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Isoflavone Contents in Germinated Soybean Seeds

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Abstract. The effect of germination on isoflavone contents in two soybean varieties (Hutcheson and Caviness) was investigated. Soybean seeds were soaked at 25 °C for 12 h, germinated at 40 °C, and freeze-dried. The isoflavone contents of dry, soaked, germinated (hypocotyl length at 0.5, 2.5, and 6.5 mm), and nongerminated seeds were determined by high performance liquid chromatography. The maximum amount of total isoflavone, genistein, and daidzein with their β-glucoside conjugates was obtained when hypocotyl length of the germinated-seed from var. Hutcheson was 0.5 mm (2.491, 1.500, and 0.671 mg/g), and from var. Caviness was 2.5 mm (2.78, 1.523, and 0.905 mg/g). A dramatic increase in malonylgenistin and malonyldaidzin (1.305 mg/g and 0.476 mg/g in Hutcheson, and 1.308 mg/g and 0.677 mg/g in Caviness, respectively) was observed at these hypocotyl lengths. A decrease was observed after this stage. Genistein and daidzein contents were highest just after soaking. Glycitein and its β-glucoside conjugates remained almost the same during germination. Controlled germination can be used to enhance isoflavone content in soybean seed.

Key words: Aglycone, Germination, Glucoside, Isoflavones, Soybean

Introduction

Soybean is a major source of protein and also provides other nutritional benefits to large parts of the world's population. Numerous epidemiological, human, animal, and in vitro studies have demonstrated that soy isoflavones are effective cancer-preventive agents for lowering risks of various cancers [1–4]. Mechanisms involved may include estrogen receptor binding, modulation of sex hormone binding globulin, antioxidants, and inhibition of protein tyrosine kinase enzymes [3, 5–8]. Evidence also points to the beneficial effects of soy isoflavones in the prevention of cardiovascular disease [9]. Other potential health benefits of soy isoflavones include prevention of osteoporosis via phytoestrogen effects of isoflavones, and prevention of neovascularization in ocular conditions [10, 11].

There are three types of isoflavones in soybean, and each type exists in four different chemical forms, which include aglycones (daidzein, genistein, and glycitein) and their β-glucoside conjugates: glucosides (daidzin, genistin, and glycitin), malonylglucosides (6″-*O*-malonyldaidzin, 6″-*O*-malonylgenistin, and 6″-*O*-malonyl glycitin), and acetylglucosides (6″-*O*-acetyldaidzin, 6″-*O*-acetylgenistin, and 6″-*O*-acetylglycitin). Most of them exist as β-glucoside, the malonylglucoside, and acetylglucoside forms. The bioavailable isoflavones (aglycones) are formed by the hydrolysis of glycosides through β-glucosidase present in soybean. Fermentation, heat treatments, and chemical and enzymatic hydrolysis were reported to induce changes in composition of isoflavone profile [12–16]. When soy protein is extracted from soy flour, glycoside forms of isoflavones can be converted into aglycones with different ratios depending on the methods used to leach the flour [17].

Germination processes have been developed to overcome the disadvantages of soybean seed used in food products. These include undesirable flavor and odor due to lipoxygenase activity and the presence of antinutritional factors such as trypsin inhibitors, phytates, and flatulent. Many studies showed the benefits of germinated soybeans, including improvement of nutritional quality by prevention of lipid oxidation, increase of the nutrients such as ascorbic acid and riboflavin contents, hydrolysis of raffinose and stachyose that are responsible for flatulence problems, and decrease of trypsin inhibitors [18–21]. However, limited information is available on the effect of soybean germination on isoflavone content and composition [22]. Terrence [23] examined the distribution of isoflavones and their conjugates in developing soybean seedling organs. They found that daidzein, genistein, and their respective conjugates were the major soluble isoflavonoids in seedling, roots, hypocotyls, and cotyledons at all time after germination. In general, the total isoflavone concentration increased after 1 day of germination and then concentration slowly decreased thereafter. Furthermore, no report is available on the isoflavone content at varying length of hypocotyl of seed during germination. The objectives of this research were to evaluate the changes in 12 isoflavones at varying length of hypocotyls during germination, and to determine the germination stage that maximizes isoflavone content for the purposes of maximizing the nutraceutical benefits of soy isoflavones consumption.

