Total Phenolics, Phenolic Acids, Isoflavones, and Anthocyanins and Antioxidant Properties of Yellow and Black Soybeans As Affected by Thermal Processing

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The effects of boiling and steaming processes on the phenolic components and antioxidant activities of whole yellow (with yellow seed coat and yellow cotyledon) and black (with black seed coat and green cotyledon) soybeans were investigated. As compared to the raw soybeans, all processing methods caused significant ($p < 0.05$) decreases in total phenolic content (TPC), total flavonoid content (TFC), condensed tannin content (CTC), monomeric anthocyanin content (MAC), DPPH free radical scavenging activity (DPPH), ferric reducing antioxidant power (FRAP), and oxygen radical absorbing capacity (ORAC) in black soybeans. Pressure steaming caused significant ($p < 0.05$) increases in TPC, CTC, DPPH, FRAP, and ORAC in yellow soybeans. The steaming resulted in a greater retention of TPC, DPPH, FRAP, and ORAC values in both yellow and black soybeans as compared to the boiling treatments. To further investigate the effect of processing on phenolic compounds and elucidate the contribution of these compounds to changes of antioxidant activities, phenolic acids, isoflavones, and anthocyanins were quantitatively determined by HPLC. The pressure steaming treatments caused significant ($p < 0.05$) increases in gallic acid and 2,3,4-trihydroxybenzoic acid, whereas all treatments caused significant ($p < 0.05$) decreases in two predominant phenolic acids (chlorogenic acid and *trans*-cinnamic acid), and total phenolic acids for both yellow and black soybeans. All thermal processing caused significant ($p < 0.05$) increases in aglucones and β-glucosides of isoflavones, but caused significant ($p < 0.05$) decreases in malonylglucosides of isoflavones for both yellow and black soybeans. All thermal processing caused significant ($p < 0.05$) decreases of cyanidin-3-glucoside and peonidin-3-glucoside in black soybeans. Significant correlations existed between selected phenolic compositions, isoflavone and anthocyanin contents, and antioxidant properties of cooked soybeans.

INTRODUCTION

Health-promoting effects of soybeans as well as their bioactive phenolic compounds (isoflavones) have been extensively studied. In recent years, antioxidant activities and phenolic compounds in raw soybeans have been reported (*1–6*). However, the health-promoting capacities of soybeans are wholly dependent on processing history. Soybeans must be processed before consumption. How processing methods affect the health-promoting phenolic phytochemicals and antioxidant activities of soybeans has not been systematically studied.

Food processing not only improves flavor and palatability of legume foods but also increases the bioavailability of nutrients,

by inactivating antinutritional factors, such as trypsin inhibitors and heme agglutinins. Boiling of soybeans is one of the traditional methods for human consumption of soybeans in the East Asian countries. Prior to cooking, soaking is a preliminary step: it helps soften the texture and shorten the cooking time. Pressure boiling and steaming can also be used for this purpose. However, no information is available in the literature regarding the changes of antioxidant activity of the whole soybeans as affected by thermal processing, such as boiling or steaming. Although Toda et al. (*7*) reported changes in isoflavones during whole soybean cooking and roasting, to our knowledge, no study has systematically investigated the influence of thermal processing on the overall phenolic compositions in whole soybean food. Thermal processing may cause complex physical and chemical reactions of phenolic compositions, including leaching of water soluble phenolics, freeing phenolics from bonded forms of phenolics, degradation of polyphenols, breakdown and trans-

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formation of phenolics, and formation of Maillard reaction products, such as formation of complex products from phenolics and proteins (*8*). It is important to elucidate the effect of processing on functional components and bioactivities of soybeans. On the basis of these considerations, the objectives of this study were to characterize the effect of boiling and steaming processes on the retention, distribution, and transformation of the total phenolics, total flavonoids, phenolic acids, isoflavones, and anthocyanins, as well as on antioxidant activities of whole soybeans.

MATERIALS AND METHODS

Soybean Materials. The soybeans used in this study were one yellow cultivar (*Glycine max* (L.) Merr. cv. Proto, with a yellow seed coat and a yellow cotyledon) and one black cultivar (*G. max* (L.) Merr. cv. C-1, with a black seed coat and a green cotyledon), obtained from a local farmer in 2005. Broken seeds, damaged seeds, and foreign materials were removed from the samples. Moisture content was determined by drying ground soybean samples in an air oven at 110 °C until a constant weight was obtained (*9*). All calculations for quantification of phenolics and determination of antioxidants activities are on dry weight basis.

Chemicals. Sixteen phenolic acids (gallic, protocatechuic, 2,3,4-trihydroxybenzoic, *p*-hydroxybenzoic, gentistic, vanillic, caffeic, chlorogenic, syringic, *p*-coumaric, *m*-coumaric, *o*-coumaric, ferulic, salicylic, sinapic, and *trans*-cinnamic acid), three aldehydes (vanillin, syringaldehyde, and protocatechualdehyde), HPLC-grade trifluoroacetic acid (TFA), 2-diphenyl-1-picrylhydrazyl radical (DPPH·), Folin−Ciocalteu reagent, sodium carbonate, 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich Inc. (St. Louis, MO). Nine isoflavone standards, including daidzin, genistin, glycitin, daidzein, genistein, glycitein, acetyldaidzin, acetylgenistin, and malonylgenistin, were purchased from LC Laboratories (Woburn, MA); malonyldaidzin, malonylglycitin, and acetylglycitin were not commercially available compounds. A mixture of six unimolar anthocyanin standards (3-*O*-β-glucosides of delphinidin, cyanidin, petunidin, pelargonidin, peonidin, and malvidin) was purchased from Polyphenols Labortories (Sandnes, Norway). 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals (Richmond, VA). HPLC-grade solvents (methanol and acetonitrile, B&J Brand), analytical grade acetic acid, and other analytical grade solvents using for extraction were purchased from VWR international (West Chester, PA).

Soaking and Determination of Hydration Ratio. The soaking procedures and determination method of hydration ratio as reported in our earlier paper (*10*) were followed. We defined the plateau phase (after 16 h of soaking) of the water adsorption curve as 100% hydration ratio. Soaking time of soybeans with desired hydration ratio was calculated by calibration through a quadratic-fit equation of respective water adsorption curve as previously described. The soaked yellow soybeans (with 100% hydration ratio) and black soybeans (with 50% hydration ratio) were drained and then boiled or steamed according to the methods described below. The reason for choosing 50% hydration ratio for soaking black soybeans was based on a preliminary study on black common bean (*Phaseolus vulgaris* L.) (*10*), in that 50% hydration was found to achieve certain softness and retained more water soluble phenolic substances than higher hydration ratios did.

Boiling, Steaming, and Cooking Times. All thermal processes were performed according to our previously described procedures (*10*). Briefly, regular boiling and regular steaming treatments were conducted using a domestic atmospheric cooker and a domestic atmospheric steaming cooker, respectively. Pressure boiling and steaming were conducted using an M-0512-H Mirro pressure cooker (Mirro Co., Manitowoc, WI), respectively. The cooking time was determined on the basis of a tactile method according to the method of Vindiola et al. (*11*) in which the heated beans are squeezed between the forefinger and the thumb with moderate pressure. A bean was deemed to be "cooked" when it could be squeezed easily. The cooking time was defined as the time duration (in minutes) of at least 90% of seeds

(submitted to the test) that were cooked. The boiling and steaming times, as well as pressure conditions, were selected from preliminary experiments. After cooking treatments, the cooked samples were drained and cooled to room temperature in covered plastic containers. Subsequently, cooked samples were frozen and freeze-dried.

Extraction of Total Phenolics from Raw and Processed Soybeans. For quantitative studies, the raw soybeans and freeze-dried cooked soybeans were ground to powder with an IKA all basic mill (IKA Works Inc., Wilmington, NC) and passed through a 60-mesh sieve. The extraction procedures of our earlier paper were followed (*10*). Briefly, yellow soybean flours (0.5 g in triplicate) were extracted twice with a total 10 mL of acetone/water (50:50, v/v), whereas black soybean flours (0.5 g in triplicate) were extracted twice with a total 10 mL of acetone/water/acetic acid (70:29.5:0.5, v/v/v). The extracts were stored at 4 °C in the dark for use.

