DOI:10.1021/jf902594m

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Phytochemical Profiles and Health-Promoting Effects of Cool-Season Food Legumes As Influenced by Thermal Processing

Baojun Xu[†] and Sam K. C. Chang*[,‡]

[†]Food Science and Technology Program, Beijing Normal University—Hong Kong Baptist University United International College, Zhuhai, Guangdong 519085, China, and [‡]Department of Cereal and Food Sciences, North Dakota State University, Fargo, North Dakota 58108

The effects of four thermal processing methods (conventional boiling, conventional steaming, pressure boiling, and pressure steaming) on phytochemical profiles, antioxidant capacities, and antiproliferation properties of commonly consumed cool-season food legumes, including green pea, yellow pea, chickpea, and lentil, were investigated. Four groups of individual phenolic compounds, including phenolic acids, anthocyanins, and flavan-3-ols, as well as flavonols and flavones were quantified using HPLC, respectively. As compared to the original raw legumes, all processing methods caused significant ($p < 0.05$) reduction in total phenolic content, procyanidin content, total saponin content, phytic acid content, chemical antioxidant capacities in terms of ferric reducing antioxidant power and peroxyl radical scavenging capacity, and cellular antioxidant activity as well as antiproliferation capacities of cool-season food legumes. Different cooking methods have varied effects on reducing total phenolics, saponins, phytic acids, and individual phenolic compounds. For all cool-season food legumes, steaming appeared to be a better cooking method than boiling in retaining antioxidants and phenolic components, whereas boiling appeared to be effective in reducing saponin and phytic acid contents. In the case of lentil, all thermal processing methods (except conventional steaming) caused significant ($p < 0.05$) decreases in gallic, chlorogenic, *p*-coumaric, sinapic, subtotal benzoic, subtotal cinnamic acid, and total phenolic acid. All thermal processing methods caused significant ($p < 0.05$) decreases in (+)-catechin and flavan-3-ols in each cool-season food legume.

KEYWORDS: Cool-season food legumes; boiling; steaming; phenolic acid; flavan-3-ol; flavonol; flavone; anthocyanin; saponin; phytic acid; antioxidant; FRAP; PRSC; cellular antioxidant activity; antiproliferation; HPLC

INTRODUCTION

Food legumes are economical dietary sources of good-quality protein, carbohydrates, dietary fiber components, and a variety of minerals and vitamins. Food legumes contain several compounds that have been traditionally considered to be antinutrients, such as protease inhibitors, phytic acids, saponins, tannins, plant sterols, and isoflavones. However, more recent information suggests that most of these compounds may actually benefit the consumer's health if used properly in the context of foods for disease prevention. Research suggests that regular dietary intake of food legumes can reduce the risk of developing nutrition-related health problems including obesity, diabetes, heart diseases, and cancers (*1*). Therefore, food legumes are recommended as an excellent food choice with health-promoting benefits.

The cool-season food legumes (CSFLs), including green pea, yellow pea, chickpea, and lentil, are traditionally low-input crops and are grown extensively in the farming system of the Indian subcontinent, the Mediterranean area, the Nile Valley, Central Europe, the Americas, and Australia (*2*). The U.S. production of CSFLs, mainly in the northwestern states, such as Washington, Idaho, Montana, and North Dakota, has increased significantly in recent years (*3*). The CSFLs have many nutritional qualities that make them attractive to food manufacturers.

Legumes must be cooked before consumption. Although antioxidant properties and phenolic compounds of raw uncooked CSFLs have been reported (*4*, *5*), how processing methods affect the phytochemicals and health-promoting activities, such as antioxidant activities and anticancer properties, has not been systematically studied. Our preliminary study showed that soaking, boiling, and steaming processes significantly affected the total phenolic contents, free radical scavenging activities, and oxygen radical absorbing capacities (ORAC) of CSFLs (*6*). To continue our study on thermal processing effects, the present study was undertaken to further investigate how thermal processing affected individual phenolic compounds (including phenolic acids and flavonoids), saponins, and phytic acids, as well as ferric reducing antioxidant power (FRAP) and peroxyl radical scavenging capacity (PRSC), cellular antioxidant activities (CAA), and antiproliferation activities of CSFLs.

*Author to whom correspondence should be addressed [telephone (701) 231-7485; fax (701) 231-6536; e-mail kow.chang@ndsu.edu].

 Published on Web 10/29/2009

MATERIALS AND METHODS

Chemicals and Standards. Sixteen phenolic acids and three aldehydes, five flavan-3-ols [(+)-catechin, (+)-epicatechin, epigallocatechin, epicatechin-gallate, epigallatecatechin-gallate (EGCG)], six flavonols or flavones (myricetin, luteolin, quercetin, apigenin, kaempferol, quercetin-3-rutinoside), soya saponin (contained a minimum of 80% saponin), phytic acid, sulfosalicylic acid, trifluoroacetic acid (TFA), Folin−Ciocalteu reagent, sodium carbonate, dimethyl sulfoxide (DMSO), 2′,7′-dichlorofluorescin diacetate (DCFH-DA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 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 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-amidinopropane) 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). Human gastric adenocarcinoma cell line AGS, human colorectal adenocarcinoma cell line SW480, and human prostate carcinoma cell line DU145 were purchased from American Type Culture Collection (ATCC, Manassas, VA). Hanks balanced salt solution (HBSS) and 0.4% trypan blue stain solution were purchased from Cambrex Bio Science Walkersville, Inc. (Walkersville, MD). Phosphate-buffered saline (PBS), trypsin−EDTA solution, penicillin−streptomycin, fetal bovine serum (FBS), and cell culture media (Eagle's MEM and F-12K) were purchased from Mediatech, Inc. (Herndon, VA).

Legume Materials. The dried cool-season food legume (CSFL) seeds used in the current study were green pea (*Pisum sativum* L. cv. Stratus), supplied by Meridian Seed LLC (West Fargo, ND); yellow pea (*P. sativum* L. cv. Golden), supplied by Steve Marman Pulse USA (Bismark, ND); and chickpea (*Cicer arietinum* L. cv. Amits) and lentil (*Lens culinaris* cv. CDC Richlea), supplied by Agricare United (Ray, ND). Broken seeds, damaged seeds, and foreign materials were removed from the samples. Moisture content was determined by drying the samples in an air-circulated oven at 110 °C until a constant weight was obtained (*7*). All calculations for determination of phenolics and quantification of antioxidant activities are on a dry weight basis.

Soaking and Hydration Ratio. The soaking procedures and determination method of hydration ratio in our earlier paper (*6*) were followed. Soaking time of CSFLs with desired hydration ratio was calculated by calibration through a quadratic fit equation of respective water adsorption curve as previously described (*6*). The soaked peas (with 100% hydration ratio) and lentils (with 50% hydration ratio) were drained and then boiled or steamed according to the methods described below.

Boiling, Steaming, and Cooking Time. All thermal processes were performed according to the procedures published in our earlier paper (*6*). Briefly, conventional 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 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 Vindiola et al. (*8*). A seed is deemed to be cooked when it can be squeezed easily. Boiling and steaming times, as well as pressure conditions, were selected from our previous paper (*6*). After cooking treatments, the legumes were drained and cooled to room temperature in covered plastic containers. Subsequently, cooked samples were frozen and then freeze-dried.

Total Phenolic Quantification. *Extraction of Polyphenols*. The original raw legumes and the freeze-dried cooked legumes were ground to flour with an IKA all basic mill (IKA Works Inc., Wilmington, NC) to pass through a 60-mesh sieve. Extraction procedures were performed according to our earlier paper (*9*). The extracts of total phenolics were used for determination of total phenolics and evaluation of antioxidant activities.

Determination of Total Phenolic Content (TPC). The TPC was determined by a Folin−Ciocalteu assay (*10*) with slight modifications (*9*) using gallic acid (GA) as the standard. The absorbance was measured using a UV−visible spectrophotometer (UV 160, Shimadzu, Kyoto, Japan) at 765 nm against a reagent blank. The TPC was expressed as milligrams of gallic acid equivalents per gram of dry legume (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 Procyanidin Content (PAC). The PAC analysis was carried out according to the method of Broadhurst and Jones (*11*) and slightly modified in our laboratory (*9*). The absorption was measured using a UV−visible spectrophotometer (UV 160, Shimadzu) at 500 nm against methanol as a blank. The PAC was expressed as milligrams of catechin equivalents per gram of dry legume (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).

Quantification of Individual Free and Conjugated Phenolic Acid by HPLC. *Extraction of Free Phenolic Acids*. The extraction of free phenolic acids was performed by modifying the method of Luthria and Pastor-Corrales (*12*). Briefly, the raw and cooked legume samples (0.5 g in triplicate) were extracted twice, each 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 then 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 papers (*12*, *13*) with slight modifications. Briefly, the raw and cooked legume samples (0.4 g in triplicate) were hydrolyzed and extracted with 10 mL of 2 N NaOH [containing 10 mM 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 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 our recent publication (*14*, *15*). 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 UV detector (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. All identified phenolic acids were quantified with external standards by using HPLC analysis as described previously (*14*). The phenolic acid contents were expressed as micrograms of phenolic acid per gram of legume (μg/g) on a dry weight basis.

Quantification of Flavan-3-ol and Flavonol by HPLC. *Extraction of Flavonols*. The cooked legume samples were freeze-dried and ground. The ground raw and cooked legumes (0.5 g in triplicate) were extracted according to the method described in our recent paper (*15*).

HPLC Analysis of Flavonols. The quantitative analysis of flavonols was performed according to the methodology of isoflavone analysis developed by Murphy et al. (*16*) with a slight modification (*15*). The same Waters Associates chromatography system as used for phenolic acid analysis was used for the analysis of flavonols with 262 nm UV detection. A YMC-Pack ODS-AM-303 C_{18} reversed-phase column (250 mm × 4.6 mm internal diameter, 5 μm particle size) was obtained from Waters and employed for chromatographic separation at 34 °C, which was maintained with a column heater.

Identification and Quantification of Flavan-3-ol and Flavonols. Five flavan-3-ols [(+)-catechin, (+)-epicatechin, epigallocatechin, epicatechin-gallate, epigallatecatechin-gallate (EGCG)] and nine flavonols or flavones (myricetin, luteolin, quercetin, apigenin, kaempferol, kaempferol-3-glucoside, kaempferol-3-rutinoside, quercetin-3-glucoside, quercetin-3-rutinoside) were commercially available and directly used to identify the sample peaks by comparing their retention times and HPLC profiles

with those of the standard mixture. In addition, a spiking method was used for peak identification of some samples. External calibration curves were obtained for each of six external standards by plotting peak area of each standard against 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 legume (μg/g) on a dry weight basis.

Quantification of Anthocyanin by HPLC. The free phenolic acid extracts were also used for anthocyanin analysis, the analysis was performed on an HP 1090 series HPLC (Hewlett-Packard, Waldbronn, Germany) equipped with filter photometric detector, using a YMC Pack ODS-AM column (4.6 mm × 250 mm, S-50 μm, 120A) according to our recent paper (*14*). The identifications and peak assignments of anthocyanins were primarily based on comparison of their retention times with those of the external standards and a blueberry reference sample. Standard curves of anthocyanins were plotted with peak areas against concentrations by duplicate injections of the six series of diluted working solutions of the standard mixture. Anthocyanin contents were expressed as micrograms of anthocyanin per gram of legume (μg/g) on a dry weight basis.

Extraction and Determination of Total Saponin. *Extraction of Saponin*. Extraction procedures were performed by modifying the method of Makkar and Becker (*17*). Briefly, the raw and freeze-dried cooked legume flours (0.5 g in triplicate) were defatted with 10 mL of petroleum ether by shaking for 4 h, and then the residues were extracted twice, each with 5 mL of 80% aqueous methanol, on an orbit shaker by shaking for 4 h each time. The extracts were stored at 4 °C in the dark for use.

