DOI:10.1021/jf900695s

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Total Phenolic, Phenolic Acid, Anthocyanin, Flavan-3-ol, and Flavonol Profiles and Antioxidant Properties of Pinto and Black Beans (*Phaseolus vulgaris* L.) as Affected by Thermal Processing

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The effects of boiling and steaming processes at atmospheric and high pressures on the phenolic components and antioxidant properties of pinto and black beans were investigated. In comparison to the original raw beans, 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) values in both pinto and black beans. Steaming processing resulted in a greater retention of TPC, DPPH, FRAP, and ORAC values than the boiling processes in both pinto and black beans. To further investigate how thermal processing affected phenolic compositions and to elucidate the contribution of individual phenolic compounds to antioxidant properties, phenolic acids, anthocyanins, flavan-3-ols, and flavonols were quantitatively analyzed by high-performance liquid chromatography (HPLC). All thermal processing significantly ($p < 0.05$) affected individual phenolic acids, anthocyanins, flavan-3-ols, and flavonols, significantly ($p < 0.05$) reduced total phenolic acid contents in both pinto and black beans and total flavonol contents in pinto beans, and dramatically reduced anthocyanin contents in black beans. Phenolic acids and flavonols may play important roles on the overall antioxidant activities of pinto beans, while anthocyanins, flavan-3-ols, and flavonols may play important roles on the overall antioxidant activities of black beans.

KEYWORDS: Pinto bean; black bean; total phenolics; phenolic acids; anthocyanins; flavan-3-ols; flavonols; processing; antioxidants; DPPH; FRAP; ORAC; HPLC

INTRODUCTION

In recent years, colored common beans, including pinto beans and black beans, have attracted a great deal of attention because of their functional pigments and health-promoting effects in relation to prevention of chronic diseases, including cancers, cardiovascular diseases, obesity, and diabetes. Nutrition scientists working at the United States Department of Agriculture (USDA) measured antioxidant levels of more than 100 antioxidant-rich foods. Interestingly, 3 types of common beans (including pinto bean) occupied the top 4 positions in the list of the top 10 antioxidant-rich foods based on per serving (*1*). *In vitro* antioxidant activities of raw pinto and black beans have been reported previously (*2*−*4*). Some major phenolic phytochemicals, such as flavonols, flavonol glycosides, anthocyanins, and procyanidins, have been isolated and identified from pinto and black beans (*2*, *5*−*7*). This research indicates that pinto and black beans may serve as excellent dietary sources of natural antioxidants for disease prevention and health promotion. However, the health-promoting capacity of beans strictly depends upon their processing history because beans must be cooked or processed before consumption.

Food processing not only improves flavor and palatability of legume foods but also increases the bioavailability of nutrients and reduces the flatulence factors (raffinose oligosaccharides) in legume foods. Cooking brings about a number of changes in physical characteristics and chemical compositions of food legumes. Food legumes are usually cooked by a boiling process before use. Pressure boiling and steaming can also be used for this purpose. Common beans (*Phaseolus vulgaris* L.) are the world's second most important legume class after soybeans and are one of the basic foods in Africa, India, and Latin American. Nutrition scientists characterize the common bean as a nearly perfect food because of its high protein content and generous amounts of fiber, complex carbohydrates, and other dietary necessities. Total per capita consumption of common beans has increased markedly over the past 2 decades (*8*) in the United States. It is important to elucidate the effect of traditional thermal processing on functional components of common beans. Currently, only two studies

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 Published on Web 05/14/2009

reported the changes of antioxidant components and antioxidant activities of the processed beans, including our previous report on black bean (*9*) and the other reported by Rocha-Guzman et al. (*10*) on pressure-cooked (autoclaved) common beans. Our preliminary study showed that soaking, boiling, and steaming processing significantly affected the total phenolic contents and antioxidant activities of black bean (*P. vulgaris* L. cv. Turtle Eclipse) (*9*) and cool season food legumes (*11*). However, how thermal processing affects individual phenolic compounds present in beans and which components are responsible for the antioxidant activity have not been studied. Therefore, the present study was undertaken to investigate the effects of boiling and steaming processes on the phenolic compounds and antioxidant activities of pinto and black beans and to elucidate the contribution of phenolic components to the antioxidant properties of pinto and black beans.

MATERIALS AND METHODS

Chemicals and Reagents. A total of 16 phenolic acids (gallic, protocatechuic, 2,3,4-trihydroxybenzoic, *p*-hydroxybenzoic, gentistic, vanillic, caffeic, chlorogenic, syringic, *p*-coumaric, *m*-coumaric, *o*-coumaric, ferullic, salicylic, sinapic, and *trans*-cinnamic acid), 3 aldehydes (vanillin, syringaldehyde, and protocatechualdehyde), 5 flavan-3-ols [(+)-catechin, (+)-epicatechin, epigallo-catechin, epicatechin-gallate, and epigallatecatechin-gallate], and 6 flavonols or flavones (myricetin, luteolin, quercetin, apigenin, kaempferol, and quercetin-3-rutinoside), high-performance liquid chromatography (HPLC)-grade trifluoroacetic acid (TFA), 2-diphenyl-1-picryhydrazyl radical (DPPH$^{\bullet}$), fluorescein disodium (FL), Folin−Ciocalteu reagent, sodium carbonate, 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ), and 6-hydroxy-2,5,7,8-tetramethlchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Kaempferol-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, and quercetin-3-*O*-glucoside were purchased from Extrasynthese S.A. (Genay, France). A mixture of six unimolar anthocyanin standards (3-*O*-*β*-glucosides of delphinidin, cyanidin, petunidin, pelargonidin, peonidin, and malvidin) was purchased from Polyphenols Laboratories (Sandnes, Norway). 2,2′-Azobis(2-amidino-propane)dihydrochloride (AAPH) was purchased from Wako Chemicals USA (Richmond, VA). HPLC-grade solvents (methanol and acetonitrile, B & J Brand) and other analytical-grade solvents used for extraction were purchased from VWR International (West Chester, PA). Polyvinylidene difluoride (PVDF) syringe filters with a pore size of 0.2 μm were purchased from National Scientific Company (Duluth, GA).

Bean Materials. The dry beans used in this study were pinto beans (*P. vulgaris* L. cv. Pinto, 2002 crop) and black beans (*P. vulgaris* L. cv. Turtle Eclipse, 2004 crop), which were obtained from Archer Daniel Midland (Casselton, ND) and the University of Idaho Foundation Seed Program Kimberly Research and Extension Center (Kimberly, ID), respectively. Broken seeds, damaged seeds, and foreign materials were removed from the samples. The moisture content was determined by drying a portion of raw beans or freeze-dried cooked bean samples in an air oven at 110 °C until a constant weight was obtained (*12*). Another portion of original raw beans (without drying) was soaked and subsequently cooked according to methods described in the following sections. All calculations for the determination of phenolics and quantification of antioxidant activities are on a dry weight basis according to the respective moisture contents in original raw beans and freeze-dried cooked beans.

Soaking and Hydration Ratio. Soaking and hydration ratio calculations were carried out according to our previous communication (*9*). The soaked beans (with a 50% hydration ratio) were drained and then boiled or steamed by the methods described below.

Boiling, Steaming, and Cooking Time. All thermal processes were performed according to our previous procedures (*9*). Briefly, regular boiling and steaming treatments were conducted using a domestic atmospheric cooker and a domestic atmospheric steam cooker, respectively. Pressure boiling and steaming were conducted using a M-0512-H Mirro pressure cooker (Mirro Co., Manitowoc, WI). The cooking time was determined on the basis of a tactile method according to Vindiola et al. (*13*). Boiling and steaming time as well as pressure conditions were selected for pinto bean from the preliminary experiments and selected for black bean from our previous report (*9*). After cooking treatments, the beans were drained and cooled to room temperature in covered plastic containers. Subsequently, the cooked samples were frozen and then freeze-dried.

Extraction of Total Phenolic Composition. The original raw beans and the freeze-dried cooked beans were ground to flour with an IKA all basic mill (IKA Works, Inc., Wilmington, NC) to pass through a 60-mesh sieve. Extraction procedures followed our earlier communication (*14*). Briefly, bean flours (0.5 g in triplicate) were extracted twice with 5 mL of acetone/water/acetic acid (70:29.5:0.5, v/v/v). The extracts were combined and used for the determination of total phenolics and antioxidant activities.

