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Analysis of Isoflavones and Phenolic Compounds in Korean Soybean [*Glycine max* (L.) Merrill] Seeds of Different Seed Weights

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The seeds of 322 Korean soybean varieties were collected from six different cultivated sites in Korea and classified into three groups based on the 100-seed weight as small, medium, and large. Seeds were analyzed for their concentrations of isoflavones and phenolic compounds. The total average isoflavones in soybean cultivated at Iksan (2.840 μmol g^{-1}) and phenolic compounds in soybean grown at Yeoncheon (9.216 μmol g^{-1}) and Iksan (9.154 μmol g^{-1}) were significantly different ($p < 0.05$). In small and medium seeds of soybeans cultivated at Yeoncheon, Yesan, and Milyang high levels of isoflavones were obtained, whereas soybeans grown in Chuncheon showed the lowest isoflavone concentrations. However, isoflavone concentrations in the large seeds of soybean cultivated at Chuncheon showed the highest level. The soybean cultivated at Yeoncheon had high levels of phenolic compounds in small, medium, and large seeds, whereas the soybean grown at Chuncheon had the lowest. On the other hand, the phenolic concentrations of large soybean cultivated at Milyang were the least. At Yeoncheon, Yesan, and Milyang, the total isoflavone and phenolic compounds levels related to their seed size was significantly different ($p < 0.05$), whereas in the soybean of different sizes cultivated at Chuncheon, the relationship to their seed size was not significantly different. The relationships of total isoflavones and phenolic compounds of small and medium soybean seeds were significantly higher than that of large soybean seeds. The hydroxybenzoic acid group in all sizes of seeds cultivated at six sites in Korea was the major phenolic compound, followed by flavonoid and hydroxycinnamic acid. The total isoflavone concentration was positively correlated with acetylglycoside and negatively correlated with malonylglycoside in the small soybean seeds cultivated at Yeoncheon. In medium soybean seeds cultivated at Yeoncheon, a significantly positive correlation was found between acetylglycoside and glycoside, between aglycone and glycoside, and between aglycone and acetylglycoside, whereas a significantly negative correlation was shown between malonylglycoside and glycoside, between acetylglycoside and malonylglycoside, and between aglycone and malonylglycoside. In large soybean seeds cultivated at Chuncheon, significantly positive and negative correlations were similar to those of medium seeds. The results presented here can improve the understanding of the relationships among the concentrations of individual chemical compounds and each chemical compound group and total chemical compounds in soybeans of different seed sizes from different cultivated sites.

KEYWORDS: Soybean; isoflavones; phenolic compounds; seed weight; HPLC

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is an important crop worldwide because it provides a good source of vegetable oils, both culinary and industrial, and high-quality protein. To overcome the risk of protein deficiency resulting from a rice-based diet, soybeans and soy foods have become popular food ingredients, and more and more consumers are looking for products that contain soybean (*1, 2*). The health benefits of soybean and soy-based foods have been well-known for many years and widely recognized around the world. Recently, the demand for healthy−functional food has increased in the West as well as in Asian countries (*3–6*).

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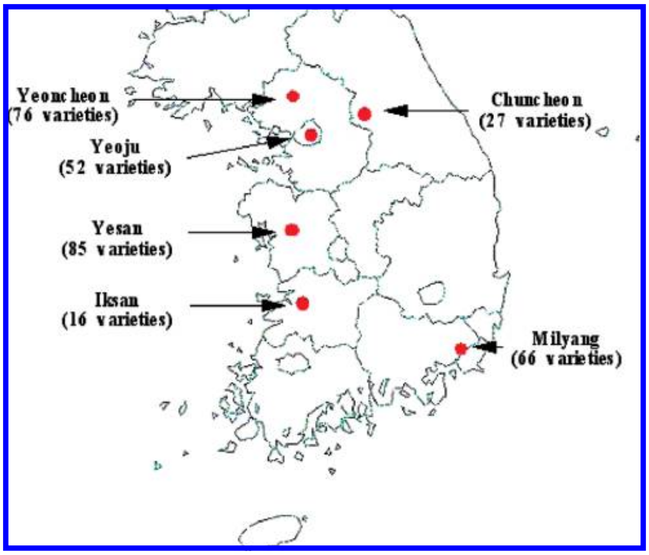

Figure 1. Six sites in Korea where soybean germplasm was grown in 2005.

Isoflavones are the active ingredients in soybeans and generally classified into daidzein, genistein, and glycitein forms and along with their glycosides and malonate conjugates are the main phenolic compounds. Much research has been concentrated on the positive aspects of various soybean isoflavones and phenolics because of their weak estrogenic and other biological properties, such as reducing the risk of cardiovascular, atherosclerotic, hemolytic, and carcinogenic diseases; improvement of bone health, osteoporosis, menopausal symptoms, and blood cholesterol levels; inhibition of the growth of hormone-related human breast cancer and prostate cancer cell lines in culture; and increased antioxidative effect in human subjects (*7–10*).

Many published papers have dealt with total phenolic concentrations, flavonoids, and phenolic acids in soybean. Kim et al. (*11*) announced that the range and mean of total phenolic compounds in nine soybean varieties was 730−1812 μmol g^{-1} over storage for 4 years. The concentration of phenolic compounds was contained in embryo, cotyledon, and seed coat of the nine soybean varieties, and the level of phenolic compounds and the antioxidant capacity showed a strong correlation (*12*).

In Korea, soybean seeds are commonly classified into three sizes, small, medium, and large, by the 100-seed weight. Generally, small soybean seeds are used for soybean sprouts, and medium and large seeds are used for cooking with rice, vegetables, soybean curd, soy milk, soy sauce, and Korean traditional fermented soybean pastes. Soybeans used for paste, soybean curd, cooking with rice, or vegetable are of medium and larger seed sizes (13−24 and 24−40 g per 100 seeds), respectively.

