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Analysis of Isoflavone, Phenolic, Soyasapogenol, and Tocopherol Compounds in Soybean [*Glycine max* (L.) Merrill] Germplasms of Different Seed Weights and Origins

Eun-Hye Kim,[†,∥] Hee-Myong Ro,[§,∥] Sun-Lim Kim,[‡] Hong-Sig Kim,[⊗] and Ill-Min Chung*[,†]

[†]Department of Applied Life Science, College of Life and Environmental Science, Konkuk University, Seoul 143-701, Korea

[§]Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Sciences, School of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea

[‡]National Institute of Crop Science, RDA, Suwon 441-857, Korea

[⊗]Department of Crop Science, College of Life and Environmental Science, Chungbuk National University, Cheongju 361-763, Korea

Supporting Information

ABSTRACT: This study investigated the functional compounds, including isoflavones, phenolics, soyasapogenols, and tocopherols, that were detected in 204 soybean [*Glycine max* (L.) Merrill] germplasms. The soybean samples were divided into three groups according to origin: America, China, and Korea. The soybean samples were also classified into three groups on the basis of 100-seed weight: small (<13 g), medium (13−24 g), and large (>24 g). Among the soybean germplasms, CSRV121 (Bosukkong) had the highest level of isoflavone content (4778.1 $\mu g\ g^{-1}$), whereas CS01316 had the lowest isoflavone content (682.4 $\mu g\ g^{-1}$). Of the soybeans from the three different countries of origin, those from Korea showed the highest average concentration of total isoflavones (2252.6 $\mu g\ g^{-1}$). The small seeds had the highest average total isoflavone concentration (2520.0 $\mu g\ g^{-1}$) of the three different seed sizes. Among the 204 soybean germplasms, CS01405 had the highest content of total phenolics (5219.6 $\mu g\ g^{-1}$), and CSRV017 (Hwangkeumkong) had the lowest phenolic content (654.6 $\mu g\ g^{-1}$). The mean concentrations of total phenolic compounds were 2729.1 $\mu g\ g^{-1}$ in American soybean seeds, 1680.4 $\mu g\ g^{-1}$ in Chinese soybean seeds, and 1977.6 $\mu g\ g^{-1}$ in Korean soybean seeds. Of the soybean seeds from the three different countries of origin, American soybean seeds had the highest average concentration of total phenolic compounds, and Korean varieties showed the second highest value. Small soybean seeds had the highest average content of total phenolic compounds (2241.7 $\mu g\ g^{-1}$), whereas medium-sized (1926.8 $\mu g\ g^{-1}$) and large (1949.9 $\mu g\ g^{-1}$) soybeans had lower concentrations of phenolic compounds. In whole soybean germplasms, the level of total soyasapogenols was higher in CS01173 (1802.3 $\mu g\ g^{-1}$) and CS01346 (1736.8 $\mu g\ g^{-1}$) than in the other types of soybeans. The mean concentrations of total soyasapogenol were 1234.0 $\mu g\ g^{-1}$ in American, 1294.5 $\mu g\ g^{-1}$ in Chinese, and 1241.5 $\mu g\ g^{-1}$ in Korean soybean varieties. Chinese soybean varieties showed the highest mean concentration of total soyasapogenol, and Korean soybean seeds showed the second highest level. The medium-seed group had the highest soyasapogenol content (1269.3 $\mu g\ g^{-1}$) of the seeds that were grouped by size. A larger amount of soyasapogenol B than soyasapogenol A was detected. In whole soybeans, CS01202 showed the highest level of total tocopherols (330.5 $\mu g\ g^{-1}$), whereas CSRV056 (Pungsannamulkong) had the lowest content (153.3 $\mu g\ g^{-1}$). Chinese soybeans had the highest average concentration of total tocopherols (255.1 $\mu g\ g^{-1}$). By comparison, the medium-sized Chinese soybean group had the highest (256.1 $\mu g\ g^{-1}$) average total tocopherol content.

KEYWORDS: *soybean germplasm, isoflavones, phenolic compounds, soyasapogenols, tocopherols*

INTRODUCTION

Legumes are present in almost every diet worldwide because they are good sources of starch, dietary fiber, protein, lipids, and essential minerals.[1] Besides their nutritional worth, legumes possess significant amounts of phenolic and polyphenolic compounds, including flavonoids, phenolic acids, and lignins.[1] These compounds have antioxidant effects, which are beneficial to human health.[2]

Soybean is a valuable crop and is the most commonly consumed legume in the world. Especially in Asia, soybean is used in various foods, such as soybean sprouts, soy paste, soy milk, tofu, and soybean oil. Soybean is considered to be a perfect crop because it contains essential amino acids, which are not synthesized in the human body.[3] Dietary ingestion of soybean is associated with decreased risk of cardiovascular disease and osteoporosis, as well as cancer, including breast and colon cancers.[2]

The term "phytochemical" refers to a naturally occurring chemical substance in plants that has biological effects. The major phytochemicals include phenolic acids, flavonoids, and other phenolics and polyphenols.[2] Soybeans contain various phytochemicals, such as isoflavones and other phenolic compounds, tocopherols, saponins, and phytic acids. Among

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these phytochemicals, isoflavones are the most common form of phytoestrogen and are found in various plants, including legumes, seeds, and whole grains. In particular, soybean and soy products are the most abundant sources of isoflavones.[4]

The main isoflavones present in the whole soybean include the following glycosides: daidzin, genistin, and glycitin. Furthermore, the major isoflavones in soybean can be classified as either malonyl or acetyl glycosides, depending on their conjugated functional groups. There are 12 kinds of isoflavones in soybeans that are divided into 4 subgroups: aglycones (daidzein, genistein, and glycitein), glycosides (daidzin, genistin, and glycitin), malonyl glycosides (malonyldaidzin, malonylgenistin, and malonylglycitin), and acetyl glycosides (acetyldaidzin, acetylgenistin, and acetylglycitin). Most soybean isoflavones exist in the glycosidic form, such as daidzin, genistin, and glycitin.[4]

Various studies have shown the positive effects of isoflavones, for example, reduction in the risk of cardiovascular disease and cancer.[5] Furthermore, recent studies have indicated that aglycones have protective effects against cancer and cardiovascular disease.[6] Consumption of isoflavones is associated with reduction of heart disease, menopausal symptoms, bone resorption, and other diseases.[7] In addition, isoflavones are reported to have health benefits because they have anticarcinogenic, antiatherogenic, antifungal, and antioxidant properties.[8]

Soybeans and soybean products have been extensively researched. Tyug et al.[9] reported the antioxidant capacity of phenolic compounds and isoflavones in soybean products. Another study found that soybean seeds are a rich source of isoflavones and phenolic compounds, including syringic, gallic, chlorogenic, and ferulic acids.[10]

Phenolic compounds are found in a variety of edible and nonedible plants, and they reportedly possess biological activities, including antioxidant activity.[10] Various phenolic compounds have been confirmed to possess physiological and pharmacological activities in humans, including prevention of low-density lipoprotein synthesis, antioxidant activities, and protection from cardiovascular and coronary heart diseases and cancers.[11] The concentrations of isoflavones and phenolic compounds are affected by environmental factors and field conditions, such as air temperature, soil moisture, soil fertility, and CO_2 levels.[7]

Saponins are naturally occurring compounds in plants. Soyasaponins constitute about 0.5% of the total soybean dry weight and are influenced by the soybean variety, cultivation year, and location.[12] They possess various physiological activities, including antiviral activity against HIV, inhibition of cell damage, antioxidant activity, and anticancer activity.[12] Soyasaponins also have been associated with cholesterol-lowering effects.[13] Soyasaponins are a type of triterpenoid glycoside with soyasapogenol (aglycone) connected to a polysaccharide.[12] Furthermore, soyasaponins are divided into two groups, A and B, on the basis of their structures. Group B soyasaponins are present in all edible seeds, whereas group A soyasaponins are present only in the hypocotyls of soybean seeds.[14] The majority of the soyasaponins found in soybeans belong to the group B soyasaponins.[13]