Determination of Total Phenolic Content (TPC). The TPC was determined by a Folin−Ciocalteu assay (*12*) with slight modifications (*5*) using gallic acid (GA) as the standard. The absorbance was measured at 765 nm against a reagent blank. The TPC was expressed as milligrams of gallic acid equivalents (mg of GAE/g of sample) through the calibration curve of gallic acid. The linearity range of the calibration curve was 50−1000 μg/mL ($r = 0.99$).

Determination of Total Flavonoid Content (TFC). The TFC was determined using a colorimetric method described previously (*13*). The absorbance was measured at 510 nm using a UV−visible spectrophotometer (UV 160, Shimadzu, Japan). The TFC were expressed as milligrams of catechin equivalents (mg of CAE/g of sample) using the calibration curve of (+)-catechin. The linearity range of the calibration curve was 10−1000 μg/mL ($r = 0.99$).

Determination of Condensed Tannin Content (CTC). The analysis of CTC was carried out according to the method of Broadhurst and Jones (*14*) and slightly modified in our laboratory (*5*). The absorption was measured at 500 nm against methanol as a blank. The amount of condensed tannin was expressed as milligrams of catechin equivalents (mg of CAE/g of sample) using the calibration curve of (+)-catechin. The linearity range of the calibration curve was 50−1000 μg/mL ($r = 0.99$).

Determination of Monomeric Anthocyanin Content (MAC). The MAC was determined using a pH differential method (*15*) without modification. A Shimadzu UV 160 double-beam spectrophotometer was used for measuring absorbance at 700 and 520 nm. The MAC was expressed as cyanidin-3-glucoside equivalents (CyE) in milligrams per gram of sample, using an extinction coefficient of 26900 L $\times$ cm^{-1} $\times$ mol^{-1} (*16*) and a molecular mass of 449.2 g $\times$ mol^{-1} of cyanidin-3-glucoside.

Determination of Radical DPPH Scavenging Activity (DPPH). The free radical scavenging capacity of extracts was evaluated according to the method of Chen and Ho (*17*) with slight modifications (*5*). The DPPH free radical scavenging activity of extracts was expressed as micromoles of Trolox equivalents per gram of sample (μmol of TE/g) using the calibration curve of Trolox. The linearity range of the calibration curve was 20−1000 μM ($r = 0.99$).

Determination of Ferric Reducing Antioxidant Power (FRAP). The FRAP assay was performed as previously described by Benzie and Strain (*18*). The FRAP value was expressed as millimoles of Fe^{2+} equivalents per 100 g of sample (mmol of FE/100 g) using the calibration curve of Fe^{2+}. The linearity range of the calibration curve was 0.1−1.0 mM ($r = 0.99$).

Determination of Oxygen Radical Absorbing Capacity (ORAC). The ORAC assay was carried out on a BMG Fluostar Optima Microplate Reader (BMG Labtech GmbH, Offenburg, Germany). The procedures were based on the previous paper by Prior et al. (*19*) with slight modifications (*5*). The ORAC value was expressed as micromoles of Trolox equivalents per gram of soybean (μmol of TE/g).

Color of Processed Soybean Flours. The color of the processed soybean flours were measured with a Minolta Color Difference Meter (model CR 310, Minolta Camera Co., Osaka, Japan) using the Hunter scale for *L*, *a*, and *b*. Calibration was conducted on a standard white plate (*Y* = 93.3). The results were expressed as tristimulus values [*L*,

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lightness (0 = black, 100 = white), a ($-a$ = greenness, $+a$ = redness), and b ($-b$ = blueness, $+b$ = yellowness)]. These values were then used to calculate chroma [$C = (a^2 + b^2)^{1/2}$], which was the color saturation.

HPLC Analysis of Phenolic Acid Contents. *Extraction of Free Phenolic Acids.* The extraction of free phenolic acids was performed according to a previous method (*20*) with slight modifications. The raw and cooked soybean samples (0.5 g in triplicate) were accurately weighed into a set of 15 mL VWR centrifuge tubes. Six milliliters of methanol/water/acidic acid/BHT (85:15:0.5: 0.2, v/v/v/w) extraction solvent was added to each tube. The tubes were capped, and the mixtures were shaken at 300 rpm at room temperature on an orbital shaker for 4 h. The mixtures were extracted for another 12 h by sitting in the dark. The extracts were filtered through Whatman no. 1 paper. An additional volume of 4 mL of the extraction solvent was added into the residues. The above-mentioned extraction procedures were repeated. The two-time extracts were combined and concentrated at 45 °C under vacuum to remove solvents. The extract was dissolved in 2.5 mL of 25% methanol, and the methanol solution was filtered through a 0.2 μm PVDF syringe filter and analyzed for free phenolic acid content by HPLC.

Extraction of Conjugated Phenolic Acids. The extraction of conjugated phenolic acids was performed according to previous methods (*20, 21*) with slight modifications. Briefly, the raw and cooked soybean samples (0.4 g in triplicate) were accurately weighed into a set of 15 mL VWR centrifuge tubes and hydrolyzed with 10 mL of 2 N NaOH [containing 10 mM EDTA and 1% vitamin C (w/v)] for 30 min at 40−45 °C. The reaction mixture was acidified by adding 2.8 mL of 7.2 N HCl. The mixture was vortexed for 5−10 s, and phenolic acids were extracted with ethyl acetate twice (2 × 10 mL). The combined organic layer was concentrated to dryness at 45 °C under vacuum to remove all solvents. The dry residue was dissolved in 1.5 mL of 75% methanol. The methanol solution was filtered through a 0.2 μm PVDF syringe filter and analyzed for conjugated phenolic acid content by HPLC.

HPLC Analysis of Phenolic Acids. The quantitative analysis of both free and conjugated phenolic acids was performed by HPLC according to the method of Robbins and Bean (*22*) with slight modifications. A Waters Associates (Milford, MA) chromatography system equipped with a model 720 system controller, a model 6000A solvent delivery system, a model 7125 loading sample injector, and a model 418 LC spectrophotometer set at 270 nm was used. A 4.6 mm × 250 mm, 5 μm, Zorbax Stablebond Analytical SB-C$_{18}$ column (Agilent technologies, Rising Sun, MD) was used for separation at 40 °C, which was maintained with a column heater. Elution was performed using mobile phase A (0.1% TFA aqueous solution) and mobile phase B (methanol); the flow rate was set to 0.7 mL/min. The solvent gradient in volumetric ratios was as follows: 5−30% B over 50 min. The solvent gradient was held at 30% B for an additional 15 min, and the gradient was increased to 100% B at 66 min. The solvent gradient was held at 100% B for an additional 10 min to clean up the column.

Identification and Quantification of Phenolic Acids. For the identification of HPLC peaks from samples, 1 mg/mL of stock solution of each individual compound was prepared and diluted to 100 μg/mL, the diluted working solutions were injected to HPLC, and the spiking method and external standard method were used for qualitative analyses by comparing peak area increases and retention times. In addition, to further identify compound peaks through their UV spectrum information, individual phenolic acids and the phenolic acid mixture as well as several typical samples were selected to perform analysis on another HPLC (HP 1090, Hewlett-Packard, Waldbronn, Germany), which was equipped with a UV-PDA detector.

All identified phenolic acids were quantified with external standards by using HPLC analysis as described previously. To prepare 1 mg/mL of the stock solution of standard mixture, 10 mg of each phenolic acid compound was mixed together and dissolved in 10 mL of 25% methanol, and then the stock solution was diluted into nine series of standard working solutions (100, 50, 25, 10, 5, 2.5, 1, 0.5, and 0.25 μg/mL) with distilled water. The standard curves of phenolic acids were plotted as peak area against concentrations of external standards by duplicate injection of nine series working solutions of standard mixture.