The main objective of this study was to analyze representative natural isoflavones and phenolic compounds in Korean soybean seeds of various seed weight characteristics grown at six different sites in Korea. The obtained results may provide a basic understanding of soybean germplasm with higher isoflavones and phenolic compounds and concentrations.

MATERIALS AND METHODS

Sample Preparation. Three hundred and twenty-two Korean soybean germplasms were cultivated at six Korean sites in 2005 (**Figure 1**): Yeoncheon (northern part of Gyeonggi, 76 varieties); Yeoju (southern part of Gyeonggi, 52 varieties); Chuncheon (Gangwon, 27 varieties); Yesan (Chungnam, 85 varieties); Iksan (Honam, 16 varieties); and Milyang (Yeongnam, 66 varieties). The climatic data for the cultivation period are exhibited in **Table 1**.

Table 1. Climatic Information of Six Sites in Korea

site	climate factor	2005 July	August	September
Yeoncheon	precipitation (mm)	364.1	335.7	231.8
	sunshine duration (h)	88.6	106.4	143.8
	temperature (°C)	24.3	24.0	20.3
	relative humidity (%)	82.0	81.5	77.6
	av wind speed (m/s)	1.5	1.7	1.4
Yesan	precipitation (mm)	451.0	381.5	225.5
	sunshine duration (h)	204.1	208.2	204.6
	temperature (°C)	25.0	24.9	21.9
	relative humidity (%)	83.3	82.2	76.5
	av wind speed (m/s)	1.3	1.5	1.7
Iksan	precipitation (mm)	465.5	431.0	101.9
	sunshine duration (h)	104.1	135.0	117.4
	temperature (°C)	26.2	26.2	23.3
	relative humidity (%)	75.6	73.7	71.3
	av wind speed (m/s)	2.1	2.2	2.1
Chuncheon	precipitation (mm)	322.2	340.5	210.5
	sunshine duration (h)	118.4	125.6	132.6
	temperature (°C)	25.4	24.5	20.3
	relative humidity (%)	77.2	76.8	73.6
	av wind speed (m/s)	1.2	1.2	1.4
Yeoju	precipitation (mm)	291.5	306.5	274.5
	sunshine duration (h)	163.5	150.9	161.6
	temperature (°C)	25.5	24.8	21.3
	relative humidity (%)	84.1	79.1	75.8
	av wind speed (m/s)	1.1	1.2	1.2
Milyang	precipitation (mm)	206.5	244.0	72.0
	sunshine duration (h)	142.0	140.6	120.6
	temperature (°C)	25.3	25.5	22.5
	relative humidity (%)	73.8	72.1	71.2
	av wind speed (m/s)	1.1	1.3	1.2

The varieties differed in 100-seed weight as shown in Table 1 of the Supporting Information. The experimental soil texture was a silt clay loam at all sites. As the preceding crop planted was soybean, no artificial inoculation was needed. The planting density was 60 cm × 15 cm per plot, and the plants were thinned to a uniform density. At 14 days after planting, agrochemicals were applied following the conventional method of soybean growth in Korea to control weeds, diseases, and insects. 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. Each plot consisted of three rows (3.75 m long and 0.6 m between rows), and the

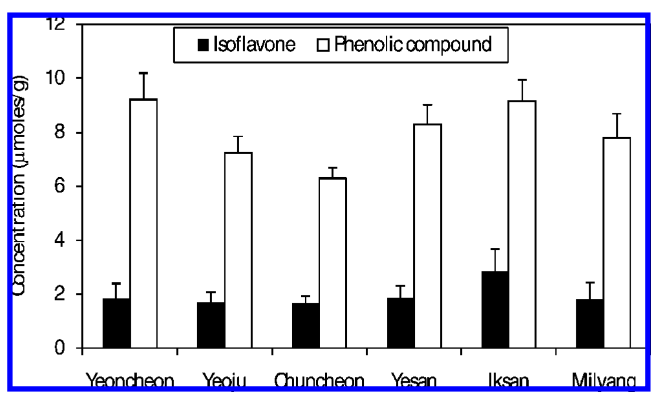

Figure 2. Total average isoflavones and phenolic compound concentrations in various soybean varieties cultivated at the six sites in Korea. Each vertical bar indicates the standard error values. Statistical significance was analyzed by the least significant difference (LSD) ($P < 0.05$).

Table 2. Comparison on Total Isoflavones and Phenolics to Small, Medium, and Large Soybeans Cultivated from the Six Sites in Korea

