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ORIGINAL ARTICLE

Changes in Phenolic Compounds (Isoflavones and Phenolic acids) and Antioxidant Properties in High-Protein Soybean (*Glycine max* L., cv. Saedanbaek) for Different Roasting Conditions

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Abstract Contents of phenolic compound including isoflavones and phenolic acids as well as antioxidant effects in high-protein soybean cultivar "*Saedanbaek*" were evaluated under different roasting conditions. The roasted soybean exhibited significantly higher antioxidant activity than unroasted soybean in the three antioxidant methods including 2,2-diphenyl-1-picrylhydrazyl, 2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid), and Ferric reducing antioxidant power. In particular, the roasted soybean at 200°C for 15 min showed the highest antioxidant activity in comparison with other conditions. The contents of phenolic compounds, isoflavone aglycones (genistein, daidzein, and glycitein), isoflavone β-glucosides (genistin, daidzin, and glycitin), and phenolic acids increased, whereas isoflavone malonyl-β-glucosides decreased during roasting process. Moreover, total phenolic and flavonoid contents as well as those of isoflavone aglycones, isoflavone-β-glucosides, and phenolic acids increased, leading to a general increase in antioxidant activity after roasting. These results suggest that the roasting soybean extracts could contribute to obtaining natural antioxidants in certain food applications.

Keywords antioxidant activity · high-protein soybean · isoflavone · phenolic acid · roasting

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Introduction

Soybeans have been consumed as an important protein source to complement grain protein in Asian countries for a long time. In addition to protein, they contain various nutritious and functional components such as isoflavones, anthocyanins, phytic acids, saponins, phenolic acids, lipids, and oligosaccharides (Kwak et al., 2007; Lee et al., 2008; Cho et al., 2011; Lee and Cho, 2012). In raw soybeans, isoflavones are present in four chemical forms: malonyl-β-glucosides (70–80%), acetyl-β-glucosides (5%), β-glucosides (25%), and aglycones (2%) (Cho et al., 2009; Lee and Lee, 2009). Several researchers have reported that isoflavones possess both antioxidant activity and metal ion-chelating properties (Kao and Chen, 2006). In addition, other studies have examined, with the exception of isoflavones, phenolic acids in soybean and soybean-based products from a physiological viewpoint (Cho et al., 2009, Cho et al., 2011; Chung et al., 2011; Kim et al., 2011).

In food processing, roasting creates both desirable and undesirable changes in the physical, chemical, and nutritional properties of seeds (Durmz and Gökmen, 2011; Youn and Chung, 2012). This processing has been used to deactivate antinutritional

components in soybeans and to give a characteristic flavor and brown color to the final products (Im et al., 1995). Conventional thermal treatment changes malonyl derivatives into glucosides via intra-conversion, whereas aglycones have a higher heat resistance (Xu et al., 2002; Shimoni, 2004). There have been only a few reports in the literature regarding changes in isoflavones and antioxidant activities caused by roasting method (Lee and Lee, 2009; Kim et al., 2011), thus little information is available on the variations of phytochemicals including isoflavones and phenolic acids and antioxidant effects on high-protein soybean, a kind of specific cultivar in different roasting conditions (Lee and Lee, 2009; Kim et al., 2011).

The main purposes of the present research were to investigate changes in phenolic compounds (isoflavones and phenolic acids) and antioxidant effects via (2,2-diphenyl-1-picrylhydrazyl, 2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid), ferric reducing antioxidant power assays) in high-protein soybean (HPS) cultivar under various roasting conditions. Moreover, variations of total phenolic and flavonoid contents as well as mallard reaction products during roasting processes were evaluated.

Materials and Methods

Plant material and chemicals. HPS cultivar "*Saedanbaek*", provided by the National Institute of Crop Science of the Rural Development Administration in Korea, was harvested in 2011. Three isoflavone-aglycones, namely, daidzein, genistein, and glycitein, were obtained from Sigma-Aldrich Chemical Co. (USA). Three isoflavone-glycosides, namely, genistin, daidzin, and glycitin, were purchased from Indofine (USA). Three malonyl- and three acetyl-isoflavone-glycosides (malonylgenistin, malonylglycitin, malonyldaidzin, acetylgenistin, acetylglycitin, and acetyldaidzin) were purchased from LC Laboratories (USA). Eleven phenolic acids, namely, gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, chlorogenic, caffeic, *p*-coumaric, ferulic, *t*-cinammic, gentisic, and sinapic acids, were supplied by Sigma-Aldrich Chemical Co. HPLC-grade H_2O, methanol, and acetonitrile were purchased from Fisher Scientific (USA). Glacial acetic acid, Folin-Cicalteu phenol reagent, DPPH, ABTS, potassium persulfate, ferric chloride, sodium acetate, 2,4,6-tripyridyl-s-triazine (TPTZ), and rutin were obtained from Sigma-Aldrich Chemical Co.. All other reagents were of analytical grade.