Tocopherols and tocotrienols are types of vitamin E compounds that play important roles in human health and nutrition.[15] Tocopherols and tocotrienols have similar structures and are derivatives of a 6-chromanol ring.[16] In addition, tocopherols have demonstrated antioxidant activity.[17] Tocopherols are found in fruits, seeds, roots, tubers, cotyledons, stems, leaves, and flowers of higher plants.[15] The main function of α-tocopherol is to break radical chains in membranes and lipoproteins.[18] There has been much research on vitamin E compounds in fruits and vegetables. Plant tissues show differences in total tocopherol content and tocopherol composition.[17] Variation in vitamin E compounds is affected by factors such as processing, storage time, and environmental conditions.[19]

In Korea, soybean seeds are generally classified into three sizes, small, medium, and large, according to their 100-seed weight. Small soybean seeds are used for soybean sprouts, and medium and large seeds are used for soybean curd, soy milk, cooking with rice or vegetables, and Korean soybean pastes.[20] Although the soybean is very common in Korea, practical research on its functional substances is lacking. In our previous study, we had investigated the functional substances such as isoflavones and phenolic compounds in soybean germplams.[20] The objective of the present study was to determine the contents of isoflavones, phenolic compounds, soyasapogenols, and tocopherols in soybean germplasms of three different origins and 100-seed weights. The results of this study may provide a basic understanding of soybean germplasms with higher concentrations of isoflavones, phenolic compounds, soyasapogenols, and tocopherols. In addition, this study will provide valuable information for plant breeders and help in developing functional food resources.

MATERIALS AND METHODS

Preparation of Soybean Germplasms. Two hundred and four soybean germplasms that were initially collected from three different countries, America (27 varieties), China (68 varieties), and Korea (109 varieties), were distributed by Chungbuk National University (Cheongju, Chungbuk, Korea) and cultivated at the Konkuk University farm. Experimental conditions, climatic conditions, and plot management are described in detail in the paper by Lee et al.[7] Briefly, soil texture was a silt clay loam at all sites. Fertilizer was applied prior to seeding at the rates recommended locally: 80, 80, and 120 kg ha^{-1} for N, P_2O_5, and K_2O, respectively. The preceding crop was soybean at all sites; thus, rhizobial inoculation was not necessary. Seeding was done at a density of 50 seeds/m^2, with plants thinned to a uniform density 14 days after planting. Each plot consisted of three rows (length = 3.75 and 0.6 m between rows); plots at each site were assigned to a completely randomized design with two replicates. Weeds, diseases, and insects were controlled using pesticides as locally recommended for soybeans. Soybean seeds of each variety were harvested from each replicate at each site. Upon harvest, the seeds were freeze-dried, ground, and stored at room temperature until isoflavone analyses. The seeds were divided into three groups on the basis of origin and differences in 100-seed weight, according to the classification method of the online crop information center of the Rural Development Administration (RDA, Suwon, Gyeonggi-Do, Korea). The soybean seeds were classified into three sizes (small seed, <13 g; medium seed, 13−24 g; and large seed, >24 g; Figure 1) on the basis of their 100-seed weights. The agronomic characteristics of the soybean germplasms are shown in the Supporting Information (Appendix 1).

Analysis of Isoflavones in Soybeans. Each soybean seed was dried in a freeze-dryer (Freezeone 4.5; Labconco, Kansas City, MO, USA) under vacuum conditions and was then ground. For this experiment, methanol (100%), acetonitrile (100%), glacial acetic acid (99.9%), and distilled water were purchased from J. T. Baker [high-performance liquid chromatography (HPLC) grade; Phillipsburg, NJ, USA], and hydrochloric acid (HCl) was purchased from Daejung Co. (Daejung Chemical & Materials Co. Ltd., Siheung, Gyeonggi-Do, Korea). Isoflavone extraction from soybean samples was performed according to the method of Wang and Murphy.[4] The extractions from soybean samples were conducted with two replications per sample.

Figure 1. Comparison of soybean seeds according to size.

The extraction solvent was composed of 10 mL of acetonitrile and 2 mL of 0.1 N HCl per sample. The ground soybean samples (2 g) were extracted using the extraction solvent; this was followed by stirring for 2 h at room temperature (Green-Sseriker; Vision Scientific Co. Ltd., Bucheon, Gyeonggi-Do, Korea). The extract was filtered through a no. 42 Whatman filter paper (125 mm × 100 circles; Maidstone, U.K.) and concentrated using a vacuum evaporator (Eyela; Tokyo Rikakikai, Co. Ltd., Japan) below 40 °C. The residues were redissolved in 10 mL of 100% aqueous methanol (HPLC grade; J. T. Baker), filtered through a 0.2 μm nylon membrane syringe filter (17 mm, Titan; Sunsri, Rockwood, TN, USA), and transferred into a 2 mL vial, followed by analysis by HPLC.

HPLC analysis was conducted using a Shimadzu product (Shimadzu Instruments Co. Ltd., Japan) with a pump model LC-10AD *VP* and detector model SPD-M10A *VP* [photodiode array (PDA) detector]. A YMC-Pack ODS AM-303 [5 μm internal diameter (i.d.), 250 mm × 4.6 mm] column was used for quantitative analysis, and the analysis was performed at a UV wavelength of 254 nm. HPLC analysis was performed according to a modified method of Lee et al.[7] The mobile phase of solvent A was 0.1% glacial acetic acid in distilled water, and solvent B was 0.1% glacial acetic acid in acetonitrile. The injection volume was 20 μL, and the gradient used in this study was as follows: 0 min, 85% A/15% B; 0−50 min, 65% A/35% B; and 50−60 min, 65% A/35% B. Run time was 60 min, and the flow rate was 1 mL min^{-1}.

Standards for the 12 isoflavones (daidzein, genistein, glycitein, daidzin, genistin, glycitin, acetyldaidzin, acetylgenistin, acetylglycitin, malonyldaidzin, malonylgenistin, and malonylglycitin) were purchased from LC Laboratories (Woburn, MA, USA). The isoflavone standards were dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA) at several concentrations (25, 50, 100, and 150 μg mL^{-1}), and high linearity (r^2 > 0.996) was obtained for each compound. The 12 isoflavones were identified by their retention times, and their concentrations were calculated by comparing the peak areas of the samples with those of the standards.

Analysis of Phenolic Compounds in Soybeans. The soybean samples were prepared according to the protocols for isoflavone extraction.[4] DMSO, gallic acid, pyrogallol, homogentisic acid, protocatechuic acid, gentisic acid, chlorogenic acid, (+)-catechin, *p*-hydroxybenzoic acid, *β*-resorcylic acid, vanillic acid, caffeic acid, syringic acid, vanillin, *p*-coumaric acid, rutin, ferulic acid, veratric acid, *m*-coumaric acid, naringin, hesperidin, *o*-coumaric acid, myricetin, resveratrol, quercetin, *trans*-cinnamic acid, naringenin, kaempferol, hesperetin, formononetin, and biochanin A were purchased from Sigma-Aldrich. All solvents used were of HPLC grade. Acetonitrile (100%), glacial acetic acid (99.9%), and distilled water were purchased from J. T. Baker.

The HPLC system used was an Agilent 1100 series system (Palo Alto, CA, USA) equipped with a PDA detector. Separation was primarily achieved using a YMC-Pack ODS AM-303 (5 μm i.d., 250 mm × 4.6 mm) column. The absorbance was measured at 280 nm. HPLC analysis was performed according to a modified method of Kim et al.[10] The mobile phases were 0.1% glacial acetic acid in distilled water (solvent A) and 0.1% glacial acetic acid in acetonitrile (solvent B). The injection volume was 20 μL, and the gradient was as follows: 0 min, 92% A/8% B; 0−2 min, 90% A/10% B; 2−27 min, 70% A/30% B; 27−50 min, 10% A/90% B; 50−51 min, 0% A/100% B; 51−60 min, 0% A/100% B; and 60−63 min, 92% A/8% B. The run time was 63 min, and the flow rate was 1 mL min^{-1}. Genuine standards of 30 phenolic compounds were made in DMSO and used to establish the calibration curves.

Phenolic compounds in soybeans were determined using the retention times of the standards, and the plotting standard concentration was obtained at several concentrations: 25, 50, 100, and 150 μg mL^{-1}. High linearity (r^2 > 0.996) was obtained from each standard calibration curve for each phenolic compound.

