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Comparison of isoflavones composition in seed, embryo, cotyledon and seed coat of cooked-with-rice and vegetable soybean (*Glycine max* L.) varieties

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Abstract

In order to study the content and composition of isoflavones retained in soybean seed component, obtained each component part the embryo, cotyledon and seed coat tissues of nine different soybean varieties were analyzed for 12 isoflavones using high performance liquid chromatography with photo diode array detector (HPLC-PDA) and were compared to each other. A total average concentration of isoflavone was 2887 μg g^{-1} in embryo, 575 μg g^{-1} in whole seed, 325 μg g^{-1} in cotyledon, and 33 μg g^{-1} in seed coat. With respect to each tissue of soybean varieties, isoflavone content was highest in Geomjeongkong 2 embryo (5701 μg g^{-1}), Geomjeongolkong whole seed (1321 μg g^{-1}), Heugcheongkong cotyledon (951 μg g^{-1}), and Keunolkong seed coat (56 μg g^{-1}). Isoflavone was least present in Keunolkong embryo (341 μg g^{-1}), Hwaeomputkong whole seed (175 μg g^{-1}), Seonheukkong cotyledon (81 μg g^{-1}), and Seoklyangputkong seed coat (5 μg g^{-1}). Overall, embryo and seed coat of all nine varieties contained isoflavones at the highest and lowest level, respectively. Isoflavones accumulated in the order of malonylglycoside, glycoside, acetylglycoside, and aglycon, among which malonylglycoside was the most abundant form ranging from 66% to 79% of the total isoflavone content in all three tissues. The embryo of cooked-with-rice soybean with black seed coat appears to be the best source of isoflavone.
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Keywords: Isoflavones; Malonylglycoside; Vegetable soybean; Cooked-with-rice soybean

1. Introduction

Soybean, which was originated from North and East Asia, has been an important highland crop in Korea. It can be cultivated during summer and autumn and may be grown on the levees around rice fields. Its gross production in Korea during 2004 was 139,000 tons with 1,373,000 tons being imported and 393,000 tons consumed as food. Consumption of soybean was about 8.5 kg per capita according to the major statistics of Agriculture and Forestry (2004). In Korea, soybean is classified into five categories according to its usage: bean paste, bean curd or milk, bean sprout, cooked-with-rice, and vegetable. The cooked-with-rice soybean varieties have a medium-sized grain with one hundred grain weight of 25–30 g. Colored soybean is sweet, having a black or brown seed coat, and an agreeable chewing texture. On the other hand, vegetable soybean varieties have a large grain seed with one hundred grain weight of 30–40 g and a high content of sugar.

Soybean is an important source of protein that is especially rich in essential amino acids such as lysine and tryp-

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tophan lacking in rice and wheat. Notably, secondary metabolites such as phenolic compounds, isoflavones, saponins, and phytic acids outstandingly serve as a healthy dietary supplement. In plants, these compounds are known to play various roles in being repellent to herbivorous insects and animals, protection against UV light and phytopathogens, signal transduction during nodulation, and attraction of pollinating animals (Koes, Verweij, & Quattrocchio, 2005). In addition, they provide pharmaceutical benefits to humans by exerting as antioxidants and anticarcinogens.

Soybean isoflavone has a phenolic structure similar to estrogen known as a phytoestrogen and provides a natural alternative to the use of postmenopausal hormone replacement therapy. Furthermore, soybean isoflavone helps to prevent osteoporosis, breast and ovarian cancer, and cardiovascular diseases (Anderson, Johnstone, & Cook-Newell, 1995; Yoshiki, Kudou, & Okubo, 1998). Isoflavone has 12 isomers that consist of aglycon forms (daidzein, glycitein and genistein), glycoside forms conjugated with sugar only (daidzin, glycitin and genistin), malonylglycoside derivatives (6″-*O*-malonyldaidzin, 6″-*O*-malonylglycitin and 6″-*O*-malonylgenistin), and acetylglycoside derivatives (6″-*O*-acetyldaidzin, 6″-*O*-acetylglycitin and 6″-*O*-acetylgenistin).