		Yeoncheon		Yeoju		Chuncheon		Yesan		Iksan		Milyang	
seed size		isoflavones	phenolics	isoflavones	phenolics	isoflavones	phenolics	isoflavones	phenolics	isoflavones	phenolics	isoflavones	phenolics
small	no. of varieties	15	15	33	33	5	5	31	31	16	16	10	10
	max (μmol g^{-1})	4.026	22.225	2.401	11.860	2.015	8.800	2.883	14.198	4.326	15.634	2.802	15.504
	min (μmol g^{-1})	0.956	3.411	1.104	3.943	1.226	3.915	1.333	6.322	1.444	5.117	0.961	2.622
	mean (μmol g^{-1})	2.491	12.818	1.753	7.902	1.621	6.358	2.108	10.260	2.885	10.376	1.882	9.063
	CV (%)	2.302	8.482	4.573	3.424	1.031	7.676	12.099	9.786	0.592	13.133		
	$LSD_{(0.05)}$	0.108	2.016	0.161	0.517	0.040	1.153	0.508	1.929	0.036	2.549		
medium	no. of varieties	19	19	19	19	3	3	25	25			32	32
	max (μmol g^{-1})	3.171	16.929	2.195	11.740	1.804	6.932	2.797	15.344			2.692	14.698
	min (μmol g^{-1})	1.288	6.217	1.179	3.827	1.183	4.378	0.886	2.858			0.962	1.747
	mean (μmol g^{-1})	2.300	11.573	1.687	7.784	1.494	5.655	1.842	9.101			1.827	8.220
	CV (%)	1.643	5.736	0.942	3.885	0.611	6.183	13.914	4.041			0.152	0.835
	$LSD_{(0.05)}$	0.071	1.257	0.032	0.560	0.028	1.039	0.539	0.675			1.885	8.336
large	no. of varieties	42	42			19	19	29	29			24	24
	max (μmol g^{-1})	2.482	14.845			2.258	9.866	2.611	7.445			2.165	10.087
	min (μmol g^{-1})	0.945	3.368			1.540	4.543	0.982	3.040			0.955	2.688
	mean (μmol g^{-1})	1.714	9.107			1.899	7.205	1.797	5.423			1.560	6.388
	CV (%)	0.567	5.857			0.549	6.011	15.574	4.133				
	$LSD_{(0.05)}$	0.018	0.940			0.020	0.827	0.531	0.598				

experiment consisted of a completely randomized design with three replicates. Soybean seeds were harvested from each replicate for each genotype at each site and stored at dehumidified room temperature (25–30 °C) for analysis of isoflavones.

Isoflavone Analysis by HPLC. All soybean seeds were dried in a freeze-dryer under vacuum Two milliliters of 0.1 N HCl and 10 mL of acetonitrile (ACN) were mixed with the ground samples (2 g) following the method of Murphy et al. (*13*), stirred for 2 h at room temperature, and filtered through a Whatman no. 42 filter paper. The filtrate was concentrated to dryness under vacuum at a temperature below 30 °C. The dried samples were redissolved in 10 mL of 80% HPLC grade methanol solution. Aliquot samples were filtered through a 0.45 μm filter unit (Titan syringe filter nylon membrane) and then analyzed by HPLC equipment. The high-performance liquid chromatography (HPLC)

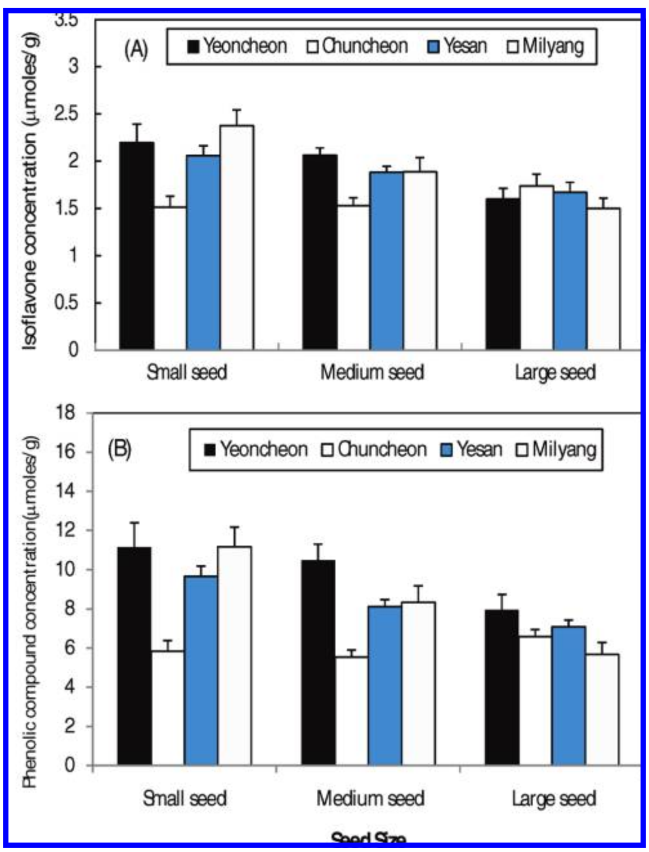

Figure 3. Comparison of isoflavone (**A**) and phenolic compound (**B**) concentrations among four cultivation sites of Korea according to different seed sizes of soybean varieties. Each vertical bar indicates the standard error values. Statistical significance was analyzed by the least significant difference (LSD) ($P < 0.05$) (small seed, <13 g; medium seed, 13−24 g; large seed, >24 g).