HPLC Analysis of Isoflavone Content. *Extraction of Isoflavones.* The isoflavones were extracted by modifying the methods of Murphy et al. (*23*) and Hou and Chang (*24*). Briefly, the ground raw and cooked soybeans (1.0 ± 0.01 g in duplicate) were accurately weighed into a set of 15 mL screw-top VWR centrifuge tubes. Five milliliters of acetonitrile and 5 mL of distilled water were added to each tube. The tubes were capped, and the mixtures were shaken at 250 rpm at room temperature on an orbital shaker for 2 h. Then the slurry was centrifuged by an Allegra 21R centrifuge (Beckman Coulter Ltd., Palo Alto, CA) at 5500 rpm for 20 min. The supernatant was filtered through Whatman no. 42 filter paper into a 125 mL flask and evaporated to dryness on a rotary evaporator at 34 °C. The residues in the flask were dissolved in 5 mL of 80% methanol and kept in a freezer (−20 °C) for <12 h before analysis. An aliquot of sample solution was filtered through a 0.2 μm PTFE syringe filter prior to HPLC assay.

HPLC Analysis of Isoflavones. The quantitative analysis of soybean isoflavones was performed by HPLC according to the method fo Hou and Chang (*24*) with slight modification. The same Waters Associates chromatography system as used for phenolic acids analysis was used for quantitative analysis of isoflavones, and a spectrophotometer set at 262 nm was used. A YMC-Pack ODS-AM-303 C$_{18}$ reversed phase column (4.6 × 250 mm i.d., 5 μm particle size) was obtained from Waters and employed for chromatographic separation at 34 °C, which was maintained with a column heater. A linear gradient mobile phase consisted of solvent A (0.1% acetic acid in water) and solvent B (0.1% acetic acid in acetonitrile). After injection of 20 μL of sample, the system was eluted with 15% of solvent B for 5 min at the flow rate of 1.0 mL/min, then increased to 29% for 31 min at the flow rate up to 1.5 mL/min, and then to 35% for 8 min at the same flow rate of 1.5 mL/min. Then the gradient increased to 50% of solvent B in 2 min and was kept at 50% of solvent B for 10 min at the flow rate of 1.5 mL/min, then recycled back to 15% B at the flow rate of 1.0 mL/min in 2 min; the column was equilibrated with initial solvent for 2 min prior to running the next sample.

Identification and Quantification of Isoflavones. Three aglucones, three β-glucosides, two acetylglucosides (acetyldaidzin, acetylgenistin), and one malonylglucoside (malonylgenistin) of isoflavones are commercially available and directly used to identify the sample peaks by comparing their retention times and HPLC profiles with standard mixture. The peak identification for noncommercially available isoflavones was confirmed by comparing the chromatograms of several identical samples performed by a well-established isoflavone analytical laboratory (Dr. Pat Murphy, Iowa State University, Ames, IA). In addition, the spiking method was also used for peak identification of some samples.

The quantification of isoflavones was performed by calibrating the peak area obtained from HPLC analyses. The external calibration curves were obtained for each of nine external standards by plotting the peak area of each standard against concentration. For the other isoflavones without commercial standards, concentrations were calculated from the standard curves that were adjusted appropriately from the standard curves of respective form of isoflavones on the basis of the differences in molecular weight of the compounds. The isoflavone contents were expressed as micrograms of isoflavone per gram of dry soybean.

HPLC Analysis of Anthocyanin Content. *HPLC Analysis of Anthocyanins.* The free phenolic acid extracts were also used for anthocyanin analysis; the analysis was performed on an HP 1090 series HPLC (HP 1090, Hewlett-Packard, Waldbronn, Germany) equipped with a filter photometric detector, using a YMC Pack ODS-AM column (4.6 × 250 mm, S-50 μm, 120A). The HPLC conditions were as follows: solvent A, 0.1% TFA/H$_2$O; solvent B, CH$_3$CN/H$_2$O/TFA (50: 50:0.1, v/v/v); linear gradient, initial percentage of B (15%) to 60 min (40%); column temperature, 40 °C; flow rate, 0.5 mL/min. The filter detector was set at 540 nm.

Identification and Quantification of Anthocyanins. The identifications and peak assignments of anthocyanins were primarily based on comparison of their retention times with those of standards, blueberry reference sample, and the literature (*25−28*). The stock solution of anthocyanins mixture was prepared by dissolving standards (unimolar mixture of 3-*O*-β-glucosides of delphinidin, cyanidin, petunidin, pelargonidin, peonidin, and malvidin) in methanol to give a concentra-
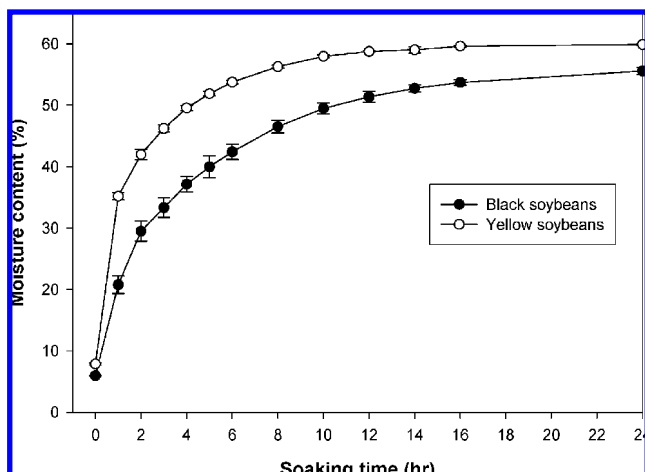
Figure 1. Water absorption curves of dry soybeans. Hydration ratio of dry beans was considered as 0, and hydration ratio of soaked beans for 24 h was considered as 100%. Desired hydration ratio of black soybean was calculated by calibration through a quadratic fit equation of water absorption curve.

tion of 1.0 mg/mL. A portion of the stock solution was then diluted using methanol to the following series of dilutions: 1 in 5, 10, 20, 40, 80, and 160. The standard curves of anthocyanins were plotted as peak area against concentrations by duplicate injection of the six series diluted working solutions of standard mixture.

Statistical Analysis. All soaking, boiling, and steaming processes were performed in triplicate. Further chemical composition analyses and antioxidant activity evaluations were performed on the basis of triplicate processed samples, and the data were expressed as mean ± standard deviation. Statistical analysis was performed using 2005 SAS (version 9.1, SAS Institute Inc., Cary, NC). An analysis of variance (ANOVA) was conducted. Duncan's multiple-range test was used to determine the differences between group means. Significant levels were defined as probabilities of ≤0.05. The Pearson correlation test was conducted to determine the correlation between variables.

RESULTS AND DISCUSSION

Hydration Ratio and Soaking, Boiling, and Steaming Time. Traditionally, dry legumes are soaked to hydrate prior to boiling, making them easier to cook. The water absorption curves of soybeans, illustrated in **Figure 1**, were characterized by an initial phase of rapid water pickup followed by an equilibrium phase, during which the soybeans approached their full soaking capacity. The soybeans were prone to saturation after soaking for 14 h, and water absorption reached a plateau phase after 16 h of soaking. In addition, the soaking water of black soybeans had a dark color. This phenomenon indicated that some soluble pigments (may include phenolic antioxidant constituents) were leached into the soaking water because anthocyanins are primarily located in the seed coat (*25−27*). To decrease potential losses of antioxidant components in the seed coat, a soaking treatment with a low hydration ratio (50%) and a short soaking time (2.5 h) was designed for black soybeans, and a 100% hydration ratio was designed for yellow soybeans in the following boiling and steaming treatments. To obtain the desired hydration ratio, soaking times of black soybeans were calculated by calibration through a quadratic fit equation of the water absorption curve.

Cooking time and cooked texture, appearance, and flavor are important processing and food quality characteristics. The cooking time for producing palatable products is one of the main criteria used in evaluating the cooking quality of dry legumes (*29*). Several methods for measuring the cooking time of

legumes have been reported. However, no universally accepted methods exist so far. In current studies, the tactile method of Vindiola et al. (*11*) was applied to evaluate the cooking time (including boiling and steaming) of all treatments. We defined the cooking time as when 90% of the beans could be squeezed easily with the forefinger and the thumb. The optimum cooking times for the different cooking conditions were selected from our preliminary experiments (results are not shown here). For further antioxidant and chemical assays, several optimum cooking times were selected to prepare samples. The optimum cooking times for regular boiling, 15 psi pressure boiling, regular steaming, and 15 psi pressure steaming were 120, 15, 100, and 60 min, respectively.