Analysis of Soyasapogenols A and B in Soybeans. The extraction of soyasapogenols A and B was performed according to the method of Rupasinghe et al.[12] The ethanol (99.5%), 1-propanol (100%), acetonitrile (100%), methanol (100%), and distilled water used in this experiment were purchased from J. T. Baker. Finely ground soybean powder (0.2 g) was extracted with 30 mL of 80% ethanol and horizontally stirred in a water bath/shaker (JS-SWS; Johnsam Corp., Bucheon, Gyeonggi-Do, Korea) at 50 °C for 2 h. The suspension was centrifuged at 3000 rpm and 4 °C for 5 min (VS-6000FN; Vision Scientific Co. Ltd.). Then, 15 mL of the supernatant liquid was transferred to a 100 mL round flask and evaporated to dryness under reduced pressure by using a rotary vacuum evaporator below 40 °C (Eyela; Tokyo Rikakikai, Co. Ltd.). The remaining residues were then reconstituted with 8 mL of 1 N HCl in methanol. The resuspended solution was horizontally stirred in a water bath/shaker (JS-SWS; Johnsam Corp.) at 75 °C for 2.5 h to allow acid hydrolysis to release soyasapogenol from the soyasaponins. The solution was placed in a C-18 Sep-Pak cartridge (Vac 6CC, 500 mg; Waters Corp., Milford, MA, USA) and washed with water, after which soyasapogenol was eluted with methanol. The samples were filtered through a 0.2 μm nylon syringe filter (17 mm, Titan; Sunsri) and analyzed by evaporative light scattering detection (ELSD).

ELSD analysis was performed according to the method of Rupasinghe et al.[12] The ELSD system consisted of an Alltech 2000 ES ELSD, a TSP (Thermo Separation Products, USA) AS 1000 autoinjector, and an ACME 9000 pump (Young-Lin, Korea). Nitrogen was used as the ELSD nebulizer gas (2 mL min^{-1}), and the tube temperature was set to 70 °C. The separation of soyasapogenols A and B was performed using an ODS C18 column (i.d., 250 × 4.6 mm). The mobile phase was as follows: acetonitrile/water/1-propanol/0.1% acetic acid = 80:13.9:6:0.1 (v/v/v/v), isocratic. The analysis time was 15 min, and the flow rate was 0.9 mL min^{-1}. The standards for soyasapogenols A and B were dissolved in methanol at several concentrations (soyasapogenol A, 12.5, 25, 50, 75, 100, and 150 μg mL^{-1}; soyasapogenol B, 37.5, 75, 150, 225, 300, and 450 μg mL^{-1}), and high linearity (r^2 > 0.996) was obtained. Determination of soyasapogenols A and B was made on the basis of retention time.

Analysis of Tocopherols in Soybeans. A modified method of Lee and Park[21] was used to analyze tocopherols and tocotrienols in the soybean samples. For this experiment, ethanol (99.5%), iso-octane (100%), hexane (100%), and distilled water were purchased from J. T. Baker (HPLC grade), and HCl was purchased from Daejung Co. Ascorbic acid and potassium hydroxide (KOH) were purchased from Samchun Co. (Samchun, Korea). Anhydrous sodium sulfate (Na_2SO_4) was purchased from Yakuri Chemical (Kyoto, Japan). Ground soybean sample (0.5 g) was added to a 50 mL Falcon tube (SPL Life Science Co. Ltd., Pocheon, Gyeonggi-Do, Korea) containing 10 mL of ethanol with 0.1 g of ascorbic acid. The tube was shaken in a hot water bath at 80 °C for 10 min, followed by the addition of 300 μL of 44% KOH for saponification and additional shaking for 10 min at the same temperature (JS-SWS; Johnsam Corp.). After saponification, the solution was quickly cooled on ice for 30 min, followed by the addition of 10 mL of hexane and distilled water; then, the solution was shaken and centrifuged for 4 min at 4000 rpm (VS-6000FN; Vision Scientific Co. Ltd.). The hexane layer was then collected, and 10 mL of hexane was added, followed by centrifugation as above. This hexane extraction process was repeated two more times, and the collected hexane layers (30 mL) were pooled and washed twice with distilled water. After removal of the water layer, the collected hexane layer was filtered through a funnel with Na_2SO_4 and concentrated using a vacuum evaporator (Eyela; Tokyo Rikakikai Co. Ltd.). The residues were redissolved in 2 mL of iso-octane and transferred to 2 mL vials.

For tocopherol analysis, tocopherol and tocotrienol standards (α-, β-, γ-, and δ-) were determined on the basis of their retention times, and the standard concentration was plotted for three concentrations: 1, 50, and 100 ppm. High linearity ($r^2 > 0.996$) was obtained from each curve. Gas chromatographic (GC) analysis was accomplished using a Shimadzu GC 2010 (Shimadzu Instruments Co. Ltd., Japan) with a 2010 autosampler equipped with a flame ionization detector. The injector and detector temperatures were 290 and 320 °C, respectively, and a Varian GC column (i.d., 50 m × 0.32 mm) was used for vitamin E separation. The GC oven temperature was initially held at 220 °C for 2 min, increased from 220 to 290 °C over a span of 14 min at 5 °C min^{-1}, and then increased again from 290 to 310 °C over 18 min at 10 °C min^{-1} to achieve the desired separation. A 2 μL sample was injected into the GC column with a 1:20 split. The standard stock solutions were made with 2,2,4-trimethylpentane (iso-octane). The tocopherol and tocotrienol standards that were dissolved in iso-octane were used to establish the calibration curves.

Statistical Analysis. In this study, statistical analyses were conducted using the general linear model (GLM) procedure of the 2005 SAS package (version 9.1; SAS Institute Inc., Cary, NC, USA). The experimental design was a completely randomized design with duplicates. The least significant difference test used with a 0.05 probability level.

RESULTS AND DISCUSSION

Isoflavones in Soybean Germplasm. Soybean seed meal is a valuable product because it contains many components that have health benefits, including soy protein, fiber, isoflavones, and other phytochemicals.[3] Soybean seeds and their products have also shown the potential to reduce the risk for certain cancers.[22] Recently, the health-promoting effects of soybeans and their bioactive phenolic compounds (phenolic acids and flavonoids) have been studied.

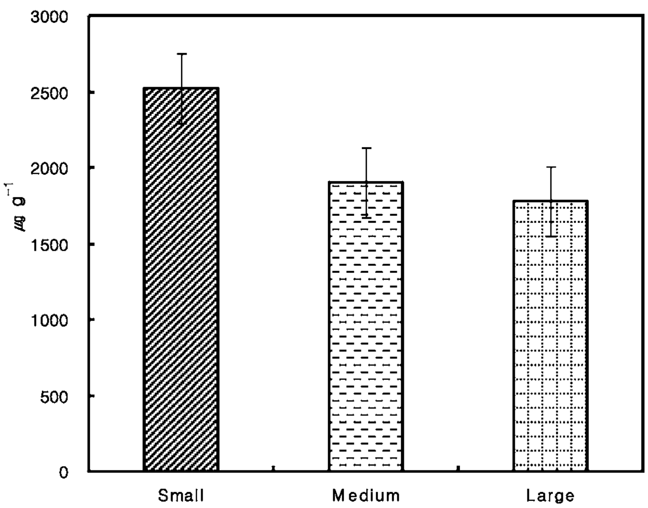

Figure 2. Average total isoflavone concentrations in soybean germplasms according to seed size. *, statistical significance was analyzed using least significant difference ($p < 0.05$).

Among all soybean germplasms, CSRV121 (Bosukkong) had the highest level of total isoflavones (4778.1 $\mu g\ g^{-1}$), whereas CS01316 had the lowest level of total isoflavones (682.4 $\mu g\ g^{-1}$). The total average content of isoflavones in the Korean soybeans was the highest (2252.6 $\mu g\ g^{-1}$) among the soybeans from the three different countries. The isoflavone concentrations were higher in the American soybean varieties (2251.0 $\mu g\ g^{-1}$) and Chinese soybean seed samples (1382.1 $\mu g\ g^{-1}$). In the soybeans from all three countries of origin, malonylgenistin was the isoflavone compound that was present in the highest concentration, whereas genistein was present at the lowest level.