Instruments. Roasting was performed using a coffee roaster (Gene Café, Genesis Co. Ltd., Korea). UV spectra were obtained using a Spectronic 2D spectrophotometer (Thermo Electron Co.,). High pressure liquid chromatography (HPLC) was performed using an Agilent 1200 series (Agilent Co., Australia) equipped with a quaternary HPLC pump, a degasser, and an Agilent 1200 series diode array detector. Isoflavones were analyzed on a LiChrospher 100 RP C18 column (4.6 × 150 mm, 5 μm; Merck, Germany). Phenolic acids were analyzed on an XTerra™ RP C8 column (4.6 × 250 mm, 5 μm, Waters Corp., USA).

Roasting. Soybean samples (50 g) were placed in the drum of a coffee roaster and roasted with hot air. The roasting temperatures and times were modified from that of the previous study (Kim et al., 2011). Three replicates each of seven different treatments were performed: unroasted and roasted at 160, 180, and 200°C for 5, 10, and 15 min, respectively. The unroasted HPS consisted of whole beans without any thermal treatment. The unroasted and roasted samples were ground to a powder and stored at −70°C before analysis.

DPPH radical-scavenging activity. The DPPH radical-scavenging activities of the samples were evaluated using the method of Blois (1958) with a slight modification. The ground soybean seeds (1 g, 60-mesh) were extracted with 50% methanol for 24 h at room temperature. The extract of soybean seeds were filtered through Whatman No. 42 filter paper, and the supernatants (0.2 mL) were examined for DPPH radical-scavenging effects of nine different concentrations including 4000, 2000, 1000, 500, 200, 100, 50, 20, and 10 μg/mL. Briefly, various concentrations of each sample extract (0.2 mL) were mixed with 0.8 mL of 1.5×10^{-4} mM DPPH solution in methanol. The mixture was vortexed and allowed to stand for 30 min at room temperature in darkness. The absorbance of the reaction mixture was determined using a spectrophotometer at 517 nm. This effect was expressed as a percentage as follows: DPPH radical-scavenging activity (%)=(1 −absorbance of sample/absorbance of control) × 100.

ABTS radical-scavenging activity. $ABTS^{\bullet+}$ (7 mM) was dissolved in methanol. This radical cation was produced by reacting the $ABTS^{\bullet+}$ stock solution with 2.45 mM potassium persulfate and allowing the mixture to stand for 12–16 h until the reaction was complete. The $ABTS^{\bullet+}$ stock solution was diluted in ethanol to an absorbance of 0.7±0.02 at 734 nm. After the addition of 0.9 mL of diluted $ABTS^{\bullet+}$ to 0.1 mL of sample (Preparation of sample: see section DPPH radical-scavenging activity), the absorbance was recorded for 3 min after the initial mixing (Choi et al., 2012). This effect was expressed as a percentage using the following formula: ABTS-scavenging activity (%)=(1−absorbance of sample/absorbance of control)×100.

Ferric reducing/antioxidant power (FRAP) assay. The FRAP assay developed by Choi et al. (2012) was used. Briefly, 1.5 mL of working, pre-warmed 37°C FRAP reagent [300 mM acetate buffer (pH 3.6) : 10 mM TPTZ in 40 mM HCl : 20 mM $FeCl_3$ = 10:1:1 (v/v/v)] was mixed with 50 ìL of samples (Preparation of sample: see section DPPH radical-scavenging activity). This mixture was vortexed, and the absorbance at 593 nm was read against a blank reagent at a pre-determined time after mixing of the sample-reagent. The test was performed at 37°C, and 0–4 min reaction time window was used.

Total phenolic contents (TPCs). A method of Cho et al. (2011) was used with slight modifications to quantify the TPCs of 50% methanol extract, which was based on gallic acid equivalents (GAE). Six gallic acid standard solutions of 100, 250, 500, 750, and 1000 mg/L were prepared in deionized water, of which 500 μL was collected and mixed with 250 μL of 2 N Folin-

Ciocalteu reagents. After mixing, the mixture to was allowed to stand at room temperature for 3 min, followed by the addition of a 500-μL aliquot of 25% sodium carbonate (Na_2CO_3) solution, and the mixture was allowed to stand at room temperature for 1 h. The absorbance of the solution was measured at 750 nm (DU650; Beckman Coulter, USA), and a standard curve was obtained by plotting concentration against absorbance. Similarly, a 500-μL aliquot of phytochemical extraction solution was collected and prepared using the same procedure as described above, and quantification was performed using the linear regression equation of the gallic acid standard curve. The results were expressed as mg gallic acid equivalents (GAE/g) dried HPS.

