation.

means for varietal and year comparisons. Interactions of variety and seed part or location were also used to estimate variability in determining LSD's. Very likely with replicated experiments the appropriate variation estimates would fall between these extremes.

RESULTS AND DISCUSSION

Defatting full-fat soybean flakes does not remove any of the isoflavones or isoflavone glucosides from soybean meal (Table I). If the values for the isoflavones in full-fat meal are corrected for the amount of oil removed by hexane (21.4%), the resulting values on a fat-free basis compare very favorably with values obtained when defatted soybean flakes are analyzed. The values in Table I agree with previously reported values for defatted soybean flours (Eldridge, 1982b). The results indicate a difference between full-fat (calculated to a fat-free basis) and defatted meals for total isoflavone and genistin content. Earlier research (Booth et al., 1960) showed that refined vegetable oils contained estrogenic compounds. Perhaps some of the free aglucons are extracted into the hexane–oil mixture. Attempts to extract the aglucons from crude soybean oil with aqueous alcohol failed to reveal any isoflavones when extracts were chromatographed. The results indicate that, in commercial practices, defatted soybean meals will contain essentially all of the isoflavones or isoflavone glucosides present in the starting soybeans.

Soybeans were cracked and separated into their anatomical parts by hand. Analyses of the hull, hypocotyl, and cotyledon for isoflavones are given in Table II. The concentration of the isoflavones on a weight basis is highest in the hypocotyl (1400–1750 mg/100 g) and lowest in the hull of the seed (10–20 mg/100 g). This is in contrast to the distribution of coumesterol in soybeans (Lookhart et al., 1979), which is concentrated in the hull or seed coat. The data in Table II also show that the hypocotyl contains ~1.5% total isoflavone and isoflavone glucoside. Why these compounds are present in the hypocotyl in such high concentrations is intriguing. The cotyledon, on the other hand, has about 20% the amount of isoflavone and isoflavone glucoside found in the hypocotyl.

The distributions of individual isoflavones are also different in the hypocotyl and cotyledon. In the hypocotyl, primarily two glucosides are found, daidzin and glycitin 7-β-glucoside, whereas in the cotyledon, there is about 20 times as much genistin as in the hypocotyl.

The isoflavone and isoflavone glucosides determined in the cotyledon are in close agreement with values found for full-fat flakes in Table I, as would be expected, because the cotyledon represents about 90% of the total seed (Bailey et al., 1935). Statistical interactions of seed part and variety were significant for daidzin, genistin, glycitein, and the total. The analytical results identified showed considerable variability between samples.

The data in Table II indicate that dehulling cracked soybeans should have little effect on the concentration of the isoflavones or isoflavone glucosides in soybean products currently produced. The data in Table II also indicate a varietal difference ($p < 0.5$) in the amount of isoflavones or isoflavone glucosides; therefore, it was of interest to study the isoflavone content of several different varieties of soybeans. Seeds were obtained, ground in a Varco mill, and analyzed without defatting. Shown in Table III is the isoflavone and isoflavone glucoside contents of four varieties of soybeans grown in Urbana, IL, in 1980. The concentrations of the isoflavones of Hardin and Amcor varieties are very similar. Century and Sprite appear to contain similar concentrations of isoflavones but have

Table IV. Isoflavone Content of Hardin and Corsoy-79 Soybeans[a] Grown in Different Locations in 1980 (mg/100 g)

isoflavone	Girard, IL		Urbana, IL		Pontiac, IL		Dekalb, IL		
	Hardin	Corsoy-79	Hardin	Corsoy-79	Hardin	Corsoy-79	Hardin	Corsoy-79	LSD[b]
daidzin	14.2	25.1	22.5	49.1	45.4	64.1	44.4	53.3	25.8
glycitin 7-β-glucoside	9.2	12.2	7.4	13.0	13.1	13.3	16.1	19.0	7.4
genistin	21.5	39.5	49.9	88.8	96.3	115.6	107.5	113.9	42.7
daidzein	1.1	2.4	0.5	3.0	2.0	1.9	2.1	3.5	3.5
glycitein									
genistein	0.9	0.8	0.06	0.6	0.03	0.4	0.06	1.0	0.8
total	46.9	79.9	81.7	154.5	156.1	195.1	170.8	190.9	71.7

[a] Parentage of varieties: Hardin = Corsoy[3] × Cutlass-71; Corsoy-79 = Corsoy[6] × Lee-68. [b] Least significant difference at 0.05 probability level, based on location-variety interaction.