The contents of the individual isoflavones were reported to differ depending on soybean variety and cultivation conditions such as area, year and temperature (Lee et al., 2003; Wang & Murphy, 1994). Moon, Jeon, and Hwang (1996) noted that soybean isoflavone concentrations differ between tissues, being higher in the embryo than in the endosperm. Kim, Kim, and Kim (2004) observed that the levels of isoflavone and anthocyanins including delphinidin-3-glucoside and cyanidin-3-glucoside are fluctuated during germination.

HPLC is a powerful tool method for soybean isoflavone analyses, and the recent chromatographic methods have been greatly improved (Coward, Smith, Kirk, & Barnes, 1998; Franke et al., 1999; Klump, McDonald, & Ballam, 2001; Setchell et al., 2001; Wang & Murphy, 1994; Wiseman, Clarke, Barnes, & Bowey, 2002). The most commonly employed mobile phases are of a gradient system of either methanol-water or acetonitrile-water along with trifluoroacetic acid or glacial acetic acid as modifier (Eldridge, 1982; Farmakalidis & Murphy, 1984; Murphy, 1981). However, all of the aforesaid methods have been unsuccessful in resolving 12 isoflavones simultaneously. A reproducible HPLC condition that permits a good well-separation of all the 12 isoflavone isomers required 90 min for a total analysis of one sample, making it cumbersome to analyze many samples (Wang & Murphy, 1994). Other protocols were also developed by Murphy, Song, Buseman, and Barua (1997) and Franke et al. (1999), though they were lengthy and insufficient in resolution. More rapid, accurate and simpler methods have been widely used for the simultaneous analysis of 12 isoflavones applying a binary gradient mobile phase despite the overlaps of both the internal standard and genistin (Hseih, Kao, & Chen, 2004; Kao & Chen, 2002; Song, Barua, Buseman, & Murphy, 1998). In this paper, we used the method of Kim, Jung, Ahn, and Chung (2005) with some modifications that permit a good separation of all 12 isoflavone isomers (Fig. 2).

So far, a very few studies have been reported on isoflavone composition of whole seed, embryo, cotyledon, and seed coat of cooked-with-rice and vegetable soybeans. The aim of this study was to compare the concentration and composition of isoflavones present in the cooked-with-rice and vegetable soybean seed components.

2. Materials and methods

2.1. Plant materials

Nine varieties of soybean used in this experiment are Galmikong, Geomjeongkong 2, Geomjeongolkong, Heugcheongkong, Hwaeomputkong, Ilpumgeomjeongkong, Keunolkong, Seoklyangputkong and Seonheukkong. All these varieties have medium to large grain size ranging from 25 to 40 g for one hundred grain count weight.

Galmikong, Geomjeongkong 2, Geomjeongolkong, Heugcheongkong and Ilpumgeomjeongkong are soybean varieties that are used for being cooked along with rice, whereas the others are vegetable varieties. With respect to seed coat, Galmikong is brown; Geomjeongkong 2, Geomjeongolkong, Heugcheongkong, Ilpumgeomjeongkong and Seonheukkong are black; Keunolkong and Seoklyangputkong are yellow; Hwaeomputkong is green. The cotyledon color of Heugcheongkong is green, and that of all the other varieties is yellow (Table 1).

All the specimens were harvested in 2004 and used for research in 2005. Heugcheongkong variety was taken from Gangwon Agricultural Research and Extension Services, and all the others were obtained from Youngnam Agricultural Research Institute (YARI) at Milyang in Korea. All of the soybean seeds were stored in a seed depository before the experiment. Heugcheongkong seed was sowed in the experimental field of Chuncheon Agricultural Technology Center on 23 May of 2004 and harvested on 24 October of the same year. The other soybean varieties were sowed in the experimental field of YARI and harvested on 20 October. The planting arrangement was 60×15 cm per plot. Appropriate pesticides were used to control weeds, diseases and insects, and fertilizers were applied prior to plowing at the recommended rates of 8, 8, and 12 kg per 1000 m^2 for N, P_2O_5, and K_2O, respectively. After freeze drying at −40 °C, the whole grain was crushed down lightly in mortar, and then components were sorted into embryo, cotyledon and seed coat.

