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Isoflavone Composition within Each Structural Part of Soybean Seeds and Sprouts

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Abstract

Isoflavone content in various parts of six soybean cultivars and soybean sprout during germination was analyzed by high performance liquid chromatography. The parts analyzed were seed coat, cotyledon, and axis for seeds and whole sprout, root, hypocotyl, and cotyledon for sprout. Two cultivars, Aga3 which is known to have the smallest seed size and the highest isoflavone content among the Korean soybean cultivars and Pungsannamulkong which is the most widely being used as soy-sprout, were selected for sampling from 1 to 10 days after germination. At the same weight, the order of isoflavone content increased from seed coat to cotyledon to axis. The highest total isoflavone (isoflavone × dry weight) content was observed in the cotyledon and the lowest in the seed coat. The cotyledon of the Aga3 variety had the highest total isoflavone content and the lowest was measured in the Pungsannamulkong variety. The highest total isoflavone content, 10,788μg/g, was observed in whole sprouts (cotyledon+hypocotyl+root) on day 7 for Aga3. After day 7, there was a decreasing trend in isoflavone content as the germination period increased. Total isoflavone content in the cotyledon of Aga3 significantly increased after seed germination, whereas the isoflavone content in the cotyledon of Pungsannamulkong decreased. However, total isoflavone content in the root of both varieties increased while isoflavone content in the hypocotyls decreased after seed germination.

Keywords: Soybean, Soybean sprout, Isoflavone

Introduction

Soybean is a popular health food in many Asian countries. Soybean is used in various forms such as soybean sprouts, soy pastes, soymilk, soybean oil, and tofu as key ingredients in cultural cuisines (Kim et al., 2006). Currently, there is an increasing consumption of soybean worldwide due to its nutritional properties and the beneficial characteristics of its constituent compounds, like isoflavone. Consumption of isoflavone is associated with human health benefits such as decreased risk of heart disease, menopausal symptoms, cardiovascular disease, and bone resorption as well as breast, prostate, and colon cancers (Adlercreutz et al., 1992; Allred et al., 2005; Anderson and Gardner, 1997; Anthony et al., 1996; Cassidy et al., 1994; Kim et al., 2005; Kennedy, 1995; Messina, 2000). The physiological function of isoflavone is mediated by a variety of mechanisms including estrogenic activity as well as inhibition of topoisomerase and protein kinases (Omoni and Aluko, 2005; Ososki and Kennelly, 2003).

Isoflavones are categorized chemically according to their functional groups. There are four subgroups including aglycones (genistein, daidzein, and glycitein), glycosides (genistin, daidzin, and glycitin), malonyl glycoside (malonyl genistin, malonyl daidzin, and malonyl glycitin), and acetyl glycosides (acetyl genistin, acetyl daidzin, and acetyl glycitin) (Eldridge, 1982; Kudou et al., 1991).

Soybean sprouts is an important year-round vegetable consumed for thousands of years in Korea, China, and Japan. They are excellent source of nutrients as well as vitamins (Lee et. 2002). Annually, more than 500,000 tons of soybean sprouts are consumed in soups, salads, and side dishes. Mass production of soybean sprouts is an important agriculture business in Korea (Hwang et al. 2004). Many studies have been conducted to improve the quality and quantity of soybean seeds and sprouts.

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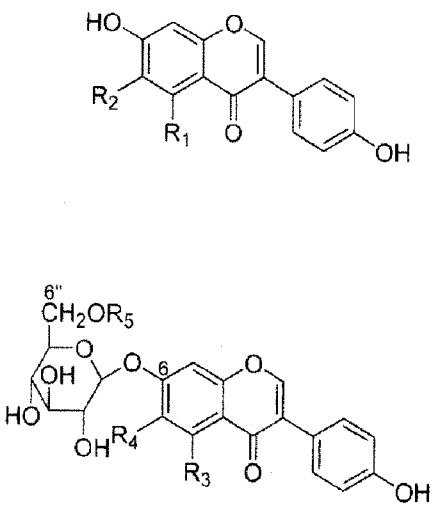

