Food Sci. Biotechnol. 25(6): 1551-1559 (2016)
DOI 10.1007/s10068-016-0240-2

Food Science
and
Biotechnology

Free and bound form bioactive compound profiles in germinated black soybean (*Glycine max* L.)

Min Young Kim, Gwi Yeong Jang, Yoonjeong Lee, Meishan Li, Yeong Mi Ji, Nara Yoon, Sang Hoon Lee[1], Kyung Mi Kim[1], Junsoo Lee, and Heon Sang Jeong*

Department of Food Science and Biotechnology, Chungbuk National University, Cheongju, Chungbuk 28644, Korea
[1]Department of Agrofood Resources, National Academy of Agricultural Science, Rural Development Administration, Jeonju, Jeonbuk 54875, Korea

Received May 10, 2016
Revised July 25, 2016
Accepted August 9, 2016
Published online December 31, 2016

*Corresponding Author
Tel: +82-43-261-2570
Fax: +82-43-271-4412
E-mail: hsjeong@chungbuk.ac.kr

pISSN 1226-7708
eISSN 2092-6456

Abstract This study investigated the transition between the free and bound forms of functional compounds in germinated black soybean. Black soybean was germinated at 25°C over 6 days and then the free and bound forms of functional compounds were extracted. Total free polyphenol, flavonoid, and phenolic acid contents in raw black soybean increased from 1.03 mg GAE/g, 0.29 mg CE/g, and 315.67 μg/g to 1.44 mg GAE/g, 0.64 mg CE/g, and 511.01 μg/g, respectively, by 4 days after germination. Changes to phenolic acid compositions can be divided into four groups, and the germination process can convert compounds to phenolic acid via anabolism and catabolism. The highest total free isoflavone content in germinated black soybean (3,724.40 μg/g) was observed at 4 days. Bound polyphenol, flavonoid, phenolic acid, and isoflavone contents decreased as the germination period increased. These results suggest that the germination process increased compound functionality in black soybean.

Keywords: black soybean, germination, free form, bound form, bioactive compound

Introduction

Black soybean [*Glycine max* L. (Merr.)] is one of the most important agricultural crops in the world. This crop is widely used because of its beneficial health effects on several chronic diseases (1). Black soybeans are associated with a wide range of the beneficial health effects, such as antimutagenicity, anti-inflammatory activities, inhibition of low density lipoprotein oxidation (2-3). Recently, a number of researchers have reported that the beneficial properties of black soybeans are due to the many phytochemicals present in the crop, including phenolic compounds, isoflavone, anthocyanin, and saponin (4).

Germination is also known to improve the nutritional value of legumes and to enhance the bioavailability of bound functional compound (5). Germination activates dormant enzymes in grain and induces the hydrolysis of high-molecular weight polymers resulting in the generation of bioactive compounds, such as vitamin E, phenolic compounds, GABA, γ-oryzanol, and useful amino acids (6).

Functional components, such as phenolics, flavonoids, and isoflavones, generally occur as soluble, conjugated forms (glycosides) and insoluble forms (7). Functional components in their insoluble form have very low bioavailability because the matrix of cell wall severely hinders their access to the necessary enzyme that contributes to their release in the human gastrointestinal tract (8). Free and soluble conjugated forms are more rapidly absorbed in the stomach and small intestine. They have physiological activities, such as inhibition activities against oxidation of LDL cholesterol and liposomes (9). Therefore, the release of bound phenolics prior to intake is necessary if health benefits, besides the prevention of colon cancer, are preferred. Several food processes, including germination, fermentation, and thermo-mechanical processes, increase the release of bound phenolics (10). Although the germination process has been suggested as an efficient way of significantly increasing the free functional components of legumes in a previous study, complex biochemical reactions occurred during the germination process, and thus, it was not clear how the transformation or biosynthesis between the free and the bound forms occurred in the black soybean matrix.

Therefore, the objectives of this study were to clarify the cause of the increased functionality of the compounds in black soybean following germination by investigating the transition between the free and bound forms of polyphenols, flavonoids, phenolic acids, isoflavones and anthocyanins at different germination points and to promote the utilization of potentially bioactive bound functional components produced in black soy bean during the germination process.

Materials and Methods

Preparation of germinated black soybean The black soybean (cv. cheongja 3, *Glycine max* L.), grown in 2015, was obtained from the National Institute of Crop Science, Rural Development Administration, Korea. The black soybean (*Glycine max* L.) seed (100 g) was soaked in distilled water (seed water ratio, 1:5, w/v) for 24 h at 20±1°C, and the water used for soaking was drained and changed every 3 h. The soaked seeds were placed on a germination tray (100×150 mm) containing wet laboratory paper. They were then covered with another wet paper and placed in a seed germinator (WGC 450; Dahan Inc., Seoul, Korea), where they were in contact with the circulating water of the germinator, which meant that the seeds were always wet through capillary action. The seeds were left in darkness at 20°C and 95% relative humidity for 1, 2, 3, 4, 5, and 6 days (11). All samples were dried for 2 days using a hot air dryer (WFO-459PD; EYELA, Tokyo, Japan), and were stored at –20°C using a deep freezer (Ultra low temperature freezer, MDF-393; SANYO, Akaiwa, Japan).

Extraction of free and bound phenolic compounds The free phenolic compounds in germinated black soybean samples were extracted using the method described by Seo *et al*. (12) and Jung *et al*. (13) with slight modifications. The powdered samples (4 g) were extracted three times with 90% methanol (40 mL) at room temperature for 1 h using an ultrasonic bath (SD-350H; Seong Dong, Seoul, Korea). The extracts were then filtered, combined, and concentrated using a rotary evaporator under vacuum, and the residue was dissolved in 5 mL of distilled water and extracted three times with 10 mL of diethyl ether/ethyl acetate solvent (50:50, v/v). The supernatant layer was evaporated using a rotary evaporator under vacuum. The residues were then dissolved in methanol and filtered through a 0.45 μm syringe filter (Millipore, Billerica, MA, USA). The bound phenolic compounds from the germinated black soybean samples were extracted according to Zielinski *et al*. (14). After extraction of the free phenolic compounds, 10 mL of 4 M NaOH was added directly to the flour residue and the suspension was sonicated for 90 min at 40°C. After alkaline hydrolysis, the solution was acidified to pH 2.0 with concentrated HCl and centrifuged at 2,200x*g* for 10 min to remove any cloudy precipitates. The liberated phenolic compounds in the clear solution were extracted three times with 20 mL of diethyl ether/ethyl acetate solvent (50:50, v/v). The supernatant layer was evaporated using a rotary evaporator under vacuum. The residues were dissolved in methanol and filtered through a 0.45 μm syringe filter (Millipore). The free and bound phenolic compound extracts were stored at –20°C until analysis.

