Evaluation of Genistin and Genistein Contents in Soybean Varieties and Soy Protein Concentrate Prepared with 3 Basic Methods

N. PANDJAITAN, N. HETTIARACHCHY, Z.Y. JU, P. CRANDALL, C. SNELLER, AND D. DOMBEK

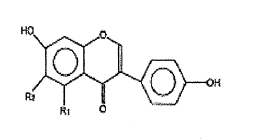
ABSTRACT: The contents of genistein and its β–glucoside form, genistin, in 13 soybean varieties were determined. Soybean variety (Var-1) with high genistein and genistin contents (0.019 mg/g and 0.420 mg/g) was used to evaluate the 3 basic soy protein concentrate (SPC) production methods (acid, alcohol, and hot-water leach) for genistin and genistein retention on a total weight basis. The acid leach method gave the highest total genistin + genistein content (0.742 mg/g) compared to SPCs prepared with the hot-water leach method (0.671 mg/g), and the alcohol leach method (0.070 mg/g). The acid leach, hot-water leach and alcohol leach methods had 20.3%, 24.2%, and 91.2% losses of total genistin + genistein, respectively.

Key Words: soy protein concentrate, genistein, genistin, β-glucosidase

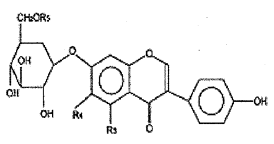
Introduction

ISOFLAVONES ARE THE MAJOR PHENOLIC COMPOUNDS IN SOY bean (Ahluwalia and others 1953). The amount of isoflavones in soybean varies according to genetics, crop year, and growth location (Wang and Murphy 1994). Concentration of isoflavone compounds can be as high as 3 mg/g in soybean (Eldridge 1983; Price and Fenwick 1985). These isoflavones exist in the form of aglucones (daidzein, genistein, glycitein) and their β-glucoside conjugates: the glucosides, the malonylglucosides, and the acetylglucosides (Fig. 1). Among the isoflavones, genistein has been reported to be the most potent inhibitor of cancer cell growth. Genistein was found most effective in inhibiting cell growth of human prostate cancer cells compared to genistin (Onozawa and others 1998). The antioxidant activity of genistein was much greater than other isoflavones (Ruiz-Larrea and others 1997).

Even though isoflavones are not classified formally as nutrients, they reportedly affect human health as much as vitamins and minerals (Messina 1997). Soy protein concentrate can act as a vehicle to carry genistein because it is incorporated at very small amounts in various food products. During soaking of soybean in water, genistin can be converted to genistein (Ha and others 1992; Matsuura and others 1995). Isoflavones have low hydrophilic property, but some isoflavones are lost during aqueous processing of soybean (Wang and Murphy 1996). The objectives of this study were to screen soybean varieties for their genistin and genistein content and evaluate 3 basic methods of SPC preparation for the retention of genistin and genistein.



R1	R2	Compound
OH	H	genistein
H	H	daidzein
H	OCH3	glycitein



R3	R4	R5	Compound
OH	H	H	genistin
H	H	H	daidzin
H	OCH3	H	glycitin
OH	H	COCH3	6”-O-acetylgenistin
H	H	COCH3	6”-O-acetyldaidzin
H	OCH3	COCH3	6”-O-acetylglycitin
OH	H	COCH2COOH	6”-O-malonylgenistin
H	H	COCH2COOH	6”-O-malonyldaidzin
H	OCH3	COCH2COOH	6”-O-malonylglycitin

Fig. 1—Structure of isoflavone isomers

Results and Discussion

Genistin and genistein contents of 13 soybean varieties

The amounts of genistin and genistein in 13 dehulled soybean varieties are shown in Table 1. The genistin contents ranged from 0.175 to 0.544 mg/g of soybean,and the genistein contents ranged from 0.004 to 0.019 mg/g. The genistin content in each variety was much higher than that of genistein. The ranges in these varieties were lower than the ranges of genistin (0.330 to 0.888 mg/g) and genistein (0.015 to 0.045 mg/g) reported by Wang and Murphy (1994) in 8 other American soybean varieties from the 1989 crop year in Iowa. The total isoflavones in Vinton