Determination of Total Saponin Content (TSC). The TSC was determined using the spectrophotometric method described by Hiai et al. (*18*). Briefly, 0.1 mL of legume extract, 0.4 mL of 80% methanol solution, 0.5 mL of freshly prepared 8% vanillin solution (in ethanol), and 5.0 mL of 72% sulfuric acid were mixed well in an ice−water bath. The mixture was warmed in a water bath at 60 °C for 10 min and then cooled in ice-cold water. Absorbance at 544 nm was recorded against the reagent blank with a UV−visible spectrophotometer (UV 160, Shimadzu). The results were expressed as milligrams of soyasaponin equivalent per gram of legume (mg of SSE/g) on a dry weight basis from a standard curve of different concentrations of crude soyasaponin (contained a minimum of 80% saponin, Sigma-Aldrich) in 80% aqueous methanol.

Extraction and Determination of Phytic Acid. *Extraction of Phytic Acid*. Phytic acid in the legume was extracted according to the method of Gao et al. (*19*). Briefly, the raw and freeze-dried cooked legume flours (0.5 g in triplicate) were defatted with 10 mL of petroleum ether by shaking on an orbit shaker for 4 h, and then the residues were extracted with 10 mL of 2.4% HCl by shaking on the orbit shaker for 16 h. The extracts were stored at 4 °C in the dark for further analysis.

Determination of Phytic Acid. The phytic acid was determined using the colorimetric (Wade Reagent) method described by Gao et al. (*19*) with slight modification. Briefly, 0.1 mL of legume extract was diluted by 2.9 mL of distilled water, and then 3 mL of this diluted sample was combined with 1 mL of freshly prepared Wade reagent (0.03% $FeCl_3 \cdot 6H_2O$ + 0.3% sulfosalicylic acid) in a 15 mL VWR tube. The contents were thoroughly mixed on a vortex and centrifuged at 5500 rpm at 10 °C for 10 min. A series of calibration standards containing 0, 5, 10, 20, 25, 50, 75, or 100 μg/mL of phytic acid were prepared by diluting 10 mg/mL of phytic acid stock solution with distilled water. Absorbance of color reaction products for both samples and standards was read at 500 nm on a UV−visible spectrophotometer (UV 160, Shimadzu) against water as blank. The results were expressed as milligrams of phytic acid per gram of legume (mg of PA/g) on a dry weight basis.

Determination of Chemical Antioxidant Capacities. *Determination of Ferric Reducing Antioxidant Power (FRAP).* The FRAP assay was performed according to the method described by Benzie and Strain (*20*). The total phenolic extract was first properly diluted with deionized water to fit within the linearity range. The absorbance was measured using a UV−visible spectrophotometer (UV 160, Shimadzu) at 593 nm against reagent blank. The FRAP value was expressed as millimoles of Fe^{2+} equivalents per 100 g of dry legumes (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 Peroxyl Radical Scavenging Capacity (PRSC). The PRSC assay was performed in a cell-free system according to the method validated by Adom and Liu (*21*) with modifications. Briefly, 1 mM DCFH-DA solution was obtained from a 20 mM stock solution (DCFH-DA dissolved in methanol) by dilution with PBS buffer. Just prior to use, an aliquot (400 μL) of 1 mM DCFH-DA solution was added into 3.6 mL of 1.0 mM KOH and hydrolyzed for 3−5 min to remove the diacetate (DA) moiety, and then DCFH was diluted to 10 μM (as final working solution) with prewarmed PBS at 37 °C. Twenty microliters of suitably diluted legume extracts, blank, and Trolox calibration solutions were loaded into clear 96-well microplates in triplicate based on a balanced layout. AAPH was used as the peroxyl generator; 27.2 mg of AAPH solid was dissolved (just before PRSC measuring) into 5 mL of warmed HBSS. Immediately after dissolving, 20 μL of AAPH solution was dispensed through a pump and an autoinjector of the plate reader into appropriate wells according to a balanced layout. The BMG Fluostar Optima Microplate Reader (BMG Labtech GmbH, Offenburg, Germany) was programmed to record the fluorescence of dichlorofluorescein (DCF) on each cycle. Kinetic readings were measured with emission at 520 nm and excitation at 485 nm for 1 h with 85 s per cycle setting. The kinetics of the fluorescence were recorded by the software BMG OPTIMA running on a PC. The areas under the average fluorescence−reaction time kinetic curve (AUC) for both control and samples were integrated and used as the basis for calculating PRSC antioxidant activity. The net AUC was obtained by subtracting the AUC of the blank from that of a sample or standard, expressed as net $AUC = AUC_{sample} - AUC_{blank}$. The quantification method is similar to the ORAC assay in our previously published paper (*11*). PRSC values were expressed as micromoles of Trolox equivalent per gram legume (μmol of TE/g) on a dry weight basis.

Cellular Antioxidant Activity (CAA) Assay. Human gastric adenocarcinoma AGS cells were grown in complete growth medium F-12K (Mediatech, Inc.) supplemented with 10% FBS and 1% penicillin−streptomycin (v/v). Cells were maintained in a humidified 5% CO_2 incubator at 37 °C. Cells used in this study were between passages 47 and 51. The CAA assay was performed by modifying the methods of Eberhardt et al. (*22*) and Wolfe and Liu (*23*) using a rapid proliferating gastric adenocarcinoma cell line AGS. Briefly, AGS cells were seeded at a density of 6×10^4/well on a 96-well microplate in 100 μL of complete growth medium. The outside wells of the plate were filled with 200 μL of PBS to maintain the temperature and prevent medium evaporation of the inner wells. After 24 h of culturing, medium was removed and wells were washed with prewarmed PBS twice. Attached AGS cells were treated with 20 μL of various concentrations of legume extracts, and 180 μL of prewarmed treatment medium (EMEM, phenol free, FBS free) contained final 25 μM DCFH-DA for 1 h. Subsequently, treatment medium was removed, and wells were washed twice with 150 μL of PBS to remove medium, extracellular sample residue, and fluorescence substance. Then 80 μL of HBSS (prewarmed at 37 °C in a water bath) was added to wells, and the microplate was incubated in the BMG Fluostar Optima Microplate Reader (BMG Labtech GmbH, Offenburg, Germany) for a minimum of 10 min to maintain the temperature evenly for each well at 37 °C. Just prior to the assay, 25 mg of AAPH dry solid was dissolved into 5 mL of prewarmed HBSS in a 15 mL of tube. Immediately after dissolving, 20 μL of AAPH solution was dispensed through a pump and an autoinjector into appropriate wells according to a balanced layout. The BMG plate reader was programmed to record the fluorescence of DCF on each cycle. Kinetic readings were measured with emission at 520 nm and excitation at 485 nm for 1 h with 85 s per cycle setting. Each plate included at least five blank and five control wells. The blank wells contained cells treated with DCFH-DA and HBSS without oxidant AAPH and antioxidant samples. The control wells contained cells treated with DCFH-DA, HBSS, and oxidant AAPH without antioxidant samples.

Quantification of CAA. The data were analyzed using Microsoft Excel (Microsoft, Roselle, IL). The area under the curve (AUC) was calculated as $AUC = [R_1/2 + \text{sum}(R_2{:}R_{n-1}) + R_n/2] \times CT$, where R_1 is the fluorescence reading at the initiation of the reaction, R_n is the last measurement, and CT = cycle time in minutes. The net AUC was obtained by subtracting the AUC of the blank from that of a sample or

standard, expressed as net AUC = $AUC_{sample} - AUC_{blank}$. The CAA unit was expressed as CAA unit = 100 − (net AUC_{sample}/net $AUC_{control}$) × 100. The median effective concentration (EC_{50}) was defined as the dose required to cause a 50% inhibition for sample extract or standard compound and calculated through the software CurveExpert (version 1.3).

Antiproliferation Assay. *Cell Lines and Cell Cultivation*. Three typical human cancer cell lines were chosen for antiproliferation assays due to their rapid proliferation properties and easy maintenance: (1) Gastric adenocarcinoma cell AGS was maintained in F-12K medium. (2) Colorectal adenocarcinoma cell SW480 was maintained in L-15 medium. (3) Prostate carcinoma cell DU145 was maintained in EMEM. All media were supplemented with 10% FBS and 1% penicillin−streptomycin. Cells were maintained at 37 °C and 5% CO_2 (except for cell line SW480 without CO_2). Cell culture medium and cultivation conditions were chosen as above according to the suggestion of ATCC. Routine observation for cell viability was performed under phase contrast inverted microscopy. Cell numbers were determined by trypan blue exclusion method and counting in a hemocytometer.

MTT Assay. The antiproliferation assay should be performed under solvent-free conditions to eliminate solvent effects. Therefore, a portion of hydrophilic total phenolic extract was freeze-dried, and then the freeze-dried extract (10 mg) was dissolved in cell culture medium as stock sample solution. The final concentrations of samples (0.125, 0.25, 0.5, 1, 2, and 5 mg/mL) were obtained by diluting sample with medium. The antiproliferation assay was performed according to a well-established MTT method (*24*). Briefly, exponentially growing cells were seeded into 96-well culture plates at a seeding density of 1×10^4 cells/well in 180 μL of medium. After 24 h, attached cells were exposed to the legume extracts with final concentrations as above for 48 h. Subsequently, 20 μL of MTT (5 mg/mL) was added to each well. The microplate was placed back on the incubator and cultured for an additional 4 h. Subsequently, the culture media were sucked from the wells, leaving cells that were adhered to the plates. Then 150 μL of DMSO was added into each well to dissolve yellow formazan (product of the reduction of tetrazolium by viable cells), and then the microplate was gently shaken on an orbit shaker for 10−15 min in the dark. The reaction resulted in the reduction of MTT by the mitochondrial dehydrogenases of viable cells to a purple formazan product, which was measured at 540 nm by the BMG microplate reader. The 50% growth inhibitory concentration (IC_{50}) was defined as the legume concentration (units in mg/mL) required to cause a 50% inhibition and was used as the basis for comparing antiproliferation activity of different samples.

Statistical Analysis. All boiling and steaming processes were performed in triplicate. 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 significant differences at $p < 0.05$.

RESULTS AND DISCUSSION

Effect of Thermal Processing on Total Phenolics and Procyanidins of CSFL's. TPC and PAC of the raw and cooked CSFLs are presented in **Figure 1**. Significant differences ($p < 0.05$) in TPC (**Figure 1A**) and PAC (**Figure 1B**) were found among most processing treatments of green pea, yellow pea, chickpea, and lentil. No significant differences in TPC existed between the two boiling treatments (regular and pressure) for green, yellow pea, and lentil, but significant differences ($p < 0.05$) existed between conventional and pressure boiling of chickpea. Significant differences ($p < 0.05$) in TPC existed between conventional and pressure steaming treatments of all tested CSFLs. Significant differences in PAC existed between conventional and pressure boiling treatments as well as between conventional steaming and pressure steaming. The TPC and PAC of cooked CSFLs were significantly reduced as compared to the respective original uncooked CSFLs. Approximately 40−60% of TPC in green pea, yellow pea, and chickpea and 60% of TPC in lentil were reduced (**Table 1**) after boiling processing, whereas about 10−30% of TPC in green pea, yellow pea, and chickpea and 50−60% of TPC in lentil were lost by steaming. Pressure processing (both boiling and steaming) lost relatively less TPC than regular processing for green pea, yellow pea, and chickpea due to shorter processing times. However, pressure steaming lost more TPC than conventional steaming in the case of lentil.