2.2. Isoflavones analysis by HPLC

Each soybean part was repeatedly freeze-dried and then was pulverized using a grinder. Two grams of each powder

Table 1
Characteristics of nine soybean varieties

Variety	100 seed weight (g)	Seed coat color	Cotyledon color	Seed use[a]
Galmikong	27.2	Brown	Yellow	CR
Geomjeongkong 2	28.3	Black	Yellow	CR
Geomjeongolkong	25.7	Black	Yellow	CR
Heugcheongkong	30.1	Black	Green	CR
Hwaeomputkong	30.7	Yellow	Yellow	VS
Ilpumgeomjeongkong	28.0	Black	Yellow	CR
Keunolkong	31.0	Yellow	Yellow	VS
Seoklyangputkong	37.6	Green	Yellow	VS
Seonheukkong	34.2	Black	Yellow	VS

[a] CR, Cooked with rice soybean; VS, Vegetable soybean.

were mixed with 10 ml of acetonitrile (ACN) and 2 ml of 0.1 N HCl, and the mixture was sonicated for 2 h at room temperature to extract constituents before filtering through Whatman (No. 42) filter paper. The extracts were freeze-dried at -40^0C. Each sample was redissolved in 10 ml of 80% methanol (MeOH) and was filtered through 0.45 μm syringe filter (TITAN, nylon).

HPLC analysis was conducted using the modified methods of Wang and Murphy (1994) and Kim, Jung, Ahn, and Chung (2005). A SHIMADZU HPLC system, together with SPD-M10A Photo Diode Array Detector equipped with YMC ODS AM-303, a column of 5 μm pore and 250 × 4.6 mm I.D. and Midas Autoinjector were employed. The wavelength of the UV detector was 254 nm. A linear HPLC gradient was engaged using solvent A (0.1% glacial acetic acid in distilled water) and solvent B (0.1% glacial acetic acid in ACN). Following the injection of 20 μl sample, solvent B was increased from 15% to 35% in 60 min, kept at 35% for 5 min, and returned to 15% in 5 min at the flow rate of 1 ml min^{-1}. After a total lapse of 85 min, equivalent treatment of the next sample was commenced. Solvent and distilled water used were of HPLC grade with a purity >99.9%, and all the solutions were preliminarily degassed by Helium gas.

2.3. Standard material and standard calibration curves

Twelve isoflavone standards were purchased from LC Laboratories (USA) and were dissolved with dimethylsulfoxide (DMSO). The concentrations to plot standard curves were 1, 50, and 100 μg ml^{-1}, and a high linearity of $r^2 > 0.998$ was obtained from each curve. All of the standards were identified by their retention times or by co-chromatography with other authentic examples, and their concentrations were calculated by comparing the peak areas of samples with those of the standards.

2.4. Statistical analysis

A statistical analysis was undertaken using the general linear model procedure (GLM) of the statistical analysis system (SAS, 2000) program The results were analyzed by means of analysis of variance (ANOVA) single factor. All of the experiments were replicated three times using a completely randomized design. The LSD (least significant difference) test was based on the 0.05 probability level.

3. Results and discussion

The representative HPLC chromatograms of soybean seed components are shown in Fig. 1, in which all 12 isoflavones are sharply resolved. Twelve isoflavones present in each sorted seed tissue of nine soybean varieties were quantified based on each peak area. As shown in Table 2, total average isoflavone concentrations of nine soybean varieties were 2887 μg g^{-1} in the embryo, 575 μg g^{-1} in the fully matured whole seed, 325 μg g^{-1} in the cotyledon, and 33 μg g^{-1} in the seed coat. Overall, embryo and seed coat of all nine varieties contained isoflavones at the highest and lowest level, respectively (Fig. 2).