Table 1—Genistin and genistein contents (mg/g) of 13 soybean varieties[A]

Variety[B]	Genistin	Genistein	Total (genistin + genistein)
Var-1	0.420^{e}	0.019^{a}	0.439^{e}
Var-2	0.521^{b}	0.016^{b}	0.537^{b}
Var-3	0.203^{l}	0.010^{c}	0.223^{i}
Var-4	0.544^{a}	0.009^{c}	0.553^{a}
Var-5	0.432^{d}	0.008^{cd}	0.440^{d}
Var-6	0.364^{h}	0.008^{cd}	0.372^{g}
Var-7	0.175^{m}	0.008^{cd}	0.183^{g}
Var-8	0.321^{i}	0.007^{d}	0.329^{h}
Var-9	0.264^{j}	0.007^{d}	0.271^{h}
Var-10	0.215^{k}	0.006^{e}	0.221^{j}
Var-11	0.419^{e}	0.005^{f}	0.424^{e}
Var-12	0.467^{c}	0.004^{g}	0.471^{c}
Var-13	0.407^{g}	0.004^{h}	0.411^{f}

[A]Values are in moisture-free basis and represent the means; n = 3. Values in a column with different superscripts were significantly different ($p < 05$).
[B]Codes (Var-1 to Var 13) were used to represent real names of 13 soy varieties' due to commercial confidence and company privacy.

Table 2—Genistin (Gin) and genistein (Gen) contents[A] (mg/g) in ground soybean and defatted soy flour[B]

	Total weight basis			Protein basis		
Material	Gin	Gen	Total (Gin + Gen)	Gin	Gen	Total (Gin + Gen)
Dehulled soybean	0.420[b]	0.019[b]	0.439[b]	1.123[a]	0.051[a]	1.174[a]
Defatted soy flour	0.602[a]	0.027[a]	0.629[a]	1.125[a]	0.050[a]	1.175[a]

[A]Values are in moisture-free basis and represent the means; n = 3. Values in a column with different superscripts were significantly different ($p < 0.05$).
[B]Prepared from Var-1

Table 3—Genistin (Gin) and genistein (Gen) contents[A] (mg/g) of SPC[B] prepared by 3 basic methods

	Total weight basis			Protein basis		
Products	Gin	Gen	Total (Gin + Gen)	Gin	Gen	Total (Gin + Gen)
SPC (acid leach)	0.289[b]	0.453[a]	0.742[a]	0.442[b]	0.693[a]	1.135[a]
SPC (alcohol leach)	0.062[c]	0.008[c]	0.070[c]	0.092[c]	0.012[c]	0.104[c]
SPC (hot-water leach)	0.570[a]	0.101[b]	0.671[b]	0.891[a]	0.158[b]	1.049[b]

[A]Values are in moisture-free basis and represent the means; n = 3. Values in a column with different superscripts were significantly different ($p < 0.05$).
[B]Prepared from defatted soy flour of Var-1

81 soybean varieties grown in Iowa in 1989 were 1.2 and 2.8 times higher than the total isoflavone amount of the same soybean variety grown in the same area in 1990 and 1991, respectively. These investigators also reported genistin contents (0.136 to 0.237 mg/g) and genistein contents (0.008 to 0.011 mg/g) in 3 Japanese soybean varieties. The genetics, crop year, location, and other environmental conditions of the soybean varieties used in our experiment may account for the differences observed in genistin and genistein contents. Var-1 had the highest genistein content (0.019 mg/g) and higher genistin content (0.420 mg/g). Hence, this variety was selected to produce SPC.