Materials and Methods

Materials

Two soybean varieties (Hutcheson and Caviness) commonly grown in Arkansas were obtained from the

Department of Crop, Soil, and Environmental Sciences, University of Arkansas (Fayetteville, AR, USA). These two varieties were selected based on their relatively high isoflavone contents [17]. Twelve isoflavone standards were used in this study. Genistein, daidzein, glycitein, genistin, daidzin, glycitin, acetylglycitin, acetyldaidzin, and acetylgenistin were purchased from LC laboratories (Woburn, MA, USA). Malonylgenistin, malonyldaidzin, and malonylglycitin were provided by Amano Enzyme Inc. (Gifu, Japan). Trifluoroacetic acid was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other HPLC-grade solvents were purchased from Fisher Scientific (Pittsburgh, PA, USA).

Soybean Germination

Soybean seed (120 g) from each of the two soybean varieties was washed three times and soaked in deionized water at ambient temperature for 12 h. The soaked soybean seed was drained and 40 g (about 25 g dry basis weight) of the seeds of each variety were removed for analysis. The remaining seeds were germinated in a Microcomputer Electric Germination Appliance (HP-30 Hatsuga Bijin, Japan) at 40 °C. As germination progressed, 100–120 germinated seeds (about 40 g) were removed when the hypocotyls from seed coat were at lengths of 0.5–1.0, 2.5–3.0, and 6.5–7.0 mm. The nongerminated seeds were removed at the last stage of germination (about 24 h). All samples were frozen, freeze-dried, ground into flour (coffee grinder model KSM 2B, Gillette Canada, Mississauga, ON, Canada), and passed through a 60-mesh sieve (W. S. Tyler Inc., Mentor, OH, USA).

Isoflavone Extraction

Isoflavones were extracted using a modified method of Xie et al. [16]. Five hundred milligrams of finely ground soybean samples were mixed with 5 ml of 80% aqueous methanol solvent, shaken for 2 h at ambient temperature, and centrifuged at 14,000 × *g* for 10 min to extract isoflavone. The supernatants were filtered through a 0.2 μm PVDF Target Syringe Filter (National Scientific, Duluth, GA, USA) and used for HPLC analysis.

HPLC Analysis of Isoflavones

Isoflavones were quantified by a Hewlett-Packard Liquid Chromatograph model 1090 equipped with a diode array ultraviolet detector. The column used was TSK-GEL Super-ODS (Supelco, Bellefonte, PA), and absorbance of the effluent was monitored at 254 nm. The mobile phase consisted of solvent A, 0.1% trifluoroacetic acid in acetonitrile, and solvent B, 0.1% trifluoroacetic acid in HPLC-grade water. Flow rate was 1.0 ml/min and column temperature was maintained at 37 °C. The initial solvent condition was 100% solvent B. A gradient was set to increase solvent A from 0 to 50% within 30 min and then returned to the initial condition in 5 min. A sample size of 6 μl was injected for the HLPC analysis. The concentrations of isoflavones in the sample were calculated from standard curves calibrated using the 12 isoflavone standards. The concentration was expressed as mg/g soy flour.

Statistical Analysis

All values are reported as means of triplicate determinations. Data were analyzed for variance and multiple mean comparisons to compare isoflavone contents of various stages of germination with JMP 5 software package [24]. Significant differences between means were determined by the Tukey Honestly Significant Difference (HSD) procedure at the 5% significance level.