As for the embryo tissue, Geomjeongkong 2 had the highest concentration of isoflavone (5701 μg g^{-1}), and Keunolkong had the lowest (341 μg g^{-1}). The five varieties including Geomjeongkong 2 accumulated isoflavones above the average concentration in the embryo. Among four groups of isoflavone, malonylglycoside (1915 μg g^{-1}) was highest, being followed by glycoside (823 μg g^{-1}), was second highest, aglycon (81 μg g^{-1}), and acetylglycoside (68 μg g^{-1}). Malonylglycoside constituted 66% of the total average isoflavone content. TDIN (sum of daidzin, malonyldaidzin, acetyldaidzin, and daidzein) and TGLY (sum of glycitin, malonylglycitin, acetylglycitin, and glycitein) all together amounted to more than 1000 μg g^{-1} or more than about 40% of a total concentration, whereas TGIN (sum of genistin, malonylgenistin, acetylgenistin, and genistein) was below 500 μg g^{-1} or about 15% of a total concentration. Among the individual isoflavones, malonyldaidzin (777 μg g^{-1}) and acetylgenistin (9.6 μg g^{-1}) accumulated at the highest and lowest level, respectively. The total sum of daidzin, glycitin, and malonylglycoside constituted more than 70% of the total isoflavone in the embryo tissue. The other individual isoflavones made up less than 40 μg g^{-1}.

With respect to the isoflavone content of whole seed, Geomjeongolkong (1321 μg g^{-1}) was highest and Hwaeomputkong (175 μg g^{-1}) was lowest. Geomjeongkong 2 (1128 μg g^{-1}) and Galmikong (737 μg g^{-1}) contained more than total average content, and the other six varieties had