Total flavonoid contents (TFCs). A method based on that of Lee et al. (2011) was used to quantify the TFCs of 50% methanol extract, which was denoted as rutin equivalents (RE). The diluted extract (1 mL) was added to a test tube containing 7 mL of methanol. After the addition of 2 mL of 90% diethylene glycol, the reaction was initiated by adding 0.1 mL of 4 M NaOH. After heating for 30 min at 50°C, the reaction mixture was incubated at room temperature for 30 min. The absorbance of the solution was measured at 420 nm (DU650; Beckman Coulter, USA). Total flavonoid content was determined using a standard curve of rutin (0, 10, 25, and 50 mg/L) and expressed as RE/mg dried HPS.

Maillard reaction products (MRPs). The MRP content was evaluated using the non-enzymatic browning measurement (Kim et al., 2011). Each extract was diluted 1:100 with HPLC-grade water to yield absorbance signals within the scale of the detector. The samples were allowed to stand for 1 h and filtered using a 0.45-μm filter prior to measuring the optical density using a spectrophotometer at 420 nm (DU650; Beckman Coulter, USA).

Preparation of calibration curves of isoflavone and phenolic acid. The peak areas of isoflavones and phenolic acids were integrated with the HPLC chromatograms at 254 and 280 nm by Agilent 1200 series (Germany). The stock solutions were dissolved in DMSO (isoflavone) and methanol (phenolic acid) to obtain a 1 mg/mL concentration. Each calibration curve was made with seven concentrations including 1, 5, 10, 20, 50, 70, and 100 μg/mL. The values of correlation coefficient (r^2) of all curves were higher than 0.998. Isoflavones and phenolic acids in samples were evaluated based on their retention times, and their contents were calculated from the calibration curves.

Isoflavone analysis. The isoflavone analysis procedure was from a previous study (Cho et al., 2011). Briefly, soybean seeds were pulverized for 3 min by a HR2860 coffee grinder (Philips, Netherlands). The ground soybean seeds (1.0 g, 60 mesh) were extracted using 50% methanol (20 mL) for 6 h at room temperature in a shaking incubator. The supernatant was centrifuged at 3000 g for 5 min and then filtered through a 0.45-μM syringe filter (Whatman, Inc., UK). A sample (20 μL) of the crude methanol extract was injected into an analytical C_{18} column, and the column temperature was set to 30°C. Isoflavones were detected by monitoring the elution at 254 nm using a diode array detector. The mobile phase was composed of 0.1% glacial acetic acid in water (solution A) and 100% acetonitrile (solution B). The gradient conditions were as follows: 0–20 min, 10% B; 30 min, 20% B; 40 min, 25% B; 50 min, 35% B. The solvent flow rate was maintained at 1 mL/min.

Phenolic acid analysis. The phenolic acid contents were measured by the method of Cho et al. (2012), using 50% methanol extract of each sample for phenolic acid content as in isoflavone analysis. A sample extract (20 μL) was injected into an analytical reverse phase C18 column of HPLC. The mobile phase was composed of 0.5% acetic acid in water (solution A) and methanol (solution B). The gradient program was as follows: 0% B to 10% B (5 min), 10% B to 20% B (10 min), 20% B to 30% B (10 min), 30% B to 40% B (20 min), 40% B to 60% B (10 min), 60% B to 80% B (5 min), 80% B to 100% B (5 min). The gradient was then maintained for 10 min before returning to the initial conditions. The column temperature was set to 30°C, and the flow rate was 1.0 mL/min. The total retention time was 60 min, and the detection was performed at 280 nm.

Statistical analysis. Data were expressed as the means±SD (standard deviation) of three replicates. The results were subjected to analysis of variance using Microsoft Excel 2007 to analyze differences.

Results and Discussion

Comparison of antioxidant activities by roasting. Several researches have revealed that various crops, fruits, and vegetables showed high antioxidant activities after roasting process (Dewanto et al., 2002; Yang et al., 2009; Gallegos-Infante et al., 2010; Locatelli et al., 2010; Chandrasekara and Shahidi, 2011; Kim et al., 2011; Durmaz and Gökmen, 2011 Choi et al., 2012). In addition, various methods have been used to determine the antioxidant effects of crops, foods and plant products (John and Shahidi, 2010; Lee et al., 2011; Alam et al., 2013). The present study used three different methods to evaluate the antioxidant effects of unroasted and roasted HPSs: DPPH radical-scavenging activity, ABTS radical-scavenging activity, and FRAP assays. Even though ordinary soybeans are consumed in several types of foods through processing method such as roasting (Lee and Lee, 2009; Kim et al., 2011), little information has been revealed on the phytochemical contents and antioxidant effects concerning special cultivar such as HPS. In other words, an improved understanding of phytochemicals and antioxidant properties from HPS may aid in their further use as food and in pharmaceutical industries. Therefore, this work documented for the first time comparison of phytochemical contents and antioxidant activities using the roasted and unroasted soybeans. In the present study, extracts of the roasted HPSs exhibited higher antioxidant activities than the unroasted ones (Fig. 1). The DPPH radical-scavenging activities of the unroasted HPS increased with increasing concentrations (>100% at 4000 μg/mL, 73.40% at 2000 μg/mL, 29.95% at 1000 μg/mL, and 16.73% at 500 μg/mL). On the other hand, roasted