Effect of Thermal Processing on Saponins and Phytic Acids of CSFLs. Figure 2 shows the saponin and phytic acid contents in raw and cooked CSFLs. The level of saponin (soyasaponin equivalent) in raw CSFLs ranged from 9.82 to 17.78 mg/g (**Figure 2A**). The level of phytic acid in raw CSFLs ranged from 7.51 to 18.92 mg/g (**Figure 2B**). Among the raw legumes, lentil and chickpea possessed higher saponin content than green pea and yellow pea. On the other hand, green pea and chickpea had higher phytic acid content than yellow pea and lentil. As compared to the raw CSFLs, all cooking treatments significantly ($p < 0.05$) reduced both saponin and phytic acid contents. Different cooking methods studied have varied effects in reducing the level of saponin and phytic acids. Among the cooking treatments, boiling appeared to effectively reduce the saponin and phytic acid levels in all CSFLs. The reduction ranges (**Table 1**) on cooking were 5.2−42.9% for saponin and 15.0−24.3% for phytic acid. The decrease in phytic acid content of lentil after cooking was 21.6−21.9%. These results are comparable to the study of Wang et al. (*25*), who found cooking caused a 15.9% reduction in phytate levels, but in contrast to those of Elhardallou and Walker (*26*), who found cooking lentils caused a 60.5% reduction. The discrepancies may be due to the differences in sample sources or processing methods. The apparent decrease in phytic acid content during cooking may be partly due either to the formation of insoluble complexes between phytic acid and other components, such as phytate−protein and phytate−protein−mineral complexes, or to the inositol hexaphosphate hydrolyzed to penta- and tetraphosphates (*27*).

Traditionally, saponins and phytic acids have been considered to be antinutritional factors. The presence of these antinutritional components in legumes impairs the digestion of protein, decreases Ca, Fe, and Zn bioavailability, and therefore reduces the nutritional value of legumes. However, recent evidence indicates that low levels of phytic acid had healthful effects as antioxidant (*28*). Reductions in glycemic response to starchy foods as well as lower plasma cholesterol and triglyceride levels have been observed with endogenous phytate consumed in foods or with the addition of purified sodium phytate. Therefore, phytate may play an important role in controlling hypercholesterolemia and atherosclerosis. In addition, phytic acid had shown anticancer effects in the colon and mammary gland in rodent models and in various tumor cell lines in vitro (*29*). Therefore, reduction of phytic acid is expected to enhance the bioavailability of proteins and dietary minerals of legumes, and at the same time the lower level of phytic acid may still have some health promotional activities. In view of these beneficial effects, the term "antinutrient" used to describe food constituents such as phytic acid needs to be re-evaluated (*30*).

Effect of Thermal Processing on Antioxidant Capacities of CSFLs. Antioxidant activity determination is reaction mechanism-dependent. The specificity and sensitivity of a single method do not lead to the complete examination of all phytochemicals in the extract. Therefore, a combination of several tests could provide a more reliable assessment of the antioxidant activity profiles of legume samples. Previously, boiling and steaming effects on antioxidant activities in terms of DPPH radical scavenging activity and oxygen radical absorbing capacity (ORAC) of CSFLs have been reported in our earlier paper (*6*). However, thermal processing effects on the ferric reducing antioxidant power (FRAP), peroxyl radical scavenging capacities

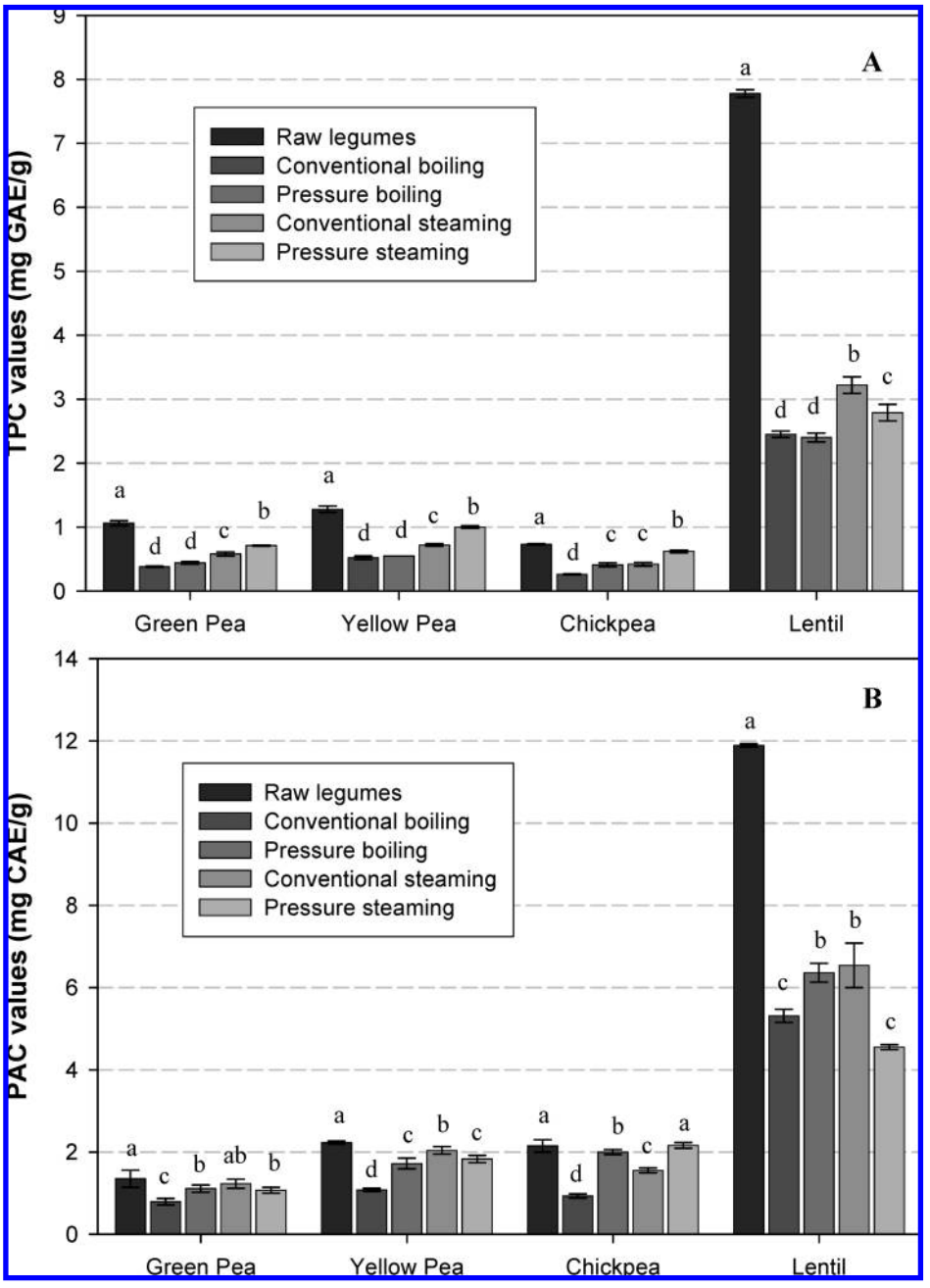

Figure 1. Effect of thermal processing on phenolics (μg/g) of cool-season food legumes. Bar data are expressed as mean ± standard deviation ($n = 3$) on a dry weight basis. The same letter above the bar indicates no significant difference ($p < 0.05$) within each group of legumes.

(PRSC), and cell-based antioxidant capacities of CSFLs have not been documented. Therefore, the current study was performed to investigate these activities of cooked CSFLs on the basis of four processing conditions selected from our previous study. The chemical antioxidant activities (FRAP and PRSC) and cellular antioxidant activities (CAA) of the raw and cooked CSFLs are presented in **Figure 3** and **Table 2**, respectively. Significant differences ($p < 0.05$) in FRAP and PRSC values were found among most treatments for all CSFLs. As compared to the raw legumes, all processing treatments caused significant ($p < 0.05$) decreases in FRAP and PRSC values. As compared to the boiling treatments, both conventional and pressure steamed legumes retained significantly ($p < 0.05$) higher FRAP values in the cases of green pea, yellow pea, and lentil. There were no significant differences in FRAP values between conventionally boiled and pressure boiled green pea, yellow pea, and lentil and no significant differences in PRSC values between the yellow pea and lentil cooked by the two boiling methods.

Among all raw CSFLs, lentil exhibited the greatest CAA with the lowest IC_{50} value (0.67 mg/mL), followed by yellow pea and green pea, whereas raw chickpea did not exhibit dose-dependent CAA. Pressure steaming reduced CAA of lentil and increased the IC_{50} value to 1.88 mg/mL. Most thermal processing methods eliminated the dose-dependent CAA, whereas dose-independent CAA was found in most cooked products. Cellular antioxidant activities of food legumes had not been reported in the literature. However, when compared to the CAA values of fruits in a previous paper (*21*), the raw CSFLs exhibited much lower EC_{50} values (ranging from 0.67 mg/mL in lentil to 1.38 mg/mL in green pea) than those of fruits (ranging from 10.81 mg/mL in blueberry to 53.01 mg/mL in green grape), which were determined by a PBS wash protocol that we also adopted. These results indicated that

Table 1. Losses of Phytochemicals and Antioxidant Capacities of CSFLs upon Thermal Processing[a]

	TPC loss (%)	PAC loss (%)	saponin loss (%)	phytic acid loss (%)	FRAP loss (%)	PRSC loss (%)
green pea						
CB, 120 min	45.9	41.5	31.9	24.3	64.2	38.2
PB, 15 psi, 15 min	42.7	17.8	34.0	21.7	58.5	29.9
CS, 70 min	25.0	8.9	15.8	18.2	45.3	56.9
PS, 15 psi, 60 min	10.5	20.7	9.5	20.7	33.0	18.6
yellow pea						
CB, 120 min	46.8	51.6	16.7	24.2	59.4	71.7
PB, 15 psi, 15 min	43.2	22.9	18.8	17.9	57.0	69.0
CS, 70 min	25.9	8.5	5.2	16.7	43.8	64.2
PS, 15 psi, 60 min	12.9	17.9	17.5	20.6	21.9	46.8
chickpea						
CB, 120 min	58.2	56.7	42.9	17.7	64.4	54.4
PB, 15 psi, 15 min	39.1	6.9	35.1	17.7	43.8	67.9
CS, 70 min	33.2	27.9	23.2	16.8	42.5	47.7
PS, 15 psi, 60 min	11.9	0	28.9	20.4	15.1	13.1
lentil						
CB, 45 min	62.1	55.3	17.2	21.6	68.5	68.7
PB, 15 psi, 5 min	61.9	46.5	34.6	21.9	69.2	66.9
CS, 15 min	49.3	45.3	7.3	16.9	58.6	34.8
PS, 15 psi, 15 min	56.1	61.7	22.3	15.0	64.1	37.8

[a] Loss percentage was calculated using original raw legumes as starting materials. TPC, total phenolic content; PAC, procyanidin content; FRAP, ferric reducing antioxidant power; PRSC, peroxyl radical scavenging capacity. CB, conventional boiling; PB, pressure boiling; CS, conventional steaming; PS, pressure steaming.

food legumes might have stronger cellular antioxidant activities than fruits. However, further comparative studies, involving legumes, fruits, and vegetables together, should be carried out to justify their activity. We cannot compare the CAA of legumes with those of vegetables reported by Eberhardt et al. (*22*), who presented CAA in a different way by using percentage decrease in DCF fluorescence.

Boiling processes decreased FRAP values about 58.5−64% in green pea, 57−60% in yellow pea, 44−64% in chickpea, and 68−70% in lentil and decreased PRSC values about 30−38% in green pea, 69−72% in yellow pea, 54−68% in chickpea, and 67−69% in lentil (**Table 1**). When compared to the boiled CSFLs, steamed products retained more FRAP values in all CSFLs and retained more PRSC values in yellow pea, chickpea, and lentil. The reduction effect of thermal processing on antioxidant activity (FRAP and PRSC values) in the current study exhibited similar trends to the reduction of DPPH and ORAC values as reported in our previous paper (*6*), as well as the reduction of DPPH and Trolox equivalent antioxidant capacity (TEAC) values as reported by Han and Baik (*31*) on cooking effect of pea, chickpea, and lentil.

Boiling and steaming processes significantly affected overall antioxidant activities (FRAP and PRSC values) in all CSFLs. The changes were dependent upon the type of legume and processing conditions. The changes in the overall antioxidant properties of processed CSFLs can be attributed to the synergistic combinations or counteracting of several types of oxidative reaction, leaching of water-soluble antioxidant compositions, formation or breakdown of antioxidant compositions, and solid losses during processes. To better understand the role and fate of natural and process-induced antioxidants on food stability and human health, the individual phenolic compounds were further quantified to investigate the molecular mechanisms responsible for the loss or formation of antioxidants and the interactions between natural and heat-induced antioxidants and their effects on the overall antioxidant properties of processed CSFLs.