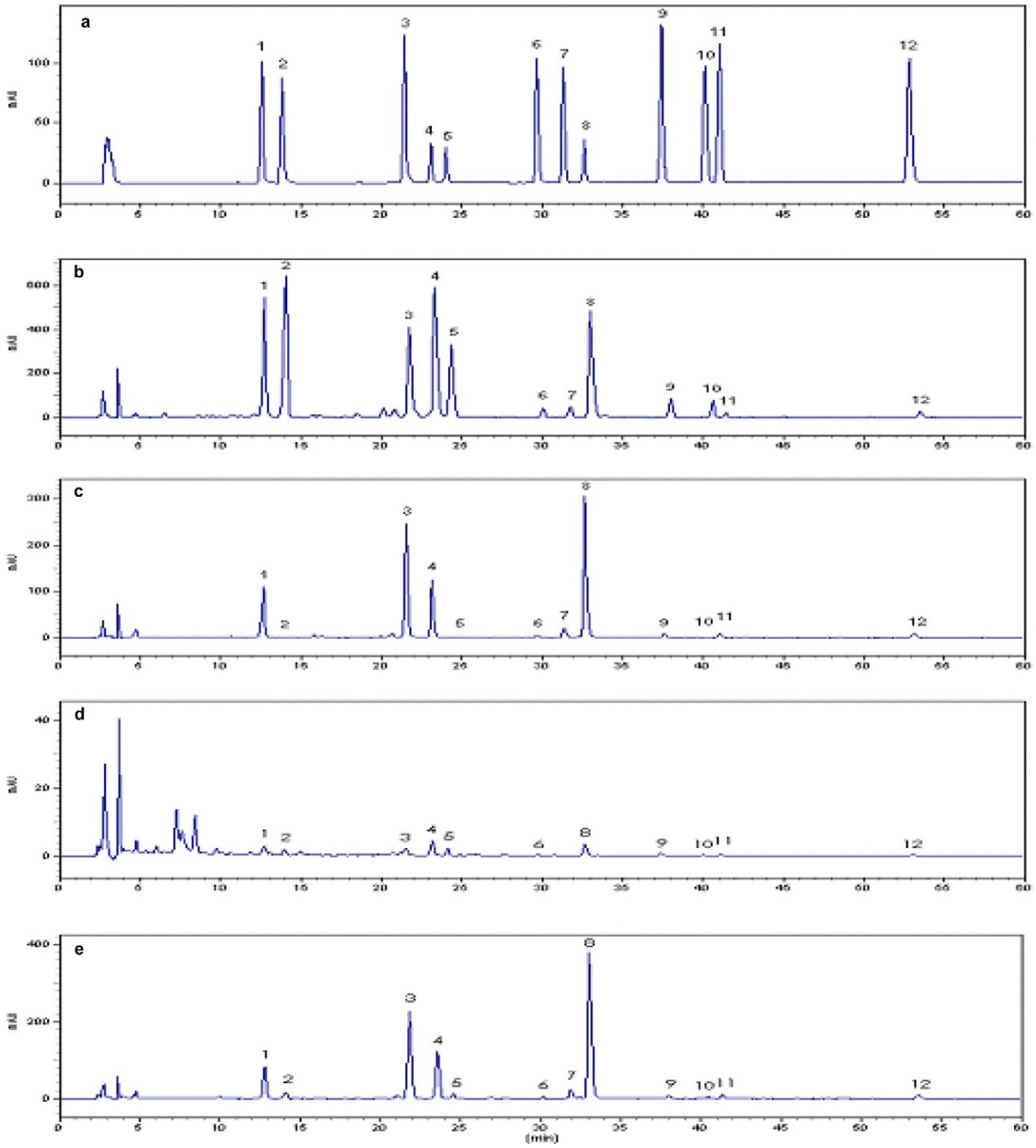

Fig. 1. HPLC chromatogram of soybean isoflavones. (a) 12 isoflavone standards; (b) Embryo of Geomjeongkong 2; (c) Cotyledon of Heugcheongkong; (d) Seed coat of Keunolkong; (e) Whole seed of Geomjeongkong 2; 1, Daidzin; 2, Glycitin; 3, Genistin; 4, Malonyldaidzin; 5, Malonylglycitin; 6, Acetyldaidzin; 7, Acetylglycitin; 8, Malonylgenistin; 9, Daidzein; 10, Glycitein; 11, Acetylgenistin; 12, Genistein.

below the total average. Malonylglycoside (450 μg g^{-1}) and TGIN (267 μg g^{-1}) constituted about 78% and 46% of the total average isoflavone level, respectively, and glycoside, acetylglycoside and aglycon accumulated in the decreasing order. Among the individual isoflavones, malonylgenistin (240 μg g^{-1}) accumulated at the highest level being equivalent to about 42% of the total concentration, and acetylglycitin was present in only trace amounts in all varieties.

As for the cotyledon tissue, Heugcheongkong (951 μg g^{-1}) was highest, being three times the total average level, whereas Seonheukkong (81 μg g^{-1}) was lowest. Among the four groups of isoflavone, malonylglycoside (258 μg g^{-1}) accumulated at the highest level, being followed by glycoside (43 μg g^{-1}), acetylglycoside (17 μg g^{-1}) and aglycon (7 μg g^{-1}). TGIN (189 μg g^{-1}), TDIN (116 μg g^{-1}), and TGLY (21 μg g^{-1}) constituted 58%, 36%, and 6% of the