Results and Discussion

Effect of Germination on Total Isoflavone Content

One hundred and twenty grams of dry soybean absorbed 120–130 g of water during soaking (12 h). The hypocotyls reached lengths of 0.5, 2.5–3.0, and 6.5–7.0 mm at 10–12 h, 14–16 h, and 20–24 h, respectively. The total isoflavone contents during germination are shown in Table 1. Total isoflavone contents in the dry seeds from soybean var. Hutcheson and Caviness were 2.190 and 2.286 mg/g, respectively. The isoflavone contents in soybean are affected not only by soybean variety but also strongly by environmental changes such as temperature, sunshine duration, and precipitation [25, 26]. The total isoflavone contents in both varieties gradually increased after soaking. The maximum total isoflavone content was obtained when hypocotyls of the seeds from soybean varieties Hutcheson and Caviness were 0.5 mm (2.491 mg/g flour) and 2.5 mm (2.78 mg/g

Table 1. Total isoflavone contents in soybean during various stages of germination (mg/g ground seed, dry basis)*

	Soybean variety	
Seed type	Hutcheson	Caviness
Dry	2.190b	2.286b
Soaked	2.235ab	2.368b
Germinated (hypocotyl 0.5 mm)	2.491a	2.700a
Germinated (hypocotyl 2.5 mm)	2.442ab	2.780a
Germinated (hypocotyl 6.5 mm)	2.304ab	2.486ab
Nongerminated	2.035b	2.174b

*Values in a column with different letters are significantly different ($p < 0.05$).

Table 2. Effect of germination on genistein and its β-glucoside conjugates (mg/g ground seed, dry basis) in soybean*

	Hutcheson					Caviness				
Seed type	Gen	Gin	M-Gin	A-Gin	Total	Gen	Gin	M-Gin	A-Gin	Total
Dry	0.019b	0.160a	1.099bc	0.009c	1.287c	0.011b	0.178a	1.020d	0.021a	1.230cd
Soaked	0.024a	0.164a	1.119bc	0.010c	1.316bc	0.022a	0.180a	1.076c	0.015ab	1.293cd
Germinated (hypocotyl 0.5 mm)	0.009c	0.166a	1.305a	0.021ab	1.500a	0.008c	0.181a	1.266ab	0.019ab	1.474ab
Germinated (hypocotyl 2.5 mm)	0.009c	0.161a	1.263a	0.025a	1.459ab	0.007c	0.186a	1.308a	0.022a	1.523a
Germinated (hypocotyl 6.5 mm)	0.007c	0.150b	1.191ab	0.016bc	1.364abc	0.007c	0.156b	1.183bc	0.018ab	1.364bc
Nongerminated	0.017b	0.146b	1.014c	0.017b	1.194c	0.020a	0.151b	0.979d	0.012b	1.163d

Gen: genistein; Gin: genistin; M-Gin: malonylgenistin; A-Gin: acetylgenistin.
*Values in a column with different letters are significantly different ($p < 0.05$).

flour), respectively. The increases of isoflavone contents at this stage may be induced by metabolic pathways of naringenin chalcone and isoliquiritigenin, the precursors of isoflavonoids commonly found in legumes [27]. Sharma [28] showed that isoflavones in chick pea (*Cicer arietinum*) increased during germination. A decrease in the total isoflavone content was observed after this stage. The percentage increase observed was higher in the seed from soybean var. Caviness (21.61%) than from var. Hutcheson (11.51%). The increase and decrease of the observed isoflavone contents could be due to the conversion of other flavonoids to isoflavones and isoflavones to other flavonoids. Terrence [23] reported that soybean primary leaf tissues underwent a programmed shift from isoflavonoid to flavonoid metabolism 3 days after germination. The total isoflavone contents were 2.235 and 2.368 mg/g flour in the soaked seeds, 2.035 and 2.174 mg/g flour in the nongerminated seeds, and 2.190 and 2.286 mg/g flour in controls (dry seed) for var. Hutcheson and Caviness, respectively.