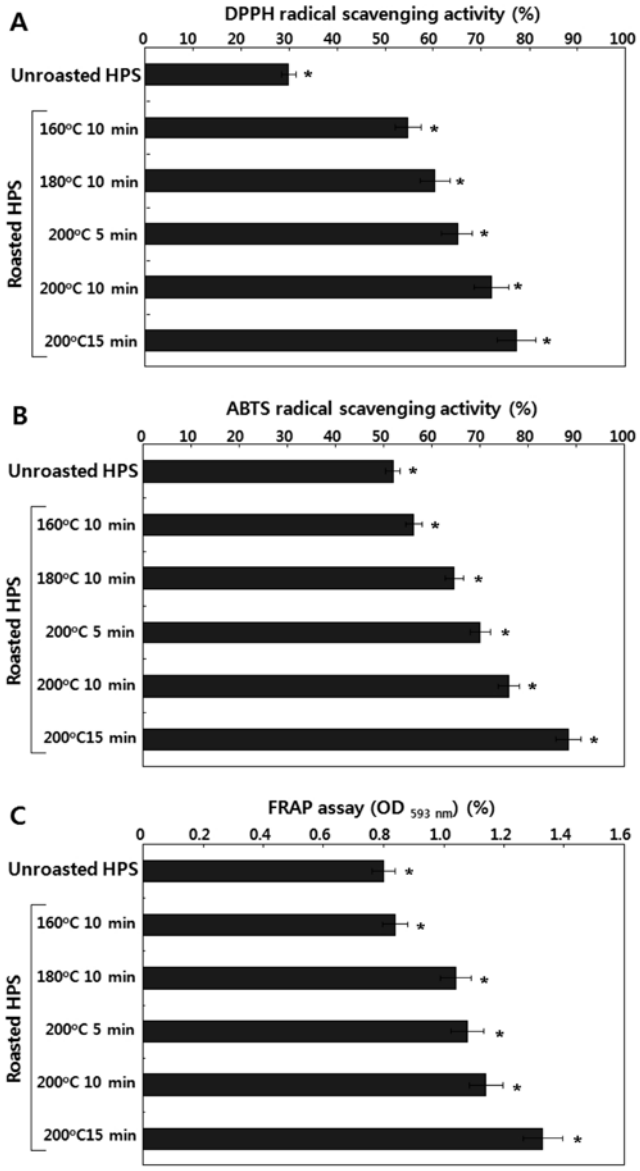


Fig. 1 Comparison of antioxidant activities through roasting conditions from the 50% methanol extract of HPS (1 mg/mL). (A) DPPH-radical scavenging activity, (B) ABTS-radical scavenging activity, and (C) FRAP assay. Each value in the table is represented as mean ± SD (n =3). Values were considered significantly different at p <0.05.