Table 2
The concentration of isoflavone in soybean components

Varieties	Part	Glycoside			Malonylglycoside			Acetylglycoside			Aglycon			TOTAL
		DIN[a] ($\mu g\ g^{-1}$)	GLY[b] ($\mu g\ g^{-1}$)	GIN[c] ($\mu g\ g^{-1}$)	MDIN[d] ($\mu g\ g^{-1}$)	MGLY[e] ($\mu g\ g^{-1}$)	MGIN[f] ($\mu g\ g^{-1}$)	AcDIN[g] ($\mu g\ g^{-1}$)	AcGLY[h] ($\mu g\ g^{-1}$)	AcGIN[i] ($\mu g\ g^{-1}$)	DEIN[j] ($\mu g\ g^{-1}$)	GLEIN[k] ($\mu g\ g^{-1}$)	GEIN[l] ($\mu g\ g^{-1}$)	
Galmikong	E[m]	235.02	205.84	5.23	582.22	357.23	115.56	9.44	11.67	1.95	22.90	10.76	2.85	1560.67
	C[n]	16.29	0.87	8.21	51.61	2.96	271.23	0.99	17.20	3.81	1.52	0.10	3.93	378.72
	S[o]	2.20	2.71	0.73	18.26	25.99	4.03	nd[*]	nd	nd	1.64	nd	0.30	55.87
	W[p]	45.93	18.80	9.89	155.96	51.67	347.28	3.51	tr[**]	88.78	5.11	4.42	5.17	736.52
Geomjeongkong 2	E	621.97	841.48	37.97	1664.57	1405.15	829.31	47.59	78.30	20.73	70.95	61.68	21.73	5701.43
	C	31.27	1.83	6.95	57.36	30.68	124.73	1.65	10.09	2.60	1.91	1.13	1.86	272.06
	S	0.01	4.33	0.19	2.85	3.60	3.56	nd	nd	5.96	0.68	0.17	0.23	21.58
	W	94.36	22.87	19.22	282.82	58.21	617.25	5.89	tr	10.60	7.46	1.87	7.07	1127.63
Geomjeongolkong	E	482.69	426.17	18.06	822.46	684.67	312.93	22.22	32.48	7.69	37.07	16.95	7.64	2871.02
	C	44.00	1.68	8.53	107.18	6.57	246.48	2.52	15.08	4.88	3.84	0.38	4.47	445.62
	S	2.52	2.76	0.14	6.33	7.65	2.95	nd	nd	5.48	0.95	0.20	0.25	29.23
	W	170.51	142.33	9.03	448.28	235.13	263.40	9.95	tr	6.00	18.35	11.53	6.66	1321.19
Heugcheongkong	E	568.87	753.08	21.65	1152.03	859.22	364.49	30.86	41.22	9.22	38.04	35.09	6.51	3880.28
	C	121.40	1.89	20.20	276.07	6.97	471.33	4.63	27.51	7.13	7.45	0.12	6.33	951.04
	S	0.01	6.02	0.13	4.55	4.33	3.00	nd	nd	5.88	0.25	0.14	0.08	24.40
	W	67.36	14.40	8.57	116.46	22.71	142.07	2.05	tr	4.52	2.06	0.53	1.43	382.16
Hwaeomputkong	E	260.77	213.54	24.99	455.62	347.25	463.28	13.56	35.19	13.00	36.86	15.46	23.32	1902.85
	C	8.54	2.94	2.97	14.76	1.75	55.73	0.94	4.07	2.15	1.11	0.01	2.59	97.56
	S	4.52	2.82	0.31	13.01	8.58	7.85	0.35	0.55	0.14	0.64	0.15	0.31	39.24