Table 1. Comparison of Total Isoflavone Concentrations in Small, Medium, and Large Soybean Seeds of Different Origins

	origin		
	America	China	Korea
Small			
no. of varieties	8		24
maximum ($\mu g\ g^{-1}$)	3071.9		4778.1
minimum ($\mu g\ g^{-1}$)	1144.6		1098.9
mean ($\mu g\ g^{-1}$)	2526.9		2563.9
Medium			
no. of varieties	17	64	46
maximum ($\mu g\ g^{-1}$)	3275.6	2441.4	4128.2
minimum ($\mu g\ g^{-1}$)	879.8	739.3	875.3
mean ($\mu g\ g^{-1}$)	2244.4	1383.2	2480.3
Large			
no. of varieties	2	4	39
maximum ($\mu g\ g^{-1}$)	1231.8	1848.4	3598.5
minimum ($\mu g\ g^{-1}$)	1175.5	683.3	814.4
mean ($\mu g\ g^{-1}$)	1203.6	1342.4	1829.6
CV (%)	30.2	29.2	33.1
$LSD_{(0.05)}$	653.9	519.4	258.4

The average total concentrations of isoflavones among the soybeans varied from 879.8 to 3275.6 $\mu g\ g^{-1}$ in the American soybeans, from 683.3 to 2441.4 $\mu g\ g^{-1}$ in the Chinese soybeans, and from 814.4 to 4778.1 $\mu g\ g^{-1}$ in the Korean soybeans. Compared to other isoflavones, malonyldaidzin and malonylgenistin contents were higher in the germplasms of the American soybeans (717.0 and 761.9 $\mu g\ g^{-1}$, respectively) than in those of the Chinese soybeans (355.2 and 433.3 $\mu g\ g^{-1}$, respectively) and Korean soybeans (617.9 and 744.6 $\mu g\ g^{-1}$, respectively).

Figure 2 shows the average total isoflavone concentrations in the soybean germplasms, according to seed size (small seed, <13 g; medium seed, 13–24 g; large seed, >24 g). Small seeds (2520.0 $\mu g\ g^{-1}$) had the highest levels of average total isoflavones. The average total isoflavone contents in medium and large seeds were 1904.4 and 1777.5 $\mu g\ g^{-1}$, respectively. The large soybean seeds (1777.5 $\mu g\ g^{-1}$) had the lowest levels of average total isoflavones. The average total isoflavone concentrations varied from 1098.9 to 4778.1 $\mu g\ g^{-1}$ in the small soybean seeds, from 739.9 to 4128.2 $\mu g\ g^{-1}$ in the medium seeds, and from 814.4 to 3598.5 $\mu g\ g^{-1}$ in the large seeds. In addition, 46% of the soybean varieties that had average total isoflavone content >2520.0 $\mu g\ g^{-1}$ were in the small soybean group. Of the medium-sized soybeans, about 42% of the soybean seeds showed higher total isoflavone content than the average total isoflavone content (1904.4 $\mu g\ g^{-1}$). Among the large soybean seeds, 49% had higher concentrations of total isoflavones than the average concentration (1777.5 $\mu g\ g^{-1}$) in their group. In comparison, the average content of malonyldaidzin was 709.4 $\mu g\ g^{-1}$ in the small seeds, 537.0 $\mu g\ g^{-1}$ in the medium seeds, and 457.9 $\mu g\ g^{-1}$ in the large soybean seeds.

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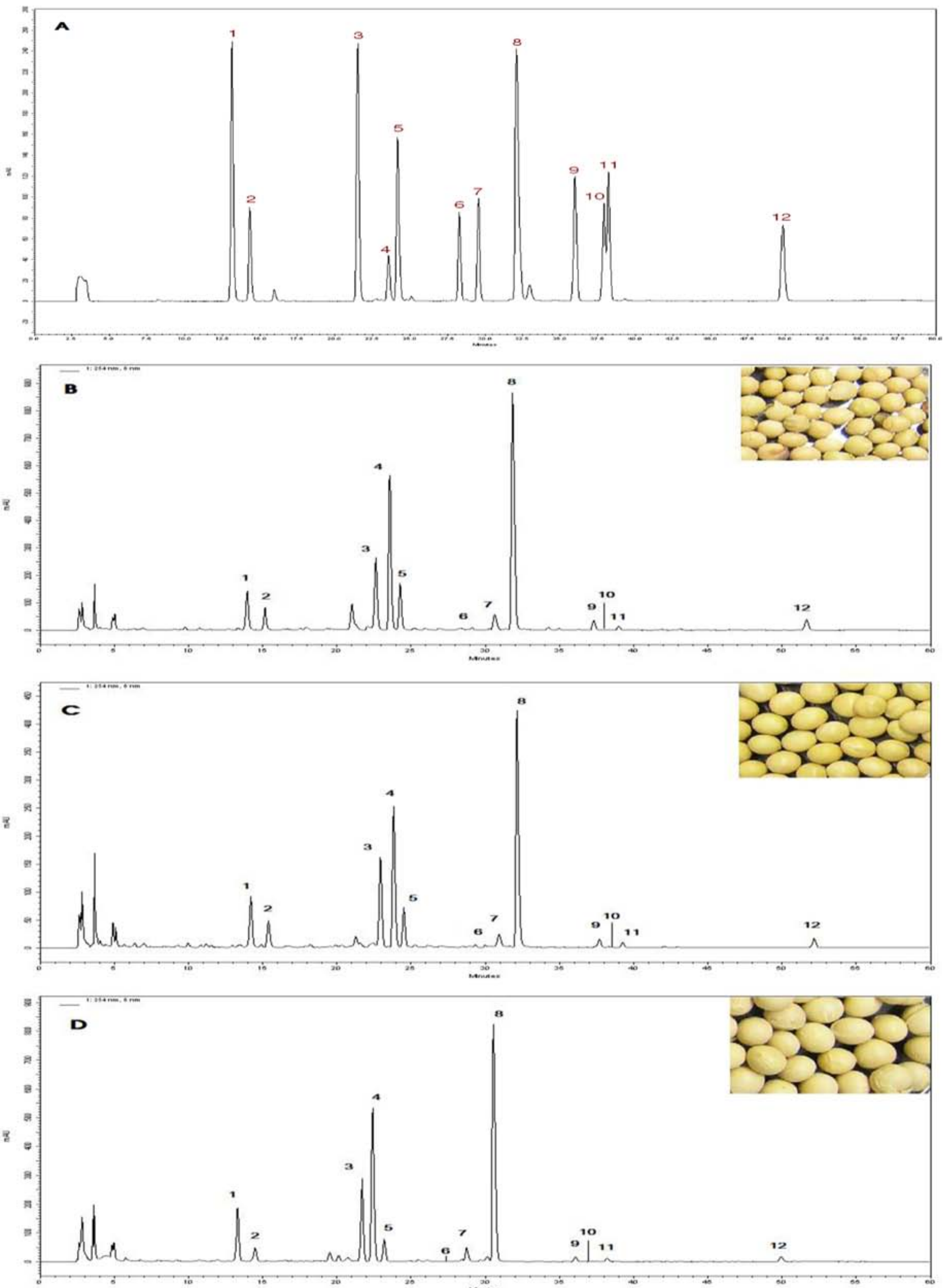

Figure 3. Chromatograms of 12 isoflavones in soybean germplasms of different seed size: (A) standard chromatogram; (B) small soybean seed; (C) medium soybean seed; (D) large soybean seed. Peaks: 1, daidzin; 2, glycitin; 3, genistin; 4, malonyldaidzin; 5, malonylglycitin; 6, acetyldaidzin; 7, acetylglycitin; 8, malonylgenistin; 9, daidzein; 10, glycitein; 11, acetylgenistin; 12, genistein.