Determination of Total Phenolic Content (TPC). The TPC was determined by a Folin−Ciocalteu assay (*15*) using gallic acid (GA) as the standard. The absorbance was measured using a UV−vis spectrophotometer (UV 160, Shimadzu, Japan) at 765 nm against a reagent blank. The TPC was expressed as milligrams of gallic acid equivalents per gram of dry bean (mg of GAE/g) 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 (*14*, *16*). The absorbance was measured at 510 nm using a UV−vis spectrophotometer (UV 160, Shimadzu, Japan). The TFC was expressed as milligrams of catechin equivalents per gram of dry bean (mg of CAE/g) 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 CTC analysis was carried out according to the method described previously (*17*), with slight modifications (*14*). The absorption was measured using a UV−vis spectrophotometer (UV 160, Shimadzu, Japan) at 500 nm against methanol as a blank. The CTC was expressed as milligrams of catechin equivalents per gram of dry bean (mg of CAE/g) 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 (*18*). A Shimadzu UV 160 double-beam spectrophotometer was used for measuring absorbance at 700 and 520 nm. The pigment content was expressed as milligrams of cyanidin-3-glucoside equivalents per gram of dry bean (mg of CyE/g), using an extinction coefficient of 26 900 L cm^{-1} mol^{-1} and molecular weight of 449.2 g mol^{-1}. Even though delphinidin-3-glucoside is the major anthocyanin in black beans (*5*), the total anthocyanin content is calculated as cyanidin-3-glucoside because of its historical usage for similar assays and its wide commercial availability (*18*).

Radical DPPH Scavenging Activity (DPPH). The free-radical scavenging capacity was evaluated according to previous reports (*14*, *19*). The DPPH value was expressed as micromoles of Trolox equivalent per gram of dry bean (μmol of TE/g) using the calibration curve of Trolox. The linearity range of the calibration curve was 20−1000 μM (r = 0.99).

Ferric-Reducing Antioxidant Power (FRAP). The FRAP assay was performed as the method described previously (*14*, *20*). The FRAP value was expressed as millimoles of Fe^{2+} equivalent per 100 g of dry bean (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).

Oxygen Radical Absorbing Capacity (ORAC). Hydrophilic ORAC analysis was carried out on a BMG Fluostar Optima Microplate Reader (BMG Labtech GmbH, Offenburg, Germany), which was equipped with two autoinjectors, an incubator, and wavelength adjustable fluorescence filters. The procedures were based on the previous report by Prior et al. (*21*), with slight modifications (*14*). The ORAC value was expressed as micromoles of Trolox equivalent per gram of dry bean (μmol of TE/g) using the calibration curve of Trolox. The linearity range of the calibration curve was 5.0−50 μM (r = 0.99).

HPLC Analysis of Phenolic Acid Contents. *Extraction of Free Phenolic Acids*. The extraction of free phenolic acids was performed by modifying the method of Luthria and Pastor-Corrales (*22*). Briefly, the original raw and freeze-dried cooked bean samples (0.5 g in triplicate) were extracted twice with 5 mL of methanol/water/acetic acid/butylated hydroxytoluene (85:15:0.5:0.2, v/v/v/w) by shaking extraction tubes on an

orbital shaker at 300 rpm at room temperature for 4 h. The extracts were concentrated at 45 °C under vacuum to remove solvents. The dry residue was dissolved in 5 mL of water and freeze-dried. The freeze-dried extracts (10 mg) were dissolved in 1 mL of 25% methanol. The methanol solution was centrifuged and 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 report (*22*), with slight modifications. Briefly, the raw and cooked bean samples (0.4 g in triplicate) were hydrolyzed and extracted with 10 mL of 2 N NaOH [contained 10 mM ethylenediaminetetraacetic acid (EDTA) and 1% vitamin C (w/v)], at 40−45 °C for 30 min. 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 solvents. The dry residue was redissolved in 1.5 mL of 25% 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 Robbins and Bean (*23*), with slight modifications (*24*). A Waters Associates (Milford, MA) chromatography system equipped with a model 720 system controller, model 6000A solvent delivery system, model 7125 loading sample injector, and model 418 LC UV detector (270 nm) was used. A 4.6 × 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), and 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 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 in the samples, 1 mg/mL stock solution of each individual standard was prepared and diluted to 100 μg/mL. The diluted working solutions were injected to HPLC. The spiking and external standard methods were used for identification of peaks by comparing the increase of peak areas and retention time. In addition, to further confirm the identities of peaks through their UV spectrum information, individual phenolic acid and phenolic acid mixture as well as several typical samples was 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 using HPLC analysis, as described previously (*24*). To prepare 1 mg/mL stock solution of the standard mixture, 10 mg of each phenolic acid compound was mixed together and dissolved in 10 mL of 25% methanol. The stock solution was diluted into nine series of standard working solution with distilled water (100, 50, 25, 10, 5, 2.5, 1, 0.5, and 0.25 μg/mL). Standard curves of phenolic acids were established by plotting peak areas against the concentrations of the standards from the averages of duplicate injections. Phenolic acid contents were expressed as micrograms per gram of bean (μg/g) on a dry weight basis.

HPLC Analysis of Flavan-3-ol and Flavonol Content. *Extraction of Flavonols*. The cooked bean samples were freeze-dried and then ground. The ground original raw and freeze-dried cooked beans (0.5 ± 0.01 g in triplicate) were extracted at room temperature with 10 mL of extraction solvent (70% acetone/29.5% water/0.5% acetic acid, v/v/v) by shaking extraction tubes on an orbital shaker at 250 rpm for 3 h. The slurry was centrifuged by an Allegra 21R Centrifuge (Beckman Coulter Ltd., Palo Alto, CA) at 5500 rpm for 20 min. A total of 5 mL of the supernatant was evaporated to dryness on a rotary evaporator at 35 °C. The residues in the flask were dissolved in 2 mL of 80% methanol. An aliquot of the sample solution was filtered through a 0.2 μm PTFE syringe filter prior to HPLC assay.

HPLC Analysis of Flavonols. The quantitative analysis of flavonols was performed according to methodology of isoflavone analysis developed by Murphy et al. (*25*), with a slight modification. The same Waters Associates chromatography system (Milford, MA) as that used for phenolic acid analysis was used for the analysis of flavonols with UV 262 nm detection. A YMC-Pack ODS-AM-303 C_{18} reversed-phase column (250 × 4.6 mm internal diameter, 5 μm particle size) was obtained from Waters (Milford, MA) 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 20 μL of sample was injected, the system was eluted with 15% of solvent B for 5 min at the flow rate of 1.0 mL/min, increased to 29% over 31 min at the flow rate up to 1.5 mL/min, and then increased to 35% over 8 min at the same flow rate of 1.5 mL/min. Then, the gradient was increased to 50% of solvent B in 2 min, kept at 50% of solvent B for 10 min at the flow rate of 1.5 mL/min, and then recycled back to 15% B at the flow rate of 1.0 mL/min in 2 min, and the column was equilibrated with the initial solvent for 2 min prior to running the next sample.

Identification and Quantification of Flavan-3-ols and Flavonols. Five flavan-3-ols [(+)-catechin, (+)-epicatechin, epigallo-catechin, epicatechin-gallate, and epigallatecatechin-gallate] and nine flavonols or flavones (myricetin, luteolin, quercetin, apigenin, kaempferol, kaempferol-3-glucoside, kaempferol-3-rutinoside, quercetin-3-glucoside, and quercetin-3-rutinoside) are commercially available and directly used to identify the sample peaks by comparing their retention times and HPLC profiles to a standard mixture as well as literature (*7*, *26*, *27*). In addition, a spiking method was used for peak identification of some samples.

The quantification of flavan-3-ols and flavonols was performed by calibrating the peak areas obtained from HPLC analyses. External calibration curves were obtained for each of six external standards by plotting the peak area of each standard against the concentration. For the other flavonols without commercial standards, concentrations were calculated from the standard curves that were adjusted appropriately from the standard curves of the respective form of flavonols based on the differences in molecular weight and molar extinction coefficients of the compounds. Flavonol contents were expressed as micrograms of flavonol per gram of dry bean (μg/g).

HPLC Analysis of Anthocyanin Content. *HPLC Conditions*. The free phenolic acid extracts were also used for anthocyanin analysis. The analysis was performed on a HP 1090 HPLC (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) according to our previous communication (*24*). HPLC conditions were as follows: solvent A, 0.1% TFA/H_2O; solvent B, CH_3CN/H_2O/TFA (50:50:0.1, v/v/v); linear gradient, initial percentage of B (15%) to 60 min (40%); column temperature, 40 °C; and flow rate, 0.5 mL/min. The filter detector was set at 540 nm.