HPS exhibited higher radical-scavenging effects when compared to the unroasted sample. Thus, for accurate comparision of antioxidant effects regarding the unroasted and roasted HPS, we evaluated the 50% methanol extracts at a concentration of 1000 μg/mL (Fig. 1). Unroasted HPS showed a DPPH radical-scavenging activity of 29.95% at 1 mg/mL, whereas the 160, 180, and 200°C roasted HPSs showed activities of 54.80, 60.39, and 72.14%, respectively. After roasting HPS at 200°C for 5, 10, and 15 min, the DPPH radical-scavenging activities at 1000 μg/mL were shown to be 65.01, 72.14, and 77.37% (Fig. 1A). For determination of the hydrogen-donating antioxidants and chain breaking antioxidants, we measured the ABTS radical-scavenging ability of soybean. The ABTS radical activity of unroasted soybean at 1000 μg/mL was 51.94%, whereas those roasted at 160, 180, and 200°C for 10 min were 56.31, 64.68, and 76.10% (increases of 7.76, 19.70, and 31.75%), respectively. After roasting at 200°C for 5, 10, and 15 min, 70.10, 76.10, and 88.46% ABTS radical activities were shown, respectively, (Fig. 1B). The FRAP assay is a direct test of total antioxidant power. This assay measures the reducing potential of an antioxidant reacting with a Fe^{3+}-TPTZ complex and producing a colored Fe^{2+}-TPTZ. The intensity of the color is related to the amount of antioxidant reductants in the extracts (Choi et al., 2012). Unroasted soybean produced a FRAP assay value of 0.80 at 1000 μg/mL, whereas the roasted HPSs at 160, 180, and 200°C for 10 min yielded values of 0.84, 1.04, and 1.14, respectively, corresponding to increases of 4.76, 23.08, and 29.82% relative to the unroasted soybean. After roasting at 200°C for 5, 10, and 15 min, FRAP assays at 1000 μg/mL yielded values of 1.02, 1.14, and 1.33, respectively, corresponding to increases of 21.57, 29.82, and 39.85% relative to the unroasted HPS (Fig. 1C). Based on our results, the scavenging effects against DPPH radical were observed with the highest variations in comparison with ABTS radical-scavenging and FRAP methods. Especially, the activities of DPPH assay showed significant differences between unroasted HPS and roasted HPS at 160°C from various roasting conditions. Our work indicates that the 50% methanol extracts of unroasted and roasted soybean samples are a potent source of antioxidants. Moreover, these results showed that soybean antioxidant activities increased with roasting time. In particular, the roasted HPS at 200°C for 15 min had significantly highest antioxidant activities than those of other extracts. In three antioxidant methods, the roasted HPS extracts showed significantly higher antioxidant activity than unroasted HPS. It is well documented that isoflavones, anthocyanins, phenolic compounds, carotenoids, and tocopherols in soybean may contribute to the various health benefits including antioxidant effects (Malencic et al., 2007). Roasting is a thermal processing method regarding dry heat treatment and causes phytochemicals to degrade (Oliviero et al., 2009). In addition, this method increased antioxidant property during the roast process due to Maillard reaction products (Lee and Lee, 2009). Therefore, the roasted soybeans may have higher activities than those of the unroasted samples. The maximum radical-scavenging ability in the ABTS assay was higher than that of the DPPH assay 77%, indicating that the ABTS assay was more sensitive to our sample contents than the DPPH assay. This higher sensitivity could be attributed to the fact that the ABTS radical dissolves in aqueous solution, such as our samples, unlike DPPH, which must be dissolved in organic solvents (Singh and Singh, 2008; Kim et al., 2011). In addition to the solvent effect, the different activities of ingredients from soybean, isoflavones, and phenolic compounds, to DPPH and ABTS assays might contribute to different radical scavenging activities (Kim et al., 2011). Furthermore, this phenomenon may be significantly

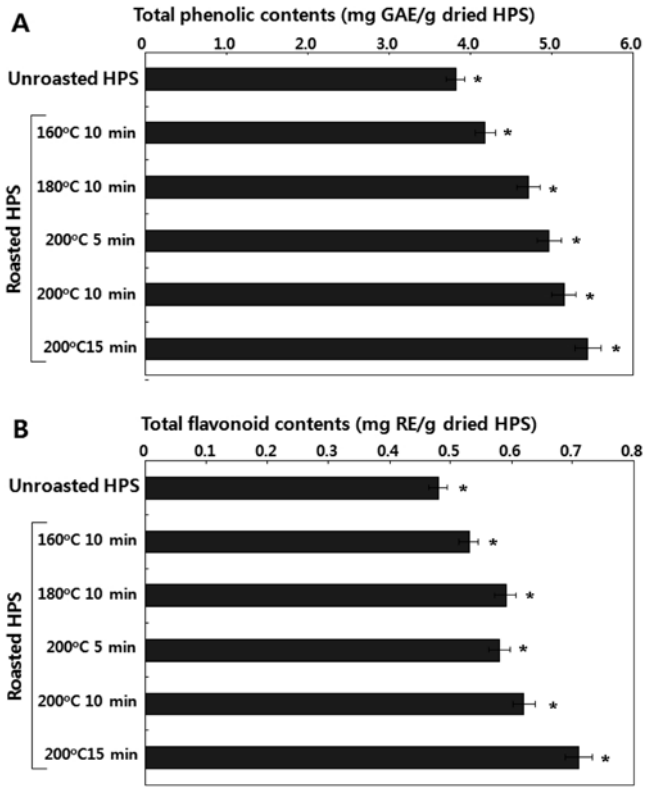


Fig. 2 Comparisons of TPC and TFC through roasting conditions from the 50% methanol extract of HPS (1 mg/mL). Each value in the table is represented as mean ± SD (n =3). Values were considered significantly different at p <0.05.

influenced by variations of other nutritional components including tocopherol and carotenoid compositions in unroasted and roasted samples as shown by Slavin et al. (2009).

Comparison of TPCs, TFCs, and MRPs by roasting. To evaluate the phenolic and flavonoid contents between unroasted and roasted HPSs, we performed TPC and TFC assays using Folin-Ciocalteu's and diethylene-glycerol reagents. There were considerable differences in the total phenolic and total flavonoid contents during roasting process. In the total phenolic content, unroasted HPS produced 3.82 mg GAE/g dried HPS, whereas the roasted HPSs at 160, 180, and 200°C for 10 min produced 34.18, 4.17, and 5.15 mg GAE/g dried HPS, respectively. Additionally, TPCs in four roasted soybean cultivars at 200°C for 5, 10, and 15 min were 4.97, 5.15, and 5.44 mg GAE/g dried HPS, respectively, corresponding to increases of 23.14, 25.83, and 29.78% relative to unroasted HPS (Fig. 2A). Among total flavonoid contents through various roasting conditions, unroasted HPS yielded a value of 0.48 mg RE/g dried HPS, whereas the roasted HPSs at 160, 180 , and 200°C for 10 min yielded values of 0.53, 0.59, and 0.62 mg RE/g dried HPS, respectively. In addition, the TFCs in HPS roasted at 200°C for 5, 10, and 15 min yielded values of 0.58 0.62, and 0.71 mg RE/g dried HPS, respectively, corresponding to increases of 17.24, 22.58, and 32.39% relative to unroasted HPS (Fig. 2B).