Determination of total polyphenol content Total polyphenol content was determined using the method described by Dewanto *et al*. (15). In a 10 mL test tube, 2 mL of 2% Na_2CO_3, 0.1 mL of extract appropriately diluted, and 0.1 mL of 50% Folin-Ciocalteu's phenol reagent (Sigma-Aldrich, St. Louis, MO, USA) were added and mixed. After exactly 30 min, the absorbance was read at 750 nm, and the phenolic content was calculated from a calibration curve (R^2=0.998) obtained using gallic acid as a standard (Sigma-Aldrich).

Determination of total flavonoids The flavonoid content in the extracts was determined using the colorimetric method described by Jia *et al*. (16) with modifications. First, 250 μL of germinated black bean extracts was mixed with 1.25 mL of distilled water and 75 μL of a 5% $NaNO_2$ solution. After 5 min, 150 μL of 10% $AlCl_3 \cdot H_2O$ solution was added. Then at 6 min, 500 μL of 1 M NaOH and 275 μL of distilled water were added. The solution was mixed well and the absorbance was read at 510 nm. A (+)-catechin standard (Sigma-Aldrich) curve was obtained and used to calculate the flavonoid content.

Determination of phenolic acid composition The phenolic acid composition of each extract was determined using an HPLC system according to the method described by Seo *et al*. (12) with slight modifications (12). The analytical column was an ODS column (5 μm, 4.6 mm×250 mm, Agilent Technologies, Santa Clara, CA, USA). A gradient elution was employed using solvent A (water containing 0.1% (v/v) acetic acid) and solvent B (acetonitrile containing 0.1% (v/v) acetic acid). The gradient program was as follows: 0–2 min, 92 to 90% A in B (gradient); 2–27 min, 90 to 70% A in B (gradient); 27–50 min, 70 to 10% A in B (gradient); 50–51 min, 10 to 0% A in B (gradient); 51–60 min, 0% A in B (isocratic); and 60–70 min, 0 to 92% A in B (gradient). The flow rate was kept at 1 mL/min and the injection volume was 20 μL. The UV detector was set at 280 nm. The phenolic acid standard mixture containing gallic acid, homogentisic acid, gentisic acid, chlorogenic acid, (+)-catechin, caffeic acid, phloretic acid, *p*-coumaric acid, ferulic acid, veratric acid, naringin, hesperidin, salicylic acid, protocatechuic acid, quercetin, *trans*-cinnamic acid, naringenin, hesperitin, and biochanin was prepared in HPLC-grade methanol. The phenolic acid concentrations were determined by standard curves obtained by injecting different concentrations of the phenolic acid standard into the HPLC system. Peaks were verified by adding the standard phenolic acids to the samples, and each peak area was calculated in relation to a standard peak area. The total phenolic acid content was calculated by adding up the different phenolic acid component amounts.

Extraction of free and bound isoflavones In order to extract the free isoflavones, 2 mL of 0.1 N HCl and 10 mL of acetonitrile were mixed with ground seed samples (2 g) following the method of Wang *et al*. (17). The samples were then 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 temperatures below 30°C. The dried samples were then redissolved in 10 mL of 80% HPLC grade methanol solution. Finally, aliquot samples were filtered through a 0.45 mm filter unit (Millipore). After extraction of

the isoflavone, 10 mL of 4 M NaOH was added directly to the flour residue and the suspension was sonicated for 90 min at 40°C. After alkaline hydrolysis, the solution was acidified to pH 2.0 with concentrated HCl. In order to extract the bound isoflavone, 2 mL of 0.1 N HCl and 10 mL of acetonitrile were mixed with samples. The samples were then 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 temperatures below 30°C. The dried samples were then redissolved in 10 mL of 80% HPLC grade methanol solution. Finally, aliquot samples were filtered through a 0.45 mm filter unit (Millipore). The free and bound isoflavone extracts were stored at –20°C until analysis.

Isoflavone content analysis HPLC analyses were carried out according to Lee *et al*. (18) on a HPLC (Shimadzu Corp., Kyoto, Japan) equipped with a photo diode array detector (model SPD-M10A VP). A YMC-Pack ODS-AM-303 (250 mm×4.6 mm I.D.) analytical HPLC column was used for the quantitative analyses. Isoflavones were identified and quantified by UV at a wavelength of 254 nm. A linear HPLC gradient was used. 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 acetonitrile. The sample injection volume was 20 μL, and 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. A high linearity of R^2>0.996 was obtained for each standard. Daidzein, genistein, glycitein, daidzin, genistin, glycitin, acetyldaidzin, acetylgenistin, acetylglycitin, malonyldaidzin, malonylgenistin, and malonylglycitin (Sigma-Aldrich) were each identified by their retention times, and their concentrations were calculated by comparing the peak areas of samples with those of the standards.

Extraction of free and bound anthocyanin The free anthocyanin in the germinated black soybean samples was extracted according to Glenda *et al*. (19) with minor modification. The ground sample (1 g) was extracted three times with 30 mL of 80% MeOH containing 0.3% HCl and assisted by ultrasound (SD-350H; Seong Dong) at 25°C for 1 h. After centrifugation at 2,200×*g* for 10 min, the combined supernatant levels were made up to 100 mL. The extraction of bound anthocyanin from the germinated black bean samples was conducted using a modified version of the method used by Glenda *et al*. (19) and Gusti and Wrolstad (20).This technique submitted the sample to controlled acid conditions with the objective of breaking the O-glycoside bonds without affecting the flavylium ion structure. This released the aglycone molecule in anthocyanin. Then 10 mL of 2 N HCl was added to the dried extracts in an inert atmosphere. The resulting solution was boiled in a water-bath for 2 h at 100°C, and then the volume was made up to 100 mL. All extracts were filtered through a 0.45 μm syringe filter (Millipore) in order to analyze for anthocyanin and anthocyanidin in their free and bound forms.

Anthocyanin and anthocyanidin contents analysis The anthocyanin contents of each extract were determined using an HPLC system according to the method described by Choung (21). The analytical column was a Mightysil RP-18 GP column (4.6×250 mm, 5 μm, Kanto Chemical, Tokyo, Japan). Gradient elution was employed using solvent A (water containing 5% (v/v) formic acid) and solvent B (acetonitrile containing 5% (v/v) formic acid). The program was as follows: 0–24 min, 10 to 40% A in B; 24–25 min, 40 to 100% A in B; 25–28 min, 100 to 100% A in B; 28–29 min, 100 to 10 A in B; and 29–40 min, 10 to 10 A in B. The flow rate was kept at 1 mL/min, the injection volume was 20 μL, and the UV detector was set at 520 nm. The standard used was delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, delphinidin, and cyanidin (Sigma-Aldrich), all samples were analyzed in triplicate. The total anthocyanin and anthocyanidin contents were calculated by adding the individual amounts of anthocyanins and anthocyanidin together, respectively.