	W	16.90	10.98	3.05	39.06	20.30	75.74	1.89	tr	2.18	2.01	0.44	2.74	175.29
Ilpumgeomjeongkong	E	383.74	636.16	28.59	723.62	903.37	495.25	19.60	47.06	12.21	58.32	39.23	27.63	3374.79
	C	25.58	1.72	6.05	45.73	3.44	122.65	1.62	8.01	3.55	4.94	0.35	6.87	230.49
	S	2.34	3.65	0.29	7.26	7.67	3.02	nd	nd	4.66	0.53	0.15	0.17	29.73
	W	30.43	18.39	5.56	67.92	36.95	137.58	1.95	tr	4.03	6.43	1.30	6.79	317.34
Keunolkong	E	55.36	45.54	4.92	79.06	62.15	71.81	2.64	6.66	2.39	5.39	2.55	2.96	341.43
	C	14.15	3.46	3.65	29.77	4.53	85.19	1.62	5.79	2.84	1.94	0.20	2.95	156.09
	S	3.41	2.38	0.19	10.42	7.74	30.72	0.34	nd	0.10	0.55	0.14	0.27	56.26
	W	23.84	11.05	4.28	57.93	21.90	117.28	2.34	tr	3.39	2.58	0.47	3.12	248.18
Seoklyangputkong	E	391.19	412.44	12.77	863.50	799.43	255.68	21.01	27.24	5.43	65.82	29.86	12.42	2896.79
	C	39.56	2.71	5.58	93.76	5.68	140.89	2.79	9.49	2.84	4.67	nd	4.96	312.93
	S	0.67	0.32	0.05	1.38	0.97	1.14	0.01	nd	0.04	0.35	0.02	0.10	5.05
	W	47.81	11.49	5.75	139.63	29.04	195.81	3.16	tr	3.56	6.50	0.49	5.33	448.58
Seonheukkong	E	202.87	489.74	25.49	647.05	1174.99	744.16	18.23	61.76	13.46	23.77	38.15	10.57	3450.24
	C	6.49	0.43	2.80	13.21	0.86	50.00	0.21	4.17	1.71	0.30	nd	0.85	81.04
	S	0.45	3.23	0.11	6.74	11.61	4.82	nd	nd	5.83	0.55	0.21	0.23	33.79
	W	18.27	12.68	7.47	63.64	42.09	262.19	1.35	tr	4.75	1.97	0.60	2.76	417.77
Average	E	355.83	447.11	19.96	776.68	732.61	405.83	20.57	37.95	9.56	39.90	27.75	12.85	2886.61
	C	34.14	1.95	7.22	76.61	7.05	174.25	1.89	11.27	3.50	3.08	0.25	3.87	325.08
	S	1.79	3.14	0.24	7.87	8.68	6.79	0.08	0.06	3.12	0.68	0.13	0.22	32.79
	W	57.27	29.22	8.09	152.41	57.56	239.84	3.57	tr	14.20	5.83	2.40	4.56	574.96
$LSD_{0.05}$	E	30.25	20.20	1.61	36.84	39.66	21.22	0.59	0.96	0.11	0.64	0.50	0.21	41.28
	C	2.89	0.68	0.64	12.18	2.42	24.32	0.90	1.78	0.13	0.11	0.15	0.06	36.16
	S	0.76	1.01	0.06	1.34	2.34	14.61	0.02	0.03	0.11	0.17	0.06	0.02	15.32
	W	6.20	2.12	0.97	12.82	6.00	18.88	0.41	nd	0.84	4.80	0.34	0.11	30.02