Table V. Isoflavone Content of Clark[a] Soybeans Grown in Urbana, IL, in Different Years (mg/100 g)

isoflavone	year 1975	1976	1978	1979	LSD[b]
daidzin	75.4	124.4	98.7	82.6	4.3
glycitin	19.9	22.8	25.5	23.5	2.3
7-β-glucoside					
genistin	153.2	210.4	157.4	135.4	7.8
daidzein	2.3	0.8	1.1	1.2	1.5
glycitein	3.0	3.2	1.9	2.0	1.3
genistein	0.7	0.9	0.2	0.6	0.7
total	254.7	362.5	284.9	245.2	14.7

[a] Clark variety = Lincoln[2] × Richland. [b] Least significant difference at 0.05 probability level, based on sample variation.

larger amounts of the isoflavones than the Hardin and Amcor, even though the four samples were grown the same year in the same location. Genistin appears to vary considerably between samples.

Further investigations into different varieties were conducted. Two varieties, Hardin and Corsoy-79, were grown the same year in four different locations in Illinois. The results of the eight samples for isoflavones and isoflavone glucosides are shown in Table IV.

The concentration of the isoflavones and isoflavone glucosides vary from variety to variety, and there are also differences when the same variety is grown in different locations. Significant variety–location interactions were observed for daidzin, glycitin 7-β-glucoside, genistin, and the total isoflavones. Varietal differences at Girard and Urbana differ from the varietal differences at Pontiac and DeKalb. These results may indicate adverse growing conditions in different locales in 1980, which was a dry year in Illinois.

Table V shows the amounts of isoflavones found in Clark soybeans when grown in Urbana, IL, in different years. Significant variation among years suggests that unknown climatic and environmental factors contribute to variation in isoflavones and isoflavone glucosides.

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Registry No. Daidzin, 552-66-9; glycitin, 40246-10-4; genistin, 529-59-9; daidzein, 486-66-8; glycitein, 40957-83-3; genistein, 446-72-0.

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Three New Ingenane Derivatives from the Latex of *Euphorbia canariensis* L.

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Three new ingenane esters, 3-*O*-acetyl-16-*O*-benzoyl-20-*O*-[(*Z*)-2-methyl-2-butenoyl]-16-hydroxyingenol (**1**), 3-*O*-[(*Z*)-2-methyl-2-butenoyl]-16-*O*-benzoyl-16-hydroxyingenol (**2**), and 3-*O*-acetyl-20-*O*-[(*Z*)-2-methyl-2-butenoyl]ingenol (**3**), were isolated from the latex of *Euphorbia canariensis* L. by using droplet countercurrent chromatography. The structures of these skin-irritant compounds were established through the interpretation of spectroscopic data. *E. canariensis* is sold in the United States as an ornamental plant and is currently under investigation for possible cultivation as a renewable energy source. Constituents **1–3** represent a health hazard for persons who contact the latex of this species with the skin or eyes.

Euphorbia canariensis L. (Euphorbiaceae) has recently been suggested as a candidate plant for cultivation in semiarid regions to produce fuel, since its latex is rich in isoprenoids (Calvin et al., 1982). This species, although native to the Canary Islands, is now available for purchase from nurseries in the United States as an ornamental houseplant. In previous work, *E. canariensis* latex has been shown to evoke severe skin inflammation in mice (Kinghorn and Evans, 1975) and, on repeated administration subsequent to a subcarcinogenic dose of 7,12-dimethylbenz[*a*]anthracene, has produced pronounced tumor-promoting effects on mouse skin (Roe and Peirce, 1961).

A number of constituents of *E. canariensis* latex has been investigated, including inositol and a phenol oxidase

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