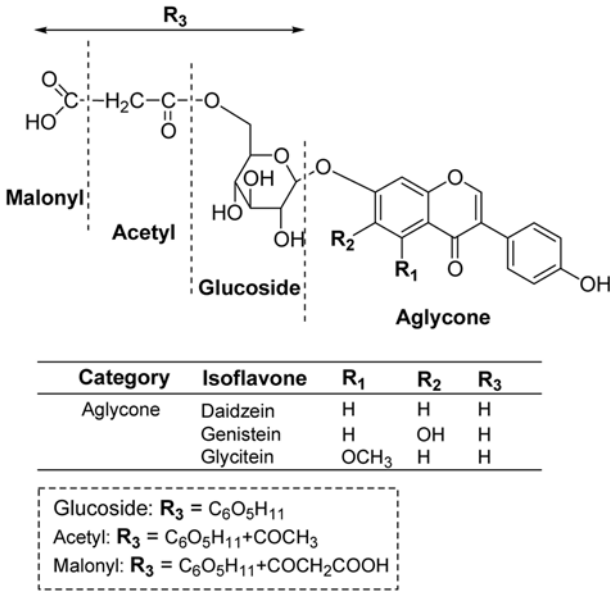
Table 1 Comparison of non-enzymatic browning degrees through roasting conditions from high-protein soybean

Roasting condition		Absorbance at 420 nm
Temperature (°C)	Time (min)	Optical density[a]
160	10	0.30±0.01
180	10	0.55±0.05
200	5	0.95±0.04
200	10	2.28±0.12
200	15	3.54±0.22

[a] Each value in the table is represented as mean ± SD (n =3).
Values were considered significantly different at p <0.05.

Based on the above results, the TPCs and TFCs of the HPS increased with increasing roasting temperature and time. The highest contents of phenolic and flavonoid compounds were obtained with a roasting temperature of 200°C and a roasting time of 15 min. The increased production of phenolics and flavonoids in the present study may be related to the increased generation of Maillard reaction products during roasting (Kim et al., 2011). To evaluate differences in MRPs between the unroasted HPS and roasted HPSs, we measured the non-enzymatic browning of HPS. For HPS roasted at 200°C, the browning degree was approximately three times higher than that of HPS roasted at 160°C. Additionally, the browning degrees increased progressively with prolonged roast processing time (Table 1). MRPs, formed due to exposure to intense heat treatment, are found in the molecular weight range of < 30 kDa and have strong antioxidant properties (Manzocco et al., 2000; Kim et al., 2011). We conducted non-enzymatic browning measurements at 420 nm to evaluate the degree of roasting and MRPs in HPS. As shown by the DPPH, ABTS, and FRAP assays, roast processing at 160, 180, and 200°C for 5, 10, and 15 min enhanced HPS antioxidant activity. Non-enzymatic browning showed the same pattern under the same conditions.

Comparison of isoflavone contents by roasting. It is well established that soybeans are have 12 isoflavone derivatives (Fig. 3), including 3 aglycones (genistein, daidzein, and glycitein) and their glucosides (3 β-glucosides, 3 acetylglucosides, and 3 malonylglucosides) (Cho et al., 2011). Among the four isoflavone groups, acetylglucosides were not observed in cultivar of the present study. This result may be significantly influenced by cultivar, environmental stress, genetic, and agronomic condition as shown in the data of previous studies (Jung et al., 2008; Lee and Choung, 2011; Riedl et al., 2007). Isoflavone contents exhibited remarkable variations at different roasting temperatures and times (Table 2). The malonylglucoside contents decreased from 1,084.6 to 601.0 μg/g, whereas the contents of glucoside and aglycone increased from 224.4 to 1,076.2 μg/g and 17.2 to 598.6 μg/g, respectively, at 160–200°C for 10 min. Moreover, isoflavones in 15 min roasted sample at 200°C decreased in comparison with those of observed in 10 min roasted sample. In other words, the roasted HPSs for 0, 5, 10, and 15 min at 200°C exhibited an increase in glucosides for up to 10 min of roasting and then a



Category	Isoflavone	R_1	R_2	R_3
Aglycone	Daidzein	H	H	H
	Genistein	H	OH	H
	Glycitein	OCH_3	H	H