Identification and Quantification of Anthocyanin. The identifications and peak assignments of anthocyanins were primarily based on the comparison of their retention times to those of standards, a blueberry reference sample, and the literature (*5*). The stock solution of anthocyanins 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 concentration of 1.0 mg/mL. A portion of the stock solution was then diluted using methanol to the following series dilutions: 1 in 5, 10, 20, 40, 80, and 160. Standard curves of anthocyanins were plotted peak areas against concentrations by duplicate injections of the six series diluted working solutions of the standard mixture. Anthocyanin contents were expressed as micrograms of anthocyanin per gram of bean (μg/g) on a dry weight basis.

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

RESULTS AND DISCUSSION

Hydration Ratio and Soaking, Boiling, and Steaming Time. Traditionally, dry legumes are soaked to hydrate prior to boiling

Table 1. Effect of Boiling and Steaming on Free Phenolic Acid (FPA) Compositions (μg/g) of Pinto and Black Beans[a]

	individual benzoic derivate							
	GA	PA	TBA	PCD	HBA	VA	VN	subtotal benzoics
				Pinto Bean				
raw	83.17 ± 7.4 b	16.08 ± 3.7 a	1.23 ± 0.2 c	49.90 ± 3.9 a	16.59 ± 0.9 a	113.16 ± 5.9 a	94.58 ± 10.8 a	374.72 ± 14.9 a
RB, 90 min	34.42 ± 2.6 cd	10.11 ± 0.6 b	2.52 ± 0.1 b	14.02 ± 0.5 bc	4.81 ± 0.3 c	28.75 ± 0.5 c	19.85 ± 1.0 b	114.48 ± 4.1 c
PB, 15 psi, 10 min	38.16 ± 2.6 c	8.94 ± 0.8 bc	1.99 ± 0.1 bc	16.66 ± 0.6 b	4.66 ± 0.5 c	38.41 ± 0.9 b	21.39 ± 8.7 b	130.24 ± 11.6 c
RS, 70 min	23.35 ± 7.8 d	5.56 ± 1.9 c	2.48 ± 0.7 b	10.15 ± 3.3 c	3.94 ± 1.4 c	15.36 ± 4.7 d	16.92 ± 5.8 b	77.77 ± 24.7 d
PS, 15 psi, 60 min	114.57 ± 11.4 a	10.45 ± 1.1 b	5.21 ± 0.7 a	10.67 ± 0.6 c	7.19 ± 0.2 b	12.08 ± 0.8 d	17.69 ± 4.3 b	177.89 ± 16.8 b
				Black Bean				
raw	89.64 ± 8.9 b	12.69 ± 0.7 b	ND[b]	ND	19.03 ± 0.7 a	58.64 ± 1.6 b	14.74 ± 0.5 b	194.73 ± 10.6 c
RB, 80 min	41.16 ± 1.5 c	ND	2.18 ± 0.2 b	ND	11.35 ± 0.3 bc	13.08 ± 0.6 c	7.42 ± 0.2 b	75.18 ± 2.4 d
PB, 15 psi, 10 min	32.92 ± 2.9 c	2.04 ± 0.3 c	1.19 ± 0.1 c	3.16 ± 0.1 c	10.74 ± 0.3 c	10.69 ± 0.9 cd	8.15 ± 0.90 b	68.89 ± 4.4 d
RS, 70 min	78.17 ± 2.7 b	66.11 ± 2.4 a	ND	55.18 ± 0.9 a	ND	7.95 ± 0.4 d	36.11 ± 16.9 a	243.53 ± 12.6 b
PS, 15 psi, 60 min	202.05 ± 11.1 a	ND	9.95 ± 0.1 a	12.22 ± 1.2 b	12.10 ± 0.4 b	62.67 ± 4.2 a	36.17 ± 8.1 a	335.17 ± 17.7 a

	individual cinnamic acid derivate							
	CFA	CLA	PCA + SD	MCA + FA	SPA	TCA	subtotal cinnamics	total phenolic acids
				Pinto Bean				
raw	ND	465.0 ± 21.2 a	ND	ND	264.0 ± 18.1a	ND	729.0 ± 38.5 a	1103.7 ± 43.2 a
RB, 90 min	ND	167.0 ± 7.1 b	18.22 ± 1.6 b	6.96 ± 0.9 a	45.18 ± 3.8 c	2.35 ± 0.1 b	239.7 ± 12.7 bc	354.2 ± 13.8 bc
PB, 15 psi, 10 min	ND	217.9 ± 6.5 b	24.00 ± 0.9 a	7.15 ± 0.9 a	60.70 ± 1.7 b	2.25 ± 0.1 b	311.3 ± 9.3 b	441.6 ± 20.4 b
RS, 70 min	ND	195.0 ± 62.2 b	7.54 ± 0.3 c	1.51 ± 0.6 b	11.00 ± 3.5 d	18.13 ± 4.8 a	232.1 ± 72.8 c	309.9 ± 97.5 c
PS, 15 psi, 60 min	ND	212.5 ± 19.3 b	6.75 ± 0.9 c	1.92 ± 0.7 b	14.32 ± 1.5 d	22.83 ± 1.9 a	258.3 ± 24.0 bc	436.2 ± 40.9 b
				Black Bean				
raw	8.59 ± 5.2 ab	226.1 ± 38.9 a	ND	9.29 ± 0.1 b	13.99 ± 0.3 d	ND	257.9 ± 43.7 b	452.7 ± 41.2 c
RB, 80 min	10.75 ± 0.6 a	111.0 ± 4.3 b	ND	2.26 ± 0.2 c	19.66 ± 0.5 c	ND	143.7 ± 4.5 c	218.9 ± 6.8 d
PB, 15 psi, 10 min	2.85 ± 0.9 b	89.9 ± 12.9 b	ND	1.42 ± 0.3 c	8.65 ± 0.5 e	ND	102.9 ± 13.0 d	171.8 ± 17.4 e
RS, 70 min	ND	259.8 ± 14.9 a	ND	9.39 ± 1.1 b	48.38 ± 2.1 b	ND	317.6 ± 13.7 a	561.1 ± 4.0 b
PS, 15 psi, 60 min	ND	250.7 ± 8.0 a	ND	13.70 ± 0.6 a	53.73 ± 3.1 a	ND	318.1 ± 10.6 a	653.3 ± 15.9 a

[a] Data are expressed as mean ± standard deviation (n = 3) on a dry weight basis. Values marked by the same letter within each bean 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; 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; TCA, *trans*-cinnamic acid. RB, regular boiling; PB, pressure boiling; RS, regular steaming; PS, pressure steaming. [b] ND = not detectable.

for ease of cooking. The water absorption curves (curves were not shown here) of pinto and black beans were characterized by an initial phase of rapid water pickup followed by an equilibrium phase, during which the beans approached their full soaking capacity. Beans were prone to saturation after soaking for 14 h, and water absorption reached a plateau after soaking for 16 h. In addition, soaking water of black beans had a dark color. This phenomenon indicated that some soluble constituents (may include phenolic antioxidant constituents) were leached into the soaking water. To decrease potential losses of antioxidant components, soaking treatments with a low hydration ratio (50%) and short soaking times were designed for the following boiling and steaming treatments. To obtain the desired hydration ratio (50%), soaking times (2.3 h for pinto bean and 4 h for black bean) were calculated by calibrating through a quadratic fit equation of the water absorption curves.

Cooking time as well as cooked texture, appearance, and flavor are important cooking quality characteristics. The cooking time for producing palatable products is one of the main criteria used in evaluating the cooking quality of dry legumes. In current studies, the tactile method of Vindiola et al. (*13*) was applied to determine the cooking time (including boiling and steaming) of all treatments. We defined the time as the cooking time 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 were not shown here). To prepare processed samples for further analysis, several optimum cooking times were selected. The optimum cooking times (shown in **Tables 1**−**4**) selected for regular boiling, 15 psi pressure boiling, regular steaming, and 15 psi pressure steaming were 90, 10, 70, and 60 min for pinto beans and 80, 10, 70, and 60 min for black beans, respectively.