Results and Discussion

Polyphenols and flavonoids The free and bound polyphenol and flavonoid contents in black soybean for each germination day are shown in Table 1. Total free polyphenol content increased from 1.03 mg GAE/g in raw black soybean to 1.44 mg GAE/g after 4 days of germination time and decreased to 1.19 and 0.89 mg GAE/g after 5 and 6 days, respectively. The total free flavonoid content in raw black soybean was 0.29 mg CE/g and the highest value (0.64 mg CE/g) was observed after 3 days germination. Lin and Lai (22) reported similar results, which showed that germination enhanced free phenolic contents in most soybean cultivars and the highest content was

Table 1. Changes in polyphenol and flavonoid contents of black soybean with germination periods

		Germination periods (day)						
	Types	0	1	2	3	4	5	6
Polyphenol (μg/g)	Free Form	1.03±0.01$^{c1)}$	1.19±0.03^{b}	1.19±0.04^{b}	1.27±0.10^{b}	1.44±0.02^{a}	1.19±0.04^{b}	0.89±0.02^{d}
	Bound Form	6.39±0.31^{ab}	6.13±0.22^{b}	5.95±0.37^{b}	6.00±0.35^{b}	6.07±0.37^{b}	6.85±0.16^{a}	6.48±0.44^{ab}
	Total	7.42±0.31^{b}	7.32±0.22^{b}	7.14±0.33^{b}	7.27±0.37^{b}	7.51±0.37^{ab}	8.04±0.12^{a}	7.37±0.46^{b}
Flavonoid (μg/g)	Free Form	0.29±0.01^{e}	0.35±0.02^{d}	0.43±0.01^{c}	0.64±0.02^{a}	0.51±0.01^{b}	0.22±0.00^{f}	0.17±0.01^{g}
	Bound Form	2.13±0.12^{b}	1.92±0.07^{c}	1.88±0.04^{c}	1.79±0.11^{c}	1.88±0.06^{c}	2.85±0.18^{a}	1.73±0.04^{c}
	Total	2.43±0.12^{b}	2.27±0.08^{b}	2.31±0.04^{b}	2.43±0.13^{b}	2.39±0.07^{b}	3.07±0.18^{a}	1.9±0.04^{a}

[1)]Values are mean±SD of replicates. Different superscripts in the same row indicate a significant difference (p<0.05) among different germination periods.

recorded in beans that had been germinating for 4 days. The reason for the increased free polyphenol and flavonoid contents during the germination period could be determined from the bound polyphenol and flavonoid content analysis results. Bound polyphenol content decreased from 6.39 mg GAE/g in raw black soybean to 6.07 mg GAE/g after 4 days germination and slightly increased to 6.85 GAE/g after 5 days. Furthermore, when the germination period reached 3 days, the flavonoid content of the bound form fell from 2.13 mg CE/g in raw black soybean to 1.79 mg CE/g. Large amounts of bound polyphenols and flavonoids incell walls are covalently conjugated to cell wall components, such as cellulose, pectin, and polysaccharides, through ester bonds (23). However, bound polyphenols and flavonoids cannot be extracted using common techniques, which means that some of the bound polyphenol has been converted into the extractable free polyphenol forms during the germination process. Interestingly, although the extraction of bound polyphenols and flavonoids is possible during the transition to the free forms during the germination, bound polyphenol content slightly increased on the final germination day. The increase in bound polyphenols may be explained by polymerization and oxidation of phenolics, and by changes in the enzymes involved in the synthesis of free or bound phenolics (24).

Phenolic acid profiles The changes in free and bound phenolic acid profiles during germination are shown in Table 2. Total free phenolic acid contents in raw black soybean increased from 315.67 to 511.01 μg/g after 4 days germination. Furthermore, total bound phenolic acid contents substantially decreased from about 126.39 to around 72.22 μg/g as the germination period increased from 1 to 6 days. These results suggest that some of the bound phenolic acid has been converted into the extractable free form during the germination process. Under the influence of germination, the maximum increase (61.88%) in the total free phenolic acid content was higher than the maximum decrease (42.85%) in total bound phenolic acid contents during germination. Therefore, the increase in total phenolic acid contents during the germination process could have other explanations that are connected to the liberation of bound phenolics. Germination effects on phenolic acid contents are based on the biosynthesis or conversion of phenolic acid to create essential secondary metabolites for plant growth (25). During the germination process, the changes in phenolic acid composition (Table 2), can be divided into groups (A–D) with the following four tendencies, A group: phenolic acids converted from the bound form into the free form; B group: phenolic acids synthesized from other types of phenolic acids or amino acid via the shikimate pathway; C group: phenolic acids synthesized in macromolecular bound forms for plant growth; and D group: phenolic acids used as precursors for other, different types of phenolic acid synthesis. The free forms of ferulic acid, salicylic acid, and biochanin contents, which belonged to A group increased, while bound forms decreased as the germination period increased. These results suggest that some of the bound forms were converted into free forms during the germination process. The bound forms of phloretic acid, *trans*-cinnamic acid, and naringenin in B group were not detected in all samples, whereas the free forms had increased during germination. The maximum contents of phloretic acid, *trans*-cinnamic acid, and naringenin were reached on day 4, day 2, and day 6 at about 130.58, 0.78, and 347.13 μg/g, respectively. The results indicate that the phenolic acids in B group are synthesized or converted by a different pathway than the free form-bound conversion pathway. The production of phloretic acid is related to the pathway for ρ-coumaric acid degradation. Furthermore, *p*-coumaric acid, which is easily synthesized from amino acid, can be metabolized into phloretic acid via the activation of phenolic acid reductase (26), and the production of naringenin is produced via a conversion from naringin. Naringin can be further hydrolyzed by the β-D-glucosidase component of naringinase into glucose and naringenin (27). The free forms of chlorogenic acid, catechin, caffeic acid, and *p*-coumaric acid in C group were not detected and free *p*-coumaric acid linearly decreased, whereas the bound forms of the C group members showed a significant increase during germination. This result demonstrated that some phenolic acids were synthesized as macromolecular bound components (i.e. polyphenol, tannin, and lignin) to support structural cell wall development. Chlorgenic acid, caffeic acid, and *p*-coumaric acid can be used as intermediates during lignin biosynthesis. Lignin is generic term for a large group of aromatic polymers resulting from the oxidative combination coupling of 4-hydroxyphenylpropanoids (28). Furthermore, the loss of (+)-catechin is associated with the tannin biosynthesis pathway and the most widely studied condensed tannins are based on the flavan-3-ols and (+)-catechin (29). Finally, as the germination period increased, the free and bound form levels of veratric acid, naringin, and protocatechuic acid in D group fell. The reduction in the levels of these compounds during the germination process is presumably due to their use as precursors or intermediates during the biosynthesis of different phenolic acids, as occurred in the A, B, and C groups. Naringin can be further hydrolyzed by the β-D-glucosidase component of naringinase into glucose and naringenin (27). Moreover, the decrease in protocatechuic acid content is related to gallic acid and hydrolysable tannin synthesis (30). In summary, the phenolic acid profile results suggest that the germination process converts various compounds into phenolic acids via various anabolic and catabolic processes. Phenolic acids are synthesized or converted by the shikimate pathway from L-phenylalanine or L-tyrosine, and various phenolic acids produced by the shikimate pathway can be used to synthesize macromolecular compounds, such as lignin, suverin, flavonoids, and tannin. These compounds are synthesized as part of the macromolecular bound component to support structural cell wall development (24). It can be concluded that increasing phenolic acid levels are based on biosynthesis; the conversion of phenolic acids to essential secondary metabolites for plant growth; or the liberation of bound phenolics.