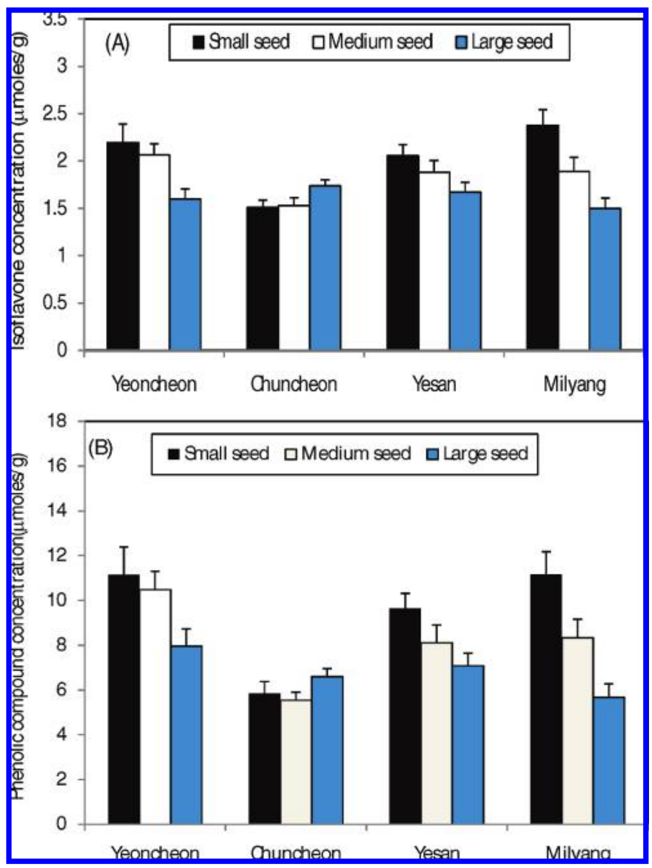

Figure 4. Comparison of isoflavone (**A**) and phenolic compound (**B**) concentrations among different seed sizes of soybean varieties among four cultivated sites of Korea. Each vertical bar indicates the standard error values. Statistical significance was analyzed by the least significant difference (LSD) ($P < 0.05$) (small seed, <13 g; medium seed, 13−24 g; large seed, >24 g).

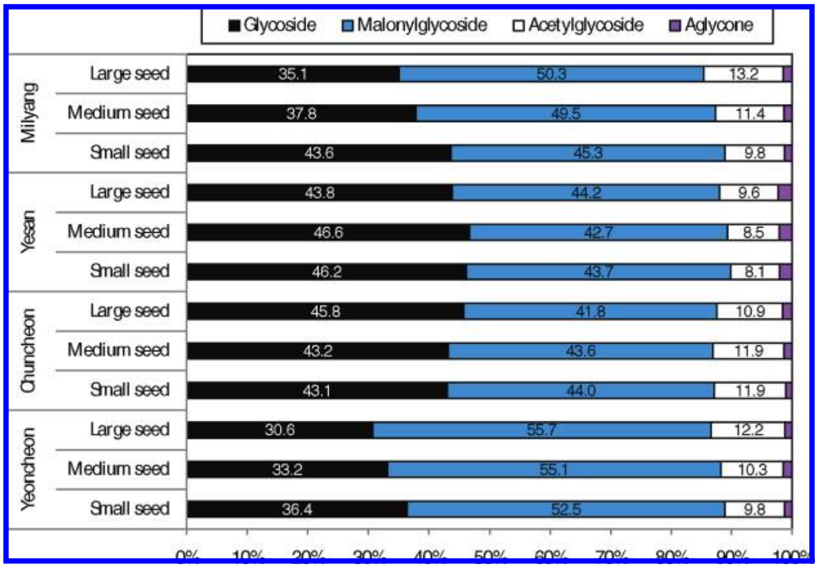

Figure 5. Ratios of each isoflavone group to total average isoflavones in different seed-size soybean varieties among four cultivation sites of Korea (glycoside = daidzin + genistin + glycitin; malonylglycoside = malonyldaidzin + malonylgenistin + malonylglycitin; acetylglycoside = acetyldaidzin + acetylgenistin + acetylglycitin; aglycone = daidzein + genistein + glycitein; small seed, <13 g; medium seed, 13−24 g; large seed, >24 g).

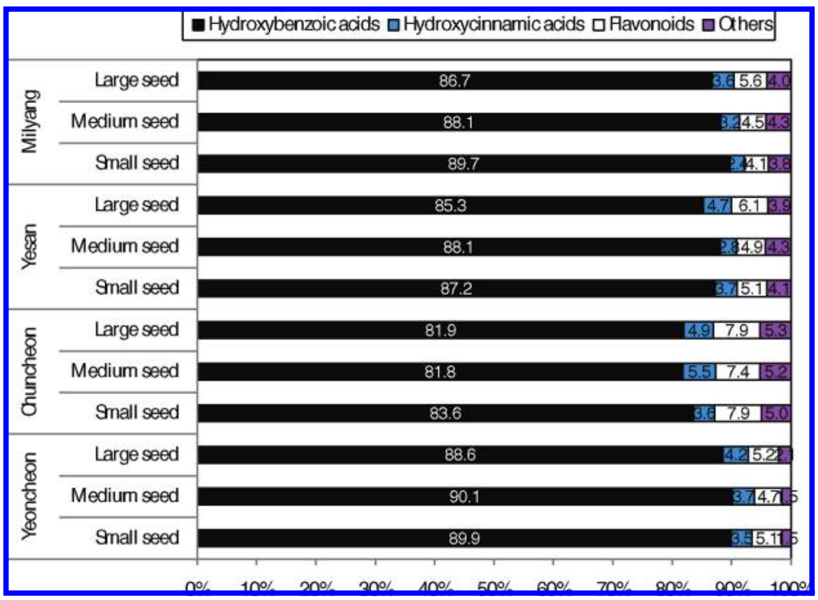

Figure 6. Ratios of each phenolic compound group to total average phenolics in different seed-size soybean varieties among four cultivation sites of Korea (hydroxybenzoic acids = gallic acid + homogentisic acid + 5-sulfosalicylic acid + protocatechuic acid + gentisic acid + *p*-hydroxybenzoic acid + vanillic acid + syringic acid + vanillin + salicylic acid + benzoic acid; hydroxycinnamic acids = chlorogenic acid + caffeic acid + *p*-coumaric acid + ferulic acid + *m*-coumaric acid + *o*-coumaric acid + *trans*-cinnamic acid; flavonoid = (+)-catechin + rutin + naringin + myricetin + quercetin + naringenin + kaempferol + hesperetin + formononetin + biochanin A; others = pyrogallol + resveratrol; small seed, <13 g; medium seed, 13−24 g; large seed, >24 g).

analysis was carried out on a product from Shimadzu Instruments Co., Ltd., with a pump model LC-10AD *VP* and a detector model SPD-M10A *VP* (photo diode array detector). A YMC-Pack ODS-AM-303 (250 × 4.6 mm i.d.) analytical HPLC column was employed for quantitative analysis. The substances being measured in the analytical procedure were monitored and determined by UV wavelength of 254 nm.