Effect of Thermal Processing on Total Phenolic Compositions of Beans. Total phenolic content (TPC), total flavonoid content (TFC), condensed tannin content (CTC), and monomeric anthocyanin content (MAC) of the extracts from the original raw and cooked pinto and black beans are presented in **Figure 1**. Significant differences (p < 0.05) in TPC, TFC, CTC, and MAC values were found among most treatments for both pinto and black beans. In the case of pinto beans, in comparison to the original raw beans, all processing treatments caused significant (p < 0.05) decreases in TPC, TFC, and CTC values, while pressure steaming did not cause significant (p < 0.05) decreases in MAC values. In the case of black beans, in comparison to the original raw beans, all heating treatments caused significant (p < 0.05) decreases in all measured phenolic indexes. Notably, all treatments caused almost complete losses of MAC values. In comparison to the boiling treatments, both regular and pressure-steaming treatments retained greater TPC and TFC values. These results indicated that processing caused complex changes in

Table 2. Effect of Boiling and Steaming on Conjugated Phenolic Acid (CPA) Compositions (μg/g) of Pinto and Black Beans[a]

	individual benzoic derivate									
	GA	PA	TBA	PCD	HBA	VA	VN	SA	SCA	subtotal benzoics
					Pinto Bean					
raw	29.06 ± 2.81 a	17.35 ± 1.95 a	84.18 ± 8.80 a	18.70 ± 0.86 a	12.69 ± 1.88 a	2.18 ± 0.20 a	11.71 ± 0.69 a	1.62 ± 0.11 a	237.92 ± 1.06 a	414.70 ± 14.27 a
RB, 90 min	16.73 ± 0.29 c	8.56 ± 0.35 c	32.63 ± 2.73 c	11.21 ± 0.64 cd	6.18 ± 0.57 d	0.61 ± 0.12 b	4.03 ± 0.39 c	ND[b]	64.79 ± 1.41 c	96.43 ± 83.53 c
PB, 15 psi, 10 min	20.04 ± 1.76 b	11.31 ± 0.21 b	35.66 ± 2.83 c	11.48 ± 1.11 c	9.58 ± 0.72 b	0.67 ± 0.08 b	4.05 ± 0.48 c	0.51 ± 0.12 c	132.35 ± 16.50 b	181.36 ± 83.15 c
RS, 70 min	15.67 ± 0.97 c	9.06 ± 0.51 c	58.30 ± 3.17 b	14.69 ± 1.19 b	9.07 ± 0.55 bc	ND	10.61 ± 0.95 a	1.19 ± 0.13 b	128.09 ± 5.97 b	251.18 ± 2.51 b
PS, 15 psi, 60 min	9.42 ± 0.61 d	4.43 ± 0.05 d	22.13 ± 0.47 d	9.48 ± 0.38 d	7.25 ± 0.16 cd	0.74 ± 0.05 b	7.94 ± 0.27 b	0.24 ± 0.03 d	18.06 ± 0.91 d	70.93 ± 14.97 c
					Black Bean					
raw	16.92 ± 1.62 c	5.69 ± 0.18 a	119.97 ± 5.47 a	11.75 ± 0.62 a	4.89 ± 0.49 a	ND	12.89 ± 0.81 a	34.74 ± 1.58 a	45.69 ± 2.52 a	252.55 ± 4.27 a
RB, 80 min	14.54 ± 0.24 c	2.61 ± 0.19 b	42.93 ± 1.89 c	6.78 ± 0.58 c	2.04 ± 0.39 c	ND	4.42 ± 0.21 c	7.01 ± 0.77 d	12.35 ± 0.35 e	92.68 ± 3.17 d
PB, 15 psi, 10 min	29.69 ± 2.69 b	2.51 ± 0.12 b	67.19 ± 5.55 b	6.96 ± 0.47 c	2.62 ± 0.45 bc	ND	4.52 ± 0.41 c	10.29 ± 1.01 c	39.36 ± 3.47 b	163.16 ± 11.15 b
RS, 70 min	15.25 ± 1.45 c	2.58 ± 0.29 b	66.68 ± 4.95 b	8.69 ± 0.41 b	3.58 ± 1.07 b	ND	13.27 ± 0.89 a	15.73 ± 1.27 b	34.67 ± 0.88 c	160.44 ± 11.21 b
PS, 15 psi, 60 min	32.96 ± 0.81 a	5.91 ± 0.22 a	34.88 ± 3.24 c	7.09 ± 0.99 c	2.87 ± 0.40 bc	0.63 ± 0.05	7.46 ± 0.38 b	7.15 ± 0.47 d	27.51 ± 0.69 d	126.47 ± 4.10 c

	individual cinnamic derivate								
	CFA	CLA	PCA + SD	MCA + FA	SPA	OCA	TCA	subtotal cinnamics	total phenolic acids
				Pinto Bean					
raw	11.07 ± 0.55 a	ND	14.49 ± 0.41 a	23.23 ± 2.08 a	24.75 ± 1.89 a	0.91 ± 0.06 a	21.46 ± 1.04 a	88.46 ± 8.94 a	503.16 ± 13.35 a
RB, 90 min	2.86 ± 0.21 c	ND	4.11 ± 0.14 d	6.17 ± 0.59 c	7.32 ± 0.45 d	ND	6.79 ± 0.04 c	27.26 ± 1.013 d	114.60 ± 75.89 c
PB, 15 psi, 10 min	4.47 ± 0.59 b	ND	8.08 ± 0.65 c	6.18 ± 1.85 c	11.66 ± 2.15 c	ND	ND	61 ± 5.40 c	209.97 ± 88.09 c
RS, 70 min	10.68 ± 0.53 a	ND	10.79 ± 0.78 b	19.81 ± 1.97 a	21.09 ± 0.76 b	1.11 ± 0.1 a	13.36 ± 0.28 b	76.27 ± 4.7 b	318.72 ± 12.64 b
PS, 15 psi, 60 min	3.66 ± 0.21 bc	ND	7.46 ± 0.28 c	13.24 ± 1.16 b	10.00 ± 0.42 cd	ND	ND	25.50 ± 17.68 d	96.44 ± 32.64 c
				Black Bean					
raw	23.43 ± 1.05 a	ND	32.44 ± 1.09 a	28.31 ± 3.39 a	25.10 ± 1.86 a	12.84 ± 0.66 a	3.30 ± 0.31 a	125.42 ± 7.14 a	377.97 ± 11.40 a
RB, 80 min	6.16 ± 0.48 e	ND	7.84 ± 0.35 d	7.33 ± 0.58 d	5.46 ± 0.46 e	4.53 ± 0.93 c	0.76 ± 0.15 c	32.23 ± 1.99 c	124.75 ± 4.6 b
PB, 15 psi, 10 min	7.81 ± 0.30 d	ND	11.84 ± 0.25 c	10.74 ± 0.17 c	8.31 ± 0.26 d	4.82 ± 0.40 c	0.87 ± 0.08 c	44.40 ± 1.33 c	207.56 ± 12.06 b
RS, 70 min	10.51 ± 1.23 c	ND	20.77 ± 0.36 c	19.23 ± 0.05 b	18.43 ± 1.52 b	7.21 ± 0.18 b	1.91 ± 0.13 b	78.07 ± 0.54 b	238.51 ± 11.76 b
PS, 15 psi, 60 min	14.68 ± 0.42 b	1.37 ± 0.03	20.84 ± 0.56 b	19.23 ± 0.97 b	13.74 ± 0.73 c	6.82 ± 0.23 b	ND	75.30 ± 2.48 b	201.77 ± 5.20 b

[a] Data are expressed as mean ± standard deviation ($n = 3$) on a dry weight basis. Values marked by the same letter within each bean 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; VA, vanillic acid; VN, vanillin; SA, syringic acid; SCA, salicylic acid; 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. RB, regular boiling; PB, pressure boiling; RS, regular steaming; PS, pressure steaming. [b] ND = not detectable.

Table 3. Effect of Boiling and Steaming on Anthocyanin Compositions (μg/g) of Pinto and Black Beans[a]

	delphinidin-3-glucose	malvidin-3, 5-diglucose[b]	petunidin-3-glucose	malvidin-3-galactoside[b]	malvidin-3-glucose
			Pinto Bean		
raw	ND[c]	ND	ND	ND	ND
			Black Bean		
raw	2196.5 ± 105.9 a	529.8 ± 8.7 a	838.2 ± 37.8 a	25.24 ± 0.53	422.4 ± 30.3 a
regular boiling, 80 min	78.52 ± 2.3 b	115.6 ± 2.1 c	50.89 ± 1.6 b	ND	44.07 ± 1.7 b
pressure boiling, 15 psi, 10 min	117.9 ± 8.7 b	149.4 ± 0.6 c	69.78 ± 11.5 b	ND	49.65 ± 3.8 b
regular steaming, 70 min	150.2 ± 3.4 b	218.3 ± 18.2 b	71.37 ± 3.0 b	ND	45.92 ± 4.9 b
pressure steaming, 15 psi, 60 min	22.98 ± 1.0 b	ND	11.16 ± 0.9 c	ND	6.39 ± 0.4 b

[a] Data are expressed as mean ± standard deviation (n = 3) on a dry weight basis. Values marked by the same letter within a column are not significantly different (p < 0.05). [b] Because of no available commercial compounds, concentrations of malvidin-3,5-diglucose, and malvidin-3-galactoside were calculated from the standard curve of malvidin-3-glucose that was adjusted on the basis of molecular-weight differences. [c] ND = not detectable.