Table 2. Changes in phenolic acid profiles of black bean with germination periods

Germination Periods (day)		Contents of phenolic compound (μg/g)																
		Gallic acid	Chlorogenic acid	(+)-Catechin	Caffeic acid	Phloretic acid	*p*-Coumaric acid	Ferulic acid	Veratric acid	Naringin	Hesperidin	Salicylic acid	Protocatechuic acid	quercetin	Cinnamic acid	Naringenin	Biochanin	Total
0	Free	1.11 ±0.11$^{a1)}$	ND	ND	ND	70 ±5.08^{d}	2.96 ±0.29^{a}	2.14 ±0.22^{b}	2.17 ±0.20^{a}	107.10 ±5.14^{c}	ND	13.01 ±1.00^{b}	85.57 ±4.30^{a}	ND	ND	31.44 ±1.72^{g}	0.17 ±0.03^{g}	315.67 ±18.06^{c}
	Bound	ND	2.35 ±0.56^{bc}	7.2 ±0.99^{e}	0.38 ±0.15^{f}	ND	3.23 ±0.10^{b}	ND	1.13 ±0.29^{a}	37.85 ±0.40^{e}	ND	3.48 ±0.01^{ab}	0.81 ±0.04^{a}	2.46 ±1.03^{ab}	ND	ND	0.88 ±0.02^{a}	59.76 ±2.79^{g}
	Total (Free+Bound)	1.11 ±0.11^{a}	2.35 ±0.56^{bc}	7.2 ±0.99^{e}	0.38 ±0.15^{f}	70 ±5.08^{d}	6.19 ±0.39^{a}	2.14 ±0.22^{c}	3.29 ±0.49^{a}	144.95 ±5.54^{c}	ND	16.48 ±0.99^{a}	86.39 ±4.34^{a}	2.46 ±1.03^{ab}	ND	31.44 ±1.72^{g}	1.05 ±0.05^{f}	375.43 ±20.68^{d}
1	Free	ND	ND	ND	ND	112.34 ±7.59^{b}	1.39 ±0.09^{b}	2.76 ±0.20^{a}	0.7 ±0.08^{bc}	158.83 ±9.58^{a}	ND	11.86 ±0.91^{c}	47.08 ±1.03^{c}	ND	0.39 ±0.08^{b}	50.73 ±3.02^{f}	0.96 ±0.11^{f}	387.67 ±22.68b^{c}
	Bound	ND	3.42 ±0.06^{b}	7.61 ±0.12^{e}	8.55 ±0.15^{e}	ND	3.35 ±0.09^{b}	1.87 ±0.06^{a}	0.75 ±0.11^{bc}	93.2 ±1.00^{a}	ND	2.64 ±1.48^{bc}	0.45 ±0.09^{bc}	4.16 ±2.41^{a}	ND	ND	0.38 ±0.03^{b}	126.39 ±3.20^{a}
	Total (Free+Bound)	ND	3.42 ±0.06^{b}	7.61 ±0.12^{e}	8.55 ±0.15^{e}	112.34 ±7.59^{b}	4.74± 0.01^{b}	4.64 ±0.26^{a}	1.45 ±0.19^{b}	252.03 ±10.58^{a}	ND	14.5 ±2.39^{b}	47.53 ±0.95^{c}	4.16 ±2.41^{a}	0.39 ±0.08^{b}	50.73 ±3.02^{f}	1.35 ±0.08^{f}	513.44 ±24.93^{b}
2	Free	ND	ND	ND	ND	75.62 ±5.3^{d}	1.07 ±0.09^{c}	2.32 ±0.10^{b}	0.38 ±0.08^{d}	101.17 ±6.28^{c}	ND	7.08 ±0.36^{d}	24.66 ±0.96^{d}	ND	0.78 ±0.13^{a}	65.86 ±3.53^{e}	2.49 ±0.11^{e}	281.44 ±16.92^{d}
	Bound	ND	2.30 ±0.20^{bc}	10.64 ±0.17^{d}	17.92 ±0.08^{b}	ND	4.54 ±0.90^{a}	1.86 ±0.06^{a}	0.75 ±0.09^{bc}	53.87 ±1.39^{b}	ND	3.59 ±0.30^{a}	1.01 ±0.12^{a}	3.74 ±1.42^{ab}	ND	ND	0.26 ±0.01^{c}	100.49 ±3.00^{c}
	Total (Free+Bound)	ND	2.30 ±0.20^{bc}	10.64 ±0.17^{d}	17.92 ±0.08^{b}	75.62 ±5.3^{d}	5.6 ±0.99^{a}	4.18 ±0.16^{b}	1.13 ±0.17^{bc}	155.04 ±7.66^{c}	ND	10.67 ±0.67^{c}	25.68 ±0.84^{d}	3.74 ±1.42^{ab}	0.78 ±0.13^{a}	65.86 ±3.53^{e}	2.76 ±0.12^{e}	381.93 ±19.61^{e}
3	Free	ND	ND	ND	ND	94.84 ±7.15^{c}	0.93 ±0.10^{c}	2.88 ±0.22^{a}	0.81 ±0.02^{b}	98.38 ±5.82^{c}	ND	6.77 ±0.41^{d}	21.53 ±0.42^{d}	ND	0.46 ±0.06^{b}	127.23 ±9.93^{d}	3.73 ±0.37^{d}	357.56 ±23.62^{c}