Aglycones	Symbol	R_1	R_2
Daidzein	De	H	H
Glycitein	Gle	H	OCH_3
Genistein	Ge	OH	H

Glucosides	Symbol	R_3	R_4	R_5
Daidzin	Di	H	H	H
Glycitin	Gly	H	OCH_3	H
Genistin	Gi	OH	H	H
Acetyldaizin	AcDi	H	H	$COCH_3$
Acetylglycitin	AcGly	H	OCH_3	$COCH_3$
Acetylgenistin	AcGi	OH	H	$COCH_3$
Malinyldaidzin	MDi	H	H	$COCH_2COOH$
Malonylglycitin	MGly	H	OCH_3	$COCH_2COOH$
Malonylgenistin	MGi	OH	H	$COCH_2COOH$

Fig. 1. Chemical structure of is isoflavone isomers in soybean.

The objective of this study was to evaluate the isoflavone content in each structure of the soybean seed and sprout to determine the germination stage that contained the highest isoflavone content for soybean sprout consumption.

Materials and Methods

Seed materials and germination method

The soybean varieties used in this study are shown in Table 1. Seed germination method was according to Lee et al. (2007a). Twenty grams of seeds of each variety were placed in a plastic container (6.0 cm *W* × 6.0 cm *L* × 15.0 cm *H*) with several small perforations at the bottom for drainage. The samples were first soaked in water at 20°C for 4 hours to initiate germination. The seeds were then removed from the water and placed in an environment-controlled chamber at 20°C with 80% humidity and exposed to light for 12 hours per day. A submersible pump connected to a nozzle was placed above the container and was set to spray about 3 liters of water per minute for 4 minutes every 3 hours.

Table 1. Hundred seed weight and seed coat color of six soybean varieties.

Variety	100seeds (g)	Seed coat color	Remark
Aga3	5.75	Green	Newly developed variety known to have the highest isoflavone content in the world
Aga4	6.69	Black	Newly developed variety
Pungsannamulkong	13.94	Yellow	A famous variety used for soy-sprout in Korea
Taekwangkong	26.83	Yellow	A famous variety used for soy-paste in Korea
Hwangkeumkong	28.22	Yellow	A famous variety used for producing soy pastes in Korea
Cheongjakong	32.14	Black	A cultivar known to have high content of isoflavone

Isoflavone extract

The extraction of isoflavone was modified from Rostageno et al (2003). Dry soybean powder 0.2g (75 × 75 μm) was added to 10 ml of 80% HPLC grade EtOH and incubated in an Ultrasonic bath (Kodo Co., Korea) at 50°C for 1 hour. The samples were then placed in a shaking (150 rpm) incubator at 50°C for 15 hours. Samples were then passed through a 0.45μm syringe filter and collected for isoflavone analysis using high performance liquid chromatography (HPLC).

Isoflavone analysis

HPLC analysis of isoflavone was based on the work of Wang and Murphy (1994). The HPLC system consisted of a TOTALCHROM V6.2.0.0.1 with LC Instrument control (PerkinElmer series 200, USA) and A COL-CHOICE C18 column 4.6 x 150 mm (5 μm) packed. A linear HPLC gradient utilized acetonitrile (solvent A) and 0.1% of acetic acid in water (solvent B). After injection of a 10-μl sample volume, solvent A was increased from 0 to 45% over 10.2 min. It further increased from 45% to 90% over 6 min, remained constant for 3.6 min, and then was reduced from 90 to 0% over 15 min. The solvent flow rate was 1.0 ml/min. The elution was monitored by UV-absorption (Series 200 uv/vis Detector) at 260 nm. Identification of the isoflavone was based on comparisons with retention times of genuine standards including daidzein, genistein, and genistin (Sigma Chemical Co, USA), as well as glycitin, daidzin, 6′-0-acetylgenistin, 6′-0-malonylgenistin, and 6′-0-acetyldaidzin (LC Laboratory, USA).

Statistical analysis

To identify significant treatment effects and interactions, analysis of variance (ANOVA) and multiple mean comparisons were carried out on the data comparing isoflavone content with the general linear model (GLM) using Statistic Analysis System (SAS 9.1). Differences among mean values were determined using Duncan's Multiple Range Test at P≤0.05 when ANOVA indicated model and treatment significances.