Table 2 shows the genistin, genistein, and total genistin + genistein contents of ground dehulled soybean and defatted soy flour. The defatted soy flour had higher amounts of genistin (0.602 mg/g) and genistein (0.027 mg/g) compared to ground soybean on a total weight basis. Ground dehulled soybean had lower genistin (0.420 mg/g) and genistein contents (0.019 mg/g). Wang and Murphy (1996) and Coward and others (1993) reported that defatting of ground soybean did not result in extraction of isoflavones. The protein contents of ground soybean and defatted soy flour were 37.4% and 53.5%, respectively. Calculations of genistin, genistein and total genistin + genistein contents on a protein basis showed that genistin and genistein contents were the same before and after defatting. This indicates that genistin and genistein were not lost during lipid extraction but were retained with protein and other components in the soy flour.

Genistin and genistein contents in SPC prepared from defatted soy flour with alcohol leach method

Preparation of SPC by alcohol leach method resulted in very low concentrations of genistin (0.062 mg/g) and genistein (0.008 mg/g) (Table 3). Ethanol is an excellent solvent for genistin and genistein and readily solubilizes genistin and genistein from soy flour during protein extraction. The protein content of SPC produced with this method was 67.2%. On a protein basis, genistin (0.092 mg/g) and genistein contents (0.012 mg/g) of the SPC were also very low (Table 3), which indicates that genistin and genistein were removed by ethanol during SPC preparation.

Tables 4 and 5 illustrate the SPC yield (Weight of prepared SPC / Total defatted soy flour weight x 100%). The yields of genistin and genistein in SPCs on total weight basis (mg of genistin and genistein in SPC prepared from per g of defatted soy flour) and on protein basis (mg of genistin and genistein in SPC prepared from per g of protein in defatted soy flour) are also given. Alcohol leach method resulted in 2.135 g of SPC from 3 g defatted soy flour (71.2% yield) (Table 4). There was 8.3% as much total genistin + genistein content in SPC prepared with this method. The SPC contained 7.7% as much genistin content (0.134mg) and 16.7% as much genistein content (0.014mg) compared to defatted soy flour (1.805 and 0.081 mg of genistin and genistein, respectively). These results are in agreement with the result of Coward and others (1993), who concluded that SPC prepared with hot aqueous ethanol had 10 to 20 fold lower isoflavone concentration.

On total weight basis, a significant loss of 91.9% of total genistin + genistein was observed. The total genistin + genistein content in SPC prepared with this method (0.153 mg) was very low in comparison with the content in defatted soy flour (1.886 mg). The total genistin + genistein loss on protein basis was 91.2% (Table 5), which indicates that genistin and genistein were not retained in SPC prepared with this method. HPLC profile of SPC prepared with alcohol leach method gave very low peaks of genistin and genistein. This is in agreement with the result of Coward and others (1993) that SPC prepared by alcohol leach method lost almost all of the isoflavones.

Genistin and genistein contents in SPC prepared from defatted soy flour with hot-water leach method

The hot-water leach method resulted in SPC with a much higher genistin and genistein content in comparison to SPC prepared by the alcohol leach method. SPC prepared with this method contained 0.570 mg/g genistin and 0.101 mg/g genistein (Table 3). The SPC had 64.0% protein content. Total genistin + genistein (1.050 mg/g), also individual genistin (0.892 mg/g) and genistein (0.158 mg/g) contents of the SPC on protein basis were high, indicating that genistin and genistein were retained with protein and other components in SPC. This method resulted in a lower total content of genistin + genistein (0.671 mg/g) compared to SPC content prepared with the acid leach method (0.742 mg/g).

Hot-water extraction at neutral pH resulted in 70.9% yield of SPC (2.127 g from 3 g defatted soy flour) (Table 4). The SPC retained most of genistein and genistin, which is in agreement with the result of Coward and others (1993). They found that the genistin and genistein amounts in SPC prepared with hot-water leach at neutral pH were similar to the amounts in defatted soy flour. This method resulted in a significant loss of 24.2% of total genistin + genistein content (1.429 mg in SPC and 1.886 mg defatted soy flour) on total weight basis. On protein basis, there was 10.6% loss of total genistin + genistein (Table 5). Thus, some genistin and genistein was leached from the protein.