	Bound	ND	6.22 ±0.24^{a}	16.64 ±0.19^{b}	25.53 ±0.37^{a}	ND	4.65 ±0.22^{a}	1.70 ±0.27^{a}	0.64 ±0.53^{cd}	45.12 ±3.23^{c}	1.17 ±0.08^{c}	2.47 ±0.19^{bc}	0.56 ±0.33^{b}	3.56 ±1.40^{ab}	ND	ND	ND	108.25 ±3.98^{b}
	Total (Free+Bound)	ND	6.22 ±0.24^{a}	16.64 ±0.19^{b}	25.53 ±0.37^{a}	94.84 ±7.15^{c}	5.58 ±0.32^{a}	4.59 ±0.49^{ab}	1.45 ±0.55^{b}	143.50 ±9.06^{c}	1.17 ±0.08^{c}	9.24 ±0.22^{c}	22.09 ±0.56^{e}	3.56 ±1.40^{ab}	0.46 ±0.06^{b}	127.23 ±9.93^{d}	3.73 ±0.37^{d}	465.82 ±27.48^{cd}
4	Free	ND	ND	ND	ND	130.58 ±7.79^{a}	0.39 ±0.04^{d}	2.21 ±0.17^{b}	0.53 ±0.15^{cd}	135.77 ±7.00^{b}	ND	15.39 ±0.69^{a}	57.36 ±2.41^{b}	ND	ND	161.58 ±8.83^{c}	7.2 ±0.49^{a}	511.01 ±27.48^{a}
	Bound	ND	5.55 ±1.47^{a}	15.09 ±0.15^{c}	15.6 ±2.06^{c}	ND	4.38 ±0.21^{a}	ND	0.61 ±0.10^{cd}	42.06 ±1.22^{d}	3.45 ±0.11^{a}	2.25 ±0.05^{c}	0.27 ±0.07^{d}	2.55 ±0.66^{ab}	ND	ND	ND	91.80 ±0.67^{d}
	Total (Free+Bound)	ND	5.55 ±1.47^{a}	15.09 ±0.15^{c}	15.6 ±2.06^{c}	130.58 ±7.79^{a}	4.77 ±0.17^{b}	2.21 ±0.17^{b}	1.15 ±0.25^{bc}	177.83 ±5.78^{b}	3.45 ±0.11^{a}	17.63 ±0.74^{a}	57.63 ±2.38^{b}	2.55 ±0.66^{ab}	ND	161.58 ±8.83^{c}	7.2 ±0.49^{a}	602.82 ±27.90^{a}
5	Free	ND	ND	ND	ND	47.81 ±2.55^{e}	0.15 ±0.01^{d}	1.14 ±0.07^{c}	0.35 ±0.01^{d}	58.79 ±2.78^{d}	ND	2.67 ±0.04^{e}	8.7 ±0.85^{e}	ND	ND	246.95 ±8.56^{b}	4.54 ±0.23^{c}	371.09 ±13.29^{c}
	Bound	ND	6.47 ±0.38^{a}	22.59 ±1.65^{a}	13.28 ±0.01^{d}	ND	4.28 ±0.28^{a}	ND	0.27 ±0.04^{d}	27.95 ±1.78^{f}	3.64 ±0.62^{a}	1.80 ±0.23^{cd}	ND	1.43 ±0.00^{b}	ND	ND	ND	81.73 ±2.51^{e}
	Total (Free+Bound)	ND	6.47 ±0.38^{a}	22.59 ±1.65^{a}	13.28 ±0.01^{d}	47.81 ±2.55^{e}	4.43 ±0.29^{b}	1.14 ±0.07^{c}	0.62 ±0.05^{cd}	86.74 ±1.01^{d}	3.64 ±0.62^{a}	4.47 ±0.19^{d}	8.7 ±0.85^{f}	1.43± 0.00^{b}	ND	246.95 ±8.56^{b}	4.54 ±0.23^{c}	452.82 ±10.78^{cd}
6	Free	ND	ND	ND	ND	24.88 ±2.23^{f}	0.18 ±0.06^{d}	0.23 ±0.03^{c}	0.07 ±0.03^{e}	29.5 ±1.36^{e}	ND	0.73 ±0.28^{f}	8.57 ±0.15^{e}	ND	ND	347.13 ±11.84^{a}	6.21 ±0.99^{b}	417.51 ±16.36
	Bound	ND	1.51 ±0.13^{c}	22 ±0.24^{a}	1.84 ±0.77^{f}	ND	4.58 ±0.04^{a}	ND	0.39 ±0.01^{cd}	36.96 ±0.25^{e}	2.18 ±0.21^{b}	0.99 ±0.17^{d}	ND	1.77 ±0.00^{ab}	ND	ND	ND	72.22 ±0.89^{f}
	Total (Free+Bound)	ND	1.51 ±0.13^{c}	22 ±0.24^{a}	1.84 ±0.77^{f}	24.88 ±2.23^{f}	4.75 ±0.02^{b}	0.23 ±0.03^{d}	0.46 ±0.05^{d}	66.46± 1.11^{e}	2.18 ±0.21^{b}	1.72 ±0.45^{e}	8.57 ±0.15^{f}	1.77 ±0.00^{ab}	ND	347.13 ±11.84^{a}	6.21 ±0.99^{b}	489.73 ±17.26^{bc}