The instrumentation for HPLC analysis was applied using the modified method of Lee et al. (*14*). In the HPLC analysis, the mobile phase consisted of solvents A and B. A linear HPLC gradient was used: solvent A was 0.1% glacial acetic acid in distilled water, and solvent B was 0.1% glacial acetic acid in ACN. The injection volume was 20 μL of the sample; solvent B was increased from 15 to 35% for 50 min and then held at 35% for 10 min. The solvent flow rate was 1 mL min^{-1}. Genuine standards of 12 isoflavones were purchased from LC Laboratories, were dissolved with dimethyl sulfoxide (DMSO), and then used for calibration curves. The plotting standard concentration was obtained at five concentrations, 1, 25, 50, 75, and 100 ppm, and the molarities of them showed high linearity of $r^2 > 0.999$ (see Table 2 of the Supporting Information). The value was steadily obtained through the whole period for preparing all standard calibration curves. This is strong proof of the precision of the HPLC equipment. Daidzein, genistein, glycitein, daidzin, genistin, glycitin, acetyldaidzin, acetylgenistin, acetylglycitin, malonyldaidzin, malonylgenistin, and malonylglycitin were each identified by their retention times, and their concentrations were calculated by comparing the peak areas of samples with those of the standards.

Phenolic Compound Analysis by HPLC. The solvent gradient HPLC analysis was applied using the modified method of Murphy et al. (*13*) and Kim et al. (*15*). The mobile phase consisted of solvents A and B. Solvent A was 0.1% glacial acetic acid in distilled water, and solvent B was 0.1% glacial acetic acid in ACN. The solvent flow rate

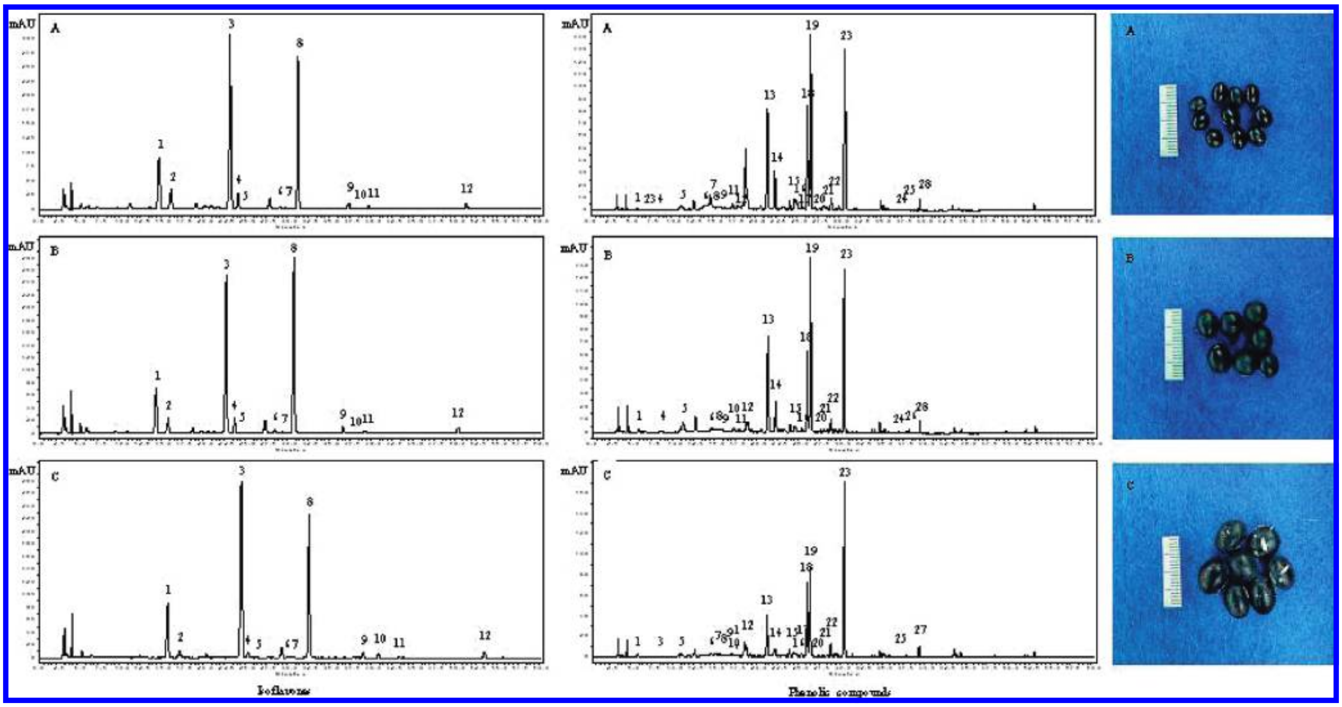

Figure 7. Comparison of HPLC chromatograms of isoflavones (1, daidzin; 2, glycitin; 3, genistin; 4, malonyldaidzin; 5, malonylglycitin; 6, acetyldaidzin; 7, acetylglycitin; 8, malonylgenistin; 9, daidzein; 10, glycitein; 11, acetylgenistin; 12, genistein) and phenolic compounds (1, gallic acid; 2, pyrogallol; 3, homogentisic acid; 4, 5-sulfosalicylic acid; 5, protocatechuic acid; 6, gentisic acid; 7, *p*-hydroxybenzoic acid; 8, (+)-catechin; 9, chlorogenic acid; 10, vanillic acid; 11, caffeic acid; 12, syringic acid; 13, vanillin; 14, *p*-coumaric acid; 15, ferulic acid; 16, rutin; 17, *m*-coumaric acid; 18, salicylic acid; 19, benzoic acid; 20, naringin; 21, *o*-coumaric acid; 22, myricetin; 23, resveratrol; 24, quercetin; 25, *trans*-cinnamic acid; 26, naringenin; 27, kaempferol; 28, hesperetin) among different seed sizes (**A**, small seeds; **B**, medium seeds; **C**, large seeds).