Table 1 shows the average total isoflavone concentrations for small, medium, and large soybeans of different origins (see also Figure 3). In the small and medium seeds, Korean soybeans had the highest levels of average total isoflavones (2563.9 and 2480.3 $\mu g\ g^{-1}$, respectively). For the large seeds, the American soybeans showed the lowest concentrations of average total isoflavones (1203.6 $\mu g\ g^{-1}$), whereas the Chinese and Korean soybeans showed 1342.4 and 1829.6 $\mu g\ g^{-1}$, respectively. Thus,

Table 2. Comparison of Concentrations of Phenolic Compounds among Soybeans of Different Origins

	origin		
	America	China	Korea
no. of varieties	27	68	109
maximum ($\mu g\ g^{-1}$)	5565.3	4208.9	5073.6
minimum ($\mu g\ g^{-1}$)	932.6	719.7	654.6
mean ($\mu g\ g^{-1}$)	2729.1	1680.4	1977.6
CV (%)	29.1	29.8	35.9
$LSD_{(0.05)}$	1668.9	999.3	1408.8

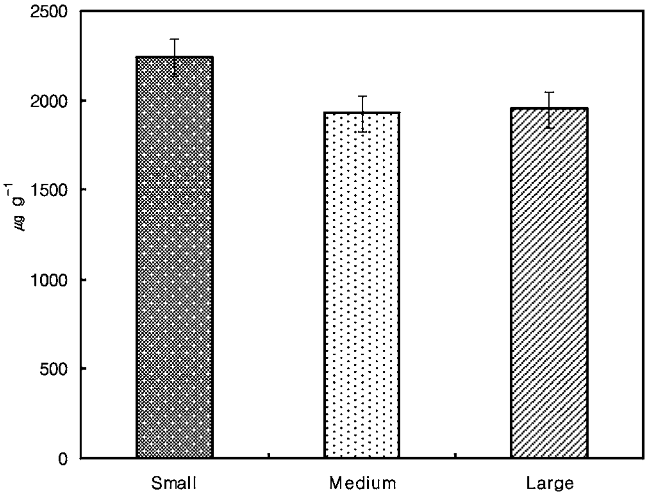

Figure 4. Average total phenolic concentrations in soybean germplasms according to seed size. *, statistical significance was analyzed using least significant difference ($p < 0.05$).

the American and Korean soybean seeds showed a similar pattern in that their small seeds contained the highest levels of isoflavones.

Table 3. Comparison of Total Phenolic Compounds in Small, Medium, and Large Soybean Seeds from Three Different Countries

	origin		
	America	China	Korea
Small			
no. of varieties	8		24
maximum ($\mu g\ g^{-1}$)	5565.0		4869.9
minimum ($\mu g\ g^{-1}$)	1614.0		765.2
mean ($\mu g\ g^{-1}$)	3985.8		1708.9
Medium			
no. of varieties	17	64	46
maximum ($\mu g\ g^{-1}$)	3943.7	4208.9	5074.5
minimum ($\mu g\ g^{-1}$)	932.6	719.7	654.6
mean ($\mu g\ g^{-1}$)	2360.5	1678.0	2083.3
Large			
no. of varieties	2	4	39
maximum ($\mu g\ g^{-1}$)	1917.1	2052.9	4829.2
minimum ($\mu g\ g^{-1}$)	1454.7	982.4	717.1
mean($\mu g\ g^{-1}$)	1685.9	1601.1	1999.2
CV (%)	39.7	50.3	57.9
$LSD_{(0.05)}$	1061.4	1086.3	394.8

Table 4. Concentrations of Soyasapogenols A and B in Soybean Seeds from Three Different Countries

	soyasapogenol A	soyasapogenol B	total
America			
maximum ($\mu g\ g^{-1}$)	446.7	1080.1	1524.7
minimum ($\mu g\ g^{-1}$)	435.2	743.9	1179.0
mean ($\mu g\ g^{-1}$)	437.6	796.4	1234.0
China			
maximum ($\mu g\ g^{-1}$)	531.2	1322.7	1802.3
minimum ($\mu g\ g^{-1}$)	435.0	742.1	1177.1
mean ($\mu g\ g^{-1}$)	448.1	846.4	1294.5
Korea			
maximum ($\mu g\ g^{-1}$)	519.9	1162.2	1682.0
minimum ($\mu g\ g^{-1}$)	434.9	741.4	1176.2
mean ($\mu g\ g^{-1}$)	443.1	798.4	1241.5
CV (%)	3.1	11.2	8.1
$LSD_{(0.05)}$	3.9	25.5	28.8

Table 5. Comparison of Total Soyasapogenols in Small, Medium, and Large Soybean Seeds from Three Different Countries

	origin		
	America	China	Korea
Small			
no. of varieties	8		24
maximum ($\mu g\ g^{-1}$)	1524.7		1682.0
minimum ($\mu g\ g^{-1}$)	1206.7		1185.0
mean ($\mu g\ g^{-1}$)	1315.2		1251.8
Medium			
no. of varieties	17	64	46
maximum ($\mu g\ g^{-1}$)	1238.1	1802.3	1592.7
minimum ($\mu g\ g^{-1}$)	1186.9	1177.1	1176.2
mean ($\mu g\ g^{-1}$)	1201.7	1230.0	1250.8
Large			
no. of varieties	2	4	39
maximum ($\mu g\ g^{-1}$)	1188.2	1249.0	1490.0
minimum ($\mu g\ g^{-1}$)	1179.0	1214.3	1181.9
mean ($\mu g\ g^{-1}$)	1183.6	1235.9	1224.6
CV (%)	5.4	10.4	6.5
$LSD_{(0.05)}$	64.2	173.9	27.8

In the small seeds, the total isoflavone concentrations varied from 1144.6 to 3071.9 $\mu g\ g^{-1}$ in the American soybeans and from 1098.9 to 4778.1 $\mu g\ g^{-1}$ in the Korean soybeans, whereas none of the Chinese soybeans were classified as having small seeds. In the medium seeds, the average total isoflavone concentrations ranged from 879.8 to 3275.6 $\mu g\ g^{-1}$ in the American soybeans, from 739.3 to 2441.4 $\mu g\ g^{-1}$ in the Chinese soybeans, and from 875.3 to 4128.2 $\mu g\ g^{-1}$ in the Korean soybeans. In the large seeds, the contents of isoflavones varied from 1175.5 to 1231.8 $\mu g\ g^{-1}$ in the American soybeans, from 683.3 to 1848.4 $\mu g\ g^{-1}$ in the Chinese soybeans, and from 814.4 to 3598.5 $\mu g\ g^{-1}$ in the Korean soybeans.

Isoflavones were divided into four groups: glycosides, malonylglycosides, acetylglycosides, and aglycones. The percentages of glycosides in the small soybean seeds from America and Korea were 19.1 and 27.6%, respectively. Furthermore, the percentages of malonylglycosides in the soybeans from America and Korea were 74.5 and 65.8%,

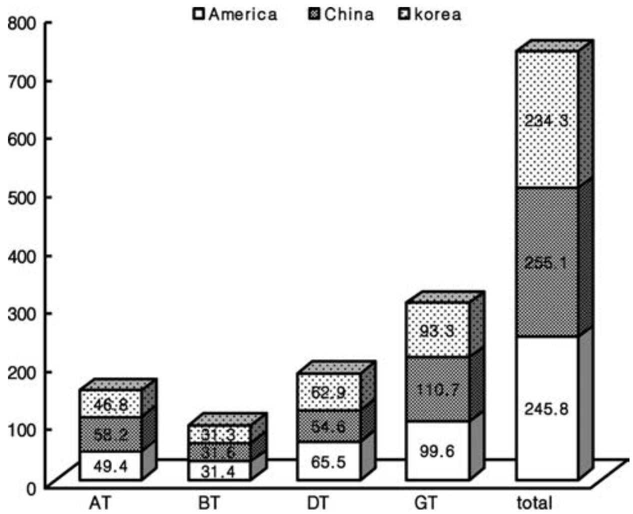

Figure 5. Comparison of the contents of four tocopherols and total tocopherols in soybean germplasms of different origin. *Y*-axis, tocopherol concentration (μg/g). Abbreviations: AT, α-tocopherol; BT, β-tocopherol; DT, δ-tocopherol; GT, γ-tocopherol.