[1)]Values are mean±SD of replicates. Different superscripts in the same row indicate a significant difference (p<0.05) among different germination periods.

Table 3. Changes in isoflavone profiles of black bean with germination periods

Types	Isoflavones	Germination periods (day)						
		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Free form (μg/g)	Daidzin	232.59±23.06$^{c1)}$	326.9±16.59^{b}	353.63±21.18^{b}	322.56±46.87^{b}	416.18±23.79^{a}	210.27±2.97^{cd}	166.73±1.94^{d}
	Glycitin	31.94±8.68^{c}	54.48±4.95^{ab}	51.12±2.52^{b}	46.42±2.91^{b}	64.88±3.42^{a}	22.64±5.33^{cd}	13.72±4.50^{d}
	Genistin	892.61±82.27^{d}	1,113.24±106.10^{ab}	1,041±36.10^{bc}	903.22±58.94^{cd}	1,251.85±63.67^{a}	613.42±63.26^{e}	489.52±11.96^{e}
	Malonyldaidzin	465.73±6.96^{b}	521.64±7.42^{a}	399.35±18.35^{c}	356.55±19.94^{d}	399.07±24.21^{c}	256.42±9.80^{d}	116.12±0.24^{e}
	Malnylglycitin	141.70±1.28^{c}	162.71±1.60^{a}	139.81±6.20^{c}	119.40±6.78^{d}	151.25±0.94^{b}	108.61±0.20^{e}	61.28±0.46^{f}
	Malonylgenistin	516.16±0.05^{b}	571.76±0.73^{a}	451.87±2.83^{d}	403.46±1.03^{e}	491.13±0.28^{c}	274.62±0.22^{f}	155.77±0.57^{g}
	Acetyldaidzin	225.61±2.03^{bc}	263.13±5.18^{a}	215.08±2.40^{cd}	207.50±4.77^{d}	231.05±5.49^{b}	145.24±10.66^{e}	93.78±7.35^{f}
	Acetylgenistin	23.47±0.20^{g}	31.86±0.31^{f}	41.82±0.51^{e}	64.60±0.19^{d}	74.69±0.23^{c}	137.16±0.24^{b}	176.64±0.36^{a}
	Daidzein	ND	ND	ND	ND	ND	ND	ND
	Glycitein	ND	ND	ND	ND	ND	ND	ND
	genistein	110.92±1.26^{g}	164.29±1.99^{f}	227.68±3.24^{e}	372.61±1.19^{d}	436.79±1.44^{c}	834.25±1.54^{b}	1,085.49±2.27^{a}
	Total β-glycosides	1,316.74±127.61^{g}	1,687.01±113.75^{f}	1,633.25±125.69^{e}	1,452.83±156.16^{d}	1,940.43±60.20^{c}	992.39±72.35^{b}	803.04±30.49^{a}
	Total malonylglycosides	1,123.58±6.90^{d}	1,256.11±9.33^{b}	991.03±17.84^{bc}	879.41±26.31^{cd}	1,041.44±26.37^{a}	639.65±11.00^{e}	333.17±0.24^{f}
	Total acetylglycosides	249.07±3.62^{b}	294.98±4.97^{a}	256.90±4.31^{d}	272.10±5.99^{e}	305.74±6.13^{c}	282.4±10.46^{f}	270.43±7.45^{g}
	Total aglycones	110.92±2.19^{d}	164.29±3.44^{a}	227.68±5.62^{d}	372.61±2.07^{bc}	436.79±2.50^{a}	834.25±2.66^{b}	1,085.49±3.94^{c}
	Total isoflavones	2,800.31±135.81^{de}	3,402.39±105.08^{b}	3,108.85±112.70^{c}	2,976.95±181.49^{cd}	3,724.40±74.20^{a}	2,748.68±90.09^{e}	2,492.13±36.42^{f}
Bound Form (μg/g)	Daidzin	77.18±1.07a	68.43±0.64b	61.76±0.05c	64.44±0.50c	52.06±0.17d	43.13±1.00e	41.64±2.74e
	Glycitein	11.23±0.69a	10.48±1.98a	14.01±2.75a	10.71±2.83a	8.87±2.17ab	10.49±1.05a	3.69±1.63b
	genistin	221.1±1.89a	209.8±1.86b	213.48±0.64b	200.21±3.68c	178.10±0.59d	163.38±4.16e	128.5±1.20f
	Total isoflavones	309.51±1.50a	288.72±2.91b	289.25±3.44b	275.36±7.02b	239.03±2.94c	217.00±6.21d	173.83±5.58e
Total (μg/g)	Daidzin	232.59±23.06^{c}	326.9±16.59^{b}	353.63±21.18^{b}	322.56±46.87^{b}	416.18±23.79^{a}	210.27±2.97^{cd}	166.73±1.94^{d}
	Glycitin	31.94±8.68^{c}	54.48±4.95^{ab}	51.12±2.52^{b}	46.42±2.91^{b}	64.88±3.42^{a}	22.64±5.33^{cd}	13.72±4.50^{d}
	Genistin	892.61±82.27^{d}	1,113.24±106.10^{ab}	1,041±36.10^{bc}	903.22±58.94^{cd}	1,251.85±63.67^{a}	613.42±63.26^{e}	489.52±11.96^{e}
	Malonyldaidzin	465.73±6.96^{b}	521.64±7.42^{a}	399.35±18.35^{c}	356.55±19.94^{d}	399.07±24.21^{c}	256.42±9.80^{d}	116.12±0.24^{e}
	Malnylglycitin	141.70±1.28^{c}	162.71±1.60^{a}	139.81±6.20^{c}	119.40±6.78^{d}	151.25±0.94^{b}	108.61±0.20^{e}	61.28±0.46^{f}
	Malonylgenistin	516.16±0.05^{b}	571.76±0.73^{a}	451.87±2.83^{d}	403.46±1.03^{e}	491.13±0.28^{c}	274.62±0.22^{f}	155.77±0.57^{g}
	Acetyldaidzin	225.61±2.03^{bc}	263.13±5.18^{a}	215.08±2.40^{cd}	207.50±4.77^{d}	231.05±5.49^{b}	145.24±10.66^{e}	93.78±7.35^{f}
	Acetylgenistin	23.47±0.20^{g}	31.86±0.31^{f}	41.82±0.51^{e}	64.60±0.19^{d}	74.69±0.23^{c}	137.16±0.24^{b}	176.64±0.36^{a}
	Daidzein	77.18±1.07a	68.43±0.64b	61.76±0.05c	64.44±0.50c	52.06±0.17d	43.13±1.00e	41.64±2.74e
	Glycitein	11.23±0.69a	10.48±1.98a	14.01±2.75a	10.71±2.83a	8.87±2.17ab	10.49±1.05a	3.69±1.63b
	genistein	332.02±0.62g	374.09±0.13f	441.16±3.88e	572.82±4.87d	614.89±2.03c	997.63±5.70b	1,213.99±1.07a
	Total β-glycosides	1,316.74±127.61^{g}	1,687.01±113.75^{f}	1,633.25±125.69^{e}	1,452.83±156.16^{d}	1,940.43±60.20^{c}	992.39±72.35^{b}	803.04±30.49^{a}
	Total malonylglycosides	1,123.58±6.90^{d}	1,256.11±9.33^{b}	991.03±17.84^{bc}	879.41±26.31^{cd}	1,041.44±26.37^{a}	639.65±11.00^{e}	333.17±0.24^{f}
	Total acetylglycosides	249.07±3.62^{b}	294.98±4.97^{a}	256.90±4.31^{d}	272.10±5.99^{e}	305.74±6.13^{c}	282.4±10.46^{f}	270.43±7.45^{g}
	Total aglycones	420.42±0.24g	453.00±1.61f	516.93±6.69e	647.97±8.21d	675.83±4.38c	1,051.25±7.75b	1,259.32±3.30a
	Total isoflavones	2,800.31±135.3de	3,402.39±91.25b	3,108.85±42.77c	2,976.95±141.33cd	3,724.4±65.45a	2,748.68±67.05e	2,492.13±12.19f

[1)]Values are mean±SD of 3 replicates. Different superscripts in the same row indicate a significant difference (p<0.05) among different germination periods.

Isoflavones The effect of germination period on the free and bound isoflavone contents of these four groups and 12 distinct forms in black soybean are shown in Table 3. The total free isoflavone contents in germinated black soybean ranged between 2,492.13 and 3,724.40 μg/g. The highest total free isoflavone content in germinated black soybean (3,724.40 μg/g) was observed after 4 days and was about 133% higher than in non-germinated black soybean. These results are in close agreement with those reported by Xiya *et al*. (31), which showed that the total isoflavone contents in soybean increased during the germination process. In contrast, the total bound isoflavone contents substantially decreased by about 309.51-173.83 μg/g as the germination period increased. These results implied that some of the bound isoflavones had been converted into their free forms during germination. Changes in the individual amounts of isoflavones in the four groups and 12 distinct forms were monitored over the 6-day germination period (Table 3). β-Glycoside and acetylglycoside levels increased during the initial germination period, but then decreased considerably during the final germination period. Both total β-glycosides and total acetyl glycoside reached their peak levels at 4 days (1,940.43 and 305.74 μg/g, respectively). A study by Fengzhong *et al.* (32) into the increase in isoflavone content during the germination of three Chinese soybean cultivars reported similar results, which showed that total β-glycosides rose during the initial period of germination in soybean and that excessive germination periods (more than 3 days) reduced total β-glycoside contents. Total malonylglycoside contents ranged from 337.17 to 1,123.58 μg/g during the germination process and were lowest at 6 days. These reductions in malonylglycoside, β-glycosides, and acetylglycoside contents during the final germination period are related to an increase in the aglycone form of isoflavone. The aglycone form was only detected as genistein, and the contents of the aglycone-type genistein changed significantly among the 12 distinct forms. The free genistein content rose from 110.92 μg/g in raw to 1,085.49 μg/g after 6 days of germination and the percentage contribution of genistein to total isoflavone content increased from 3.96% at day 0 to 43.56% on day 6. According to Kim *et al*. (33), no aglycone isoflavone was detected in the non-germinated seeds of seven soybean cultivars. However, after 120 h of germination, the average aglycone