Table 4. Effect of Boiling and Steaming on Flavan-3-ols and Flavonols Compositions (μg/g) of Pinto and Black Beans[a]

	flavan-3-ols			flavonols			
	(+)-catechin	(+)-epicatechin	epicatechin gallate	kaempferol-3-*O*-glucoside	kaempferol-3-*O*-acetylglucoside	myricetin	total flavonols
				Pinto Bean			
raw	ND[b]	ND	ND	147.91 ± 10.5 a	29.99 ± 1.0 a	ND	177.90 ± 11.5 a
RB, 90 min	ND	ND	ND	52.17 ± 1.6 c	9.25 ± 0.2 d	ND	61.42 ± 1.8 c
PB, 15 psi, 10 min	ND	ND	ND	54.53 ± 4.4 c	12.39 ± 0.6 c	ND	66.92 ± 3.9 c
RS, 70 min	ND	ND	ND	80.55 ± 6.5 b	16.35 ± 0.1 b	ND	96.89 ± 6.6 b
PS, 15 psi, 60 min	ND	ND	ND	56.86 ± 4.3 c	ND	ND	56.87 ± 4.3 c
				Black Bean			
raw	246.9 ± 5.9 b	214.8 ± 16.9 b	89.57 ± 3.6 a	27.37 ± 1.2 a	ND	98.76 ± 0.8 a	677.36 ± 17.3 b
RB, 80 min	126.1 ± 15.0 c	177.9 ± 0.3 c	40.65 ± 0.2 d	ND	ND	45.23 ± 0.5 e	389.97 ± 14.5 d
PB, 15 psi, 10 min	103.5 ± 0.3 d	178.9 ± 3.1 c	58.31 ± 3.8 c	2.24 ± 0.1 b	ND	54.98 ± 1.7 d	397.95 ± 8.3 d
RS, 70 min	240.5 ± 5.4 b	207.8 ± 14.5 b	57.33 ± 0.1 c	ND	ND	77.18 ± 0.6 c	582.79 ± 20.5 c
PS, 15 psi, 60 min	296.2 ± 7.5 a	294.1 ± 9.5 a	68.66 ± 1.6 b	ND	ND	88.20 ± 1.2 b	747.09 ± 19.8 a

[a] Data are expressed as mean ± standard deviation (n = 3) on a dry weight basis. Values marked by the same letter within each bean in each column are not significantly different (p < 0.05). [b] ND, not detectable; RB, regular boiling; PB, pressure boiling; RS, regular steaming; PS, pressure steaming.

chemical compositions. Thermal processing might cause degradation of polyphenols and release bound phenolic compositions. The differences in phenolic profiles caused by thermal processing between pinto and black beans might be due to the differences in the distributions and compositions of individual phenolic components in seed coat and cotyledon. In addition, it has been extensively accepted that phenolic determinations based on color reaction may sometimes overestimate phenolic substance content because of certain nonphenolic substances giving a positive reaction. For example, vitamin C and proteins may give a positive reaction in the Folin−Ciocalteu assay. Trace color substances (non-anthocyanin) in pinto bean extract gave a false-positive reaction in the MAC assay of pinto bean, in which anthocyanins were not detected using HPLC analysis. However, we realize that all methods have limitations. For instance, HPLC cannot quantify all phenolic substances under the specific wavelength. That is why we use multiple methods to determine phenolic substances by combining colorimetric and HPLC methods in this report.

Data on phenolics in cooked legumes are very limited. Bressani and Elias (*28*) observed that about 30−40% of phenolics could be removed from common beans by cooking and discarding the cooking water. In the present study, it was found that about 63−77% of TPC, 67−84% of TFC, 32−54% of CTC, and 0−100% of MAC were reduced in pinto beans. Both regular boiling and pressure boiling lost much more TPC (about 71−77%) than steaming treatments in pinto beans. In the case of black beans, about 61−74% of TPC, 61−79% of TFC, 68−73% CTC, and 88−100% MAC losses were found in thermal-processed black beans. In addition, boiling treatment reduced more TPC and TFC than steaming processing. Pressure steaming reduced more TPC, TFC, CTC, and MAC values than regular steaming. These results on the variations of TPC by processing are in good agreement with those reported by Ismail et al. (*29*), who found that thermal treatment decreased the TPC in all vegetables. These results also exhibited trends similar to those of our previous papers (*9*, *11*, *24*), in which we showed that thermal processing lost 50−70% of TPC in peas, 70−80% of TPC in black common beans, and 43−63% of TPC in black soybean. These significant losses might be attributed to those water-soluble phenolics that were leached into soaking and cooking water before and during thermal processing as well as the breakdown of phenolics during processing.

Effect of Thermal Processing on Antioxidant Activities of Beans. Antioxidant activity determination is reaction-mechanism-dependent. The ORAC test reaction in removing reactive oxygen species uses the hydrogen-transfer mechanism, whereas the DPPH and FRAP use the electron-transfer mechanism (*30*). The specificity and sensitivity of one method does not lead to the complete examination of all phenolic compounds in the extract. Therefore, a combination of several tests could provide a more reliable assessment of the antioxidant activity profiles of bean

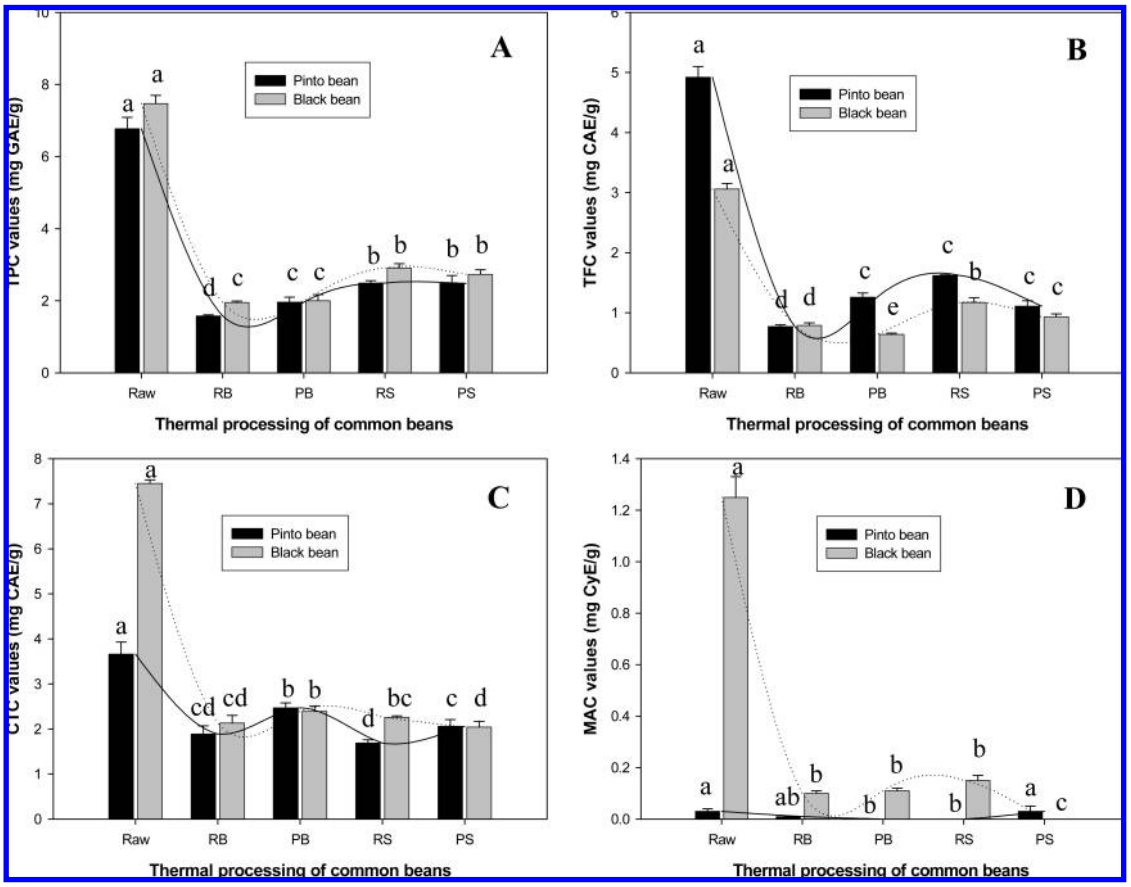

Figure 1. Effect of boiling and steaming on total phenolics (A, TPC; B, TFC; C, CTC; and D, MAC) of pinto and black beans. Bar data are expressed as mean ± standard deviation ($n = 3$) on a dry weight basis. Values marked above the same color bars with the same letter are not significantly different ($p < 0.05$). RB, regular boiling for 90 min on pinto beans and for 80 min on black beans; PB, pressure (15 psi) boiling for 10 min; RS, regular steaming for 70 min; and PS, pressure (15 psi) steaming for 60 min.