Table 6. Comparison of Total Tocopherols in Small, Medium, and Large Soybean Seeds from Three Different Countries

	origin		
	America	China	Korea
	Small		
no. of varieties	8		24
maximum (μg g^{-1})	266.8		271.0
minimum (μg g^{-1})	211.1		153.3
mean (μg g^{-1})	246.0		221.2
	Medium		
no. of varieties	17	64	46
maximum (μg g^{-1})	272.9	330.5	303.9
minimum (μg g^{-1})	227.2	190.8	181.3
mean (μg g^{-1})	248.1	256.1	240.0
	Large		
no. of varieties	2	4	39
maximum (μg g^{-1})	228.7	242.3	284.9
minimum (μg g^{-1})	222.7	226.2	177.1
mean (μg g^{-1})	225.7	238.1	235.7
CV (%)	5.9	9.1	10.9
$LSD_{(0.05)}$	13.9	29.9	8.9

respectively. The percentages of malonylglycosides in the medium seeds were 71% in the American soybean seeds and 68.2% in the Korean soybean seeds. The proportion of glycosides in the Korean soybean seeds (25.9%) was higher than that in the American soybean seeds (20.6%). In contrast, the percentage of acetylglycosides was 5.7% in the American small seeds and 5.9% in the Korean small seeds. In all of the soybean groups, aglycones had the lowest concentration among the four types of isoflavones. Chung et al.[23] reported that the concentration of aglycones was lower than that of glycosides in soybeans. This result indicated that major isoflavones existed in soybean seeds in the form of glycosides and not as aglycones.

In this study, the concentrations of isoflavones in soybean germplasms were affected by seed weight and the country of origin. In particular, small soybean seeds had the highest levels of total isoflavones. On the basis of this finding, our conclusion is that isoflavones are compressed in small soybean seeds and diffused in large seeds. Thus, we suggest that isoflavone synthesis and accumulation are related to seed size. A previous study[20] showed that isoflavone concentrations had significant variation in soybeans of different seed size. According to Lee et al.,[7] the total isoflavone concentration in small soybean seeds is higher than that in large soybean seeds. In addition, Kim et al.[24] reported that total isoflavone concentrations in soybean seeds showed significant differences between varieties of different seed size. Furthermore, the isoflavone concentration is influenced by the country of origin. Therefore, environmental factors and other characteristics can affect isoflavone concentration and synthesis in soybean seeds. Studies have also shown that the isoflavone content in soybeans may be altered by the growing, processing, and storage conditions, as well as the genotype. A previous study showed that the isoflavone contents of soybeans vary according to environmental factors such as cultivation regions, temperatures, and rainfall.[20] In addition, isoflavone contents are affected by plant genetic characteristics, such as variety, seed weight, seed color, disease tolerance, and resistance to insects.[25,26]

In conclusion, our results are similar to those of previous studies that have observed that isoflavone concentrations are influenced by the genotype and, in particular, seed weight. On the basis of this study, our conclusion is that isoflavone content is affected by seed weight, environmental factors, and other characteristics, such as origin. In other words, isoflavone concentrations depend on the origin and size of soybean seeds. Therefore, these results will be useful for the selection of soybean germplasms.

Phenolic Compounds in Soybean Germplasm. Phenolic compounds show various physiological activities, such as antioxidant, anti-inflammatory, antiallergenic, and cardioprotective activities.[10] Among the 204 soybean germplasms that we analyzed, CS01405 (Appendix 1 in the Supporting Information) had the highest content of total phenolics (5219.6 μg g^{-1}), and CSRV017 (Hwangkeumkong) had the lowest content of total phenolics (654.6 μg g^{-1}). The average total phenolic content in soybean germplasms from the three countries of origin are shown in Table 2. The average total phenolic contents were 2729.1 μg g^{-1} in the American soybean seeds, 1680.4 μg g^{-1} in the Chinese soybean seeds, and 1977.6 μg g^{-1} in the Korean soybean seeds. The average total concentration of phenolic compounds in the soybean seeds varied from 932.6 to 5565.3 μg g^{-1} in the American soybean seeds, from 719.7 to 4208.9 μg g^{-1} in the Chinese soybean seeds, and from 654.6 to 5073.6 μg g^{-1} in the Korean soybean seeds. In particular, the American soybean seeds had the highest concentration of total phenolic compounds among the seeds from the three countries of origin; Korean seeds had the second highest concentration of total phenolic compounds.

Figure 4 shows the average total phenolic content in soybean germplasm on the basis of seed size. Small seeds contained the highest levels of phenolic compounds (2241.7 μg g^{-1}), whereas medium (1926.8 μg g^{-1}) and large seeds (1949.9 μg g^{-1}) contained lower levels. The average total phenolic concentrations ranged from 765.2 to 5565.3 μg g^{-1} in the small soybean seeds, from 654.6 to 5074.5 μg g^{-1} in the medium seeds, and from 717.1 to 4829.2 μg g^{-1} in the large seeds. Table 3 shows the average total phenolic concentrations in the small, medium, and large soybeans from the three countries of origin. For the small and medium seeds, the American soybeans

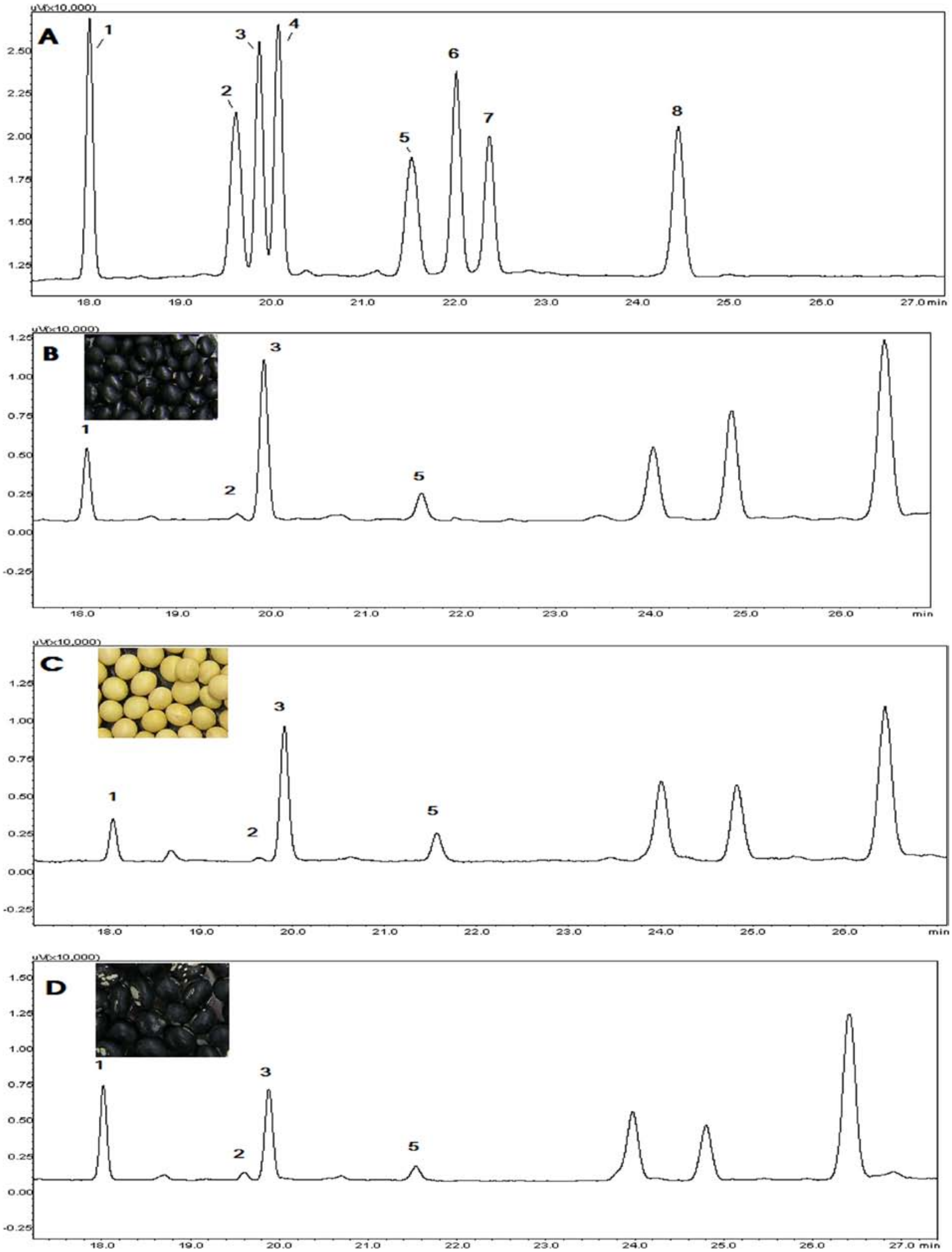

Figure 6. Chromatogram of four tocopherols in soybean germplasms of different size: (A) standard chromatogram; (B) small soybean seed; (C) medium soybean seed; (D) large soybean seed. Peaks: 1, δ-tocopherol; 2, β-tocopherol; 3, γ-tocopherol; 4, δ-tocotrienol; 5, α-tocopherol; 6, β-tocotrienol; 7, γ-tocotrienol; 8, α-tocotrienol.