Effect of Germination on Genistein and Its β-Glucoside Conjugate Contents

Genistein and its β-glucoside conjugate contents during various stages of germination are given in Table 2. The total genistein and its β-glucoside conjugates increased significantly during soybean germination. The maximum amount of genistein and its β-glucoside conjugates were obtained at hypocotyl length of 0.5 mm (1.500 mg/g flour) for var. Hutcheson and 2.5 mm (1.523 mg/g flour) for var. Caviness. After this stage a decrease in isoflavone content of the seeds from both varieties was observed. Genistein and its β-glucoside conjugate contents of the seeds from var. Caviness decreased faster than those from var. Hutcheson. Malonylgenistin showed higher percentage of increase than genistein and its other β-glucoside conjugates. The percentage increases in malonylgenistin contents in the germinated seeds from var. Hutcheson and Caviness were 18.74 and 28.24%, respectively. Significant differences of genistein and its β-glucoside conjugates in germinated seeds compared to dry, soaked, and nongerminated seeds were observed ($p < 0.05$). The highest amount of genistein was observed soon after soaking (12 h) the seeds from both varieties, which were significantly different in comparison to dry, germinated, and nongerminated seeds ($p < 0.05$). Percent increases of genistein content in soaked seeds from var. Hutcheson and Caviness were 26.32 and 100%, respectively, compared with dry seeds. The reason is that dry seed from var. Caviness had a lower genistein content. However, a decrease in genistein content was observed between soaked seeds and germinated seeds. The maximum amount of acetylgenistin was obtained at hypocotyls lengths of 2.5 mm in both varieties.

The observed differences in genistein content among dry seeds, soaked seeds, and germinated seeds may be due to physiological changes during soaking and germination. Hydrolysis of glucoside during soaking contributed to an increase in genistein. As germination progressed, the observed decrease in genistein content may have been due to the conversion of genistein to other isoflavone.

Effect of Soybean Germination on Daidzein and Its β-Glucoside Conjugates

Effect of soybean germination on daidzein and its β-glucoside conjugates is given in Table 3. Both varieties had similar daidzein content. A small amount of daidzein was present in soybean seed in comparison to its glucosides. Graham [29] showed that daidzein was predominantly present as its malonylated conjugate in the soybean seed but significantly high in the root. The maximum amounts of daidzein (0.028 and 0.029 mg/g flour) were observed after soaking, which was significantly different ($p < 0.01$) from other treatments of seeds from both varieties. The percent increase of daidzein contents in soaked seeds from var. Hutcheson and Caviness were 27.27 and 31.81%, respectively, compared to dry seeds. For both varieties, daidzein content in the germinated seeds was lower than in the dry and soaked seeds. However, the rate of

Table 3. Effect of germination on daidzein and its β-glucoside conjugates (mg/g ground seed, dry basis) in soybean*

	Hutcheson					Caviness				
Seed type	Den	Din	M-Din	A-Din	Total	Den	Din	M-Din	A-Din	Total
Dry	0.022b	0.073a	0.405b	0.087c	0.587bc	0.022b	0.100a	0.531c	0.088c	0.740bc
Soaked	0.028a	0.068ab	0.407b	0.092b	0.594abc	0.029a	0.099a	0.560b	0.090b	0.778bc
Germinated (hypocotyl 0.5 mm)	0.016c	0.071a	0.476a	0.108a	0.671a	0.012c	0.102a	0.655a	0.108a	0.878a
Germinated (hypocotyl 2.5 mm)	0.017c	0.069ab	0.462a	0.106a	0.653a	0.012c	0.106a	0.677a	0.111ab	0.905a
Germinated (hypocotyl 6.5 mm)	0.014c	0.067ab	0.451a	0.102ab	0.634ab	0.010c	0.093ab	0.611ab	0.100b	0.815ab
Nongerminated	0.022b	0.060b	0.363b	0.088c	0.532c	0.025b	0.087b	0.520c	0.084c	0.716c

Den: daidzein; Din: daidzin; M-Din: malonyldaidzin; A-Din: acetyldaidzin.
*Values in a column with different letters are significantly different ($p < 0.05$).