The genistein content increased 1.6 times in SPC (0.215 mg) in comparison to the defatted soy flour (0.081mg). It is possible that genistin was converted to genistein by endogenous β–glucosidase in soybean. Soaking of soybean in water has been known to increase the genistin conversion to genistein due to the hydrolytic activity of β–glucosidase in soybean. HPLC profiles of SPC prepared with both hot-water and acid leach methods gave high levels of genistin and genistein, which indicated that SPCs prepared with the 2 methods resulted in better retention of the 2 compounds compared to the alcohol leach method.

Genistin and genistein contents in SPC prepared from defatted soy flour with acid leach method

SPC prepared with the acid leach method had the highest

Table 4—Yields and genistin (Gin) and genistein (Gen) contents[A] in defatted soy flour and SPC[B] prepared with 3 basic methods (total weight basis)

Materials	Yield (%)[C]	Yield (g)[D]	Gin (mg)	Gen (mg)	Total (Gin + Gen) (mg)
Defatted soy flour	100.0	3.000[a]	1.805[a]	0.081[c]	1.886[a]
SPC (acid leach)	67.1	2.012[d]	0.581[c]	0.912[a]	1.493[b]
SPC (alcohol leach)	71.2	2.135[b]	0.134[d]	0.014[d]	0.153[d]
SPC (hot-water leach)	70.9	2.127[c]	1.214[b]	0.215[b]	1.429[c]

[A]Values are in moisture-free basis and represent the means; n = 3. Values in a column with different superscripts were significantly different ($p < 0.05$).
[B]Prepared from defatted soy flour of Var-1
[C]Calculated as percentage of amount of prepared SPC in total defatted soy flour weight used to prepare the SPC
[D]Represent amount of SPC prepared from 3 g of defatted soy flour

Table 5—Yields and genistin (Gin) and genistein (Gen) contents[A] in defatted soy flour and SPC[B] prepared with 3 basic methods (protein basis)

Materials	Yield (%)[C]	Yield (g)[D]	Gin (mg)	Gen (mg)	Total (Gin + Gen) (mg)
Defatted soy flour	100.0	1.605[a]	1.125[a]	0.050[c]	1.175[a]
SPC (acid leach)	82.0	1.316[d]	0.442[c]	0.693[a]	1.135[b]
SPC (alcohol leach)	89.4	1.435[b]	0.093[d]	0.010[d]	0.103[d]
SPC (hot-water leach)	84.8	1.361[c]	0.892[b]	0.158[b]	1.050[c]

[A]Values are in moisture-free basis and represent the means; n = 3. Values in a column with differentsuperscripts were significantly different ($p < 0.05$).
[B]Prepared from defatted soy flour of Var-1
[C]Calculated as percentage of weight of prepared SPC in total defatted soy flour weight used to prepare the SPC
[D]Represents weight (g) of SPC prepared from 3 g of defatted soy flour

genistin content (0.289 mg/g), genistein content (0.453 mg/g), and total genistin + genistein content (0.742 mg/g) compared to SPCs prepared with the other 2 methods (Table 3). The protein content of SPC prepared with this method was 65.4% on moisture-free basis. The total genistin + genistein content (1.135 mg/g) as well as individual genistin content (0.442 mg/g) and genistein content (0.693 mg/g) of the SPC on protein basis were also the highest in comparison with the other 2 methods.

The yield of 67.1% SPC was obtained with acid leach method (2.012 g from 3 g defatted soy flour) (Table 4). A significant loss of 20.3% of total genistin + genisteinbased on total weight basis was observed with this method. The loss of total genistin + genistein on protein basis in SPC was only 2.9%, which was low compared to the loss observed in SPCs prepared with the other 2 methods. Because SPC prepared with the acid leach method resulted in the best retention of genistin and genistein, it was selected as the method for SPC preparation.

There was some conversion of genistin to genistein during SPC preparation with the acid leach method, which may be due to the hydrolytic activity of the endogenous β–glucosidase in soybean or acid hydrolysis during SPC preparation. The SPC contained 32.6% less genistin (0.589 mg) and 11 times as much genistein (0.913 mg) content when compared to the defatted soy flour.