Table 3. Relationships among Isoflavone Groups in Small, Medium, and Large Soybean Seeds at the Sites with the Maximum and Minimum Isoflavone Concentrations[a]

seed size	site	isoflavone	glycoside	malonylglycoside	acetylglycoside	aglycone
small	max (Yeoncheon)	malonylglycoside	0.580***			
		acetylglycoside	0.236	−0.176		
		aglycone	0.308*	0.410**	0.186	
		total isoflavones	−0.119	−0.670***	0.835***	0.009
	min (Chuncheon)	malonylglycoside	−0.842***			
		acetylglycoside	0.878***	−0.916***		
		aglycone	0.620**	−0.584*	0.607**	
		total isoflavones	0.363	−0.133	0.512*	0.116
medium	max (Yeoncheon)	malonylglycoside	−0.701***			
		acetylglycoside	0.751***	−0.770***		
		aglycone	0.686***	−0.613***	0.736***	
		total isoflavones	0.001	0.380**	0.274*	−0.016
	min (Chuncheon)	malonylglycoside	−0.806***			
		acetylglycoside	0.799***	−0.917***		
		aglycone	0.419	−0.425	0.512	
		total isoflavones	0.365	−0.254	0.603*	0.223
large	max (Chuncheon)	malonylglycoside	−0.812***			
		acetylglycoside	0.865***	−0.936***		
		aglycone	0.702***	−0.852***	0.840***	
		total isoflavones	0.040	0.314	0.015	−0.189
	min (Milyang)	malonylglycoside	−0.588***			
		acetylglycoside	0.855***	−0.755***		
		aglycone	0.860***	−0.774***	0.839***	
		total isoflavones	0.561***	0.111	0.144	0.417*

[a] ***, $P > 0.001$; **, $P > 0.01$; *, $P > 0.05$.

was 1 mL min^{-1}, and the wavelength of the PDA was 280 nm. The injection volume was 20 μL of the sample, the linear gradient of HPLC solvent was as follows: B was increased from 8 to 10% for 2 min, then from 10 to 30% for 25 min, from 30 to 90% for 23 min, from 90 to 100% for 2 min, and kept at 100% for 5 min, before a return to the initiation state. The next sample was injected after 15 min. The genuine standards of 30 phenolic compounds were purchased from Sigma and Extrasynthese and were dissolved with DMSO and then used for calibration curves. The plotting standard concentration was obtained at five concentrations, 1, 25, 50, 75, and 100 ppm, and the molarities of them showed high linearity of $r^2 > 0.999$ (see Table 3 of the Supporting Information). The value was steadily obtained through the whole period for preparing all standard calibration curves. This is strong proof of the precision of the HPLC equipment. In phenolic compound

Table 4. Relationships among Phenolic Compound Groups in Small, Medium, and Large Soybean Seeds at the Sites with the Maximum and Minimum Phenolic Compound Concentrations[a]

seed size	site	phenolic compound	hydroxybenzoic acid	hydroxycinnamic acid	flavonoid	others
small	max (Milyang)	phenolic acids	0.046			
		flavonoids	0.133	−0.360		
		others	0.445*	0.416*	−0.637***	
		total phenolics	0.696***	0.212	−0.204	0.699***
	min (Chuncheon)	phenolic acids	−0.154			
		flavonoids	0.578*	−0.266		
		others	−0.058	0.538*	−0.623**	
		total phenolics	0.539*	−0.309	0.487*	0.121
medium	max (Yeoncheon)	phenolic acids	−0.065			
		flavonoids	0.341	−0.472**		
		others	0.601***	0.027	0.397*	
		total phenolics	0.448**	0.247	0.051	0.547**
	min (Chuncheon)	phenolic acids	−0.502			
		flavonoids	0.410	−0.597*		
		others	−0.394	0.863***	−0.483	
		total phenolics	0.173	0.008	0.665**	0.103
large	max (Yeoncheon)	phenolic acids	0.333*			
		flavonoids	0.511***	−0.110		
		others	0.456***	0.240	0.275	
		total phenolics	0.809***	0.582***	0.219	0.532***
	min (Milyang)	phenolic acids	0.216			
		flavonoids	0.602***	−0.106		
		others	0.492**	0.377*	−0.149	
		total phenolics	0.752***	0.341*	0.145	0.624***

[a] ***, $P > 0.001$; **, $P > 0.01$; *, $P > 0.05$.

analysis, 19 free phenolic acids, 9 flavonoids (including 2 glycosylic bound compounds, rutin and naringin), and 2 others were classified. Benzoic acid, caffeic acid, chlorogenic acid, *trans*-cinnamic acid, *m*-coumaric acid, *o*-coumaric acid, *p*-coumaric acid, ferulic acid, gallic acid, gentisic acid, homogentisic acid, *p*-hydroxybenzoic acid, protocatechuic acid, pyrogallol, resveratrol, rutin, salicylic acid, 5-sulfosalicylic acid, syringic acid, vanillic acid, vanillin, biochanin A, (+)-catechin, formononetin, hesperetin, kaempferol, myricetin, naringenin, naringin, and quercetin were each identified by their retention times or by cochromatography with other authentic examples, and their concentrations were calculated by comparing the peak areas of samples with those of the standards.