Results and Discussion

Total isoflavone content in seed components

The seed of soybean cultivar has a composition of 85.3-91.6% cotyledon, 1.7-3.8% axis, and 6.4-12% seed coat. Similarly, Ribeiro et al. (2006) reported that the BRS 213 soybean cultivar was composed of 86.8% cotyledons, 3.2% axis, and 10% seed coat. Content of the seed components depended on the size of seed. Big seeds had 91.3, 91.6, and 90.2% cotyledon; 2.22, 1.7, and 2.0% axis; and 6.4, 8.7, and 7.9% seed coat (Taekwangkong, Hwangkeumkong, and Cheongjakong, respectively). In the case of small and medium sized seeds, axis and seed coat percentages were higher than big seeds, which were 3.8, 3.0, and 2.8% radicle and 11.0, 12.0, and 8.5% seed coat (Aga3, Aga4, and Pungsannamulkong, respectively). Nevertheless, the cotyledon of small and medium seeds was smaller than that of big seeds, which were only 85.3, 84.9, and 88.8% for Aga3, Aga4, and Pungsannamulkong, respectively.

When compared within the same weight, the order of isoflavone content increased from seed coat to cotyledon to axis (Table 2). This observation was consistent with Kudou et al. (1991), which reported the total isoflavone content of the hypocotyls (containing the plumule and radicle) was 5.5 to 6.0 times higher than that of the cotyledon. Isoflavone was not found in the seed coat of soybean. However, our study found isoflavone content in the seed coat, but only very small amount.

Total isoflavone content (isoflavone x dry weight per single seed) in different seed components (axis, cotyledon, and seed coat) and between varieties (Aga3, Aga4, Pungsannamulkong, Taekwangkong, Hwangkeumkong, and Cheongjakong) were significantly difference (P<0.01) as shown in Table 6. In each variety, the highest total isoflavone content was in the cotyledon, which was 487, 232, 207, 303, 383, and 214 μg per seed for Aga3, Aga4, Pungsannamulkong, Taekwangkong, Hwangkeumkong, and Cheongjakong, respectively. The isoflavone content of seed coat was the lowest, which was 4, 1, 7, 4, 2, and 3μg per seed for Aga3, Aga4, Pungsannamulkong, Taekwangkong, Hwangkeumkong, and Cheongjakong, respectively. The cotyledon of Aga3 had the highest total isoflavone content and the lowest was in Pungsannamulkong, which were 487 and 207μg per seed, respectively. The axis of Taekwangkong showed the highest total isoflavone content and the lowest was observed in Hwangkeumkong, which were 64 and 20μg, respectively. The seed coat of Pungsannamulkong had the highest total isoflavone content while the lowest was in Aga4, which were 7 and 1μg per seed, respectively.

Table 2. The isoflavone content in different soybean seed components.

Variety	Seed components	Dry weight (g)(A)	Isoflavone (μg)(B)	Total (AxB)
Aga3	Radicle	0.002	20,304a	43b
	Cotyledon	0.048	10,203b	487a
	Seed coat	0.006	617c	4c
	Total	0.056		534A
Aga4	Radicle	0.003	14,855a	45b
	Cotyledon	0.042	5,524b	232a
	Seed coat	0.009	94c	1c
	Total	0.054		278DE
Pungsannamulkong	Radicle	0.005	10,154a	50b
	Cotyledon	0.097	2,127b	207a
	Seed coat	0.014	494c	7c
	Total	0.116		264E
Taekwangkong	Radicle	0.004	14,489a	64b
	Cotyledon	0.202	1,502b	303a
	Seed coat	0.018	213c	4c
	Total	0.224		371C
Hwangkeumkong	Radicle	0.002	13,131a	20b
	Cotyledon	0.238	1,608b	383a
	Seed coat	0.006	266c	2c
	Total	0.248		405B
Cheongjakong	Radicle	0.005	11,525a	52b
	Cotyledon	0.207	1,035b	214a
	Seed coat	0.018	156c	3c
	Total	0.230		269D
Variety				**
Seed component				**

** = Significant (P<0.01). + Value with different letters (capital letter A-F) is significantly different at 5% level by DMRT between varieties.
+ Value with different letters (small letter a-c) is significantly different at 5% level by DMRT of seed component within a variety.