Effect of Thermal Processing on Total Phenolic Compositions of Soybeans. The total phenolic content (TPC), total flavonoid content (TFC), condensed tannin content (CTC), and monomeric anthocyanin content (MAC) of the extracts from raw and cooked soybeans are presented in **Table 1**. Significant differences ($p < 0.05$) in TPC, TFC, CTC, and MAC values were found among most treatments for both yellow and black soybeans. In the case of yellow soybeans, as compared to the original raw beans, regular and pressure boiling and regular steaming caused significant ($p < 0.05$) decreases in TPC, but significant increases in TFC, CTC, whereas pressure steaming caused significant ($p < 0.05$) increases in TPC, TFC, and CTC. In the case of black soybeans, as compared to the original raw soybeans, all heating treatments caused significant ($p < 0.05$) decreases in all measured phenolic indexes; especially, all treatments caused almost complete losses of monomeric anthocyanin content. As compared to the boiling treatments, both regular and pressure steaming treatments retained greater TPC and TFC values in the black soybeans, but slightly lower CTC values. These results indicated that processing caused complex changes on chemical compositions; thermal processing might cause degradation of polyphenols and release of bound phenolic compositions. The differences in changing phenolic profiles caused by thermal processing between yellow and black soybeans might be due to the differences in distribution and content of individual phenolic compounds in seed coat and cotyledon.

Data on phenolics in cooked legumes are very limited. Bressani and Elias (*30*) observed that about 30−40% of phenolics could be removed from common beans (*P. vulgaris*) by cooking and discarding the cooking water. In the present study, about 43−62% of TPC, 68−78% of TFC, 28−36% of CTC, and 88−95% of MAC were decreased in cooked black soybeans. Both regular boiling and pressure boiling caused greater TPC (about 62%) and TFC (73−78%) losses than steaming treatments in black soybeans. These results on the variations of TPC by processing are in good agreement with those reported by Ismail et al. (*31*), who found that thermal treatment decreased the TPC in all vegetables; these results also exhibited trends similar to those of our previous papers (*10, 32*), in which thermal processing lost 50−70% of TPC in peas and 70−80% of TPC in black common beans (*P. vulgaris*). These significant losses could be attributed to water soluble phenolics leaching into soaking and cooking water before and during thermal processing as well as breakdown of phenolics during processing. In the case of yellow soybeans, about 10−30% of TPC losses were found in the two boiling treatments and the regular steaming treatment. It is widely believed that many food antioxidant components can be significantly lost as a consequence of industrial sterilization, pasteurization, and dehydration, as well as home-cooking (*33, 34*). However, processing does

Table 1. Effect of Boiling and Steaming on Total Phenolic Compositions and Color Values of Soybeans[a]

	TPC (mg of GAE/g)	TPC loss (%)	TFC (mg of CAE/g)	TFC loss (%)	CTC (mg of CAE/g)	CTC loss (%)	MAC (mg of CyE/g)	MAC loss (%)	color values			
									L	a	b	chroma
					Yellow Soybean							
raw	2.15 b		0.68 d		1.78 c		ND		86.9 a	−2.1 d	16.5 e	16.6 e
RB, 120 min	1.56 e	27.4	1.30 a	−91.2	2.17 b	−21.9	NS		80.3 b	1.2 b	16.9 d	16.9 d
PB, 15 psi, 15 min	1.78 d	17.2	1.09 b	−60.3	2.78 a	−56.2	NS		79.6 c	0.8 c	18.5 c	18.5 c
RS, 100 min	1.94 c	9.8	1.09 b	−60.3	2.74 a	−53.9	NS		78.9 d	1.1 b	19.2 b	19.2 b
PS, 15 psi, 60 min	2.90 a	−34.9	1.35 a	−98.5	2.74 a	−53.9	NS		72.1 e	3.6 a	19.6 a	19.9 a
					Black Soybean							
raw	6.96 a		3.43 a		7.31 a		0.43 a		75.1 a	−5.8 e	15.6 a	16.7 a
RB, 120 min	2.62 d	62.4	0.75 e	78.1	5.94 b	18.7	0.03 d	93.0	63.9 c	1.1 b	11.3 c	11.4 d
PB, 15 psi, 15 min	2.58 d	62.9	0.92 d	73.2	5.99 b	18.1	0.05 b	88.4	62.5 d	0.9 c	11.5 c	11.5 d
RS, 100 min	3.49 c	49.9	1.21 b	74.7	5.52 c	24.5	0.04 c	90.7	64.5 b	−0.0 d	12.6 b	12.6 c
PS, 15 psi, 60 min	3.95 b	43.2	1.07 c	68.8	5.35 c	26.8	0.02 e	95.3	61.9 e	3.2 a	15.8 a	16.1 b

[a] Data are expressed as means of triplicate experiments on dry weight basis. Values marked by the same letter within each soybean in each column are not significantly different ($p < 0.05$). ND, not detected; NS, not determined because yellow soybean contains little anthocyanins; RB, regular boiling; PB, pressure boiling; RS, regular steaming; PS, pressure steaming.

not always result in the destruction of the antioxidant components. In some cases, processing factors can induce the formation of compounds (*35, 36*). Cooking (included boiling and steaming) was found to give rise to an increase in phenolics in green beans, pepper, and broccoli (*37*). Contrary to the effect of boiling, steam cooking had been reported to increase the content of total phenolics in broccoli (*38*). In addition, steaming treatments increased the total phenolic contents 2−13 times in all six genotypes of sweet potatoes as compared to raw sweet potatoes (*39*). In the current study on the thermal processing of yellow soybeans, pressure steaming increased TPC about 35%. All thermal processing increased TFC about 60−90% and CTC about 20−60%. The increased total phenolic values might be related to the release of phenolic or phenolic analogue substances with reactivity toward phenolic detection reagents (such as Folin−Ciocalteu reagent) from polymerized structural substances (such as lignin) in cell walls upon thermal processing. Lignin is covalently bound to cellulose in the cell walls.

Lignins, a category of phenolic derivates, are formed via polymerization of a mixture of three monolignols, *p*-coumaryl, sinapyl, and coniferyl alcohols (*40*). A steam explosion technique, high-pressure steaming followed by rapid decompression, had been applied to the degradation of lignocellulose as a promising process method to separate and increase the accessibility of the main components of lignocellulosic biomass (*41*). The typical effects of this treatment are the substantial breakdown of lignocellulosic structure, depolymerization of the lignin components, and defibration. As a consequence, water soluble phenolic compounds were increased (*41, 42*). To some extent, pressure steaming of soybeans in the current study might be similar to the steam explosion process. Therefore, the increased phenolic values in pressure-steamed yellow soybeans might partly be attributed to depolymerization of the lignin components.

Effect of Thermal Processing on Antioxidant Capacities of Soybeans. The antioxidant activities of the raw and cooked soybeans, including DPPH free radical scavenging capacity (DPPH), ferric reducing antioxidant power (FRAP), and oxygen radical absorbing capacity (ORAC) are presented in **Figure 2**. Significant differences ($p < 0.05$) in DPPH, FRAP, and ORAC values were found among most treatments for both yellow and black soybeans. In the case of yellow soybeans, as compared to the raw beans, both regular and pressure steaming caused significant ($p < 0.05$) increases in DPPH, FRAP, and ORAC values, and pressure boiling caused significant increases in DPPH values, whereas regular boiling caused significant ($p < 0.05$) decreases in FRAP and ORAC values. A similar pattern had been reported for heated ginseng (steamed ginseng at 100 and 120 °C, respectively), which showed better radical scavenging activity than air-dried ginseng (*43*). The increased activities in steamed yellow soybeans and ginseng may be contributed by the improvement of antioxidant properties or formation of novel compounds having antioxidant activity upon steaming treatments. This hypothesis had been verified by the fact that the content of maltol in heated ginsengs was remarkably increased in a temperature-dependent manner by steaming processing (*43*). Interestingly, as compared to the boiling treatments, steaming treatments caused significant ($p < 0.05$) increases in antioxidant activities by all assays. In addition, pressure boiling and steaming treatments caused significant ($p < 0.05$) increases in DPPH and ORAC values as compared to their respective regular (atmospheric) treatments. In the case of black soybeans, as compared to the raw beans, all cooking treatments caused significant ($p < 0.05$) decreases in antioxidant activities by all assays. The steaming treatments (both regular and pressure) preserved greater antioxidant values as compared to the boiling treatments. Similar to the yellow soybeans, pressure boiling treatments retained significantly ($p < 0.05$) higher DPPH, FRAP, and ORAC values as compared to their respective regular treatments.