Effect of Thermal Processing on Anticancer Properties of CSFLs. Cell proliferation was analyzed 48 h after cancer cells had been exposed to various concentrations of CSFL extracts. The antiproliferation properties of the raw and cooked CSFLs against three human cancer cell lines are summarized in **Table 2**. In the case of green pea, the raw pea exhibited dose−response antiproliferation effects against both gastric (AGS) and colorectal (SW480) adenocarcinoma cell lines. Boiled green pea exhibited higher antiproliferation activities against these two cell lines (with lower IC_{50} values) as compared to the raw pea. Steamed green peas did not exhibit dose−response antiproliferation effects. Neither raw nor cooked green pea exhibited dose−response antiproliferation effects against prostate carcinoma cell line DU145. In the case of green pea, losses of TPC and chemical antioxidant capacities (**Figure 3**) did not correlate with antiproliferation capacities (**Table 2**). Conventionally and pressure boiled green pea had lower TPC, PAC, FRAP, and PRSC but higher antiproliferation capacities against AGS and SW480 cells as compared to those of pressure steamed green pea. In the case of yellow pea, the raw pea exhibited antiproliferation activity in a dose-dependent manner against colorectal cell line SW480 only, but not for AGS and DU145. No cooked yellow pea exhibited dose−response antiproliferation activity against any of the cell lines tested. In the case of chickpea, the raw chickpea exhibited dose−response antiproliferation effects against AGS and SW48 cells, but cooked chickpea did not. In the case of lentil, the raw lentil exhibited dose−response antiproliferation activities against all cell lines tested. However, thermal processing reduced antiproliferation activities of lentil (with increased IC_{50} values) as compared to the raw lentil. Among all CSFLs, the raw lentil possessed the strongest antiproliferation capacities by comparing their IC_{50} values. In summary, the antiproliferation effect of CSFLs was dependent on legume type, processing method, and cell line properties. Most cooked CSFLs possessed lower antiproliferation capacities as compared to the raw CSFLs or tended to have non-dose-dependent activity.

The anticancer effects of food legumes as well as their bioactive components, such as soybeans, common beans, isoflavones, and soyasaponins, had been extensively investigated (*32*). However, the anticancer potential of pea, chickpea, and lentil had not been fully studied. Only a recent epidemiological study showed that the consumption of bean and lentil was related to a lower incidence of breast cancer among several common fruits and vegetables (*33*). However, the anticancer mechanisms of these legumes are unknown. The current in vitro cell culture system involved with three different cell lines verified the anticancer potential of lentil,

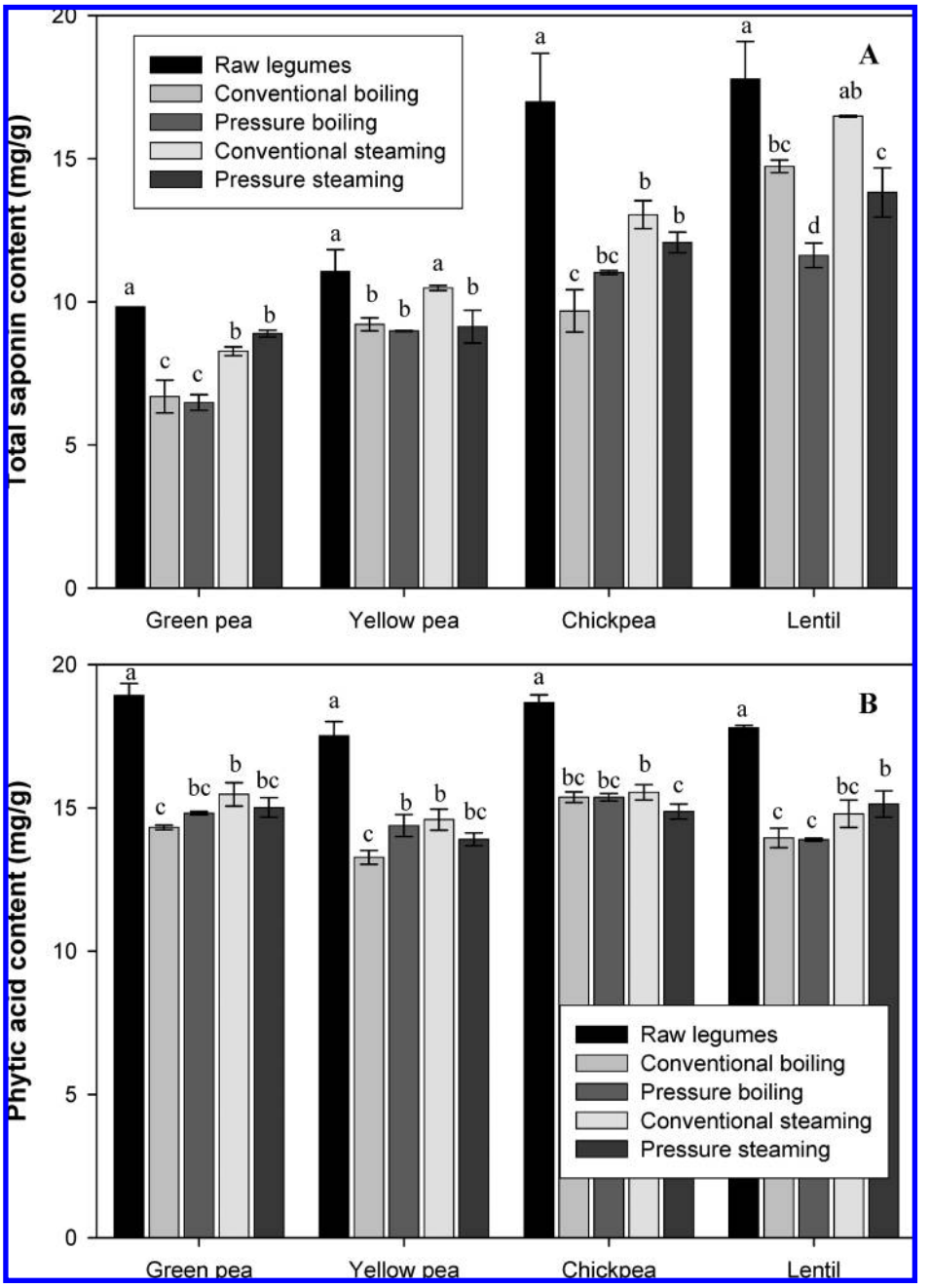

Figure 2. Effect of thermal processing on saponin (**A**) and phytic acid (**B**) of cool-season food legumes. Bar data are expressed as mean ± standard deviation (n = 3) on a dry weight basis. The same letter above the bar indicates no significant difference ($p < 0.05$) within each group of legumes.

and the phytochemical and antioxidant analyses showed that phenolic antioxidant components may partly contribute to the anticancer activity of lentil, because tremendous phenolic components and antioxidant capacities were lost in cooked lentil coupled with a reduction in the antiproliferation capacities (with the exception of pressure steamed lentil tested on SW480 cells).

Effect of Thermal Processing on Phenolic Acids of CSFLs. The free phenolic acid contents of the raw and cooked CSFLs are presented in **Table 3**. Gallic, 2,3,4-trihydroxybenzoic, and chlorogenic acid were detected in all raw and cooked CSFLs. In the case of green pea, three phenolic acids of the benzoic types (gallic, protocatechuic, and 2,3,4-trihydroxybenzoic acid) and one phenolic acid of the cinnamic type (chlorogenic acid) were detected in both raw and cooked pea (**Table 3**). Chlorogenic acid and gallic acid were the predominant phenolic acids among the compounds detected in both raw and cooked green pea. In the case of yellow pea, two phenolic acids of the benzoic type (gallic and 2,3,4-trihydroxybenzoic acid) and two phenolic acids of the cinnamic type (chlorogenic and *p*-coumaric acid) were detected in both raw and cooked yellow pea. Chlorogenic acid and gallic acid were also the predominant phenolic acids among the compounds detected in both raw and cooked yellow pea. In the case of chickpea, three phenolic acids of the benzoic type (gallic, 2,3,4-trihydroxybenzoic acid, and protocatechualdehyde) and three phenolic acids of the cinnamic type (chlorogenic, *p*-coumaric, and *m*-coumaric acid) were detected in both raw and cooked chickpea. Chlorogenic, gallic, *p*-coumaric acid and protocatechualdehyde were the predominant phenolic acids among the compounds detected in both raw and cooked chickpea. In the case of lentil, two phenolic acids of the benzoic type (gallic and 2,3,4-trihydroxybenzoic acid) and four phenolic acids of the cinnamic type (chlorogenic, *p*-coumaric, *m*-coumaric, and sinapic acid) were detected in both raw and

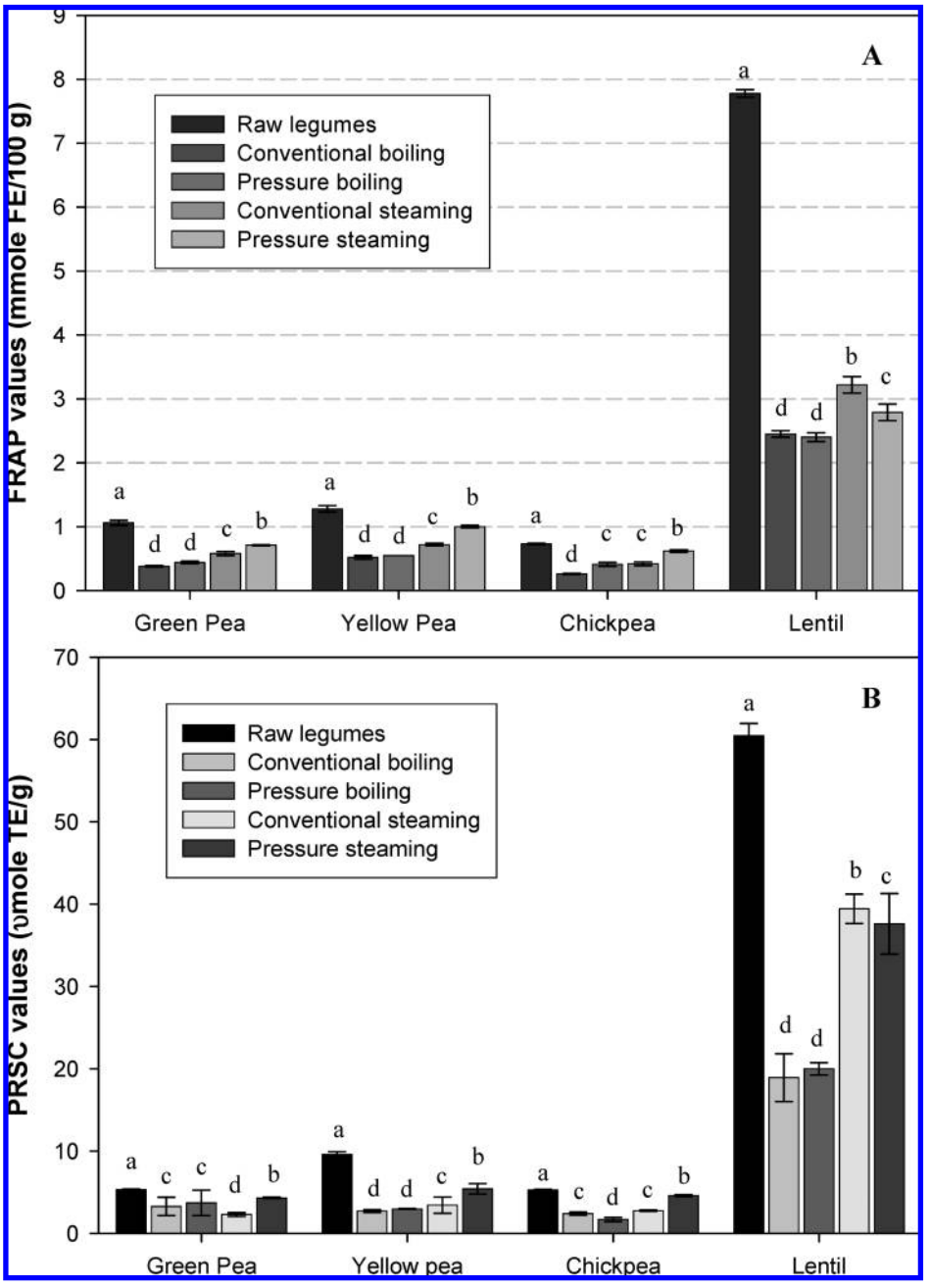

Figure 3. Effect of thermal processing on antioxidant activities (**A**, FRAP; **B**, PRSC) of cool-season food legumes. Bar data are expressed as mean ± standard deviation ($n = 3$) on a dry weight basis. The same letter above the bar indicates no significant difference ($p < 0.05$) within each group of legumes.

cooked lentil. Sinapic, chlorogenic, gallic, and *p*-coumaric acids were the predominant phenolic acids among the compounds detected in both raw and cooked lentil.