daidzein decrease between soaked seeds and germinated seeds with 0.5 mm cotyledons was faster for Caviness than Hutcheson. After 24 h (6.5 mm hypocotyls length) of exposure to sprouting conditions, nongerminated seeds had higher levels of daidzein (0.022 mg/g in var. Hutcheson and 0.025 mg/g in var. Caviness), which were not significantly different ($p > 0.05$) from dry seeds (0.022 mg/g), but significantly higher than germinated seeds (0.014–0.017 mg/g from var. Hutcheson and 0.010–0.012 mg/g from var. Caviness at $p < 0.05$). Daidzein and genistein were not converted into their glucoside conjugates. The inability for this conversion may account for nongermination. The increase was observed in malonyldaidzin of the germinated seed, which was much higher than daidzin and acetyldaidzin. The maximum amounts of malonyldaidzin in the germinated seeds from var. Hutcheson and Caviness were obtained when hypocotyls had reached lengths of 0.5 mm (0.476 mg/g) and 2.5 mm (0.677 mg/g), respectively. The percentage of increase in malonyldaidzin contents of the germinated seeds was 17.53 and 27.50%, respectively, compared to dry seeds.

The total amounts of daidzein and its β-glucoside conjugates were significantly higher in germinated seeds ($p < 0.05$). Soybean var. Caviness had higher content of daidzein and its β-glucoside conjugates than did soybean var. Hutcheson. The maximum amounts of daidzein and its β-glucoside conjugates observed during germination were 0.671 mg/g in the seed from var. Hutcheson, and 0.905 mg/g in the seed from var. Caviness.

Effect of Germination on Glycitein and Its β-Glucoside Conjugates

Effect of germination on the contents of glycitein and its β-glucoside conjugates is given in Table 4. Germination had little effect on glycitein and its β-glucoside conjugate contents in comparison to the other two types of isoflavones. The total glycitein and its β-glucoside conjugate contents were 0.297–0.351 mg/g. A significant difference was observed only in acetylglycitin contents of germinated seed from soybean var. Caviness. The main reason is that most of glycitein and its β-glucoside conjugates were in the hypocotyl axis. The hypocotyl axis is only 2% of the whole soybean seed [30]. Since whole germinated soybeans were used for analysis, this difference may not have been evident due to the insignificant amount of hypocotyls in soybean.

Conclusions

The total isoflavone content increased rapidly during the early stage of germination. The maximum amounts of total isoflavone were observed when the hypocotyl length of seeds from soybean var. Hutcheson and Caviness was

Table 4. Effect of germination on glycitein and its β-glucoside conjugates (mg/g ground seed, dry basis) in soybean*

	Hutcheson					Caviness				
Seed type	Glye	Gly	M-Gly	A-Gly	Total	Glye	Gly	M-Gly	A-Gly	Total
Dry	0.019a	0.093ab	0.161b	0.044a	0.316ab	0.013a	0.090ab	0.172ab	0.041b	0.316b
Soaked	0.017ab	0.089b	0.174a	0.044a	0.324a	0.015a	0.082b	0.158b	0.042ab	0.297c
Germinated (hypocotyl 0.5 mm)	0.015ab	0.088b	0.171a	0.045a	0.320a	0.013a	0.101a	0.188a	0.047a	0.349a
Germinated (hypocotyl 2.5 mm)	0.019a	0.096a	0.170a	0.044a	0.330a	0.014a	0.102a	0.189a	0.046a	0.351a
Germinated (hypocotyl 6.5 mm)	0.011b	0.088b	0.167ab	0.041ab	0.307b	0.012a	0.088ab	0.168ab	0.039bc	0.307bc
Nongerminated	0.016ab	0.086b	0.171a	0.036b	0.309b	0.013a	0.084b	0.164b	0.033c	0.295c

Glye: glycitein; Gly: glycitin; M-Gly: malonylglycitin; A-Gly: acetylglycitin.
*Values in a column with different letters are significantly different ($p < 0.05$).

0.5 and 2.5 mm, respectively. A decrease in isoflavone content was observed after this stage. The increase was dominated by β-glucoside conjugates, especially their malonylglucoside. The contents of aglycones, genisteins, and daidzeins, reached the highest just after soaking. Controlled germination can be used to enhance isoflavone content in soybean. The maximal benefits of soybeans as nutraceuticals can be achieved when the hypocotyl length is approximately 0.5–2.5 mm in length.

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