Boiling is generally regarded as being destructive to antioxidant compositions (*44*). As verified by our antioxidant assays, the results from yellow soybeans showed that regular boiling processes lost about 33% of FRAP values and about 35% of ORAC values. The results from black soybeans showed that boiling (both regular and pressure) lost about 53−58% DPPH values, about 65−70% FRAP values, and about 47−60% of ORAC values. Similar trends were found in the boiling processes of peas (*Pisum sativam*), lentils (*Lens culinaris*), and black common beans (*P. vulgaris*) in our previous papers (*10, 32*). However, the antioxidant properties of the same type of food might be influenced by different processing methods. The steaming processing significantly ($p < 0.05$) increased the antioxidant activities of yellow soybeans: increased by about 75−140% DPPH values, by 3−50% FRAP values, and by 40−100% ORAC values as compared to the raw yellow soybeans. Similar positive heat effects were found in the pasteurization of tea extracts (*35*), which caused an increase in the antioxidant activity of tea. Halvorsen et al. (*45*) reported large increases in antioxidant activities for several vegetables such as carrots, spinach, mushrooms, asparagus, broccoli, cabbage, red cabbage, green and red peppers, potatoes, and
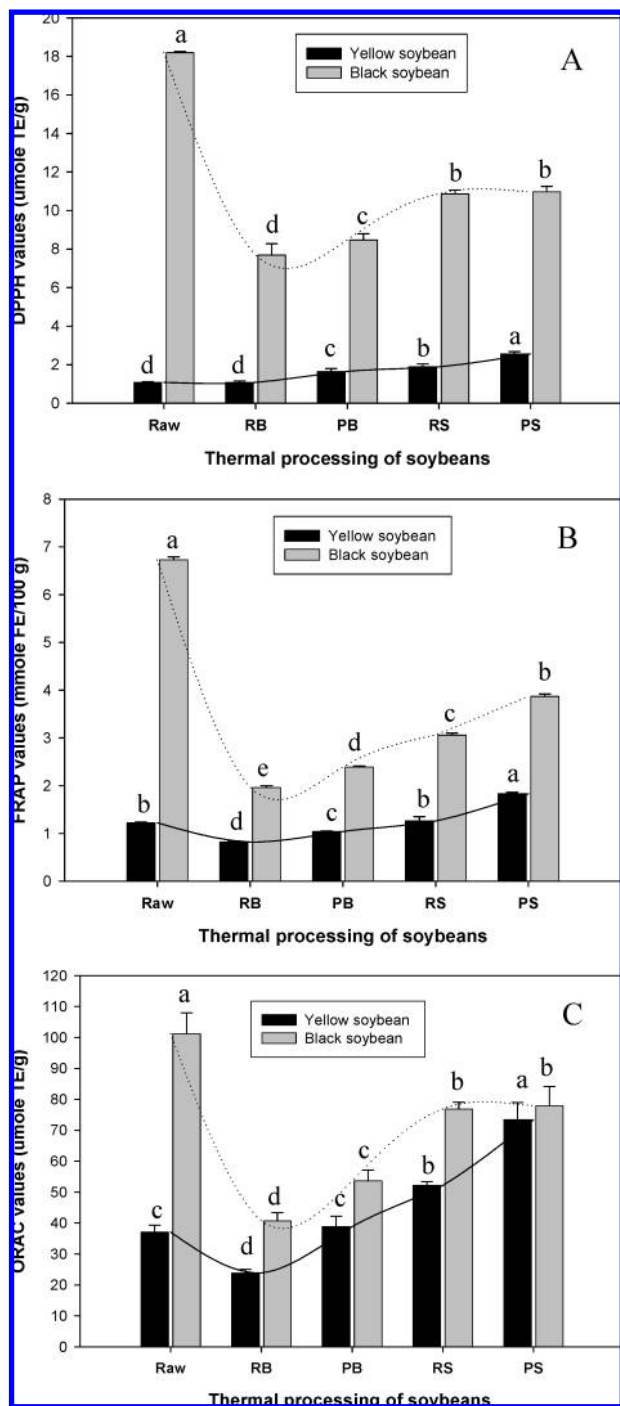
Figure 2. Effect of boiling and steaming on antioxidant properties (**A**, DPPH; **B**, FRAP; **C**, ORAC) of soybeans. Bar data are expressed as mean ± standard deviation ($n = 3$) on dry weight basis. Values marked above the same color bar with the same letter are not significantly different ($p < 0.05$). RB, regular boiling for 120 min; PB, pressure (15 psi) boiling for 15 min; RS, regular steaming for 100 min; PS, pressure (15 psi) steaming for 60 min.

tomatoes after steaming and boiling. Furthermore, thermal processing could lead to the formation of novel compounds with antioxidant activity or the release of bound phenolic compounds (*36, 46, 47*).

Pressure boiling and pressure steaming of both yellow and black soybeans yielded darker color products than regular cooking and regular steaming (**Table 1**). Both pressure-steamed yellow and black soybeans exhibited significantly darker color (lower *L* values and higher *a* and chroma values) than regular

steamed soybeans. These darker color products might be from the Maillard reactions. Previous research on fruits and vegetables indicated that thermal processing increased antioxidant potential due to formation of novel compounds such as Maillard reaction products (MPRs) having antioxidant activity (*34, 48*). In particular, Samaras et al. found that antioxidant activity increased with the intensity of heating, in parallel with color formation, and that MPRs were responsible for the majority of the antioxidant activity in highly roasted malts (*49*). Therefore, the MPRs may have partly contributed to the increase of antioxidant capacity of steamed and pressure-boiled soybeans.

The correlation analyses between total phenolics, total flavonoids, condensed tannins, monomeric anthocyanins, and antioxidant activities among all soybean samples were performed. In the case of yellow soybeans, there were no significant linear correlations between different types of phenolics, whereas there were significant linear correlations between TPC and DPPH ($r = 0.76$, $p < 0.05$), between TPC and FRAP ($r = 0.97$, $p < 0.0001$), and between TPC and ORAC ($r = 0.89$, $p < 0.0001$), as well as between CTC and DPPH ($r = 0.77$, $p < 0.05$) and between CTC and ORAC ($r = 0.57$, $p < 0.05$). In the case of black soybeans, there were significant linear correlations between different types of phenolics: TPC and TFC ($r = 0.97$, $p < 0.0001$), CTC ($r = 0.75$, $p < 0.05$), MAC ($r = 0.93$, $p < 0.0001$) and between phenolics and antioxidant activities. These results indicated that all antioxidant assay methods were well-correlated; meanwhile, different phenolics contents might have different degrees of contribution to overall antioxidant activities.

The boiling and steaming processes significantly affected the total phenolics and antioxidant activities in both yellow and black soybeans. The changes depended on the type of soybeans and processing conditions. The steaming processes caused smaller losses in total phenolic compositions and antioxidant activities for black soybeans and retained more phenolic composition content and antioxidant activities than the boiling processes. Therefore, steaming is recommended for soybeans in domestic and industrial processes to preserve antioxidant components. The changes in the overall antioxidant properties of processed soybeans can be attributed to the synergistic combinations or counteracting of several types of chemical reactions, leaching of water soluble antioxidant compositions, and formation or breakdown of antioxidant compositions. To better understand the role and fate of natural and heat-induced antioxidants on food stability and human health, the following chemical composition research was performed to investigate the molecular mechanisms responsible for loss or formation of antioxidants and interactions between natural and heat-induced antioxidants and their effects on the overall antioxidant properties of cooked soybeans.