The conjugated phenolic acid contents of the raw and cooked CSFLs are presented in **Table 4**. Six conjugated phenolic acids of the benzoic type (gallic, protocatechuic, 2,3,4-trihydroxybenzoic, *p*-hydroxybenzoic, gentisic, and syringic acid and protocatechualdehyde) and one conjugated phenolic acid of the cinnamic type (*p*-coumaric acid) were detected in all raw and cooked CSFLs. As compared to free phenolic acid assay, more types of conjugated phenolic acids were detected, such as *p*-hydroxybenzoic, gentisic, syringic, salicylic, caffeic, and *o*-coumaric acid, and vanillin. However, the predominant phenolic acid compositions (such as chlorogenic acid and sinapic acid) in free phenolic acid assay became undetectable or decreased greatly in both raw and cooked CSFLs. These phenomena might be attributable 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 free and conjugated phenolic acid contents were found among most processing treatments for CSFLs. In the case of the free phenolic acid assay of green pea (**Table 3**), as compared to raw pea, conventional and pressure boiling and regular steaming caused significant ($p < 0.05$) decreases in gallic, protocatechuic, and subtotal benzoic acids, but significant increases in 2,3,4-trihydroxybenzoic, chlorogenic, and subtotal cinnamic acids, whereas pressure steaming caused significant ($p < 0.05$) increases in all detected individual phenolic acids, subtotal benzoic acids, subtotal cinnamic acids, and total phenolic acids of green pea. In the case of the free phenolic acid assay of yellow pea, as compared to raw pea, conventional and pressure boiling caused significant ($p < 0.05$)

Table 2. Cellular Antioxidant Properties and Antiproliferation Capacities of CSFLs against Cancer Cell Lines[a]

	antiproliferation						CAA
	gastric adenocarcinoma cell AGS		colorectal adenocarcinoma cell SW480		prostate carcinoma cell DU145		gastric adenocarcinoma cell AGS
	activity[b]	IC_{50} values (mg/mL)	activity	IC_{50} values (mg/mL)	activity	IC_{50} values (mg/mL)	EC_{50} value (mg/mL)
green pea							
raw	DDI	3.25	DDI	4.52	NDD	NA	1.38
CB, 120 min	DDI	3.12	DDI	1.67	NDD	NA	NDDA[c]
PB, 15 psi, 15 min	DDI	2.50	DDI	1.99	NDD	NA	NDDA
CS, 70 min	NDD	NA[d]	NDD	NA	NDD	NA	NDDA
PS, 15 psi, 60 min	NDD	NA	NDD	NA	NDD	NA	NDDA
yellow pea							
raw	NDD	NA	DDI	5.13	NDD	NA	0.78
CB, 120 min	NDD	NA	NDD	NA	NDD	NA	NDDA
PB, 15 psi, 15 min	NDD	NA	NDD	NA	NDD	NA	NDDA
CS, 70 min	NDD	NA	NDD	NA	NDD	NA	NDDA
PS, 15 psi, 60 min	NDD	NA	NDD	NA	NDD	NA	NDDA
chickpea							
raw	DDI	3.23	DDI	5.71	NDD	NA	NDDA
CB, 120 min	NDD	NA	NDD	NA	PP[e]	NA	NDDA
PB, 15 psi, 15 min	NDD	NA	NDD	NA	PP	NA	NDDA
CS, 70 min	NDD	NA	NDD	NA	PP	NA	NDDA
PS, 15 psi, 60 min	NDD	NA	NDD	NA	PP	NA	NDDA
lentil							
raw	DDI	1.27	DDI	1.91	DDI	1.47	0.67
CB, 45 min	DDI	5.13	NDD	NA	NDD	NA	NDDA
PB, 15 psi, 5 min	DDI	4.16	NDD	NA	NDD	NA	NDDA
CS, 15 min	NDD	NA	DDI	2.22	NDD	NA	NDDA
PS, 15 psi, 15 min	DDI	6.10	DDI	1.67	NDD	NA	1.88

[a] Data were obtained from triplicate wells for each sample and duplicate running on 96-well plates. CB, conventional boiling; PB, pressure boiling; CS, conventional steaming; PS, pressure steaming. [b] DDI, dose-dependent inhibition; NDD, nondose-dependent inhibition. [c] NDDA, non-dose-dependent antioxidant activity. [d] NA, not available. [e] PP, promoting proliferation.

decreases in gallic acid, *p*-coumaric acid + syringaldehyde, subtotal benzoic acid, subtotal cinnamic acid, and total phenolic acids, whereas pressure steaming caused significant ($p < 0.05$) increases in gallic, 2,3,4-trihydroxybenzoic, chlorogenic acid, subtotal benzoic acids, subtotal cinnamic acids, and total phenolic acids of yellow pea. In the case of the free phenolic acid assay of chickpea, as compared to raw chickpea, all processing caused significant ($p < 0.05$) decreases in individual phenolic acids (except for pressure streaming increasing gallic and 2,3,4-trihydroxybenzoic acid), subtotal, and total phenolic acids. In the case of the free phenolic acid assay of lentil, as compared to raw lentil, conventional boiling, pressure boiling, and pressure steaming caused significant ($p < 0.05$) decreases in gallic, chlorogenic, sinapic, *p*-coumaric acid, subtotal, and total phenolic acids, but caused significant increases in 2,3,4-trihydroxybenzoic acid. On the other hand, regular steaming did not cause significant changes in the chlorogenic and sinapic acid and subtotal cinnamic acid and total phenolic acid content.

In the case of conjugated phenolic acid assay of CSFLs, there were no obvious trends for phenolic acid changes upon thermal effects, except that 2,3,4-trihydroxybenzoic acid and *p*-hydroxybenzoic acid contents were significantly ($p < 0.05$) reduced by all processing methods for all cases of CSFLs as compared to those of raw legumes. In addition, protocatechuic acid contents were significantly reduced in all cooked chickpea and lentil.

In summary, thermal effects on phenolic acid profiles of CSFLs were very complex; they depended on legume type and processing method as well as phenolic type. Overall, all processing methods reduced total phenolic acid content of chickpea and lentil. Both conventional and pressure boiling reduced the total phenolic acid content in all CSFLs as compared to raw pea. Interestingly, pressure boiling increased the total phenolic acid content of green pea and yellow pea as compared to raw peas. According to the description of Fleuriet and Macheix (*34*), the changes of phenolic acids in CSFLs upon boiling and steaming processing 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 acids and other chemicals, in particular, proteins and tannins.

Among the four varieties of raw CSFLs, lentil possessed the highest total free phenolic acid (2818.6 μg/g), followed by chickpea (1285.7 μg/g), yellow pea (253.3 μg/g), and green pea (154.4 μg/g). Among all detected free phenolic acids, sinapic acid and chlorogenic acid occupied 80 and 13.6% of total phenolic acid in lentil, respectively. Chlorogenic acid occupied 83.9, 60.7, and 51.5% of total phenolic acid in chickpea, green pea, and yellow pea, respectively.

The literature on phenolic acid content in peas, chickpeas, and lentils is very limited and contradictory. Phenolic acid contents in raw pea and lentil grown in Europe have been reported in several earlier studies (*35*−*39*). However, quantification of phenolic acid was based on either separated parts (seed coats and cotyledon) or germinated or enzyme-treated lentils or peas. An analysis on colored and white pea grown in Poland showed that dominant phenolic acid compositions in peas were different between varieties, in which protocatechuic, gentisic, and vanillic acid were found in colored seed coat, whereas ferulic and coumaric acid were found in the white seed coat (*35*). Here we also found that different free phenolic acid profiles existed between green pea and yellow pea. However, in terms of phenolic acid concentration and constituents, there were big variations between different varieties of peas when compared to the current U.S. peas investigated and the European peas reported in the literature (*35*, *36*, *38*). There were also large variations between different varieties in the

Table 3. Effect of Boiling and Steaming on Free Phenolic Acid Compositions (Micrograms per Gram) of CSFLs [a]

	individual benzoic acid and derivate					
	GA	PA	TBA	PCD	HBA	subtotal benzoics
green pea						
raw	73.22 ± 2.40b	1.21 ± 0.54ab	0.48 ± 0.07e	ND	ND	74.91 ± 1.94b
CB, 120 min	26.49 ± 1.13d	1.69 ± 0.28a	1.04 ± 0.17d	ND	ND	29.21 ± 0.99d
PB, 15 psi, 15 min	39.09 ± 2.19c	0.54 ± 0.27c	1.32 ± 0.12c	ND	ND	40.96 ± 1.90c
CS, 70 min	20.43 ± 1.16e	0.86 ± 0.13bc	1.62 ± 0.09b	ND	ND	22.91 ± 1.34e
PS, 15 psi, 60 min	125.57 ± 1.57a	1.43 ± 0.07ab	2.65 ± 0.14a	ND	ND	129.65 ± 1.47a
yellow pea						
raw	83.67 ± 4.06b	ND[b]	0.70 ± 0.04c	ND	ND	84.38 ± 4.07b
CB, 120 min	34.09 ± 2.86c	ND	0.71 ± 0.12c	ND	ND	34.81 ± 2.85c
PB, 15 psi, 15 min	40.48 ± 3.46c	ND	1.04 ± 0.10b	ND	ND	41.52 ± 3.54c
CS, 70 min	23.67 ± 2.27d	ND	1.21 ± 0.05b	ND	ND	24.89 ± 2.21d
PS, 15 psi, 60 min	120.64 ± 5.97a	ND	1.81 ± 0.15a	ND	ND	122.46 ± 6.04a
chickpea						
raw	67.57 ± 4.29b	0.89 ± 0.39a	1.11 ± 0.10d	85.86 ± 8.51a	10.11 ± 1.02a	165.6 ± 12.68a
CB, 120 min	44.32 ± 3.89c	0.19 ± 0.01b	2.66 ± 0.05b	35.79 ± 2.72d	0.91 ± 0.04b	83.88 ± 6.61d
PB, 15 psi, 15 min	39.99 ± 6.02c	ND	1.61 ± 0.24c	35.31 ± 4.99d	ND	76.91 ± 11.25d
CS, 70 min	41.79 ± 1.65c	0.46 ± 0.19ab	3.45 ± 0.08a	57.52 ± 1.61b	ND	103.1 ± 0.83c
PS, 15 psi, 60 min	75.21 ± 3.85a	0.33 ± 0.14b	2.82 ± 0.19b	45.51 ± 5.61c	ND	123.8 ± 9.37b
lentil						
raw	133.9 ± 8.39a	ND	2.19 ± 0.19d	ND	ND	136.1 ± 8.34a
CB, 45 min	46.53 ± 3.79c	ND	3.48 ± 0.22b	ND	ND	50.01 ± 3.98c
PB, 15 psi, 5 min	52.73 ± 4.65c	ND	2.83 ± 0.17c	ND	ND	55.56 ± 4.47c
CS, 15 min	79.32 ± 8.88b	ND	5.32 ± 0.04a	ND	ND	84.64 ± 8.90b
PS, 15 psi, 15 min	47.59 ± 3.13c	ND	3.63 ± 0.18b	ND	ND	51.23 ± 3.29c