samples. Antioxidant activities of the original raw and cooked pinto and black beans, including DPPH, FRAP, and ORAC, are presented in **Figure 2**. Significant differences ($p < 0.05$) in DPPH, FRAP, and ORAC values were found among most treatments for both pinto and black beans. In comparison to the original raw beans (both pinto and black beans), all processing treatments caused significant ($p < 0.05$) decreases in DPPH, FRAP, and ORAC values. In comparison to the boiling treatments, both regular and pressure-steaming treatments preserved greater antioxidant values. In comparison to the regular (atmospheric) heat treatments, pressure boiling treatments retained significantly ($p < 0.05$) higher DPPH, FRAP, and ORAC values. Pressure boiling achieved palatable cooked beans within a shorter cooking time (10 min) than regular boiling (90 min); therefore, pressure boiling leached relatively fewer phenolic antioxidant substances into cooking water than regular boiling.

Boiling is generally regarded as being destructive to antioxidant compounds (*31*). This was verified by our antioxidant assays. The results from pinto beans showed that boiling processes decreased about 60.1−66.6% of DPPH values, 69.5−74.3% of FRAP values, and about 60−70% of ORAC values. The results from black beans showed that boiling (both regular and pressure) decreased DPPH values by about 46%, FRAP values by about 72%, and ORAC values by about 70−82%. However, steaming processing preserved significantly ($p < 0.05$) higher antioxidant activities (DPPH, FRAP, and ORAC values) as compared to the boiling treatments in both pinto and black beans.

The linear correlation among total phenolics, total flavonoids, condensed tannins, monomeric anthocyanins, and antioxidant activities among all processed pinto and black bean samples were analyzed. In the case of pinto beans, there were significant ($p < 0.0001$) correlations between different types of phenolics (TPC and TFC, TPC and CTC, TFC and CTC) but there were no significant correlations between MAC and other types of phenolics (TPC, TFC, and CTC). There were significant ($p < 0.0001$) correlations between total phenolics (TPC, TFC, and CTC) and antioxidant values (r values ranged from 0.84 to 0.99), whereas there were no significant ($p < 0.0001$) correlations between MAC and antioxidant values.

In the case of black beans, there were significant correlations between different types of phenolics: TPC and TFC ($r = 0.99$, $p < 0.0001$), TPC and CTC ($r = 0.97$, $p < 0.0001$), TPC and MAC ($r = 0.97$, $p < 0.0001$), TFC and CTC ($r = 0.97$, $p < 0.0001$), TFC and MAC ($r = 0.97$, $p < 0.0001$), as well as CTC and MAC ($r = 0.97$, $p < 0.0001$). Significant ($p < 0.0001$) correlations existed between all phenolic indexes and antioxidant activity values. These correlation results indicated that all antioxidant assay methods were well-correlated and that different phenolics contents might have different degrees of contributions to overall antioxidant activities.

The changes in the overall antioxidant properties of processed beans can be attributed to synergistic combinations or counteractions of several types of chemical reactions, leaching of water-soluble antioxidant compositions, and the 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 research was performed to investigate the molecular mechanisms responsible for the loss or formation of antioxidants and interactions between natural

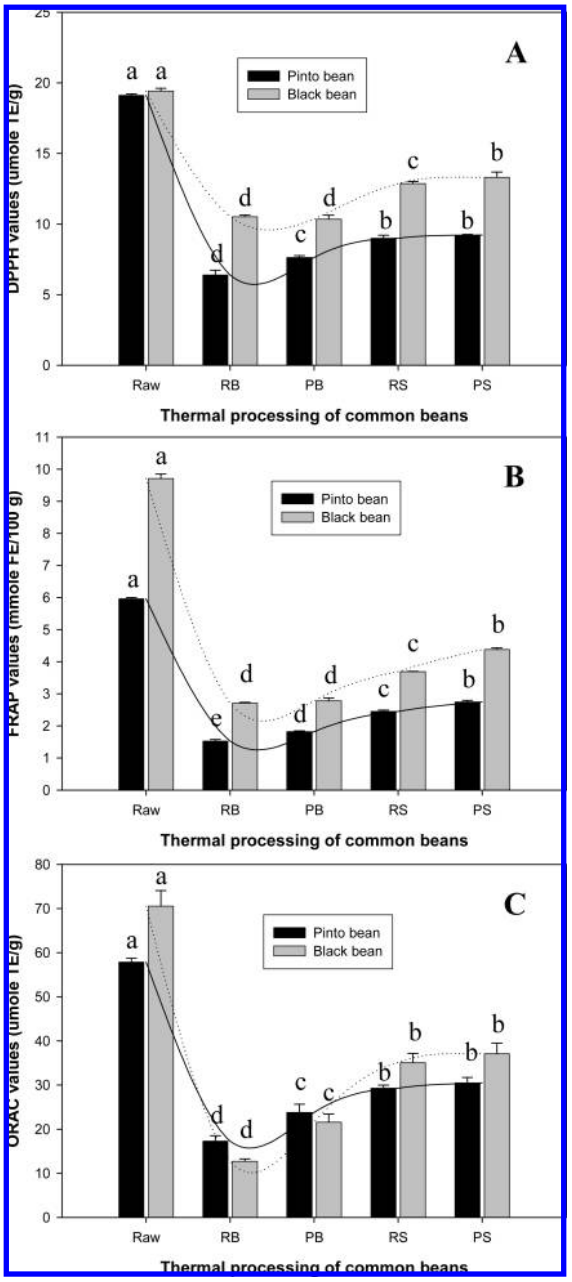

Figure 2. Effect of boiling and steaming on antioxidant properties (A, DPPH; B, FRAP; and C, ORAC) of pinto and black beans. Bar data are expressed as mean ± standard deviation (n = 3) on a dry weight basis. Values marked above the same color bars with the same letter are not significantly different ($p < 0.05$). RB, regular boiling for 90 min on pinto beans and for 80 min on black beans; PB, pressure (15 psi) boiling for 10 min; RS, regular steaming for 70 min; and PS, pressure (15 psi) steaming for 60 min.

and heat-induced antioxidants and their effects on the overall antioxidant properties of cooked beans.

Effect of Thermal Processing on Phenolic Acid Compositions. Phenolic acids have great importance as precursors for synthesizing many other phenolic molecules in plants. The most important biological activity of phenolic acids is their antioxidant properties (*32*). To the best of our knowledge, phenolic acid profiles in processed common beans have not been systematically investigated. The free phenolic acid (FPA) contents and conjugated phenolic acid (CPA) contents of the original raw and processed pinto and black beans are presented in **Tables 1** and **2**, respectively. Because of the instrumental limitation of current HPLC and the performance of the column, *p*-coumaric acid (PCA) and syringaldehyde (SD) as well as *m*-coumaric acid (MCA) and ferullic acid (FA) did not become separated completely in both standard mixtures and samples. Four compounds contributed two peaks. Therefore, PCA and SD were estimated as one compound (PCA + SD), using one standard curve by plotting integrated peak area contributed by these two compounds against the concentration (PCA + SD in a 1:1 mass ratio). MCA and FA were estimated as one compound (MCA + FA) in a similar manner. Seven benzoic phenolic acids and their derivates (gallic, protocatechuic, 2,3,4-trihydroxybenzoic, *p*-hydroxybenzoic, and vanillic acids, protocatechualdehyde, and vanillin) and two phenolic acids of the cinnamic types (chlorogenic and sinapic acid) were detected in both raw and processed pinto beans (**Table 1**). Among the compounds detected, gallic, vanillic, chlorogenic, and sinapic acids, protocatechualdehyde, and vanillin were the predominant phenolic acids in the raw pinto beans. Three benzoic phenolic acids and their derivates (gallic and vanillic acids and vanillin) and three phenolic acids of the cinnamic types (chlorogenic, MCA + FA, and sinapic acids) were detected in both raw and processed black beans. Gallic, vanillic, and chlorogenic acids were also the predominant phenolic acids among the compounds detected in the raw black beans.