Statistical Analysis. Statistical analysis was undertaken using the general linear model procedure (GLM) of the statistical analysis program of SAS (*16*). All of the experiments were repeated three times using a completely randomized design. The least significant difference (LSD) test was based on the 0.05 probability level. The SAS program was used to test for correlations among the individual isoflavone classes, phenolic compounds, and total isoflavones and phenolic compounds.

RESULTS AND DISCUSSION

Isoflavone and Phenolic Compound Concentrations in the Korean Soybean Seeds pf Different Sizes. The seeds of 322 Korean soybean varieties, which were cultivated at six different sites in Korea, differed in seed weight as shown in the Supporting Information. In this study, Korean soybean seeds were classified into three types on the basis of seed size: small (below 13 g), medium (13−24 g), and large (above 24 g) according to traditional Korean soybean classification based on the 100-seed weight.

The concentrations of isoflavones and phenolic compounds were expressed as micromoles per gram (μmol g^{-1}) for all samples in this experiment. Total average isoflavones and phenolics for 322 varieties grown at the six sites are given in **Figure 2** and **Table 2**. The total average concentration of isoflavones in soybean cultivated at Iksan (2.840 μmol g^{-1}) was significantly different at the 0.05 probability level. Soybean varieties cultivated at Iksan were all small seeds, which have 100-seed weight of <13 g, and are used with sprout soybean. According to this result, it can be said that the chemical compound concentration is high.

The results compared small, medium, and large seeds for soybeans produced at the six sites. In the small seeds, the total isoflavone concentrations of soybean ranged from 0.956 to 4.206 μmol g^{-1} at Yeoncheon, from 1.104 to 2.401μmol g^{-1} at Yeoju, from 1.226 to 2.015 μmol g^{-1} at Chuncheon, from 1.333 to 2.883 μmole g^{-1} at Yesan, from 1.444 to 4.326 μmol g^{-1} at Iksan, and from 0.961 to 2.802 μmol g^{-1} at Milyang. In the medium seeds, the total concentrations of isoflavones varied from 1.288 to 3.171 μmol g^{-1} (Yeoncheon), from 1.179 to 2.195 μmol g^{-1} (Yeoju), from 1.183 to 1.804 μmol g^{-1} (Chuncheon), from 0.886 to 2.797 μmol g^{-1} (Yesan), and from 0.963 to 2.962 μmol g^{-1} (Milyang). For large seed soybean varieties, the concentrations of isoflavone were 0.945−2.482 μmol g^{-1} at Yeoncheon, 1.540−2.258 μmol g^{-1} at Chuncheon, 0.982−2.611 μmol g^{-1} at Yesan, and 0.955−2.165 μmol g^{-1} at Milyang. In small and medium seeds, the soybeans cultivated at Yeoncheon, Yesan, and Milyang had high levels of isoflavone, whereas those cultivated at Chuncheon had the lowest. According to CV values of small, medium, and large soybean seeds (11.9, 13.9, and 15.9%, respectively) grown at Yesan, varietal difference of all soybean germplasms was the highest. The HPLC chromatograms of isoflavones and phenolic compounds for small, medium, and large seeds are shown in **Figure 7**.

The total average concentrations of phenolic compounds in soybeans cultivated at Yeoncheon (9.216 μmol g^{-1}), Iksan (9.154 μmol g^{-1}), and Chuncheon (6.296 μmol g^{-1}) were significantly different at the 0.05 probability level. In the small seeds, the total phenolic compound concentration of soybean ranged from 3.411 to 22.225 μmol g^{-1} at Yeoncheon, from 3.943 to 11.860 μmol g^{-1} at Yeoju, from 3.915 to 8.800 μmol g^{-1} at Chuncheon, from 6.322 to 14.198 μmol g^{-1} at Yesan, from 5.117 to 15.634 μmol g^{-1} at Iksan, and from 2.622 to 15.504 μmol g^{-1} at Milyang. In the medium seeds, the total concentrations of phenolic compounds varied from 6.217 to 16.929 μmol g^{-1} at Yeoncheon, from 3.837 to 11.740 μmol g^{-1} at Yeoju, from 4.378 to 6.932 μmol g^{-1} at Chuncheon, from 2.858 to 15.344 μmol g^{-1} at Yesan, and from 1.747 to 14.578 μmol g^{-1} at Milyang. For large-seed soybean varieties, the concentrations of phenolic compounds were 3.368−14.845 μmol g^{-1} at Yeoncheon, 4.543−9.866 μmol g^{-1} at Chuncheon, 3.040−7.445 μmol g^{-1} at Yesan, and 2.688−10.087 μmol g^{-1} at Milyang. In small, medium, and large seeds, the soybeans cultivated at Yeoncheon had the highest levels of phenolic compounds, whereas those grown at Chuncheon had the lowest. On the other hand, the isoflavone concentrations of soybeans cultivated at Chuncheon having large seeds were the highest, and the phenolic compound concentrations of soybeans cultivated at Milyang having large seeds were the lowest (**Figure 3**). Total average isoflavones and phenolics among different seed sizes of soybean among four cultivated sites in Korea are shown in **Figure 4**. At Yeoncheon, Yesan, and Milyang, the total isoflavones and phenolics according to their seed size were significantly different ($P < 0.05$), whereas for the soybeans of different sizes cultivated at Chuncheon their seed size was not significantly different. The total isoflavones and phenolics of small and medium seeds of soybean were significantly higher than those of large soybean seeds. In soybean seeds cultivated at Chuncheon, the total phenolic concentrations of large seeds were slightly higher than those of small and medium seed varieties; however, the differences were not statistically significant. **Figure 5** shows the ratios for glycoside, malonylglycoside, acetylglycoside, and aglycone groups in soybean germplasm of different seed sizes. The malonylglycoside group of small and medium soybean seeds from Yeoncheon and Milyang was higher than that in seeds from Chuncheon and Yesan. **Figure 6** shows the ratios for hydroxybenzoic acid, hydroxycinnamic acid, flavonoids, and other groups in soybean germplasm of different seed sizes. The hydroxybenzoic acid group in all sizes of seeds cultivated at six sites in Korea was the major phenolic compounds followed by flavonoids and hydroxycinnamic acid. Phenolic acid, which contains both hydroxybenzoic and hydroxycinnamic acid derivative forms, possessed the major component of total phenolics; particularly, the concentration of total phenolic acid was much higher than those of flavonoids or others.