	individual cinnamic acid and derivate					
	CLA	PCA + SD	MCA + FA	SPA	subtotal cinnamics	total phenolic acids
green pea						
raw	79.46 ± 3.47e	ND	ND	ND	79.46 ± 3.47e	154.4 ± 2.97c
CB, 120 min	95.26 ± 0.80d	ND	ND	ND	95.26 ± 0.80d	124.5 ± 1.76d
PB, 15 psi, 15 min	110.1 ± 1.38c	ND	ND	ND	110.1 ± 1.38c	151.1 ± 1.59c
CS, 70 min	154.9 ± 6.19b	ND	ND	ND	154.9 ± 6.19b	177.8 ± 7.53b
PS, 15 psi, 60 min	177.7 ± 3.02a	ND	ND	ND	177.7 ± 3.02a	307.3 ± 4.44a
yellow pea						
raw	153.9 ± 3.50c	15.02 ± 0.33a	ND	ND	168.9 ± 3.58c	253.3 ± 6.57b
CB, 120 min	122.9 ± 9.14d	2.88 ± 0.05d	ND	ND	125.8 ± 9.16e	160.6 ± 11.49e
PB, 15 psi, 15 min	140.9 ± 7.13c	3.21 ± 0.18d	ND	ND	144.1 ± 7.29d	185.6 ± 10.54d
CS, 70 min	183.3 ± 16.58b	4.36 ± 0.26c	ND	ND	187.7 ± 16.81b	212.6 ± 18.98c
PS, 15 psi, 60 min	206.4 ± 1.56a	4.84 ± 0.08b	ND	ND	211.3 ± 1.52a	333.7 ± 4.79a
chickpea						
raw	1079.3 ± 77.44a	37.92 ± 3.54a	2.94 ± 0.22a	ND	1120.2 ± 80.93a	1285.7 ± 93.35a
CB, 120 min	454.2 ± 16.49c	20.55 ± 1.66c	2.32 ± 0.09b	ND	477.1 ± 18.09c	560.9 ± 24.27 cd
PB, 15 psi, 15 min	434.3 ± 74.67c	18.31 ± 2.11c	1.52 ± 0.48c	ND	454.1 ± 77.08c	531.0 ± 87.49d
CS, 70 min	668.4 ± 40.20b	26.29 ± 1.67b	1.74 ± 0.12c	ND	696.4 ± 41.66b	799.5 ± 42.33b
PS, 15 psi, 60 min	524.9 ± 55.91c	22.02 ± 2.87bc	1.03 ± 0.18d	ND	548.0 ± 58.37c	671.8 ± 67.46c
lentil						
raw	383.0 ± 16.88a	18.99 ± 1.28b	9.85 ± 0.72b	2270.6 ± 57.49a	2682.5 ± 45.99a	2818.6 ± 39.46a
CB, 45 min	199.2 ± 11.44b	11.28 ± 0.49c	10.61 ± 0.35b	1405.9 ± 52.31d	1627.0 ± 46.93c	1677.0 ± 50.82d
PB, 15 psi, 5 min	191.9 ± 18.88b	8.60 ± 0.75d	8.29 ± 1.05c	1522.2 ± 42.58c	1731.1 ± 48.15c	1786.7 ± 52.46c
CS, 15 min	344.5 ± 38.63a	22.41 ± 1.96a	12.50 ± 1.04a	2257.9 ± 58.41a	2637.3 ± 87.11a	2721.9 ± 87.35a
PS, 15 psi, 15 min	228.7 ± 2.27b	13.31 ± 0.63c	9.68 ± 0.29bc	1808.3 ± 43.89b	2059.9 ± 46.91b	2111.2 ± 49.02b

[a] Data are expressed as mean ± standard deviation ($n = 3$) on a dry weight basis; values marked by the same letter within each legume type 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; CLA, chlorogenic acid; PCA + SD, *p*-coumaric acid + syringaldehyde; MCA + FA, *m*-coumaric acid + ferulic acid; SPA, sinapic acid. CB, conventional boiling; PB, pressure boiling; CS, conventional steaming; PS, pressure steaming. [b] ND, not detectable.

literature. The occurrence of phenolic acids as protecting substances against plant stress environments may be affected by both genetic and growth environments. The phenolic acid profile of raw lentil (cultivar CDC Richlea grown in North Dakota) in our current investigation was different from those of Spanish lentils (*36*, *39*), in which fewer phenolic acids (protocatechuic, *p*-hydroxybenzoic, *p*-coumaric, and ferulic acid) with lower content (ranging from 0.1 to 7.5 μg/g) were detected. The major phenolic acid compositions (gallic, chlorogenic, and sinapic acid) found in the current lentil samples were not reported in the Spanish lentils (*37*−*39*). The differences may be attributed to the differences of sample sources or extraction and quantification methods.

Effect of Thermal Processing on Anthocyanin Compositions of CSFLs. The five most common naturally occurring anthocyanins, namely, delphinidin-3-glucoside, malvidin-3,5-diglucoside, petunidin-3-glucoside, malvidin-3-galactoside, and malvidin-3-glucoside, were used as external standards to detect and quantify individual anthocyanin content in CSFLs as described under

Table 4. Effect of Boiling and Steaming on Conjugated Phenolic Acid Compositions (Micrograms per Gram) of CSFLs[a]

	GA	PA	TBA	PCD	HBA	GTA	VN	SA	SCA
green pea									
raw	7.36 ± 0.41bc	2.02 ± 0.21c	44.15 ± 2.09a	2.23 ± 0.29b	6.82 ± 0.37a	153.25 ± 2.58a	2.83 ± 0.21a	15.91 ± 2.71b	41.92 ± 2.65a
CB, 120 min	10.56 ± 0.77a	2.52 ± 0.04b	14.66 ± 0.72c	1.60 ± 0.05c	1.43 ± 0.01c	101.88 ± 3.66c	0.99 ± 0.02c	2.97 ± 0.07c	14.85 ± 0.82b
PB, 15 psi, 15 min	7.55 ± 0.53b	0.69 ± 0.21d	16.00 ± 0.87bc	1.69 ± 0.13c	0.93 ± 0.03c	153.98 ± 8.80a	0.65 ± 0.06d	3.32 ± 0.15c	7.58 ± 0.13d
CS, 70 min	6.38 ± 0.48c	0.74 ± 0.21d	18.81 ± 1.89b	1.59 ± 0.04c	1.20 ± 0.38c	116.27 ± 25.77bc	0.78 ± 0.04 cd	2.35 ± 0.46c	14.30 ± 1.09b
PS, 15 psi, 60 min	10.79 ± 0.42a	4.23 ± 0.34a	16.47 ± 0.96bc	3.68 ± 0.16a	2.02 ± 0.35b	135.78 ± 12.78ab	1.56 ± 0.04b	31.34 ± 2.12a	10.72 ± 0.43c
yellow pea									
raw	6.90 ± 0.35c	1.76 ± 0.34a	29.15 ± 1.86a	2.58 ± 0.55ab	3.04 ± 0.46a	261.10 ± 31.65a	2.25 ± 0.26b	3.17 ± 0.34b	27.15 ± 2.37a
CB, 120 min	5.86 ± 0.46d	1.21 ± 0.07b	10.62 ± 0.39b	2.13 ± 0.28bc	1.05 ± 0.01c	162.78 ± 19.4b	1.61 ± 0.12c	0.77 ± 0.15c	11.95 ± 0.51b
PB, 15 psi, 15 min	2.98 ± 0.29e	ND[b]	6.10 ± 0.12c	1.76 ± 0.14c	0.26 ± 0.03d	48.98 ± 4.41c	0.89 ± 0.00d	1.19 ± 0.15c	ND
CS, 70 min	8.89 ± 0.22b	1.05 ± 0.06b	11.64 ± 1.20b	1.82 ± 0.10c	1.44 ± 0.19bc	15.58 ± 0.96c	2.36 ± 0.19ab	ND	15.19 ± 0.05b
PS, 15 psi, 60 min	10.32 ± 0.33a	1.95 ± 0.06a	10.20 ± 0.71b	3.09 ± 0.11a	1.70 ± 0.18b	42.49 ± 1.47c	2.78 ± 0.34a	4.38 ± 0.05a	12.03 ± 1.45b
chickpea									
raw	8.73 ± 0.72c	2.64 ± 0.11a	108.11 ± 6.42a	4.29 ± 0.16b	8.89 ± 0.16a	54.20 ± 10.87b	0.71 ± 0.06b	0.79 ± 0.19bc	ND
CB, 120 min	11.66 ± 1.18b	1.94 ± 0.19bc	30.33 ± 2.85d	2.03 ± 0.26c	4.04 ± 0.31e	37.35 ± 2.31c	0.59 ± 0.07c	0.62 ± 0.02 cd	ND
PB, 15 psi, 15 min	15.60 ± 0.88a	1.62 ± 0.20c	39.99 ± 0.84c	5.39 ± 0.71a	6.98 ± 0.31c	35.75 ± 3.69c	0.72 ± 0.01b	0.47 ± 0.01d	ND
CS, 70 min	12.36 ± 0.25b	2.14 ± 0.17b	50.13 ± 1.75b	5.79 ± 0.47a	6.15 ± 0.71d	35.10 ± 9.02c	1.01 ± 0.06a	0.81 ± 0.08b	ND
PS, 15 psi, 60 min	16.99 ± 0.71a	1.81 ± 0.04c	34.79 ± 2.37 cd	5.13 ± 0.39a	7.86 ± 0.25b	80.02 ± 8.55a	1.02 ± 0.01a	1.19 ± 0.05a	ND
lentil									
raw	16.35 ± 0.76b	6.00 ± 0.30a	62.71 ± 1.06a	15.44 ± 0.19a	3.79 ± 0.45a	18.62 ± 4.41b	1.41 ± 0.20a	1.54 ± 0.25a	ND
CB, 45 min	20.80 ± 0.90a	3.61 ± 0.34 cd	40.37 ± 1.13b	9.58 ± 0.77b	2.07 ± 0.03c	2.40 ± 1.44c	ND	ND	ND
PB, 15 psi, 5 min	13.03 ± 1.44c	4.24 ± 0.45c	33.23 ± 2.65c	6.35 ± 0.55c	2.24 ± 0.11c	1.67 ± 1.28c	ND	ND	ND
CS, 15 min	16.91 ± 1.19b	3.15 ± 0.41d	30.51 ± 1.47c	9.35 ± 0.28b	2.21 ± 0.16c	31.27 ± 4.81a	1.04 ± 0.10b	1.26 ± 0.10a	ND
PS, 15 psi, 15 min	19.78 ± 0.99a	5.07 ± 0.37b	42.75 ± 3.50b	9.18 ± 0.23b	2.87 ± 0.25b	32.48 ± 5.13a	1.21 ± 0.09ab	1.22 ± 0.06a	ND