Total isoflavone content in different parts of germinated soybean seeds

As shown in Table 3, the isoflavone content of Aga3 seeds, at 0 days after germination, was about 6 times higher than that of Pungsannamulkong when it was measured on the basis of weight, μg/g. Aga3 and Pungsannamulkong were sampled from 1 to 10 days after germination. Samples were taken from whole sprouts and their parts such as cotyledon, root, and hypocotyl. Germinated soybean seed components were separated after 4 to

10 days because components were not distinguishable at 3 days.

Dry weights of whole sprout and cotyledons decreased after seed germination. In contrast, hypocotyls and root dry weights increased in Aga3 and Pungsannamulkong. Total isoflavone content in germinated soybean seed components and days after germination were significantly different ($P<0.01$) (Table 3-6).

The isoflavone content of germinated soybean seeds at different time intervals showed a significant change ($P<0.05$). The highest isoflavone content was observed in the whole sprout on the day 7 for Pungsannamulkong and Aga3. After 7 days, there was a decreasing trend in isoflavone content as the germination period increased (Table3). Terrence (1991) observed that soybean primary leaf tissues underwent a programmed shift from isoflavonoid to flavonoid metabolism after 3 days of germination and became largely dominated by glycosides of flavonols, kampferol, quercetin, and isorhamnetin after 5 days. Regression analysis showed a quadratic polynomial trend for both Aga3 and Pungsannamulkong. The results followed a similar trend as reported by Kim et al. (2002), which reported that isoflavone increased 20-50% after 24 hours of germination. Danhua et al. (2005) reported that isoflavone content from seeds of 3-day-old Caviness and Hutcheson varieties demonstrated 21.61% and 11.51% increases in isoflavone content, respectively. In addition, Xu et al. (2005) showed that ascorbic acid in soybeans also increased during germination primarily because of its increased biosynthesis.

At the same weight, isoflavone content in root was higher than cotyledon, a gradual increase 1 to 6 days after germination in Aga3, thereafter isoflavone content in the cotyledon was higher than in the root. However, isoflavone content in the root was higher than in the cotyledon due to a gradual increase 10 days after germination for Pungsannamulkong. Hypocotyl demonstrated the lowest isoflavone content. Our result agree with Kim et al. (2003), which found that the isoflavone content of bean sprouts increased gradually during the cultivation period and were the highest in the roots, then cotyledon, and then hypocotyl.

Our data indicate that isoflavone content in different parts of soybean sprouts were varied according to genotype and date of germination. Similarly, Lee et al. (2007b) reported that in soybean sprout at 5 days after germination the roots and hypocotyls contained the highest and lowest isoflavone levels, respectivly. Nevertheless, some varieties had isoflavone levels higher in cotyledon than in roots. In agreement with this observation, Kim et al. (2004) found that isoflavone content in roots was 2.9-fold higher than cotyledons of Junjori, at 7 days post germination. Whereas, for Myoungjoonamulkong and Chinese black soybeans, isoflavone content in cotyledons were 1.38 and 4.18 fold greater than the roots, respectively.

Total isoflavone (isoflavone _ dry weight per single sprout) content in cotyledons of Aga3 significantly increased after seed germination, whereas in the cotyledons of Pungsannamulkong it decreased. However, total isoflavone content in roots of both varieties increased while the content in hypocotyls decreased after seed germination (Table 4-6).

Whole sprouts, cotyledons, and roots of Aga3 and whole sprouts of Pungsannamulkong were observed to reach maximum isoflavone content at 7 days after seed germination, which were 578, 500, 68, and 335μg per single sprout, respectively. Whereas, the highest total isoflavone content in the cotyledons of Pungsannamulkong was 294μg per single sprout at 2 days after seed germination. The maximum total isoflavone content in roots of Pungsannamulkong was 100μg per sprout at 9 days after seed germination. For hypocotyls of both varieties, total isoflavone content increased 2-3 days after seed germination and then progressively declined as shown in Table 6.

Table 3. Total isoflavone content in whole sprout.