showed the highest levels of phenolic compounds (3985.8 and 2360.5 $\mu g\ g^{-1}$, respectively). Medium-sized Chinese soybean seeds showed the lowest level of phenolic compounds (1678.0 $\mu g\ g^{-1}$). Of the large seeds, the Korean soybeans showed the highest level (1999.2 $\mu g\ g^{-1}$) of phenolic compounds, whereas the Chinese soybeans showed the lowest level (1601.1 $\mu g\ g^{-1}$); the American soybeans contained an intermediate concentration of phenolic compounds (1685.9 $\mu g\ g^{-1}$).

In the small-seed group, the total phenolic contents in the soybeans varied from 1614.0 to 5565.0 $\mu g\ g^{-1}$ in the American varieties and from 765.2 to 4869.9 $\mu g\ g^{-1}$ in the Korean varieties. In the medium-seed group, the total average phenolic contents ranged from 932.6 to 3943.7 $\mu g\ g^{-1}$ in the American soybeans, from 719.7 to 4208.9 $\mu g\ g^{-1}$ in the Chinese soybeans, and from 654.6 to 5074.5 $\mu g\ g^{-1}$ in the Korean soybeans. In the large-seed group, the total phenolic contents ranged from 1454.7 to 1917.1 $\mu g\ g^{-1}$ in the American soybeans, from 982.4 to 2052.9 $\mu g\ g^{-1}$ in the Chinese soybeans, and from 717.1 to 4829.2 $\mu g\ g^{-1}$ in the Korean soybeans (Table 3). These results indicate that the small soybean seeds from America had the highest levels of total phenolic compounds (3985.8 $\mu g\ g^{-1}$) among the seeds from the three different countries, and the average concentrations of total phenolic compounds in the small soybean seeds were the highest among the three different sizes. In addition, our previous studies[25] have shown that the amount of phenolic compounds significantly varied with seed weight. In particular, the small soybean seeds from America had the highest levels of total phenolic compounds (3985.8 $\mu g\ g^{-1}$). We concluded that phenolic compounds were compressed in small soybean seeds, whereas they were diffused at a low density in large soybean seeds. Therefore, we suggest that the synthesis and accumulation of phenolic compounds are related to seed size. Accordingly, the concentration of phenolic compounds in the medium-sized Korean seeds was higher than that of phenolic compounds in the small seeds. We concluded that the concentration of phenolic compounds may be affected by environmental factors and genetic characteristics such as seed weight. Our previous paper indicated that the phenolic compound contents differed according to variety, environmental factors, and cultivation locations.[10] Our previous results also showed that the concentrations of isoflavones and phenolic compounds in soybean sprouts varied with light conditions.[10]

In this study, the small soybean seeds showed the highest levels of total phenolics, and phenolic compounds varied according to origin. Thus, we concluded that genetic differences and place of origin can affect the total content and synthesis of phenolic compounds in soybean seeds. In conclusion, the contents of phenolic compounds and other functional materials, such as isoflavones, are dependent on seed size.

Soyasapogenols in the Soybean Germplasm. In this study, soyasapogenols A and B were analyzed using ELSD. In whole soybean germplasm, levels of total soyasapogenols were higher in CS01173 and CS01346 (1802.3 and 1736.8 $\mu g\ g^{-1}$, respectively) than in the other soybeans. The mean concentrations of total soyasapogenols were 1234.0 $\mu g\ g^{-1}$ in American, 1294.5 $\mu g\ g^{-1}$ in Chinese, and 1241.5 $\mu g\ g^{-1}$ in Korean soybean seeds. Among the three countries of origin, the Chinese soybeans showed the highest average concentration of total soyasapogenols (1294.5 $\mu g\ g^{-1}$). The average total concentrations of soyasapogenols in soybeans varied from 1179.0 to 1524.7 $\mu g\ g^{-1}$ in American, from 1177.1 to 1802.3 $\mu g\ g^{-1}$ in Chinese, and from 1176.2 to 1682.0 $\mu g\ g^{-1}$ in Korean soybean seeds.

The average concentrations of soyasapogenol A ranged from 435.2 to 446.7 $\mu g\ g^{-1}$ in American, from 435.0 to 531.2 $\mu g\ g^{-1}$ in Chinese, and from 434.9 to 519.9 $\mu g\ g^{-1}$ in Korean soybeans. Soyasapogenol B varied from 743.9 to 1080.1 $\mu g\ g^{-1}$ in American, from 742.1 to 1322.7 $\mu g\ g^{-1}$ in Chinese, and from 741.4 to 1162.2 $\mu g\ g^{-1}$ in Korean soybean seeds (Table 4). The content of soyasapogenol B in the soybean seeds was about 2-fold higher than that of soyasapogenol A. Two previous studies have also shown that the content of soyasapogenol B was 2.5–4.5-fold higher than that of soyasapogenol A in soybean seeds.[12,27] With respect to the different countries of origin, the Chinese soybean varieties had the highest concentrations of total soyasapogenols, whereas the Korean soybean seeds had the second highest level. Among the different-sized soybean seeds, the average total soyasapogenol contents were 1260.6 $\mu g\ g^{-1}$ in small seeds, 1269.3 $\mu g\ g^{-1}$ in medium seeds, and 1223.8 $\mu g\ g^{-1}$ in large seeds. Thus, the medium-sized soybean seeds had the highest concentration of average total soyasapogenols (1269.3 $\mu g\ g^{-1}$).

Table 4 shows the individual concentrations of soyasapogenols A and B, according to the country of origin. The concentration of soyasapogenol B was higher than that of soyasapogenol A, which was highest in the Chinese soybean seeds. There were no differences in their contents. The mean concentrations of soyasapogenol A were 437.6 $\mu g\ g^{-1}$ in American, 448.1 $\mu g\ g^{-1}$ in Chinese, and 443.1 $\mu g\ g^{-1}$ in Korean soybean seeds. The concentration of soyasapogenol B was about 2-fold higher than that of soyasapogenol A.

Of the small soybean seeds, the soyasapogenol concentration in the American soybeans was higher (1315.2 $\mu g\ g^{-1}$) than that in the Korean soybeans (1251.8 $\mu g\ g^{-1}$). In contrast, the total soyasapogenol contents in the medium (1250.8 $\mu g\ g^{-1}$) and large (1224.6 $\mu g\ g^{-1}$) Korean soybeans were higher than those in the American soybeans. The concentration of soyasapogenols shows a difference between the medium and large soybeans from America and Korea. The small American soybean seeds had the highest level of total soyasapogenols (1315.2 $\mu g\ g^{-1}$), whereas the small Korean soybeans contained 1251.8 $\mu g\ g^{-1}$. The Korean soybean seeds contained a higher average content of total soyasapogenols than either medium or large American soybeans.