[*] Not detected.
[**] Trace (peak have not integral value at 254 nm).
[a] Daidzin.
[b] Glycitin.
[c] Genistin.
[d] Malonyldaidzin.
[e] Malonylglycitin.
[f] Malonylgenistin.
[g] Acetyldaidizin.
[h] Acetylglycitin.
[i] Acetylgenistin.
[j] Daidzein.
[k] Glycitein.
[l] Genistein.
[m] Embryo.
[n] Cotyledon.
[o] Seed coat.
[p] Whole seed.

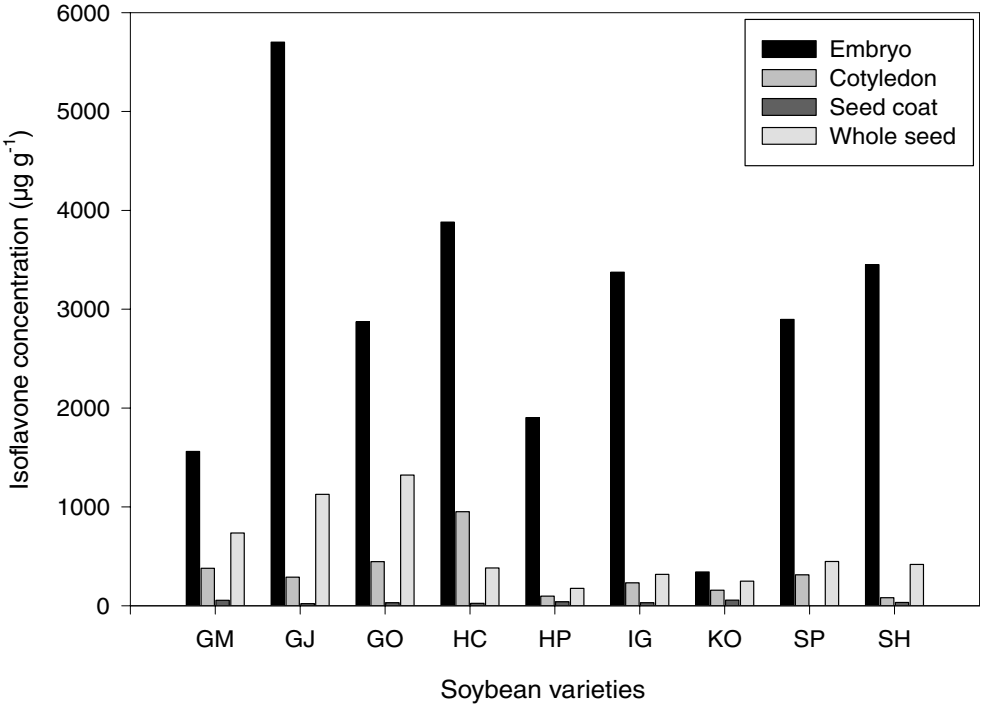

Fig. 2. Comparison of isoflavone contents in whole seed and its components of nine soybean varieties. GM, Galmikong; GJ, Geomjeongkong 2; GO, Geomjeongolkong; HC, Heugcheongkong; HP, Hwaeomputkong; IG, Ilpumgeomjeongkong; KO, Keunolkong; SP, Seoklyangputkong, SH, Seonheukkong.

total average content, respectively. Notably, malonylgenistin (174 μg g^{-1}) and malonyldaidzin (77 μg g^{-1}) combined together to constitute about 79% of the total average level.

Seed coat contained considerably lower amounts of isoflavone as compared to other soybean parts. Keunolkong (56 μg g^{-1}) and Galmikong (56 μg g^{-1}) were highest and Seoklyangputkong (5 μg g^{-1}) was lowest in seed coat. Accumulation levels of TDIN, TGLY and TGIN were similar to each other. Isoflavone accumulation was diminished in the order of malonylglycoside (23 μg g^{-1}), glycoside (5 μg g^{-1}), acetylglycoside (3 μg g^{-1}) and aglycon. Despite the low level of isoflavones in seed coat, malonylglycoside made up 71% of the total average content.

Lee, Park, Oh, and Kwak (2002) reported that Geomjeongolkong (1145 μg g^{-1}) was highest in total isoflavone content of whole seed among the black soybean seed cultivars, being consistent with our result. However, they noted that Ilpumgeomjeongkong seed (751 μg g^{-1}) contained more isoflavones than Geomjeongkong 2 seed (498 μg g^{-1}). This is in contrast to our result showing that Ilpumgeomjeongkong seed (317 μg g^{-1}) had much less isoflavones than Geomjeongkong 2 seed (1128 μg g^{-1}). This could be due to a difference in extraction method and cultivation conditions. Kim, Hong, and Kim (1997) reported that black soybean seeds have more isoflavone on average than yellow soybean seeds. The same observation was also made in the present study.

In conclusion, the levels of soybean isoflavonoids vary widely depending on tissue types, varieties, and growth conditions in habitat. All nine varieties accumulated isoflavones at the highest and lowest level in embryo and seed coat tissue, respectively. Isoflavones accumulated in the order of malonylglycoside, glycoside, acetylglycoside and aglycon, among which malonylglycoside was the most abundant form ranging from 66 to 79% of the total isoflavone content in all three tissues. The embryo of cooked-with-rice soybean with black seed coat appears to be the best source of isoflavone.

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