Effect of Thermal Processing on Phenolic Acid Compositions. The free phenolic acid contents (FPA) and conjugated phenolic acid contents (CPA) of the extracts from original unprocessed and processed soybeans are presented in **Tables 2** and **3**, respectively. Four benzoic type phenolic acids (gallic acid, 2,3,4-trihydroxybenzoic acid, vanillic acid, and protocatechualdehyde) and three cinnamic type phenolic acids (chlorogenic, sinapic, and *trans*-cinnamic acid) were detected in both raw and processed yellow soybeans (**Table 2**). The chlorogenic acid and *trans*-cinnamic acid were the predominant phenolic acids among these compounds in the raw yellow soybeans. Five benzoic type phenolic acids (gallic acid, protocatechuic acid, 2,3,4-trihydroxybenzoic acid, vanillic acid, and protocatechualdehyde) and four cinnamic type phenolic acids (chlorogenic,

Table 2. Effect of Boiling and Steaming on Free Phenolic Acid Compositions (Micrograms per Gram) of Yellow and Black Soybeans[a]

	individual benzoic acid derivate					subtotal benzoics	individual cinnamic acid derivate					subtotal cinnamics	total phenolic acids
	GA	PA	TBA	PCD	VA		CLA	PCA + SD	MCA + FA	SPA	TCA		
						Yellow Soybean							
raw	79.00 b	ND	0.93 e	21.49 a	20.34 b	121.77 b	736.4 a	17.35	ND	11.76 a	261.28 a	1022.9 a	1144.7 a
RB, 120 min	24.24 d	ND	1.95 d	3.57 d	4.04 d	33.80 d	307.5 d	ND	ND	12.43 a	21.06 c	340.9 d	374.8 d
PB, 15 psi, 15 min	36.61 c	ND	2.67 b	13.97 c	5.15 c	58.40 c	417.7 c	ND	ND	10.78 a	49.77 b	478.3 c	536.7 c
RS, 100 min	33.29 c	ND	3.67 b	16.64 b	10.48 c	64.08 c	461.4 c	ND	ND	12.35 a	46.76 b	520.5 c	584.6 c
PS, 15 psi, 60 min	109.59 a	ND	8.27 a	15.34 bc	24.42 a	149.49 a	607.1 b	ND	ND	9.55 a	14.11 c	627.6 b	777.1 b
						Black Soybean							
raw	61.29 b	62.34 a	0.78 c	64.85 c	6.29 b	195.57 b	846.9 a	22.37 a	ND	28.62 a	151.9 a	1042.3 a	1237.9 a
RB, 120 min	31.47 d	15.05 c	3.55 c	18.09 d	2.46 c	70.63 d	334.3 d	4.15 e	3.57 b	15.65 bc	11.28 cd	368.9 d	439.6 c
PB, 15 psi, 15 min	28.82 d	10.72 c	4.43 c	23.41 d	3.46 b	70.83 d	394.3 d	7.81 d	2.43 c	12.66 c	15.76 c	432.9 d	503.7 c
RS, 100 min	43.07 c	13.12 c	5.74 b	41.36 b	5.58 a	108.87 c	767.1 b	12.47 c	2.70 c	13.36 c	24.27 b	819.9 b	928.8 b
PS, 15 psi, 60 min	152.17 a	32.18 b	15.10 a	33.13 c	6.38 a	238.97 a	652.8 c	20.66 b	4.79 a	16.96 b	4.69 d	699.9 c	938.8 b

[a] Data are expressed as means of triplicate experiments on dry weight basis. Values marked by the same letter within each soybean in each column are not significantly different ($p < 0.05$). Phenolic acids: GA, gallic acid; PA, protocatechuic acid; TBA, 2,3,4-trihydroxybenzoic acid; PCD, protocatechualdehyde; VA, vanillic acid; CLA, chlorogenic acid; PCA + SD, p-coumaric acid + syringaldehyde; MCA + FA, m-coumaric acid + ferullic acid; SPA, sinapic acid; TCA, *trans*-cinnamic acid. ND, not detectable; RB, regular boiling; PB, pressure boiling; RS, regular steaming; PS, pressure steaming.

Table 3. Effect of Boiling and Steaming on Conjugated Phenolic Acid Compositions (Micrograms per Gram) of Yellow and Black Soybeans[a]

	individual benzoic acid derivate								subtotal benzoics	individual cinnamic acid derivate							subtotal cinnamics	total phenolic acids
	GA	PA	TBA	PCD	HBA	SA	VA	VN		CFA	CLA	PCA + SD	MCA + FA	SPA	OCA	TCA		
									Yellow Soybean									
raw	2.79 a	0.79 b	158.6 a	0.69 d	5.80 b	47.60 c	28.48 b	1.02 a	245.8 a	1.24 b	ND	51.72 a	2.86 c	51.28 a	1.35 b	0.92 d	109.4 b	355.2 a
RB, 120 min	1.12 c	0.64 b	39.46 d	1.12 c	3.28 d	32.12 d	16.60 c	0.59 b	94.93 e	0.49 c	ND	14.17 e	2.80 c	24.09 c	0.71 c	1.18 d	43.43 e	138.4 d
PB, 15 psi, 15 min	1.55 c	0.49 b	67.19 b	0.78 cd	5.87 b	27.74 d	15.76 c	0.65 b	119.9 d	0.56 c	ND	25.52 d	3.87 a	24.06 c	0.83 c	3.27 b	57.01 d	176.9 c
RS, 100 min	2.06 b	0.59 b	57.99 c	1.89 b	4.24 c	91.75 b	28.41 b	1.07 a	188.0 c	1.24 b	ND	35.00 c	2.99 c	39.93 b	1.03 c	2.69 c	82.83 c	270.8 b
PS, 15 psi, 60 min	1.29 c	3.49 a	46.15 d	2.97 a	9.23 a	113.82 a	46.58 a	0.59 b	224.1 b	6.72 a	ND	44.66 b	3.45 b	56.60 a	2.29 a	5.92 a	119.8 a	343.9 a
									Black Soybean									
raw	65.01 a	12.41 a	236.3 a	22.73 a	3.97 a	42.45 a	33.47 a	1.76 a	418.1 a	11.55 a	ND	32.28 a	3.46 c	38.71 a	ND	11.12 a	97.08 a	515.1 a
RB, 120 min	19.66 c	11.46 ab	62.37 c	10.53 c	1.12 d	30.59 bc	19.09 b	1.38 b	156.2 d	1.47 b	ND	8.87 c	3.32 c	23.86 c	ND	1.13 b	38.64 d	194.9 d
PB, 15 psi, 15 min	24.97 b	8.19 c	90.26 b	10.09 c	2.18 c	27.08 c	22.62 b	1.24 b	186.6 c	0.84 c	ND	15.37 b	3.63 c	26.53 c	0.09	0.89 b	47.26 c	233.9 c
RS, 100 min	26.09 abc	9.98 abc	95.42 c	13.11 b	2.78 b	42.50 a	25.01 b	1.17 b	216.1 b	1.66 b	ND	17.88 b	4.96 b	32.81 b	ND	0.93 b	58.25 b	274.3 b
PS, 15 psi, 60 min	11.12 d	9.43 bc	64.14 c	6.51 d	2.84 b	38.32 ab	22.99 b	1.11 b	156.5 d	1.63 b	ND	18.38 b	5.92 a	17.91 d	ND	0.81 b	44.66 cd	201.1 d

[a] Data are expressed as means of triplicate experiments on dry weight basis. Values marked by the same letter within each soybean in each column are not significantly different ($p < 0.05$). Phenolic acids: GA, gallic acid; PA, protocatechuic acid; TBA, 2,3,4-trihydroxybenzoic acid; PCD, protocatechualdehyde; HBA, p-hydroxybenzoic acid; SA, syringic acid; VA, vanillic acid; VN, vanillin; CFA, caffeic acid; CLA, chlorogenic acid; PCA + SD, p-coumaric acid + syringaldehyde; MCA + FA, m-coumaric acid + ferullic acid; SPA, sinapic acid; OCA, o-coumaric acid; TCA, *trans*-cinnamic acid. ND, not detectable; RB, regular boiling; PB, pressure boiling; RS, regular steaming; PS, pressure steaming.

p-coumaric, sinapic, and *trans*-cinnamic acid) were detected in both raw and cooked black soybeans. The chlorogenic acid and *trans*-cinnamic acid were also the predominant phenolic acids among these compounds in the raw black soybeans. **Table 3** shows that nine benzoic type conjugated phenolic acids (gallic, protocatechuic, 2,3,4-trihydroxybenzoic, p-hydroxybenzoic, gentistic, syringic, and vanillic acid, protocatechualdehyde, and vanillin) and six cinnamic type of conjugated phenolic acids (caffeic, p-coumaric, m-coumaric, o-coumaric, sinapic, and *trans*-cinnamic acid) were detected in both yellow and black soybeans (raw and cooked). However, chlorogenic acid and *trans*-cinnamic acid (predominant phenolic acid compositions in free phenolic acid assay) were not detected or decreased greatly in both yellow and black soybeans, and more types of conjugated phenolic acids were detected. These phenomena might be attributed to alkaline hydrolysis, which released more types of phenolic acids from the bound form to the free form. In addition, alkaline hydrolysis partly broke down some original free phenolic acids.