CPA analyses (**Table 2**) showed that seven phenolic acids of the benzoic types (gallic, protocatechuic, 2,3,4-trihydroxybenzoic, *p*-hydroxybenzoic, and salicylic acids, protocatechualdehyde, and vanillin) and five phenolic acids of the cinnamic types (caffeic, PCA + SD, MCA + FA, *o*-coumaric, and sinapic acids) were detected in both raw and processed pinto beans. Eight benzoic phenolic acids and their derivates (gallic, protocatechuic, 2,3,4-trihydroxybenzoic, *p*-hydroxybenzoic, syringic, and salicylic acids, vanillin, and protocatechualdehyde) and five phenolic acids of the cinnamic types (caffeic, PCA + SD, MCA + FA, *o*-coumaric, and sinapic acids) were detected in both raw and processed black beans. However, chlorogenic and *trans*-cinnamic acids [the predominant phenolic acid compositions in the FPA assay (**Table 1**)] were not detected or decreased greatly in both pinto and black beans. More types of phenolic acids were detected in the CPA assay (**Table 2**) than the FPA assay. This phenomenon might be attributed to sample alkaline hydrolysis, which released more types of phenolic acids from the bonded forms to the free forms. In addition, alkaline hydrolysis partly broke down some original free phenolic acids, such as chlorogenic acid and the constituent aglycone (caffeic acid) (*33*). That is why chlorogenic acid was the predominant phenolic acid in beans in the FPA analyses (**Table 1**) but became undetectable in beans in the CPA analyses (**Table 2**).

Significant differences ($p < 0.05$) in FPA and CPA were found among most treatments for both pinto and black beans. In the case of FPA compositions of pinto beans (**Table 1**), in comparison to the original raw beans, regular and pressure boiling, regular steaming caused significant ($p < 0.05$) decreases in all benzoic acids and their derivates (except for 2,3,4-trihydroxybenzoic acid), subtotal benzoic acids, chlorogenic acid, sinapic acid, subtotal cinnamic acids, and total phenolic acids content but caused significant increases in 2,3,4-trihydroxybenzoic acid, PCA + SD, and MCA + FA. Pressure steaming caused significant ($p < 0.05$) increases in gallic, 2,3,4-trihydroxybenzoic, and *trans*-cinnamic acids but caused significant decreases in the other individual phenolic acids, subtotal benzoic acids, subtotal cinnamic acids, and total phenolic acids.

In the case of FPA compositions of black beans (**Table 1**), in comparison to the original raw beans, both regular and pressure boiling caused significant ($p < 0.05$) decreases in all detected

individual phenolic acids and their derivates (except for caffeic acid), subtotal benzoic acids, subtotal cinnamic acids, and total phenolic acids but regular boiling treatment caused significant increases in caffeic acid content. Regular steaming caused significant ($p < 0.05$) increases in protocatechuic, chlorogenic, and sinapic acids, protochechualdehyde, vanillin, subtotal benzoic acids, subtotal cinnamic acids, and total phenolic acids but caused significant decreases in the other individual phenolic acids. Pressure steaming caused significant ($p < 0.05$) increases in gallic, 2,3,4-trihydroxybenzoic, chlorogenic, MCA + FA, and sinapic acids, protochechualdehyde, vanillin, subtotal benzoic acids, subtotal cinnamic acids, and total phenolic acids but caused significant decreases in the other individual phenolic acids.

Free total phenolic acids of pinto beans were reduced by all thermal-processing treatments. Total phenolic acids in pinto beans were reduced by 67.9% after regular boiling, by 59.9% after pressure boiling, by 71.9% after regular steaming, and by 60.4% after pressure steaming. Total phenolic acids in black beans were reduced by 51.7% after regular boiling, reduced by 62.1% after pressure boiling, increased by 23.9% after regular steaming, and increased by 44.3% after pressure steaming. Therefore, steaming processing retained or yielded (by releasing from bond form) more total phenolic acids in black bean than boiling processing.

In the analysis of CPA in pinto beans (**Table 2**), in comparison to the original raw beans, all processing treatments caused significant ($p < 0.05$) decreases in most individual phenolic acids and their derivates, subtotal benzoic acids, subtotal cinnamic acids, and total phenolic acids, with exception of the cases of vanillin, caffeic acid, and MCA + FA of pinto beans, to which regular steaming produced no significant differences for these compound as compared to the raw pinto beans. In the analysis of CPA in black beans, in comparison to the raw black beans, regular and pressure boiling, regular steaming treatments caused significant ($p < 0.05$) decreases in most phenolic acids, subtotal benzoic acids, subtotal cinnamic acids, and total phenolic acid. However, pressure-steaming treatments caused significant increases in gallic acid and caused significant decreases in the other phenolic acids, subtotal benzoic acids, subtotal cinnamic acids, and total phenolic acids.

Thermal treatments could cause changes in the phenolic substances in the food products. For example, the roasting of coffee beans induced a significant loss in chlorogenic content (*34*) that was due to chemical degradation or oxidation. According to the description of Fleuriet and Macheix (*32*), the changes of phenolic acids in pinto and black beans upon boiling and steaming in the current study might mainly result from three sets of reactions: (1) the oxidative degradation of phenolic acids, including enzymatic browning, (2) the release of free acids from conjugate forms, and (3) the formation of complex structures of phenolic substances from related compounds, such as proteins, tannins, and anthocyanins.

The literature on phenolic acid content in common beans is very limited. Only one work quantified phenolic acids in 15 raw common beans (*22*). Three cultivars of pinto bean and three cultivars of black bean (including Eclipse) were involved in this investigation. However, the phenolic acid profiles and contents of our current investigation on raw pinto and black beans are completely different from those of Luthria and Pastor-Corrales (*22*), who did not detect free phenolic acids in the raw beans analyzed, and only four phenolic acids were detected after sequential hydrolysis sample treatments. In the present study, free phenolic acids were detected in both raw and processed beans analyzed and more types of conjugated phenolic acids were detected in both raw and cooked beans than that report (*22*). The differences could be attributed to the differences of extraction methods.

Effect of Thermal Processing on Anthocyanin Composition. The anthocyanin contents of the original raw and cooked pinto and black beans are presented in **Table 3**. Anthocyanins were not detectable in the raw and cooked pinto beans. Five anthocyanins, namely, delphinidin-3-glucoside, malvidin-3,5-diglucoside, petunidin-3-glucoside, malvidin-3-galactoside, and malvidin-3-glucoside, were detected in the raw black beans. The dominant components were delphinidin-3-glucoside, malvidin-3,5-diglucoside, petunidin-3-glucoside, and malvidin-3-glucoside. These findings are in accordance with those of Takeoka et al. (*5*) and Wu and Prior (*35*), who found that delphinidin-3-glucoside, petunidin-3-glucoside, and malvidin-3-glucoside were the major anthocyanins in black common beans. The discrepancy was that malvidin-3,5-diglucoside as the third major component after delphinidin-3-glucoside and petunidin-3-glucoside was detected in our pinto bean sample at a higher content than malvidin-3-glucoside. However, malvidin-3,5-diglucoside was reported as the minor anthocyanin in black beans in previous studies (*5*, *35*). The reason may partly be due to the age of the beans (harvested in 2004 and analyzed in 2007), in which a glycosylation process might occur during postharvest storage.

There was a significant impact on the retention of anthocyanins as a result of thermal processing; it exhibited similar trends to those of black soybeans in our previous paper (*24*). All thermal-processing treatments significantly ($p < 0.05$) reduced the contents of each individual anthocyanin in black beans. In comparison to the other thermal processing methods, regular steaming retained a higher content of delphinidin-3-glucoside, malvidin-3,5-diglucoside, and petunidin-3-glucoside, while pressure steaming eliminated all malvidin-3-glucoside and malvidin-3-galactoside. Only trace amounts of delphindin-3-glucoside (22.9 μg/g), petunidin-3-glucoside (11.2 μg/g), and malvidin-3-glucoside (6.4 μg/g) were retained in the pressure-steamed black beans. There were no significant differences in anthocyanin contents between regular and pressure boiled black beans. In comparison to the raw black beans, thermal processing lost 93.3−99% of delphinidin-3-glucoside, 58.8−100% malvidin-3,5-diglucoside, 91.5−98.7% petunidin-3-glucoside, 100% malvidin-3-galactoside, and 88.2−98.5% malvidin-3-glucoside. These results indicated the degradation of anthocyanin compositions in black beans upon thermal processing.