Days after germination	Aga3			Pungsannamulkong		
	Dry wt (g)(A)	Isoflavone (μg/g) (B)	Total (A + B)	Dry wt (g)(C)	Isoflavone (μg/g) (D)	Total (C + D)
0	0.065	7,242	471e	0.100	1,236	124j
1	0.064	8,451	544bcd	0.100	1,353	135i
2	0.062	9,085	567a	0.098	1,875	185h
3	0.060	8,918	535d	0.097	2,819	275f
4	0.058	9,806	569a	0.097	2,962	287e
5	0.056	10,146	568a	0.096	2,970	285e
6	0.054	10,379	564ab	0.095	3,493	331b
7	0.054	10,788	578a	0.094	3,556	335a
8	0.053	10,545	559abc	0.092	3,440	317c
9	0.052	10,364	542cd	0.091	2,892	262g
10	0.051	9,400	481e	0.089	3,253	289d

+ Value with different letters in the same column within sprout component is significantly different at 5% level by DMRT.
A and C = dry weight of single sprout's components.

Table 4. Total isoflavone content in cotyledon.

Days after germination	Aga3			Pungsannamulkong		
	Dry wt (g)(A)	Isoflavone (μg/g) (B)	Total (A + B)	Dry wt (g)(C)	Isoflavone (μg/g) (D)	Total (C + D)
0	0.046	10,196	473b	0.094	2,137	201de
1	0.045	10,255	461c	0.093	3,040	284a
2	0.041	11,696	475b	0.086	3,426	294a
3	0.039	10,500	409e	0.082	3,265	267b
4	0.036	12,026	435d	0.078	2,863	223c
5	0.035	12,551	434d	0.066	3,419	226c
6	0.033	13,329	437d	0.069	3,342	229c
7	0.031	15,930	500a	0.060	3,718	225c
8	0.027	14,601	397f	0.054	3,100	167f
9	0.024	13,260	318g	0.055	3,852	211d
10	0.026	9,224	244h	0.053	3,708	197e

+ Value with different letters in the same column within sprout component is significantly different at 5% level by DMRT.
A and C = dry weight of single sprout's components

Table 5. Total isoflavone content in root.

Days after germination	Aga3			Pungsannamulkong		
	Dry wt (g)(A)	Isoflavone (μg/g) (B)	Total (A + B)	Dry wt (g)(C)	Isoflavone (μg/g) (D)	Total (C + D)
0	0.002	20,268	44d	0.003	9,492	30h
1	0.001	28,417	40e	0.003	10,737	28h
2	0.002	21,921	44d	0.004	12,133	44ef
3	0.003	9,788	33f	0.007	7,094	52cd
4	0.002	19,129	46d	0.004	9,095	35gh
5	0.003	13,251	37e	0.005	7,817	39fg
6	0.004	14 ,100	51c	0.006	8,209	49de
7	0.005	12,550	68a	0.007	9,489	70b
8	0.007	8,849	60b	0.011	8,691	99a
9	0.006	9,293	59b	0.011	8,739	100a
10	0.007	6,317	47d	0.010	5,485	57c

+ Value with different letters in the same column within sprout component is significantly different at 5% level by DMRT.
A and C = dry weight of single sprout's components.

Table 6. Total isoflavone content in hylpocotyl.

Days after germination	Aga3			Pungsannamulkong		
	Dry wt (g)(A)	Isoflavone (μg/g) (B)	Total (A + B)	Dry wt (g)(C)	Isoflavone (μg/g) (D)	Total (C + D)
0	0.002	20,268	44a	0.003	9,492	30c
1	0.001	28,417	40b	0.003	10,737	28c
2	0.002	21,921	44a	0.004	12,133	44b
3	0.003	9,788	33c	0.007	7,094	52a
4	0.003	9,038	23d	0.006	3,179	18d
5	0.004	4,221	17e	0.008	1,484	12e
6	0.008	1,921	16e	0.014	606	9f
7	0.008	1,100	9g	0.016	592	9f
8	0.009	1,697	15e	0.018	502	9f
9	0.009	1,068	9g	0.019	606	12e
10	0.012	856	11f	0.022	337	7g

+ Value with different letters in the same column within sprout component is significantly different at 5% level by DMRT.
A and C = dry weight of single sprout's components.

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