Glucoside: $R_3 = C_6O_5H_{11}$
Acetyl: $R_3 = C_6O_5H_{11}+COCH_3$
Malonyl: $R_3 = C_6O_5H_{11}+COCH_2COOH$

Fig. 3 Chemical structures of isoflavones in soybean.

slight decrease at 15 min. The total isoflavone contents increased from 1,362.2 to 1,981.5 μg/g after roast processing. In particular, the HPS isoflavone contents such as daidzin, glycitin, genistin, daidzein, and glycitein increased after roasting. Many food researchers are searching for natural sources with high isoflavone contents to develop functional foods and nutraceuticals with benefits to human health. For example, Coward et al. (1998) reported that the total isoflavone content in food is not reduced by normal cooking conditions, whereas food under excessive heating exhibited increased aglycones and decreased total isoflavones. Furthermore, Toda et al. (2000) roasted soybeans for 0, 5, 10, and 20 min at 200°C and reported a significant increase of glucosides for up to 10 min of roasting and a slight decrease at 20 min. Lee and Lee (2009) reported that roasting at 200°C for 7, 14, and 21 min decreased malonyl derivatives, whereas increasing glucoside derivatives. Recently, Lee and Choung (2011) reported that all isoflavone glucosides in soybean were completely converted to their aglycones after 120 min in a drying oven or 50 min in a microwave using an acid hydrolysis condition. Our results suggest that changes in isoflavone contents through roasting process are similar to those of the previous studies mentioned above. In general, the DPPH radical-scavenging activity was not affected by the various isoflavone standards and mixtures of two and four isoflavone standards were more effective in scavenging DPPH free radicals (yielding 5.6 and 10.5% scavenging activities, respectively) (Kao and Chen, 2006). Similarly, many isoflavones were reported to have low scavenging activity for DPPH free

Table 2 Comparison of isoflavone contents through roasting conditions from high-protein soybean.

Isoflavone (μg/g)[a]	Unroasted soybean	Roasted soybean				
		160°C 10 min	180°C 10 min	200°C 5 min	200°C 10 min	200°C 15 min
β-Glucosides						
Daidzin	69.4±1.2	182.3±2.6	170.3±1.0	157.9±0.2	227.7±2.5	210.7±2.6
Glycitin	75.0±1.1	228.9±3.0	353.9±4.0	298.1±3.1	426.6±3.8	385.9±4.1
Genistin	80.0±1.6	374.9±2.9	373.0±3.2	343.9±3.5	421.9±4.0	373.4±3.9
Total	224.4	786.1	897.2	799.9	1,076.2	970.0
Malonyl-β-glucosides						
Malonyldaidzin	417.6±4.2	201.6±2.8	89.8±1.7	119.1±2.0	100.0±1.9	89.2±1.6
Malonylglycitin	95.0±2.0	117.5±2.1	55.6±1.5	50.3±1.6	49.6±1.3	48.1±1.4
Malonylgenistin	572.0±5.0	442.5±4.6	418.4±4.3	419.9±3.9	451.4±4.1	393.4±4.0
Total	1,084.6	761.6	563.8	589.3	60.1	530.7
Acetyl-β-glucosides						
Acetyldaidzin	nd[b]	nd	nd	nd	nd	nd
Acetylglycitin	nd	nd	nd	nd	nd	nd
Acetylgenistin	nd	nd	nd	nd	nd	nd
Total	0	0	0	0	0	0
Aglycones						
Daidzein	4.3±0.4	294.1±3.1	305.9±3.2	338.9±3.9	327.0±3.7	279.3±3.2
Glycitein	5.3±0.5	16.4±0.7	27.5±0.4	21.0±0.3	38.1±0.9	28.6±0.3
Genistein	7.6±0.6	226.0±0.2	223.4±0.1	223.6±0.1	233.5±0.3	218.7±0.1
Total	17.2	536.5	556.8	583.5	598.6	526.6

[a] Each value in the table is represented as mean ± SD (n =3).
[b] nd: not detected (Acetylglucoside isoflavones were not detected in the 50% methanol extracts of the unroasted and roasted HPS.)
Values were considered significantly different at p <0.05.

radicals, with scavenging effects of only half that of α-tocopherol and one-third that of epicatechin (Lee et al., 2005). However, isoflavones have direct free radical quenching ability, with daidzein and genistein being particularly effective (Shon et al., 2007; Cho et al., 2009; Cho et al., 2011). These results suggested that an increase in isoflavone aglycones may be contributed to the enhancement of the antioxidant activity of soybean after roasting.