Significant differences ($p < 0.05$) in FPA and CPA were found among most treatments for both yellow and black soybeans. In the case of FPA assay of both yellow and black soybeans (**Table 2**), as compared to the original raw beans, regular and pressure boiling and regular steaming caused significant ($p < 0.05$) decreases in gallic acid, protocatechualdehyde, vanillic acid, chlorogenic acid, p-coumaric acid, *trans*-cinnamic acid, subtotal benzoic acids, subtotal cinnamic acid, and total phenolic acids content, but significant increases in 2,3,4-trihydroxybenzoic acid, whereas pressure steaming caused significant ($p < 0.05$) increases in gallic acid, 2,3,4-trihydroxybenzoic acid, vanillic acid, and subtotal benzoic acids but significant decreases in protocatechualdehyde, chlorogenic acid, sinapic acid, *trans*-cinnamic acid, subtotal cinnamic acid, and total phenolic acids. In the case of CPA assay of yellow soybeans (**Table 3**), as compared to the original raw beans, all thermal treatments caused significant ($p < 0.05$) decreases in gallic acid, 2,3,4-trihydroxybenzoic acid, and p-coumaric acid. In the case of CPA assay of black soybeans, all thermal treatments caused significant ($p < 0.05$) decreases in gallic acid, protocatechuic acid, 2,3,4-trihydroxybenzoic acid, protocatechualdehyde, p-hydroxybenzoic acid, vanillic acid, vanillin, caffeic acid, p-coumaric acid, sinapic acid, and *trans*-cinnamic acid as compared to the raw black soybeans.

Literature data on phenolic acid content in soybeans are very limited. In the present study, we found that total phenolic acids of both yellow and black soybeans were reduced by thermal

Table 4. Effect of Boiling and Steaming on Isoflavone Compositions (Micrograms per Gram) of Yellow and Black Soybeans[a]

	β-glucosides			malonylglucosides			acetylglucosides			aglucones			total individuals[b]			total isoflavones[c]
	Din	Gin	Gly	MDin	MGin	MGly	ADin	AGin	AGly	Dein	Gein	Glein	T-Dein	T-Gein	T-Glein	
							Yellow Soybean									
raw	152.6 d	424.3 d	50.36 d	1122.9 a	1264.2 a	126.2 a	147.9 a	ND	30.04 b	4.91 a	3.26 c	ND	769.3 a	117.1 c	962.2 b	1848.6 b
RB, 120 min	445.5 bc	1441.1 b	66.93 c	50.18 c	153.3 c	11.64 c	34.74 c	ND	19.02 c	6.60 d	6.00 b	34.98 c	300.5 d	89.62 e	986.6 b	1376.7 c
PB, 15 psi, 15 min	403.6 c	1242.5 c	60.99 c	162.4 b	364.9 b	16.81 b	112.4 b	ND	4.16 d	8.75 b	6.83 b	53.41 b	399.7 c	103.7 d	973.7 b	1477.0 c
RS, 100 min	484.2 b	1448.4 b	93.51 b	ND	258.0 c	17.49 b	30.66 c	ND	82.30 a	7.78 c	5.96 b	29.80 c	320.4 d	146.7 b	1045.7b	1512.8 c
PS, 15 psi, 60 min	826.9 a	2283.5 a	153.4 a	ND	20.97 e	ND	148.9 a	ND	20.68 c	15.20 a	14.22 a	121.2 a	602.7 b	231.1 a	1452.3 a	2286.0 a
							Black Soybean									
raw	70.03 e	311.6 e	54.84 e	468.9 a	45.63 c	200.0 a	87.28 b	ND	7.18 e	4.81 b	2.24 c	ND	333.3 a	146.1 c	527.8 b	1007.2 c
RB, 120 min	266.1 c	750.4 c	152.1 c	46.40 b	13.30 c	15.66 d	36.35 e	ND	22.04 c	5.06 b	3.63 b	17.73 c	211.2 bc	135.9 c	518.4 b	865.5 d
PB, 15 psi, 15 min	208.3 d	576.1 d	123.3 d	59.16 b	17.33 b	25.06 c	56.17 d	ND	30.96 c	5.04 b	3.40 b	31.49 b	193.3 c	141.5 c	424.1 c	758.9 e
RS, 100 min	302.5 b	937.9 b	186.9 b	ND	12.98 c	42.68 b	72.21 c	ND	44.35 b	5.33 b	3.39 b	26.18 b	230.1 b	194.1 b	664.1 a	1088.2 b
PS, 15 psi, 60 min	427.4 a	1029.2 a	238.8 a	ND	0.60 d	3.96 e	117.9 a	ND	59.26 a	8.66 a	6.27 b	75.50 a	335.0 a	264.4 a	653.4 a	1252.8 a

[a] Data are expressed as means of triplicate experiments on dry weight basis. Values marked by the same letter within each soybean in each column are not significantly different ($p < 0.05$). Din, daidzin; Gin, genistin; Gly, glycitin; MDin, malonyldaidzin; MGin, malonylgenistin; MGly, malonylglycitin; ADin, acetyldaidzin; AGin, acetylgenistin; AGly, acetylglycitin; Dein, daidzein; Gein, genistein; Glein, glycitein; T-Dein, subtotal daidzein; T-Gein, subtotal genistein; T-Glein, subtotal glycitein. ND, not detected; RB, regular boiling; PB, pressure boiling; RS, regular steaming; PS, pressure steaming. [b] Subtotal individuals = moles of isoflavone × molecular weight of aglycone form isoflavone. [c] Total isoflavones = sum of subtotal individuals of aglycones.

processing. In the case of yellow soybeans, total phenolic acids were reduced by 67.3% after regular boiling, by 53.1% after pressure boiling, by 48.9% after regular steaming, and by 32.1% after pressure steaming. In the case of black soybeans, total phenolic acids were reduced by 64.5% after regular boiling, by 59.3% after pressure boiling, by 24.9% after regular steaming, and by 24.2% after pressure steaming. In addition, we found that steaming processing retained more total phenolic acids than boiling processing.

Effect of Thermal Processing on Isoflavone Compositions. The chemical changes of isoflavone compositions in processed yellow soybeans and soy foods, such as soy milk, tofu, and tempeh, have been investigated in several earlier papers (7, 50, 51). However, no systematic studies had been reported for both whole yellow and black soybeans cooked under atmospheric and pressure thermal processing conditions. The isoflavone contents of the original raw and thermal processed yellow and black soybeans are presented in **Table 4**. The results in this table were expressed in two ways: (1) Individual isoflavone contents were directly measured from HPLC chromatograms for all 12 forms. (2) Subtotal isoflavone contents of aglucones (aglucone equivalents) for each of the three types of isoflavones were calculated by converting the malonylglucosides, acetyl-glucosides, and β-glucosides weight into the aglucone weight using the respective molecular weight factors prior to summation. Total isoflavone contents were the sum of the adjusted sums of total genistein + total daidzein + total glycitein according to the method of Murphy et al. (23). Therefore, the total isoflavone values were not the simple addition of the mean individual values.

The total isoflavones contained in the raw yellow and black soybeans were about 1850 and 1000 μg/g, respectively. Most isoflavones existed as glucosides. The highest proportion at more than 76% of the total was 6″-*O*-malonyl-β-glucosides, followed by β-glucosides at 19%, whereas 6″-*O*-acetyl-β-glucosides and aglucones occurred in only very small proportions.