Table 4. Changes in anthocyanin and anthocyanidin of black bean with germination periods

Germination Periods (day)	Types	Contents of anthocynins and anthocyanidins (g/g)						
		Cyanidin-3-Glusose	Delphinidin-3-Glucose	Petunidin-3-Glucose	Cyanidin	Delphinidin	Total anthocyanin	Total anthocyanidin
0	Free	76.54±4.88[a1)]	39.77±3.5[a]	0.75±0.33[a]	ND	ND	117.06±8.57[a]	ND
	Bound	ND	ND	ND	53.49±0.90[a]	1.93±0.03[ab]	ND	55.43±0.92[a]
	Total (Free+Bound)	76.54±4.88[a]	39.77±3.5[a]	0.75±0.33[a]	53.49±0.90[a]	1.93±0.03[ab]	117.06±8.57[a]	55.43±0.92[a]
1	Free	65.67±0.8[b]	38.57±3.61[a]	0.8±0.15[a]	ND	ND	105.04±4.51[b]	ND
	Bound	ND	ND	ND	35.94±0.03[b]	1.94±0.01[ab]	ND	37.88±0.03[b]
	Total (Free+Bound)	65.67±0.8[b]	38.57±3.61[a]	0.8±0.15[a]	35.94±0.03[b]	1.94±0.01[ab]	105.04±4.51[b]	37.88±0.03[b]
2	Free	46.6±0.71[c]	32.72±2.28[b]	ND	ND	ND	79.32±1.79[c]	ND
	Bound	ND	ND	ND	28.83±0.18[c]	1.85±0.13[bc]	ND	30.67±0.05[c]
	Total (Free+Bound)	46.6±0.71[c]	32.72±2.28[b]	ND	28.83±0.18[c]	1.85±0.13[bc]	79.32±1.79[c]	30.67±0.05[c]
3	Free	29.5±0.88[d]	32.42±3.21[b]	ND	ND	ND	61.91±3.89[d]	ND
	Bound	ND	ND	ND	26.9±0.20[d]	2.22±0.03[a]	ND	29.12±0.23[d]
	Total (Free+Bound)	29.5±0.88[d]	32.42±3.21[b]	ND	26.9±0.20[d]	2.22±0.03[a]	61.91±3.89[d]	29.12±0.23[d]
4	Free	27.35±5.67[d]	30.02±2.63[bc]	ND	ND	ND	57.37±7.86[d]	ND
	Bound	ND	ND	ND	18.92±0.08[e]	1.69±0.07[c]	ND	20.61±0.15[f]
	Total (Free+Bound)	27.35±5.67[d]	30.02±2.63[bc]	ND	18.92±0.08[e]	1.69±0.07[c]	57.37±7.86[d]	20.61±0.15[f]
5	Free	11.85±3.83[e]	26.58±1.44[cd]	ND	ND	ND	38.43±5.26[e]	ND
	Bound	ND	ND	ND	19.98±0.03[f]	2.22±0.04[a]	ND	22.2±0.08[e]
	Total (Free+Bound)	11.85±3.83[e]	26.58±1.44[cd]	ND	19.98±0.03[f]	2.22±0.04[a]	ND	22.2±0.08[e]
6	Free	10.22±2.43[e]	23.91±0.78[d]	ND	ND	ND	34.12±1.87[e]	ND
	Bound	ND	ND	ND	15.15±0.09[g]	1.62±0.21[c]	ND	16.77±0.12[g]
	Total (Free+Bound)	10.22±2.43[e]	23.91±0.78[d]	ND	15.15±0.09[g]	1.62±0.21[c]	34.12±1.87[e]	16.77±0.12[g]

[1)]Values are mean±SD of 3 replicates. Different superscripts in the same row indicate a significant difference (p<0.05) among different germination periods.

content was 0.558 and 0.383 mol/g, respectively. These results indicated that isoflavones were not only produced by biosynthetic pathways, but were also produced by metabolizing glycosides to aglycone during germination through the activation of β-glycosidase in soybean. Furthermore, certain types of malonyl and acetyl esterase can also be induced and expressed during the germination process (34).

Anthocyanin and anthocyanidin The changes in the free and bound forms of the total anthocyanin and anthocyanidin profiles during germination are shown in Table 4. Some of the anthocyanins and anthocyanidins identified, which included cyanidin-3-glucose, delphinidin-3-glucose, petunidin-3-glucose, cyanidin, and delphinidin. The total anthocyanin and anthocyanidin contents of black soybean decreased during germination. Table 4 shows that the only free forms detected were anthocyanins. The contents of two different anthocyanins, i.e., cyanidin-3-glucose and delphinidin-3-glucose, linearly decreased as the germination period increased, and their contents ranged from 10.22 to 76.52 μg/g, and 23.91 to 39.77 μg/g, respectively. The bound anthocyanidin forms detected were produced by acid-hydrolysis, and the cyanidin, and delphinidin contents decreased from 53.49 and 1.93 μg/g to 15.15 and 1.62 μg/g after 6 days germination, respectively. Anthocyanins are water soluble, polar molecules, so the solvents used in most extractions are aqueous mixtures that are less stable than liposoluble pigments, such as carotenoids (35). Furthermore, the enzymes present in plant tissues, such as anthocyanase (anthocyanin-β-glucosidase) and polyphenoloxidase, could play an important role in the degradation of anthocyanins (35). Therefore, these results suggested that the decrease in anthocyanin and anthocyanidin are due to hydration or loss of water molecules during soaking and germination or enzymatic activation of anthocyanase (anthocyanin-β-glucosidase) and polyphenoloxidase during the germination process.

In this study, it has been suggested that germination is an efficient way of increasing the free functional components of legumes and promotes the utilization of the bound functional components of black soybean. Also, this study are investigated that the transition between the free and the bound forms of polyphenols, flavonoids, phenolic acids, isoflavones, and anthocyanins was investigated at different points during the germination process to clarify the cause of their increased functionality during the germination process. Free functional components, such as total polyphenol, total flavonoid, phenolic acids, and isoflavones, significantly increased during germination. Bound functional components substantially decreased as the germination period increased (1–6 days). These results suggest that some bound forms have been converted into extractable free forms during the germination process. The rates of increase or decrease (%) in free and bound forms, and the functional profiles of compounds, such as phenolic acids and isoflavones, suggest that the cause of the increase in functional component contents during germination could have other explanations apart from the liberation of bound forms. In conclusion, the germination effects on functional compounds are based on biosynthesis or conversion to essential secondary metabolites that are needed for plant growth.

Acknowledgment This research was supported by National Academy of Agricultural Science in Rural Development Administration (PJ0109132015), South Korea.

Disclosure The authors declare no conflict of interest.