Comparison of phenolic acid contents by roasting. HPLC analysis showed the changes in the benzoic acid, cinnamic acid, and total phenolic acid contents by roasting temperature and time (Table 3). The HPSs roasted at 200°C for 15 min showed the highest contents of phenolic acid than other conditions. The contents of phenolic acid compounds, such as gallic acid, gentisic acid, protocatechuic acid, *t*-cinnamic acid, and ferulic acid increased after roasting. The highest contents of gallic acid (212.8 μg/g), gentisic acid (18.3 μg/g), *p*-hydroxylbenzoic acid (21.6 μg/g), protocatechuic acid (294.4 μg/g), and vanillic acid (46.5 μg/g) were observed for HPS at 200°C for 15 min. HPS roasted at 200°C for 5 min exhibited the highest *t*-cinnamic acid content (52.0 μg/g). Finally, the ferulic acid content (53.2 μg/g) was highest for HPS roasted at 160°C for 10 min, and unroasted HPS exhibited the highest *p*-coumaric acid (45.2 μg/g) and sinapic acid (15.1 μg/g) contents (Table 3). Phenolic acids are widely distributed in plants and have gained much attention due to their antioxidant activities and free radical scavenging abilities, which have potential benefits on human health (Choi et al., 2012). Gallic acid, protocatechuic acid, *p*-coumaric acid, and ferulic acid are the main components of soybean phenolic acids, and they possess strong antioxidant activity (Cho et al., 2009; Cho et al., 2011). In the present study, the TPC and TFC results as well as each phenolic content showed that the roasted HPSs exhibited higher antioxidant activity than unroasted HPS. We presumed that the increase in phenolic contents was primarily due to the increased release of phytochemicals from the cell matrix as phenolic acids. Thermal processing disrupts the cell membranes and cell walls and releases soluble phenolic contents from the insoluble ester bonds (Kim et al., 2011; Choi et al., 2012). Accordingly, the increase in solubilized phenolic content increased the soybean antioxidant activity during roasting. Previous studies on corn (Dewanto et al., 2002), maize bran (Saulnier et al., 2001), and small black soybean (Kim et al., 2011) reported that increases in solubilized ferulic acid and chlorogenic acid increase the total antioxidant activity; however, significant solubilization occurs only at temperatures above 180°C. Recently, Choi et al. (2012) reported that the increase in the solubilized gallic acid, protocatechuic acid, vanillic acid, and ferulic acid contents from bitter melon increases total antioxidant activity; significant solubilization occurs only at temperature higher than 200°C. These previous reports support our finding that there is a significant increase in antioxidant activity after roasting at 200°C for 5–15 min. These results suggest that the increase in phenolic contents could be one factor that enhances the antioxidant activity of HPS during roasting.

Consequently, the present study has evaluated phenolic compound contents (isoflavones and phenolic acids) and antioxidant properties through roasting process of HPS cultivar "*Saedanbaek*". The contents of isoflavone-β-glucosides, isoflavone aglycones and

Table 3 Comparison of phenolic acid contents through roasting conditions from high-protein soybean.

Phenolic acids (μg/g)[a]	Unroasted soybean	Roasted soybean				
		160°C 10 min	180°C 10 min	200°C 5 min	200°C 10 min	200°C 15 min
Benzoic acids						
Gallic acid	31.3±0.82	96.4±1.59	129.9±2.05	119.2±2.02	180.1±2.56	212.8±3.03
Gentisic acid	9.7±0.35	12.7±0.42	16.7±0.46	10.4±0.39	16.4±0.45	18.3±0.38
p-Hydroxylbenzoic acid	nd[b]	nd	nd	nd	15.5	21.6
Protocatechuic acid	137.3±2.32	173.5±2.88	180.5±3.12	190.2±2.85	209.3±3.22	294.4±3.11
Salicylic acid	nd	nd	nd	nd	nd	nd
Vanillic acid	nd	nd	nd	nd	nd	46.5
Total	178.3	282.6	327.1	319.8	421.3	593.6
Cinnamic acids						
Caffeic acid	nd	nd	nd	nd	nd	nd
Chlorogenic acid	nd	nd	nd	nd	nd	nd
t-Cinnamic acid	28.2±0.48	42.8±0.64	48.3±0.70	52.0±0.72	43.2±0.62	44.2±0.58
p-Coumaric acid	45.2±0.64	41.3±0.52	30.7±0.50	36.2±0.42	22.9±0.46	20.3±0.40
Ferulic acid	nd	53.2±0.59	52.0±0.64	52.9±0.70	49.8±0.61	49.3±0.69
Sinapic acid	15.1±0.22	15.1±0.28	13.5±0.36	14.2±0.24	13.7±0.30	14.4±0.32
Total	88.5	152.4	144.5	155.3	129.6	128.2

[a] Each value in the table is represented as mean ± SD (n =3).
[b] nd: not detected (The content of each compound was not detected in the 50% methanol extracts of the unroasted and roasted HPS.)
Values were considered significantly different at p <0.05.

phenolic acids were markedly increased. Furthermore, the roasted HPS showed high antioxidant effects through various methods. We demonstrated that the roasted soybean at 200°C for 15 min resulted in the highest antioxidant activity. Thus, our works suggest that the roasted HPS may be a potential source of natural antioxidants in certain food applications.

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