From this study, we can consider that isoflavones and phenolic compounds were compressed in small seeds, whereas they were dispersed by low density in large seeds, suggesting that isoflavone synthesis and accumulation are related to embryo and cotyledon size. Recently, Kim et al. (*17*) reported that the isoflavone concentrations and its distribution showed significant variation among soybean varieties when classified by the seed size. According to Kim et al. (*17*), total isoflavone concentrations were higher in small and medium soybean seeds, compared to large size ones. Similarly, Choung et al. (*18*) announced that the total isoflavone concentration of small soybean line (below 13 g per 100 seeds) was the highest among those of other soybean germplasm lines. Consequently, our study is similar to the results of previous studies that isoflavone and phenolic compound concentrations are influenced by various environmental factors such as cultivated sites, temperatures, and rainfall as well as by genetic characteristics such as variety, seed weight, seed color, disease tolerance, and insect resistance (*19–22*).

Relationships among Isoflavones and Phenolic Compounds in Korean Soybean Seed Types. Statistical analysis of the correlations among the concentrations of total isoflavones and each group of isoflavones in small, medium, and large seeds of the soybean germplasm is shown in **Table 3**.

As shown in **Table 3**, in small seeds of soybean cultivated at Yeoncheon, total isoflavone concentration was positively correlated with acetylglycoside and negatively correlated with malonylglycoside. In medium soybean seeds cultivated at Yeoncheon, a significantly positive correlation was found between acetylglycoside and glycoside, between aglycone and glycoside, and between aglycone and acetylglycoside, whereas a significantly negative correlation was shown between malonylglycoside and glycoside, between acetylglycoside and malonylglycoside, and between aglycone and malonylglycoside. In large seeds of soybean cultivated at Chuncheon, total isoflavone was not correlated with malonylglycoside; significantly positive and negative correlations were similar to those of medium soybean seeds.

Generally, the malonylglycoside, which is highly thermally unstable and is converted into glycoside when heated at 80 °C with 70% aqueous ethanol, is the major isoflavone constituent in soybean seeds (*23*). Kim et al. (*20*) reported that total isoflavone was correlated with the concentration of the malonylglycoside ($r = 0.88$***) and glycoside ($r = 0.80$***) groups, but not with the acetylglycoside group. Our study could not, however, reveal that the total isoflavone was strongly positively correlated with the malonylglycoside isoflavone, whereas a significantly negative correlation ($r = -0.67$***) in small seeds grown at Yeoncheon was observed. Also, Kim et al. (*17*) reported that the isoflavone concentration showed significant difference among cultivars according to the seed size. Genistein concentration was established as the major factor determining the relationship between isoflavone concentrations and seed size ($r = -0.21$*). Daidzein and glycitein showed no significant relationship with seed size. Kim et al. (*12*), however, carried out correlation analysis only between total isoflavone and aglycone, such as daidzein, genistein, and glycitein.

Correlations among the concentrations of total phenolic compound and each phenolic compound group in small, medium, and large seeds of soybean are shown in **Table 4**. In small soybean seeds cultivated at Milyang, total phenolic concentrations were positively correlated with hydroxybenzoic acid and other groups, whereas the flavonoid group showed a negative correlation with other groups. In medium soybean seeds cultivated at Yeoncheon, total phenolic concentrations were positively correlated with hydroxybenzoic acid and other groups, whereas flavonoid was negatively correlated with hydroxycinnamic acid. In large soybean seeds cultivated at Yeoncheon, a significantly positive correlation was found between flavonoid and hydroxybenzoic acid, between others and hydroxybenzoic acid, betweeb total phenolics and hydroxybenzoic acid, and between hydroxycinnamic acid and others. Likewise, in this study, Kim et al. (*11*) reported that total phenolic compounds were highly positively correlated with hydroxybenzoic acid, such as gentisic acid ($r = 0.88$***) and salicylic acid ($r = 0.95$***).

From the results obtained in this study, we can advance an appreciation of the close relationships among the individual chemical compounds, each chemical compound group, and total chemical compounds in soybeans having different seed sizes grown at different cultivation sites. It is suggested that the results reflect the influence of inheritance and variable environmental factors, such as climatic, temperature, and cultivation soil condition factors, on the isoflavone and phenolic compound composition and concentrations of soybean.

Supporting Information Available: Tables providing characteristics of Korean soybean germplasm and molarities of isoflavone and phenolic compound standard solutions. This material is available free of charge via the Internet at http://pubs.acs.org.

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