Effect of Thermal Processing on Flavonol Compositions. Chemical profiles of flavonoids had been investigated in several earlier reports on raw common beans (*2*, *6*, *26*, *36*) and processed beans (*7*). However, no systematic studies were carried out on both pinto and black beans that were cooked under atmospheric and high-pressure conditions. The flavan-3-ol and flavonol contents of the original raw and thermal processed pinto and black beans are presented in **Table 4**. Kaempferol-3-glucoside and kaempferol-3-acetylglucoside, as the two major flavonols, were detected in both raw and processed pinto beans. Three flavan-3-ols [(+)-catechin, (+)-epicatechin, and epicatechin-gallate] and two flavonols (kaempferol-3-glucoside and myricetin) were detected in both raw and processed black beans. In contrast to previous studies (*7*, *27*), we did not detect the aglycone form of kaempferol in our pinto beans, which may be due to the aged pinto bean materials used (harvested in 2002 and analyzed in 2007), in which an after-darkening process occurred during the storage. The unglucosylated kaempferol was known to combine with tannins in the seed coat during an after-darkening process, reducing the kaempferol concentration as compared to non-aged beans (*27*). The total flavonol contents were about 177.9 μg/g in the raw pinto beans and 677.4 μg/g in the raw black beans. From a

quantitative point of view, the flavonol contents in the raw beans are comparable to the Italian common beans in the previous report (*26*) but much less than that reported (>500 μg/g) in yellow−orange-colored beans (*37*).

All thermal processing significantly ($p < 0.05$) decreased kaempferol-3-glucoside and kaempferol-3-acetylglucoside contents in pinto beans as compared to the raw pinto beans. Both regular and pressure boiling significantly ($p < 0.05$) decreased flavan-3-ols [(+)-catechin, (+)-epicatechin, and epicatechin-gallate] and flavonol (myricetin) contents in black beans as compared to the raw black beans. Regular steaming did not affect (+)-catechin and (+)-epicatechin contents but significantly ($p < 0.05$) reduced epicatechin-gallate and myricetin contents. Pressure steaming significantly ($p < 0.05$) increased (+)-catechin and (+)-epicatechin contents but significantly ($p < 0.05$) reduced epicatechin-gallate and myricetin contents as compared to the raw black beans.

In terms of total flavonol contents (the sum of individual flavonols), in comparison to the raw beans, all thermal processing significantly ($p < 0.05$) reduced total flavonol contents in the pinto beans. There were no obvious different trends between the regular and pressure boiling treatments in terms of total flavonol contents in both pinto and black beans. Regular and pressure boiling and regular steaming significantly ($p < 0.05$) reduced total flavonol contents in the black beans, while pressure steaming significantly ($p < 0.05$) increased contents of total flavonols in the black beans. In comparison to the regular steaming, pressure-steaming processes yielded significantly ($p < 0.05$) higher flavan-3-ols, myricetin (flavonol), and the total flavonols in the cooked black beans. The increased total flavonols in the cooked black beans were predominantly contributed by the increases of (+)-catechin, which may be due to the release of the basal structural compound (catechin) from condensed tannins through a depolarization process upon thermal and pressure conditions. There was a significant impact on the retention of flavonols as a result of different processing methods. This finding is different from the previous report on green beans, which was not much affected by the heat treatment (*38*). The differences may be attributed to the difference between dry beans in the current study and fresh beans used in the literature.

Correlations of Phenolic Compounds and Antioxidant Activities. The linear correlation coefficients between selected predominant phenolic compounds (found in pinto and black beans) and overall antioxidant activities of beans are presented in **Table 5**. In the case of pinto beans, the most predominant phenolic acids (chlorogenic, sinapic, and vanillic acids, protocatechualdehyde, and vanillin) and flavonols (kaempferol-3-glucoside and kaempferol-3-acetylglucoside), subtotal benzoic acids, subtotal cinnamic acids, total phenolic acids, and total flavonols exhibited significant ($p < 0.05$ or 0.0001) correlations with the overall antioxidant activities (DPPH, FRAP, and ORAC values). In the case of black beans, a few part of individual phenolic acids (vanillic and chlorogenic acids), all anthocyanins, all detected flavonols (kaempferol-3-glucoside and myricetin), and one flavan-3-ol (epicatechin-gallate) exhibited significant ($p < 0.05$ or 0.0001) correlations with the overall antioxidant activities (DPPH, FRAP, and ORAC values). However, no significant correlations existed between the other individual phenolic acids, subtotal benzoic acids, total phenolic acids, and (+)-epicatechin and antioxidant activities (DPPH, FRAP and ORAC values) in black beans. These results indicated that both phenolic acids and flavonols play an important role on the overall antioxidant activities (DPPH, FRAP, and ORAC) of pinto beans. Unlike anthocyanins, flavan-3-ols and flavonols may play an important role on overall antioxidant activities (DPPH, FRAP, and ORAC) of black beans. Thermal processing reduced these compounds dramatically in black beans and therefore decreased the overall antioxidant activities of cooked black beans.

Table 5. Correlations between Antioxidant Activities and Predominant Phenolic Compounds Found in Beans

	pinto beans ($N = 15$)[a]			black beans ($N = 15$)		
correlation coefficients (r)	DPPH	FRAP	ORAC	DPPH	FRAP	ORAC
gallic acid	0.44	0.49	0.56	0.31	0.23	0.34
protocatechualdehyde	0.93[b]	0.91[b]	0.90[c]			
vanillic acid	0.90[b]	0.87[b]	0.85[c]	0.71[c]	0.70[c]	0.71[c]
vanillin	0.95[c]	0.93[b]	0.93[b]			
chlorogenic acid	0.96[b]	0.95[b]	0.97[b]	0.58[c]	0.46	0.63[c]
sinapic acid	0.92[b]	0.89[b]	0.86[c]			
subtotal benzoic acids	0.94[b]	0.93[b]	0.93[b]	0.41	0.29	0.47
subtotal cinnamic acids	0.95[b]	0.93[b]	0.93[b]	0.50	0.37	0.53[c]
total phenolic acids	0.95[b]	0.94[b]	0.94[b]	0.46	0.33	0.51
delphinidin-3-glucose				0.92[b]	0.96[b]	0.88[b]
malvidin-3,5-diglucose				0.99[b]	0.99[b]	0.78[b]
petunidin-3-glucose				0.91[b]	0.96[b]	0.87[b]
malvidin-3-glucose				0.90[b]	0.94[b]	0.85[b]
kaempferol-3-glucoside	0.96[b]	0.95[b]	0.96[b]	0.99[c]	0.99[c]	0.86[c]
kaempferol-3-acetylglucoside	0.99[b]	0.99[b]	0.75[c]			
(+)-catechin				0.62	0.52	0.67[c]
(+)-epicatechin				0.30	0.23	0.37
(+)-epicatechin gallate				0.89[c]	0.91[c]	0.95[b]
myricetin				0.86[c]	0.81[c]	0.92[b]
total flavonols	0.95[b]	0.93[b]	0.94[b]	0.69[c]	0.62	0.75[c]

[a] Values were from triplicate determinations on raw beans and the beans cooked by four cooking methods. [b] The correlation is significant at $p = 0.0001$ (two-tailed). [c] The correlation is significant at $p = 0.05$ (two-tailed).

In general, boiling and steaming processes significantly affected the total phenolics, individual phenolic compounds, and antioxidant activities on both pinto and black beans. The changes depend upon the type of beans and processing conditions. Steamed beans preserved higher antioxidant activities because of smaller losses in total phenolic compositions, individual anthocyanins, individual flavan-3-ols, flavonols, and total flavonols than the boiled beans.

ABBREVIATION USED

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

ACKNOWLEDGMENT

This project is a part of ND AES Hatch project 1905. Mrs. Angie Ostenson assisted in sample freeze drying. Mrs. Gloria Nygard and Mr. Cook Leword assisted in the HPLC operation and maintenance.

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Received February 28, 2009. Revised manuscript received April 15, 2009.