	CFA	CLA	PCA + SD	MCA + FA	SPA	OCA	TCA
green pea							
raw	2.87 ± 0.29b	4.11 ± 0.65a	2.42 ± 0.34a	2.73 ± 0.16b	ND	3.69 ± 0.24b	ND
CB, 120 min	1.77 ± 0.04c	3.07 ± 0.58b	0.17 ± 0.04d	1.19 ± 0.05c	ND	3.22 ± 0.07c	ND
PB, 15 psi, 15 min	1.40 ± 0.00d	3.62 ± 0.00ab	0.35 ± 0.04d	0.82 ± 0.02d	ND	4.35 ± 0.11a	ND
CS, 70 min	3.75 ± 0.05a	3.09 ± 0.29b	1.06 ± 0.20c	1.17 ± 0.24c	ND	2.98 ± 0.15c	ND
PS, 15 psi, 60 min	1.52 ± 0.05 cd	4.19 ± 0.17a	1.49 ± 0.09b	3.06 ± 0.08a	ND	2.34 ± 0.22d	ND
yellow pea							
raw	ND	ND	2.31 ± 0.47a	4.68 ± 0.32a	2.73 ± 0.15a	2.62 ± 0.23a	ND
CB, 120 min	1.63 ± 0.20b	6.82 ± 0.56a	1.18 ± 0.09bc	3.19 ± 0.26ab	2.69 ± 0.56a	1.58 ± 0.17c	ND
PB, 15 psi, 15 min	3.04 ± 0.02a	6.13 ± 0.29a	0.30 ± 0.00d	1.00 ± 0.05c	ND	2.08 ± 0.13b	ND
CS, 70 min	1.05 ± 0.07c	3.55 ± 0.39b	0.81 ± 0.10 cd	1.81 ± 1.58bc	ND	ND	ND
PS, 15 psi, 60 min	1.38 ± 0.25bc	4.21 ± 0.62b	1.36 ± 0.11b	3.69 ± 0.19a	ND	ND	ND
chickpea							
raw	1.52 ± 0.13a	ND	1.31 ± 0.05c	ND	2.61 ± 0.01a	0.79 ± 0.06	ND
CB, 120 min	0.92 ± 0.03b	ND	2.47 ± 0.21b	ND	1.48 ± 0.17b	ND	ND
PB, 15 psi, 15 min	ND	ND	2.91 ± 0.09a	ND	ND	ND	ND
CS, 70 min	ND	ND	1.41 ± 0.21c	ND	ND	ND	ND
PS, 15 psi, 60 min	1.62 ± 0.11a	ND	2.56 ± 0.03b	ND	1.01 ± 0.10c	ND	ND
lentil							
raw	1.25 ± 0.05	8.51 ± 1.71a	8.50 ± 0.73a	0.25 ± 0.07	ND	ND	5.16 ± 0.39a
CB, 45 min	ND	4.05 ± 0.06b	5.17 ± 0.25b	ND	ND	ND	2.10 ± 0.04b
PB, 15 psi, 5 min	ND	10.07 ± 0.24a	4.45 ± 0.22b	ND	ND	ND	0.51 ± 0.03c
CS, 15 min	ND	4.76 ± 0.06b	4.76 ± 0.47b	ND	ND	ND	0.59 ± 0.06c
PS, 15 psi, 15 min	ND	3.22 ± 0.14b	4.67 ± 0.47b	ND	ND	0.46 ± 0.04	1.80 ± 0.04b

[a] Data are expressed as mean ± standard deviation ($n = 3$) on a dry weight basis; values marked by the same letter within each legume type 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; GTA, gentistic 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 + ferulic acid; SPA, sinapic acid; OCA, *o*-coumaric acid; TCA, *trans*-cinnamic acid. CB, conventional boiling; PB, pressure boiling; CS, conventional steaming; PS, pressure steaming. [b] ND, not detectable.

Materials and Methods. However, these compounds were not detectable in all raw and cooked CSFLs. In addition, there were no other unknown peaks beyond standard compound peaks when detected at 540 nm by HPLC. The information indicated that no identifiable anthocyanins existed in green pea, yellow pea, chickpea, and lentil. In the literature, anthocyanins had only been reported in a lentil cultivar of Beluga Black (*40*).

Effect of Thermal Processing on Flavan-3-ol Composition of CSFLs. A systematic HPLC quantification was performed to investigate thermal effects on flavan-3-ol profiles of CSFLs. The flavan-3-ol profiles of the raw and cooked CSFLs are presented in **Table 5**. (+)-Catechin as a major flavan-3-ol was detected in all raw and cooked CSFLs. In addition, epicatechin, epigallocatechin, and EGCG were detected in chickpea and lentil. The (+)-catechin contents were significantly ($p < 0.05$) reduced upon all thermal processing methods in all CSFLs. The epigallocatechin contents were significantly ($p < 0.05$) increased by all thermal processing in chickpea and lentil.

In terms of total flavan-3-ol contents (the sum of total individual flavan-3-ol), as compared to raw legumes, all thermal processing significantly ($p < 0.05$) reduced total flavan-3-ol contents in all CSFLs. More total flavan-3-ols were lost in

Table 5. Effect of Boiling and Steaming on Flavan-3-ol Contents (Micrograms per Gram) of CSFLs[a]

	individual flavan-3-ols				
	(+)-catechin	epigallocatechin	epicatechin	EGCG[b]	total flavan-3-ols
green pea					
raw	205.3 ± 8.12a	ND[c]	ND	ND	205.3 ± 8.12a
CB, 120 min	61.19 ± 2.47d	ND	ND	ND	61.19 ± 2.47d
PB, 15 psi, 15 min	96.17 ± 5.59c	ND	ND	ND	96.17 ± 5.59c
CS, 70 min	129.9 ± 4.95b	ND	ND	ND	129.9 ± 4.95b
PS, 15 psi, 60 min	130.9 ± 0.94b	ND	ND	ND	130.9 ± 0.94b
yellow pea					
raw	282.9 ± 52.57a	ND	ND	ND	282.9 ± 52.57a
CB, 120 min	121.3 ± 5.78d	ND	ND	ND	121.3 ± 5.78d
PB, 15 psi, 15 min	143.0 ± 18.10c	ND	ND	ND	143.0 ± 18.10c
CS, 70 min	186.3 ± 7.61b	ND	ND	ND	186.3 ± 7.61b
PS, 15 psi, 60 min	135.8 ± 3.79c	ND	ND	ND	135.8 ± 3.79c
chickpea					
raw	1507.6 ± 122.6a	23.95 ± 0.49c	145.5 ± 2.01b	16.79 ± 0.62	1693.7 ± 124.5a
CB, 120 min	397.6 ± 10.65c	176.9 ± 4.92b	52.94 ± 2.14e	ND	627.4 ± 7.88d
PB, 15 psi, 15 min	353.8 ± 19.87c	189.9 ± 0.33b	72.39 ± 2.94d	ND	616.2 ± 16.61d
CS, 70 min	634.4 ± 13.05b	281.8 ± 20.07a	102.4 ± 2.31c	ND	1018.6 ± 35.43c
PS, 15 psi, 60 min	756.17 ± 2.57b	271.4 ± 9.68a	164.0 ± 3.82a	ND	1191.5 ± 3.29 b
lentil					
raw	695.5 ± 19.72a	10.09 ± 1.88d	3819.4 ± 297.81a	ND	4524.9 ± 317.5a
CB, 45 min	380.7 ± 21.16b	66.6 ± 4.93b	2354.9 ± 24.02c	35.71 ± 0.74b	2837.9 ± 47.05c
PB, 15 psi, 5 min	129.2 ± 1.74d	60.44 ± 0.47b	2687.5 ± 67.62bc	45.76 ± 0.24a	2922.9 ± 69.6c
CS, 15 min	121.7 ± 3.90d	53.44 ± 1.87c	3619.7 ± 34.84a	42.47 ± 3.11a	3837.3 ± 25.9b
PS, 15 psi, 15 min	253.7 ± 8.78c	86.95 ± 2.16a	2842.3 ± 205.09b	ND	3182.9 ± 216.0c

[a] Data are expressed as mean ± standard deviation ($n = 3$) on a dry weight basis,; values marked by the same letter within each legume type in each column are not significantly different ($p < 0.05$). CB, conventional boiling; PB, pressure boiling; CS, conventional steaming; PS, pressure steaming. [b] EGCG, epigallatecatechin gallate. [c] ND, not detectable.

Table 6. Effect of Boiling and Steaming on Flavonol and Flavone Compositions (Micrograms per Gram) of CSFLs[a]

	flavonols						flavones	
	rutin	Q-3-G[b]	K-3-R[b]	K-3-G[b]	kaempferol	myricetin	luteolin	apigenin
green pea								
raw	ND[c]	ND	ND	3.80 ± 0.12a	ND	36.24 ± 0.78	ND	ND
CB, 120 min	ND	ND	ND	ND	ND	ND	ND	ND
PB, 15 psi, 15 min	ND	ND	ND	ND	ND	ND	ND	ND
CS, 70 min	ND	ND	ND	ND	ND	ND	ND	ND
PS, 15 psi, 60 min	ND	ND	ND	3.28 ± 0.04b	ND	ND	ND	ND
yellow pea								
raw	ND	ND	ND	ND	ND	36.71 ± 0.41	ND	ND
CB, 120 min	ND	ND	ND	ND	ND	ND	ND	ND
PB, 15 psi, 15 min	ND	ND	ND	ND	ND	ND	ND	ND
CS, 70 min	ND	ND	ND	ND	ND	ND	ND	ND
PS, 15 psi, 60 min	ND	ND	ND	ND	ND	ND	ND	ND
chickpea								
raw	ND	ND	ND	ND	18.11 ± 1.68a	32.09 ± 0.35	ND	ND
CB, 120 min	ND	ND	ND	1.64 ± 0.17a	4.93 ± 0.29c	ND	ND	ND
PB, 15 psi, 15 min	ND	ND	ND	0.19 ± 0.07b	5.84 ± 0.19c	ND	ND	7.88 ± 0.06b
CS, 70 min	ND	ND	ND	1.76 ± 0.01a	6.92 ± 0.17c	ND	ND	13.51 ± 0.43a
PS, 15 psi, 60 min	ND	ND	ND	ND	10.74 ± 0.33b	ND	ND	4.64 ± 0.06c
lentil								
raw	ND	ND	ND	ND	2.78 ± 0.04b	33.31 ± 0.01a	9.69 ± 0.01a	ND
CB, 45 min	21.50 ± 0.17a	7.81 ± 0.45a	8.12 ± 0.12a	2.38 ± 0.11a	4.68 ± 0.04a	31.29 ± 0.40a	8.22 ± 0.90b	ND
PB, 15 psi, 5 min	15.91 ± 0.49c	3.53 ± 0.07b	1.00 ± 0.04d	0.29 ± 0.01b	1.39 ± 0.05c	31.31 ± 0.01a	7.99 ± 0.74b	ND
CS, 15 min	19.34 ± 1.23b	4.51 ± 0.38b	3.34 ± 1.31c	2.85 ± 0.15a	ND	31.87 ± 0.29a	ND	ND
PS, 15 psi, 15 min	16.91 ± 1.40c	ND	5.29 ± 0.60b	ND	ND	32.09 ± 0.35	ND	ND

[a] Data are expressed as mean ± standard deviation ($n = 3$) on a dry weight basis; values marked by the same letter within each legume type in each column are not significantly different ($p < 0.05$). CB, conventional boiling; PB, pressure boiling; CS, conventional steaming; PS, pressure steaming. [b] Q-3-G, quercetin-3-glucoside; K-3-R, kaempferol-3-rutinoside; K-3-G, kaempferol-3-glucoside. [c] ND, not detectable.

conventionally cooked products than in pressure-cooked products for both green pea and yellow pea, whereas there were no obvious different trends between the total flavan-3-ol contents of the conventionally and pressure boiled products for both chickpea and lentil. Both conventional and pressure steaming retained more flavan-3-ol in green pea, chickpea, and lentil as compared to the two boiling methods. Among the four raw CSFLs studied, lentil contained the highest flavan-3-ols, followed by chickpea, yellow pea, and green pea.