The average total soyasapogenol concentrations in the small, medium, and large soybeans from the three different countries are shown in Table 5. In the small seeds, the total soyasapogenol concentrations in the soybeans varied from 1206.7 to 1524.7 $\mu g\ g^{-1}$ in the American soybeans and from 1185.0 to 1682.0 $\mu g\ g^{-1}$ in the Korean soybeans. In the medium soybeans, the average total soyasapogenol concentrations ranged from 1186.9 to 1238.1 $\mu g\ g^{-1}$ in American, from 1177.1 to 1802.3 $\mu g\ g^{-1}$ in Chinese, and from 1176.2 to 1592.7 $\mu g\ g^{-1}$ in Korean seeds. In the large seeds, the concentrations of phenolic compounds varied from 1179.0 to 1188.2 $\mu g\ g^{-1}$ in American, from 1214.3 to 1249.0 $\mu g\ g^{-1}$ in Chinese, and from 1181.9 to 1490.0 $\mu g\ g^{-1}$ in Korean soybeans (Table 5).

Soyasaponins are classified into two major groups, soyasaponins A and B, according to their structures. Soyasaponin A is responsible for undesirable, bitter, and astringent tastes in soy foods.[28] Soyasaponin B is monodesmodic and may be responsible for the health-beneficial effects of soyasaponins.[14] Thus, soybeans that contain soyasapogenol B are more valuable than those that contain soyasapogenol A.

In this study, the average soyasapogenol concentration in the soybean germplasm was influenced by seed weight and the country of origin. The small American soybean seeds had the highest levels of soyasapogenols (1315.2 $\mu g\ g^{-1}$), and the small Korean soybean seeds also showed high levels (Table 5). Thus, we can conclude that the soyasapogenols were compressed in the small soybean seeds and diffused at low density in the large seeds. We suggest that the synthesis and accumulation of soyasaponins are related to seed size. Our previous study[27] showed that the soyasapogenol concentrations of soybean seeds varied according to seed size and other characteristics.

We found that the total concentration of soyasapogenols was influenced by genetic characteristics such as origin and seed weight. In addition, we concluded that environmental factors such as growth location, cultivation year, seasonal change, and environmental conditions such as light, temperature, humidity, and soil fertility affect the content and accumulation of soyasapogenols in soybean seeds. A previous study has shown that the local climate, seasonal changes, and external conditions such as light, temperature, and soil fertility affect the qualitative composition of saponins.[29] The results of this study may be useful for plant breeding and food industries.

Tocopherols in Soybean Germplasm. In this study, the concentrations of vitamin E compounds (tocopherols and tocotrienols) were measured using GC to detect four tocopherols and tocotrienols. Tocotrienols were not detected in whole soybean germplasm. In whole soybeans, CS01202 had the highest level of total tocopherols (330.5 $\mu g\ g^{-1}$), whereas CSRV056 (Pungsannamulkong) had the lowest content (153.3 $\mu g\ g^{-1}$). Among the three different countries of origin, the Chinese soybean seeds had the highest average concentration of total tocopherols (255.1 $\mu g\ g^{-1}$), the American seeds had the second highest content (245.9 $\mu g\ g^{-1}$), and the Korean soybean samples had the lowest level (234.3 $\mu g\ g^{-1}$) of total tocopherols.

In this study, α-, β-, γ-, and δ-tocopherol compounds were detected in the soybean seed samples. However, tocotrienols were not detected in any of the soybean seeds. Among the four tocopherols, γ-tocopherol was present at the highest level (American seeds, 99.6 $\mu g\ g^{-1}$; Chinese seeds, 110.7 $\mu g\ g^{-1}$; and Korean seeds, 93.3 $\mu g\ g^{-1}$), whereas β-tocopherol was present at the lowest level (American seeds, 31.4 $\mu g\ g^{-1}$; Chinese seeds, 31.6 $\mu g\ g^{-1}$; and Korean seeds, 31.3 $\mu g\ g^{-1}$). δ-Tocopherol was the second most abundant compound among the tocopherols (American seeds, 65.5 $\mu g\ g^{-1}$; Chinese seeds, 54.6 $\mu g\ g^{-1}$; Korean seeds, 62.9 $\mu g\ g^{-1}$; Figure 5).

Table 6 compares the total tocopherol contents among small, medium, and large soybeans from the three different countries (see also Figure 6). In the small soybean seeds, the average total tocopherol concentrations were 246.0 $\mu g\ g^{-1}$ in the American soybeans and 221.2 $\mu g\ g^{-1}$ in the Korean soybeans. The average total tocopherol concentration in the medium seeds was 248.1 $\mu g\ g^{-1}$ in American, 256.1 $\mu g\ g^{-1}$ in Chinese, and 240.0 $\mu g\ g^{-1}$ in Korean soybean seeds. The mean concentrations of tocopherols in the large soybean samples were 225.7 $\mu g\ g^{-1}$ in American, 238.1 $\mu g\ g^{-1}$ in Chinese, and 235.7 $\mu g\ g^{-1}$ in Korean soybeans. In this comparison, the medium Chinese soybean group had the highest level of average total tocopherols (256.1 $\mu g\ g^{-1}$). In the small soybeans, the total tocopherol concentration ranged from 211.1 to 266.8 $\mu g\ g^{-1}$ in the American seeds and from 153.3 to 271.0 $\mu g\ g^{-1}$ in the Korean seeds. In the medium seeds, the average total tocopherol concentration varied from 227.2 to 272.9 $\mu g\ g^{-1}$ in American, from 190.8 to 330.5 $\mu g\ g^{-1}$ in Chinese, and from 181.3 to 303.9 $\mu g\ g^{-1}$ in Korean soybeans. In the large seeds, the average total tocopherol concentration ranged from 222.7 to 228.7 $\mu g\ g^{-1}$ in American, from 226.2 to 242.3 $\mu g\ g^{-1}$ in Chinese, and from 177.1 to 284.9 $\mu g\ g^{-1}$ in Korean seeds (Table 6).

The percentage of γ-tocopherol in the Korean soybean seeds was the highest among the four individual tocopherols (α-, β-, γ-, and δ-tocopherols) in the three seed-size groups (39.6%). The percentage of δ-tocopherol was 26.6%, and the percentage of β-tocopherol was about half that of δ-tocopherol. In the small Korean soybeans, the percentage of γ-tocopherol was 38.8%, δ-tocopherol was 25.5%, α-tocopherol was 21.6%, and β-tocopherol was 14.1%.

A previous study has shown that γ-tocopherol has biological activity, which protects against chronic diseases such as inflammation.[30] Both γ- and δ-tocopherols may be necessary for preventing lipid oxidation, and a mixture of tocopherols containing γ-, δ-, and α-tocopherols (5:2:1) has been shown to have antioxidant and anti-inflammatory activities.

In our results, the medium Korean soybeans had the highest proportion of γ-tocopherol (41.1%) and contained 26.6% δ-tocopherol, 19.4% α-tocopherol, and 12.9% β-tocopherol. β-Tocopherol was present in the lowest proportion among the four tocopherols. Previous studies have shown that most of the tocopherols in soybean are γ- and δ-tocopherols.[31] In common soybean seeds, the main forms of tocopherols are γ- and δ-tocopherols, which account for about 60 and 25% of total tocopherol content, respectively.[32]

Genetic variability in both the total contents and proportions of α-, β-, γ-, and/or δ-tocopherols as well as their related tocotrienols in soybeans[31] and other species has been reported. In addition, the distribution of tocopherol concentrations is influenced by environmental effects.[32] A previous study showed that the tocopherol concentrations in soybean seeds were affected by crop management, including seeding date and cultivar, and were also influenced by cultivation regions.[33] In this study, the medium soybeans had the highest level of total tocopherols. Thus, we concluded that smaller seeds contained the highest contents of tocopherols. In a previous study, the concentrations of functional compounds were higher in small soybean seeds than in large seeds.[27] This study indicates that tocopherol content depends on seed size. Furthermore, the concentration of total tocopherols depends on the country of origin. Consequently, our study indicated that the tocopherol concentrations of soybeans can be affected by genetic differences such as seed weight and variety.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Agronomic characteristics of soybean germplasms. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +82-2-450-3730. Fax: +82-2-446-7856. E-mail: imcim@konkuk.ac.kr.

Author Contributions

‖Eun-Hye Kim and Hee-Myong Ro equally contributed to this study.

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Notes

The authors declare no competing financial interest.

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