There was a significant impact on the retention and distribution of isoflavones as a result of different processing methods. The malonyl glucosides decreased dramatically with an increase in β-glucosides and aglucones after thermal processing. Significant differences ($p < 0.05$) in isoflavone contents were found among most processing treatments for both yellow and black

soybeans. As compared to the raw soybeans, all processing conditions significantly ($p < 0.05$) increased the content of β-glucoside form (daidzin, glycitin, genistin) and aglucone (daidzein, glycitein, genistein) isoflavones and significantly ($p < 0.05$) decreased the content of the malonylglucoside form (malonyldaidzin, malonylglycitin, malonylgenistin) isoflavones in both yellow and black soybeans. The boiling treatments significantly decreased acetyldaidzin content in both yellow and black soybeans, whereas pressure steaming significantly increased the contents of acetyldaidzin and acetylglycitin in the case of black soybeans as compared to their raw beans. In terms of the content of total individuals of each isoflavone group (glucosides and conjugated forms adjusted to the respective aglucone weight of that group), regular boiling, pressure boiling, and regular steaming significantly ($p < 0.05$) reduced the contents of total individuals of daidzein group in both yellow and black soybeans as compared to the raw soybeans, whereas both regular and pressure steaming significantly ($p < 0.05$) increased contents of total individuals of genistein and glycitein group in black soybeans. The pressure steaming significantly ($p < 0.05$) increased the contents of total individuals of genistein and glycitein group in yellow soybeans as compared to the raw soybeans. In terms of the contents of total isoflavones (sum of all 12 isoflavone forms, which were adjusted to their respective aglucone weight), as compared to the raw soybeans, both regular and pressure boiling significantly ($p < 0.05$) reduced contents of total isoflavones in both yellow and black soybeans. The regular steaming significantly ($p < 0.05$) reduced the contents of total isoflavones in yellow soybeans, whereas pressure steaming significantly ($p < 0.05$) increased the contents of total isoflavones in both yellow and black soybeans.

As compared to the boiling processing, steaming processing yielded significantly ($p < 0.05$) higher β-glucoside forms and relatively higher aglucone forms, as well as significantly ($p < 0.05$) higher total individuals and significantly ($p < 0.05$) higher total isoflavones in both yellow and black soybeans. As compared to the regular steaming processing, pressure steaming processing yielded significantly ($p < 0.05$) higher β-glucosides, acetylglucosides, and aglucones, as well as higher total individuals (daidzein and genistein) and higher total isoflavones in both yellow and black soybeans, but yielded significantly ($p < 0.05$) lower malonylglucoside forms of isoflavones in both

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Table 5. Effect of Boiling and Steaming on Anthocyanin Compositions (Micrograms per Gram) of Soybeans[a]

	cyanidin-3-glucose	peonidin-3-glucose
	Yellow Soybean	
raw	ND	ND
	Black Soybean	
raw	365.8 a	62.79
RB, 120 min	9.64 b	ND
PB, 15 psi, 15 min	12.40 b	ND
RS, 100 min	12.58 b	ND
PS, 15 psi, 60 min	7.73 b	ND

[a] Data are expressed as means of triplicate experiments on dry weight basis. Values marked by the same letter within each soybean in each column are not significantly different ($p < 0.05$). ND, not detectable; RB, regular boiling; PB, pressure boiling; RS, regular steaming; PS, pressure steaming.

yellow and black soybeans. There were no obvious pattern differences between regular and pressure boiling in terms of the content of individual, total individual, and total isoflavones in both yellow and black soybeans.

Our findings confirmed that the isoflavones contained in soybeans are mainly 6″-O-malonyl-β-glucosides, but are transformed to β-glucosides, 6″-O-acetyl-β-glucosides and aglucones upon thermal processing (*7, 50–53*). In addition, we found that steaming processing transformed more 6″-O-malonyl-β-glucosides into β-glucosides, 6″-O-acetyl-β-glucosides, and aglucones than boiling, but retained more total isoflavones, in cooked yellow and black soybeans. The pressure steaming transformed more 6″-O-malonyl-β-glucosides into β-glucosides, 6″-O-acetyl-β-glucosides, and aglucones than regular steaming and yielded higher total isoflavones than regular steaming, even higher than in the raw yellow and black soybeans. The exact reason for the increase of total isoflavones in both yellow and black soybeans upon pressure steaming remains unclear. A possible reason may be attributed to the release of more isoflavones, which complexed with structural protein or with other types of substances.

Effect of Thermal Processing on Anthocyanin Compositions. The anthocyanin contents of the original raw and thermally processed yellow and black soybeans are presented in **Table 5**. The anthocyanins were not detectable in the raw and processed yellow soybeans, but two anthocyanins (cyanidin-3-glucoside and peonidin-3-glucoside) were detected in the raw black soybeans. The dominant component was cyanidin-3-glucoside. These findings are in accordance with those of Yoshida et al. (*25*), Choung et al. (*26*), and Katsuzaki et al. (*27*), who found that cyanidin-3-glucoside was the major anthocyanin in black soybeans.

There was a significant impact on the retention of anthocyanins as a result of thermal processing. Only trace (8–13 μg/g) amounts of cyanidin-3-glucoside were detected in thermally processed black soybeans, whereas no peonidin-3-glucoside was detected in thermally processed black soybean products. There were no significant differences between different processing methods in terms of anthocyanin contents in cooked black soybeans. The thermal processing lost 96–98% of cyanidin-3-glucoside and 100% of peonidin-3-glucoside as compared to the raw black soybeans (365.8 and 62.8 μg/g, respectively). The lost anthocyanins in black soybeans might be attributed to degradation or decomposition of anthocyanins upon thermal treatments. Previously, the thermal degradation of anthocyanins, both in extracts and in model systems, was reported to follow first-order reaction kinetics in all studies. The stability of

anthocyanins and all pigments found in foods decreased with increase temperature (*54*). These results indicated the fate of anthocyanins during thermal processing.

Correlations of Phenolic Compounds and Antioxidant Activities. The correlations between selected predominant phenolic compounds that existed in soybeans and overall antioxidant activities of soybeans were analyzed. In the case of yellow soybeans, a few individual phenolic compounds (gallic acid, daidzin, glycitin, genistin), subtotal individual compositions (subtotal glycitein, subtotal genistein), and the total compositions (total isoflavones) exhibited significant ($p < 0.05$ or 0.0001) linear correlations with the overall antioxidant activities (DPPH, FRAP, ORAC). However, no significant correlations existed between the other compounds and antioxidant activities. The steaming process caused more free gallic acids to be released from the bound form, yielded higher content of subtotal benzoic acids, transformed most malonylglucoside isoflavones into β-glucosides and aglucone isoflavones, and yielded higher contents of subtotal glycitein, subtotal genistein, and total isoflavones and, therefore, increased overall antioxidant activities (DPPH, FRAP, ORAC) of steamed yellow soybean products. In the case of black soybeans, the most predominant individual phenolic acids (chlorogenic acid, *trans*-cinnamic acid), malonylglucoside isoflavones (malonyldaidzin, malonylglycitin, malonylgenistin), the predominant anthocyanin (cyanidin-3-glucoside), subtotal individual compositions (subtotal benzoic acids, subtotal cinnamic acids, subtotal daidzein), and total compositions (total phenolic acids) exhibited significant ($p < 0.05$ or 0.0001) linear correlations with antioxidant activities (DPPH, FRAP, ORAC). However, no significant correlations existed between the other compounds and antioxidant activities. All cooking methods broke down chlorogenic acid, *trans*-cinnamic acid, and cyanidin-3-glucoside and decreased the content of subtotal cinnamic acids, total phenolic acids, and anthocyanins in black soybeans and, therefore, decreased overall antioxidant activities (DPPH, FRAP, ORAC) of cooked black soybean products. In summary, the different correlation patterns between phenolic compositions and antioxidant properties of yellow and black soybeans helped explain the different effects of cooking methods on the antioxidant properties of these two bean types. Thermal processing affected not only the content of phenolic compounds in soybeans but may also affect the beneficial biological effects associated with these compounds. A greater quantity of phenolic compounds and antioxidant capacity could be provided by consuming steamed soybeans, especially pressure steamed, as compared with soybeans prepared by other cooking processes.

ABBREVIATIONS USED

TPC, total phenolic content; TFC, total flavonoid content; CTC, condensed tannin content; MAC, monomeric anthocyanin content; DPPH, 2-diphenyl-1-picrylhydrazyl radical; FRAP, ferric reducing antioxidant power; ORAC, oxygen radical absorbing capacity; FPA, free phenolic acid; CPA, conjugated phenolic acid; MPRs, Maillard reaction products.

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