Conclusions

SOYBEAN VARIETIES INVESTIGATED IN THIS EXPERIMENT HAD 0.004 to 0.019 mg genistein and 0.175 to 0.544 genistin per g of dehulled soybean. The genistin and genistein contents of soy protein concentrate (SPC) were affected by processing steps. Evaluation of genistin and genistein distribution during soy protein concentrate production showed that the acid leach method was the preferred method for soy protein concentrate because it had the least genistin and genistein losses compared to alcohol leach and hot-water leach methods. SPC prepared with acid leach, hot-water leach, and alcohol leach methods had 20.3%, 24.2%, and 91.9% of total genistin + genistein losses, respectively.

Materials and Methods

Materials

Thirteen soybean varieties (Var-1 to Var13, Table1) were obtained from the Department of Agronomy, University of Arkansas. Genistein and genistin standards were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). The reagents were analytical grade and purchased from Fisher Scientific (Pittsburgh, Pa., U.S.A.) and Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Codes (Var-1 to Var13) were used to represent real name of 13 soy varieties due to company privacy.

Soy protein concentrate preparation with 3 basic methods

Three basic methods for soy protein concentrate (SPC) preparation consisting of acid leach, hot-water leach and alcohol leach methods were evaluated for genistin and genistein retention. The concentration of total genistin + genistein and the concentration of individual genistin and genistein were calculated. The amount of total genistin + genistein obtained from each method of SPC preparation was compared with the total genistin + genistein present in defatted soy flour used to prepare the SPC in order to evaluate the total loss of genistin + genistein during SPC preparation.

Soybeans were dehulled and ground to obtain soy flour. Defatted soy flour was prepared by the extraction of lipids from soy flour with hexane. The suspension of hexane and soy flour was shaken for 30 min with a shaker (Damon/IEC model CRU-5000, Needham Heights, Mass., U.S.A.) at ambient temperature and centrifuged at 5000 x g for 10 min; this process was repeated twice. After removing the hexane phase by centrifugation, the soy flour was air-dried overnight under a hood to remove residual hexane.

Acid leach method to prepare SPC

Finely ground defatted soy flour was dispersed in 1:10 deionized water (w/v) and stirred for 1 h at ambient temperature. The dispersion was adjusted to pH 4.5(isoelectric point of protein) with 1N hydrochloric acid. The precipitated protein was removed by centrifugation with a Beckman model J2-21 centrifuge (Palo Alto, Calif., U.S.A.) at 13,000 x *g* for 10 min, washed once, neutralized to pH 7.0, and freeze-dried.

Hot-water leach method to prepare SPC

The soy protein concentrate was prepared according to the method of Circle and Smith (1972). Finely ground defatted soy flour was dispersed in 1:10 deionized water (w/v) and stirred for 1 h at ambient temperature. The suspension was heated to

80 °C, then the precipitated protein was separated with centrifugation with a Beckman model J2-21 centrifuge (Palo Alto, Calif., U.S.A.) at 13,000 x *g* for 10 min, washed, and freeze-dried.

Alcohol leach method to prepare SPC

The soy protein concentrate was prepared according to the method of Campbell and others (1985). Finely ground defatted soy flour was dispersed in 1:10 (w/v) of 60% aqueous ethanol and stirred for 1 h at ambient temperature. The dispersion of soy flour was centrifuged with a Beckman model J2-21 centrifuge (Palo Alto, Calif., U.S.A.) at 13,000 x g for 10 min, and the residue was washed and freeze-dried.

HPLC analysis

Two grams of finely ground soybean seeds, defatted soy flour, or ground freeze-dried sample of soy protein concentrate (SPC) were stirred in 10 mL of 80% aqueous methanol solvent for 2 h at ambient temperature. The extract was filtered through Whatman no. 42 filter paper. A rotatory evaporator was used to dry the filtrate under vacuum at a temperature below 37 °C. The residue was redissolved in 10 mL of 80% MeOH and filtered through a 0.45 µm filter.