References

1. Anderson JW, Major AW. Pulses and lipaemia, short and long-term effect: Potential in the prevention of cardiovascular disease. Brit. J. Nutr. 3: 263–271 (2002)
2. AstadiIR, Astuti M, SantosoU, Nugraheni PS. *In vitro* antioxidant activity of anthocyanins of black soybean seed coat in human low density lipoprotein (LDL). Food Chem. 112: 659–663 (2009)
3. Takahashi R, Wakabayashi K. Antioxidant activities of black and yellow soybeans against low density lipoprotein oxidation. J. Agr. Food Chem. 53: 4578–4582 (2005)
4. Correa CR, Li L, Aldini G, Carimi M, Chen CYO, Chun HY. Composition and stability of phytochemicals in five varieties of black soybeans (*Glycine max*). Food Chem. 123: 1176–1184 (2010)
5. Subba Rao MV, Muralikrishna G. Evaluation of the antioxidant properties of free and bound phenolic acids from native and malted finger millet (ragi, Eleusinecoracana Indaf-15). J. Agr. Food Chem. 50: 889–892 (2002)
6. Yao S, Yang T, Zhao L, Xiong S. The variation of c-aminobutyric acid content in germinated brown rice among different cultivars. Sci. Agric. Sinica 41: 3974–3982 (2008)
7. Nardini M, Ghiselli A. Determination of free and bound phenolic acids in beer. Food Chem. 84: 137–143 (2004)
8. Wong DWS. Feruloyl esterase: A key enzyme in biomass degradation. Appl. Biochem. Biotech. 133: 87–112 (2006)
9. Chandrasekara A, Shahidi F. Bioactivities and antiradical properties of millet grains and hulls. J. Agr. Food Chem. 59: 9563–9571 (2011)
10. Beatriz A, Acostaa E, Janet AG, Sergio OS. Bound phenolics in foods, a review. Food Chem. 152: 46-55 (2014)
11. Ana L, Tarek E, Montserrat D, Teresa O, Isabe E, Teresa H, Pilar G, Olga P, Emilia C. Effect of cooking and germination on phenolic composition and biological properties of dark beans (*Phaseolus vulgaris* L.). Food Chem. 138: 547-555 (2013)
12. Seo MC, Ko JY, Song SB. Antioxidant compounds and activities of foxtail millet, 93 proso millet and sorghum with different pulverizing methods. J. Korean Soc. Food Sci. Nutr. 40: 790-797 (2011)
13. Jung KH, Hong HD, Cho CW. Phenolic acid composition and antioxidative activity of red ginseng prepared by high temperature and high pressure process. Korean J. Food Nutr. 25: 827-832 (2011)
14. Zielinski H, Kozlowska H, Lewczuk B. Bioactive compounds in the cereal grains before and after hydrothermal processing. Innov. Food. Sci. Emerg. 2: 159–169 (2001)
15. Dewanto V, Wu X, Liu RH. Processed sweet corn has higher antioxidant activity. J. Agr. Food Chem. 50: 4959-4964 (2002)
16. Jia Z, Tang M, Wu J. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64: 555-559 (1999)
17. Wang YI, Sheen LY, Chou CC. Storage effects on the content of anthocyanin, mutagenicity, and antimutagenicity of black soybean koji. LWT-Food Sci. Technol. 43: 702–707 (2010)
18. Lee SJ, Kim JJ, Moon HI, Ahn JK, Chun SC, Jung WS, Lee OK, Chung IM. Analysis of isoflavones and phenolic compounds in Korean soybean [*Glycine max* (L.) Merrill] seeds of different seed weights. J. Agr. Food. Chem. 56: 2751–2758 (2008)
19. Glenda AP, Julian CR, Jose JP, Ana MG. Natural occurrence of free anthocyanin aglycones in beans (*Phaseolus vulgaris* L.). Food Chem. 94: 448-456 (2006)
20. Gusti MM, Wrolstad R. Characterization of red radish anthocyaninins. J. Food Sci. 61: 322–326 (1996)
21. Choung MH. Optimal HPLC condition for simultaneous determination of anthocyanins in black soybean seed coats. Korean J. Crop. Sci. 54: 359-468 (2008).
22. Lin PY, Lai HM. Bioactive compounds in legumes and their germinated

products. J. Agr. Food. Chem. 54: 3807–3814 (2006)
23. Naczk M, Shahidi F. The effect of methanol–ammonia–water treatment on the content of phenolic acids of canola. Food Chem. 31: 159–164 (1989)
24. Huihui T, Ruifen Z, Mingwei Z, Qing L, Zhencheng W. Dynamic changes in the free and bound phenolic compounds and antioxidant activity of brown rice at different germination stages. Food Chem. 161: 337–344 (2014)
25. Gross GG. Biosynthesis and metabolism of phenolic acids and monolignols. pp. 229–271. In: Biosynthesis and Biodegradation of Wood Components. Higuchi T (ed). Elsevier, Amsterdam, Netherlands (1985)
26. Lise B, Charles D, Jean-Francois C. Knockout of the *p*-coumarate decarboxylase gene from *Lactobacillus plantarum* reveals the existence of two other inducible enzymatic activities involved in phenolic acid metabolism. Am. Soc. Microb. J. 66: 3368-3375 (2002)
27. Puri M, Banerjee U. Production, purification, and characterization of the debittering enzyme naringinase. Biotechnol. Adv. 18: 207–217 (2000)
28. Mattila P, Pihlava JM, Hellström J. Contents of phenolic acids, alkyl- and alkenylresorcinols, and avenanthramides in commercial grain products. J. Agr. Food. Chem. 53: 8290-8295 (2005)
29. Temple NJ. Antioxidants and disease: More questions than answers. Nutr. Res. 20: 449-459 (2000)
30. Charlton AJ, Baxter NJ, Lilley TH, Haslam E, McDonald CJ, Williamson MP. Tannin interactions with a full-length human salivary proline-rich protein display a stronger affinity than with single proline-rich repeats. FEBS Lett. 382: 289–292 (1996)
31. Xiya H, Weixi C, Baojun X. Kinetic changes of nutrients and antioxidant capacities of germinated soybean (*Glycine max* L.) and mung bean (*Vigna radiata* L.) with germination time. Food Chem. 143: 268–276 (2014)
32. Fengzhong W, Hifang W, Conghui W, Fang F, Jixiang L, Tao W, Rong T. Isoflavone, γ-aminobutyric acid contents and antioxidant activities are significantly increased during germination of three Chinese soybean cultivars. J. Funct. Foods 14: 596–604 (2015)
33. Kim JS, Kim JG, Kim WJ. Changes in isoflavone and oligosaccharides of soybeans during germination. Korean J. Food Sci. Technol. 36: 294–298 (2004)
34. Hendrich S. Bioavailability of isoflavones. J. Chromatogr. B 777: 203–210 (2002)
35. Long-Ze L, James MH, Marcia SP, Devanand LL. The polyphenolic profiles of common bean (*Phaseolus vulgaris* L.). Food Chem. 107: 399–410 (2008)