Effect of Thermal Processing on Flavonol and Flavone Compositions CSFLs. The flavonol and flavone contents of the raw and cooked CSFLs are presented in **Table 6**. Six flavonols including

rutin, quercetin-3-glucoside, kaempferol-3-rutinoside, kaempferol-3-glucoside, kaempferol, and myricetin and one flavone (luteolin) were detected in either raw or cooked lentil. Three flavonols including kaempferol-3-glucoside, kaempferol, and myricetin and one flavone (apigenin) were detected in either raw or cooked chickpea. However, only two flavonols (kaempferol-3-glucoside and myricetin) were detected in the raw green pea, and only one flavonol (myricetin) was detected in the raw yellow pea, whereas no flavones were detected in either green pea or yellow pea. After thermal processing, rutin, quercetin-3-glucoside, kaempferol-3-rutinoside, and kaempferol-3-glucoside became detectable in cooked lentil. This indicated that thermal processing released these compounds from bonded forms. After thermal processing, myricetin became undetectable in green pea, yellow pea, and chickpea. In addition, thermal processing significantly ($p < 0.05$) reduced kaempferol content in chickpea. Besides these effects, there was no significant impact on the retention of other flavonols and flavones as a result of different processing methods.

The results from current lentil and pea studies verified previous findings (*36*, *39*) about the existence of (+)-catechin, epicatechin, and luteolin in lentil and pea. However, as compared to the previous studies (*37*−*39*), there was no apigenin glycoside or myricetin glycoside existing in lentil in the current investigation. The discrepancies may be attributed to the differences of sample sources or extraction methods.

Taken together, thermal processes significantly affected not only phytochemical profiles of CSFLs but also beneficial biological effects (antioxidant, antiproliferation) associated with these compounds. The changes depended upon the types of legumes and processing conditions. Steaming appeared to be the best cooking method for retaining antioxidants and phenolic components. Boiling appeared to effectively reduce the saponin and phytic acid levels. Except in the SW480 colon tumor cell system, thermal processing eliminated or reduced the antiproliferation capacities of all cool-season legumes tested. However, some antiproliferation capacities remained after cooking of green pea and lentil. This information suggests that raw legumes may be better material than cooked legumes for the nutraceutical industry to produce certain anticancer agents. Because legumes must be cooked before consumption, the selection of cooking methods becomes important for retaining antioxidant or antiproliferation effects, and such effects might be legume−tumor-type dependent. Furthermore, our research indicates that the potential for reducing cancer risks by long-term consumption of legume food may be associated with not only phenolic antioxidants but also other types of phytochemicals.

ABBREVIATIONS USED

AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; AUC, area under curve; CAA, cellular antioxidant activity; CSFLs, cool-season food legumes; DCF, dichlorofluorescein; DCFH-DA, 2′,7′-dichlorofluorescin diacetate; FBS, fetal bovine serum; FRAP, ferric reducing antioxidant power; HBSS, Hank's balanced salt solution; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PAC, total procyanidin content; PBS, phosphate-buffered saline; PRSC, peroxyl radical scavenging capacity; TPC, total phenolic content; TSC, total saponin content.

ACKNOWLEDGMENT

Angie Ostenson assisted in sample freeze-drying. Gloria Nygard and Cook Leword assisted in HPLC operation and maintenance. U.S. Dry Pea and Lentil Association, North Dakota Pea & Lentil Association, and mentioned companies provided legume samples for this study.

LITERATURE CITED

(1) Kushi, L. H.; Meyer, K. A.; Jacobs, D. R. Cereal, legumes, and chronic disease risk reduction: evidence from epidemiologic studies. *Am. J. Clin. Nutr.* **1999**, *70* (Suppl.), 451S−458S.

(2) Singh, K. B., Saxena, M. C., Eds. *Breeding for Stress Tolerance in Cool-Season Food Legumes*; Wiley: Chichester, U.K., 1993; pp 474.

(3) Janzen, E.; Flakerud, G.; Fisher J.; Bartsch, E. *Pulse Crop Marketing Guide, NDSU Extension Service*, **2006**; www.ag.ndsu.edu.

(4) Xu, B. J.; Yuan, S. H.; Chang, S. K. C. Comparative analyses of phenolic composition, antioxidant capacity, and color of cool season legumes and other selected food legumes. *J. Food Sci.* **2007**, *72*, S167–S177.

(5) Xu, B. J.; Yuan, S. H.; Chang, S. K. C. Comparative studies on the antioxidant activities of nine common food legumes against copper-induced human low-density lipoprotein oxidation *in vitro*. *J. Food Sci.* **2007**, *72*, S522–S527.

(6) Xu, B. J.; Chang, S. K. C. Effects of soaking, boiling, and steaming on total phenolic content and antioxidant activities of cool season food legumes. *Food Chem.* **2008**, *110*, 1–13.

(7) Association of Official Analytical Chemists. AOAC Method 925.10 (air oven method) for moisture in flour. In *Official Methods of Analysis*, 17th ed.; Gaithersburg, MD, 2000.

(8) Vindiola, O. L.; Seib, P. A.; Hoseney, R. C. Accelerated development of the hard-to-cook state in beans. *Cereal Foods World* **1986**, *31*, 538–552.

(9) Xu, B. J.; Chang, S. K. C. A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *J. Food Sci.* **2007**, *72*, S159–S166.

(10) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolic with phosphomolybdic−phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158.

(11) Broadhurst, R. B.; Jones, W. T. Analysis of condensed tannin using acified vanillin. *J. Sci. Food Agric.* **1978**, *29*, 788–794.

(12) Luthria, D. L.; Pastor-Corrales, M. A. Phenolic acids content of fifteen dry edible bean (*Phaseolus vulgaris* L.) varieties. *J. Food Compos. Anal.* **2006**, *19*, 205–211.

(13) Nardini, M.; Cirilo, E.; Natella, F.; Mencarelli, D.; Comisso, A.; Scaccini, C. Detection of bound phenolic acids: prevention by ascorbic and ethylenediamine tetracetic acid of degradation of phenolic acids during alkaline hydrolysis. *Food Chem.* **2002**, *79*, 119–124.

(14) Xu, B. J.; Chang, S. K. C. Total phenolics, phenolic acids, isoflavones, and anthocyanins and antioxidant properties of yellow and black soybeans as affected by thermal processing. *J. Agric. Food Chem.* **2008**, *56*, 7165–7175.

(15) Xu, B. J.; Chang, S. K. C. 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. *J. Agric. Food Chem.* **2009**, *57*, 4754–4764.

(16) Murphy, P. A.; Song, T.; Buseman, G.; Barua, K. Isoflavones in soy-based infant formulas. *J. Agric. Food Chem.* **1997**, *45*, 4635–4638.

(17) Makker, H. P. S.; Becker, K. Nutrients and antiquality factors in different morphological parts of the *Moringa oleifera* tree. *J. Agric. Sci. Cambridge* **1997**, *128*, 311–322.

(18) Hiai, S.; Oura, H.; Nakajima, T. Color reaction of some sapogenins and saponins with vanillin and sulfuric acid. *Planta Med.* **1976**, *29*, 116–122.

(19) Gao, Y.; Shang, M. A.; Maroof, S.; Biyashev, R. M.; Grabau, E. A.; Kwanyuen, P.; Burton, J. W.; Buss, G. R. A modified colorimetric method for phytic acid analysis in soybean. *Crop Sci.* **2007**, *47*, 1797–1803.

(20) Benzie, I. F. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.* **1996**, *239*, 70–76.

(21) Adom, K. K.; Liu, R. H. Rapid peroxyl radical scavenging capacity (PSC) assay for assessing both hydrophilic and lipophilic antioxidants. *J. Agric. Food Chem.* **2005**, *53*, 6572–6580.

(22) Eberhardt, M. V.; Kobira, K.; Keck, A. S.; Juvik, J. A.; Jeffery, E. H. Correlation analyses of phytochemical composition, chemical, and cellular measures of antioxidant activity of broccoli (*Brassica oleracea* L. var. *italica*). *J. Agric. Food Chem.* **2005**, *53*, 7421–7431.

(23) Wolfe, K. L.; Liu, R. H. Cellular antioxidant activity (CAA) assay for assessing antioxidants, foods, and dietary supplements. *J. Agric. Food Chem.* **2007**, *55*, 8896–8907.
(24) Carmichael, J.; Degraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. *Cancer Res.* **1987**, *47*, 936–942.
(25) Wang, N.; Hatcher, D. W.; Toews, R.; Gawalko, E. J. Influence of cooking and dehulling on nutritional composition of several varieties of lentils (*Lens culinaris*). *LWT* **2009**, *42*, 842–848.
(26) Elhardallou, S. B.; Walker, A. F. Phytic acid content of three legumes in the raw, cooked and fibre forms. *Phytochem. Anal.* **1994**, *5*, 243–246.
(27) Siddhuraju, P.; Becker, K. Effect of various domestic processing methods on antinutrients and in vitro protein and starch digestibility of two indigenous varieties of Indian pulses, *Mucuna pruries* var Utilis. *J. Agric. Food Chem.* **2001**, *49*, 3058–3067.
(28) Graf, E.; Eaton, J. W. Antioxidant functions of phytic acid. *Free Radical Biol. Med.* **1990**, *8*, 61–69.
(29) Shamsuddin, A. M. Anti-cancer function of phytic acid. *Int. J. Food Sci. Technol.* **2002**, *37*, 769–782.
(30) Rickard, S. E.; Thompson, L. U. Interactions and biological effects of phytic acid. *ACS Symp. Ser.* **1997**, *No. 662*, 294−312.
(31) Han, H.; Baik, B. K. Antioxidant activity and phenolic content of lentils (*Lens culinaris*), chickpeas (*Cicer arietinum* L.), peas (*Pisum sativum* L.) and soybeans (*Glycine max*), and their quantitative changes during processing. *Int. J. Food Sci. Technol.* **2008**, *43*, 1971–1978.
(32) Messina, M. J. Legumes and soybeans: overview of their nutritional profiles and health effects. *Am. J. Clin. Nutr.* **1999**, *70*, 439S–450S.
(33) Adebamowo, C. A.; Cho, E. Y.; Sampson, L.; Katan, M. B.; Spiegelman, D.; Willett, W. C.; Holmes, M. D. Dietary flavonols and flavonol-rich foods intake and the risk of breast cancer. *Int. J. Cancer* **2005**, *114*, 628–633.
(34) Fleuriet, A.; Macheix, J. J. Phenolic acids in fruits and vegetables. In *Flavonoids in Health and Disease*; Rice-Evans, C., Packer, L., Eds.; CRC Press: Boca Raton, FL, 2003; pp 1−42.
(35) Troszynska, A.; Ciska, E. Phenolic compounds of seed coats of white and colored varieties of pea (*Pisum sativum* L.) and their total antioxidant activity. *Czech J. Food Sci.* **2002**, *20*, 15–22.
(36) Duenas, M.; Hernandez, T.; Estrella, I. Phenolic composition of the cotyledon and seed coat of lentils (*Lens culinaris* L.). *Eur. Food Res. Technol.* **2002**, *215*, 478–483.
(37) Bartolome, B.; Estrella, I.; Hernandez, T. Changes in phenolic compounds in lentils (*Lens culinaris*) during germination and fermentation. *Z. Lebensm. Unters. Forsch. A* **1997**, *205*, 290–294.
(38) López-Amorós, M. L.; Hernández, T.; Estrella, I. Effect of germination on legume phenolic compounds and their antioxidant activity. *J. Food Compos. Anal.* **2006**, *19*, 277–283.
(39) Duenas, M.; Hernandez, T.; Estrella, I. Changes in the content of bioactive polyphenolic compounds of lentils by the action of exogenous enzymes. Effect on their antioxidant activity. *Food Chem.* **2007**, *101*, 90–97.
(40) Takeoka, G. R.; Dao, L. T.; Tamura, H.; Harden, L. A. Delphinidin 3-*O*-(2-*O*-*β*-D-glucopyranosyl-α-L-arabinopyranoside): a novel anthocyanin identified in Beluga black lentils. *J. Agric. Food Chem.* **2005**, *53*, 4932–4937.

Received for review July 26, 2009. Revised manuscript received October 6, 2009. Accepted October 12, 2009. This work was supported by Grant FAR100773 from the USDA-CSREES Cool Season Legume Research Program administered through the University of Idaho. This publication was made possible by Grants P20RR015566 and P20RR016741 from the National Institutes of Health (NIH). Its contents are solely the responsibility of the authors and do not necessarily represent the official view of NCRR or NIH.