A high performance liquid chromatography (HPLC) solvent gradient system was used according to method of Wang and Murphy (1994). For linear HPLC gradient, solvent A was 0.1% glacial acetic acid in H_2O, and solvent B was 0.1% glacial acetic acid in acetonitrile. Injection of a 20-µL sample was followed by the increase of solvent B from 15% to 35% and the decrease of solvent A from 85% to 65% in 26 min. The solvent flow rate was 1.0 mL/min. A TSK-Gel Super-ODS HPLC column (4.6 x 100 mm) was used (Hewlett-Packard, Wilmington, Del., U.S.A.). The eluting components were detected from their absorbency at 254 nm. Each sample was spiked to confirm identification of genistein and genistin. Concentrations of genistin and genistein were calculated from standard curves. The concentrations were expressed as mg per g of soybean, defatted soy flour, or soy protein concentrate.

Statistical analysis

The experiments were performed in triplicate, and the data was analyzed with the general linear model procedure of SAS Institute Inc. to determine differences of the means (SAS 1990). The significance of differences between means was determined by the least significant difference test procedure at $P < 0.05$.

References

Ahluwalia VK, Bhasin MM, Seshadri TR. 1953. Isoflavones of soybeans. Current Sci 22:263-265.

Campbell M.F. 1985. In: Altschul AM, Wilcke HL, editors. New Protein Foods. Vol. 5. New York: Academic Press. P 301-307.

Coward L, Barnes NC, Setchell KDR, Barnes S. 1993. Genistein, daidzein, and their _-glucoside conjugates: antitumor isoflavones in soybean foods from American and Asian diets. J Agric Food Chem 41:353-355.

Eldrige A, Kwolek W. 1983. Soybean isoflavones: Effect of environment and variety on composition. J. Agric. Food Chem. 31:394-396.

Ha EYW, Morr CV, Seo A. 1992. Isoflavone aglucones and volatile organic compounds in soybeans; effects of soaking treatments. J Food Sci 57:414-417.

Matsuura M, Obata A, Murao S. 1995. Studies on _-glucosidases from soybeans that hydrolyze daidzin and genistin: Isolation and characterization of an isozyme. Biosci Biotech Biochem 59:1623-1627.

Messina MJ. 1997. Soy foods: Their role in disease prevention and treatment. In: Liu K, editor. Soybeans: Chemistry, Technology, and Utilization. New York: Chapman & Hall. P 443.

Onozawa M, Fukuda K, Ohtani M, Akaza H, Sugimura T, Wakabayashi K. 1998. Effects of soybean isoflavones on cell growth and apoptosis of the human prostatic cancer cell line LNCaP. Jpn J Clin Oncol 28:360-363.

Price KR, Fenwick GR. 1985. Naturally occuring oestrogens in foods-a review. Food Addit Contam 2:73-106.

Ruiz-Larrea MB, Mohan AR, Paganga G, Miller NJ, Bolwell GP, Rice-Evans CA. 1997. Antioxidant activity of phytoestrogenic isoflavones. Free Rad Res 26:63-70.

SAS 1990. User's Guide: Statistics. . Cary, N.C.: SAS Institute Inc.

Wang HJ, Murphy PA. 1994. Isoflavone composition in American and Japanese soybeans in Iowa: Effects of variety, crop year, and location. J Agri Food Chem 42:1674-1677.

Wang HJ, Murphy PA. 1996. Mass balance study of isoflavones during soybean processing. J Agri Food Chem 44:2377-2383.

MS 19990632 received 6/21/99; revised 2/8/00; accepted 3/30/00.

We thank the Arkansas Soybean Research and Promotion Board for financial support.

Authors Pandjaitan, Hettiarachchy, Ju, and Crandall are with the Department of Food Science, University of Arkansas, 272 Young Ave., Fayetteville, AR 72704. Authors Sneller and Dombek are with the Department of Agronomy, University of Arkansas, 272 Young Ave., Fayetteville, AR 72704. Direct correspondence to Dr. N.S. Hettiarachchy (E-mail: nhettiar@comp.uark.edu).