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Characterization of Phenolic Substances and Antioxidant Properties of Food Soybeans Grown in the North Dakota−Minnesota Region

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Phenolic profiles and antioxidant properties of a total of 30 soybean samples, including 27 grown in the North Dakota−Minnesota region and three soybeans from the other regions, were investigated. The total phenolic content (TPC), total flavonoids content (TFC), phenolic acids, flavonols, anthocyanins, and isoflavones were quantified. Antioxidant properties of soybean extracts were assessed using 2-diphenyl-1-picryhydrazyl free radical scavenging activity (DPPH), ferric reducing antioxidant power (FRAP), and oxygen radical absorbance capacity (ORAC) methods. Results showed that black soybean cultivars possessed significantly higher TPC, TFC, DPPH, FRAP, and ORAC values than all yellow soybean cultivars. However, black soybean cultivars did not exhibit significantly higher individual phenolic contents (except for anthocyanins), such as phenolic acids and isoflavones, than the yellow soybean cultivars. The isoflavone profiles of North Dakota soybean cultivars were similar to those of South Dakota, but average values of total isoflavone (TI) contents were higher than soybeans grown in the other states and Korea and Japan according to the U.S. Department of Agriculture−Iowa State University Database on the isoflavone contents of foods. Correlation assays showed that TPC, TI, total phenolic acids, daidzin, genistin, malonyldaidzin, daidzein, genistein, and *trans*-cinnamic acid significantly (r = 0.73, 0.62, 0.49, 0.68, 0.59, 0.59, 0.56, 0.47, and 0.76, respectively, $p < 0.0001$) correlated with ORAC values of yellow soybeans. Both isoflavones and phenolic acids contributed to the ORAC values of yellow soybeans. These data suggest that some selected soybean cultivars may be used as high-quality food-grade soybeans for providing high phenolic phytochemicals and antioxidant activities.

KEYWORDS: North Dakota soybean cultivars; total phenolics; isoflavones; phenolic acids; flavonols; anthocyanins; antioxidants; DPPH; FRAP; ORAC; HPLC

INTRODUCTION

Soybean originated in North and East Asia. Nowadays, however, more soybeans are grown in the United States than anywhere else in the world. The U.S. production of soybeans, mainly in the Western Corn Belt (including the states of Iowa, Kansas, Missouri, Nebraska, South Dakota, North Dakota, and Minnesota) and the Eastern Corn Belt (including the states of Illinois, Indiana, Michigan, Ohio, and Wisconsin), has increased significantly in recent years. U.S. farmers produced their largest-ever soybean crop in 2006, according to the Crop Production 2006 Summary released by the U.S. Department of Agriculture's National Agricultural Statistics Service (*1*).

Health-promoting effects of soybeans as well as their bioactive phytochemicals, especially isoflavones, have been extensively studied around the world. To a lesser degree, antioxidant activities of soybeans have been reported (*2*–*5*). Whole soybeans and soy-based foods are extensively consumed in the Asian diets. Tofu and soymilk are the most popular soy food items in China, Korea, Japan, Singapore, and Thailand. Fermented soy-based foods are also popular flavor ingredients in traditional Oriental diets, such as soy sauce, douchi, and bean paste in China; soy sauce, miso, and natto in Japan; chungkujang and denjang in Korea; and tempeh in Indonesia. Epidemiological studies have shown a low incidence of several hormone-dependent diseases, such as breast and prostate cancers and postmenopausal symptoms, in these Asian countries, due to the high consumption of soy and soy-based foods (*6*). However, Westerners consume little soy foods. One strategy to increase the use of soy is to add soy ingredients into processed food products (*2*). Selecting for soybean cultivars that possess high phenolic contents (phenolic acids, isoflavones, and anthocyanins) and high antioxidant capacity may help in the production and international trade of soybeans from farmers to the food/nutraceutical industry.

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It is commonly known that the composition of phenolic substances is affected by variety, planting location, and cropping year. North Dakota and northern Minnesota regions are two of the highest latitude geographical zones in the United States for growing warm season legume soybeans. The particular geographical area may yield special properties of secondary metabolites in soybeans. However, the phenolic profiles and antioxidant properties of the food soybean (defined as soybeans used for soymilk and tofu making) cultivars produced in the North Dakota−Minnesota region have not been studied. Our objectives were to systematically assess total phenolics, phenolic acids, anthocyanins, and isoflavone profiles and the antioxidant capacities of food soybeans grown in the North Dakota−Minnesota region and to investigate the relationships between phenolic compounds and antioxidant capacities.

MATERIALS AND METHODS

Chemicals. Sixteen phenolic acids [gallic acid (GA), protocatechuic acid (PA), 2,3,4-trihydroxybenzoic acid (TBA), *p*-hydroxybenzoic acid (HBA), gentistic acid, vanillic acid (VA), caffeic acid (CFA), chlorogenic acid (CLA), syringic acid (SA), *p*-coumaric acid (PCA), *m*-coumaric acid (MCA), *o*-coumaric acid (OCA), ferulic acid (FA), salicylic acid, sinapic acid (SPA), and *trans*-cinnamic acid (TCA)], three aldehydes [vanillin (VN), syringaldehyde (SD), and protocatechualdehyde], (+)-catechin, high-performance liquid chromatography (HPLC)-grade trifluoroacetic acid (TFA), 2-diphenyl-1-picryhydrazyl radical (DPPH$^{\bullet}$), fluorescein disodium (FL), Folin−Ciocalteu reagent, sodium carbonate, 6-hydroxyflavone (HFL), 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 Inc. (St. Louis, MO). Nine isoflavone standards, including daidzin, genistin, glycitin, daidzein, genistein, glycitein, acetyldaidzin, acetylgenistin, and malonylgenistin, were purchased from LC Laboratories (Woburn, MA). A mixture of six unimolar anthocyanin standards (3-*O*-β-glucosides of delphinidin, cyanidin, petunidin, pelargonidin, peonidin, and malvidin) was purchased from Polyphenols Labortories (Sandnes, Norway). 2,2′-Azobis (2-amidino-propane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA (Richmond, VA). 2,4,4′-Trihydroxybenzoin (THB, one of internal standards for isoflavone quantification) was synthesized and purified in our laboratory. HPLC-grade solvents (methanol and acetonitrile, B & J Brand), analytical grade acetic acid, and other analytical grade solvent used for extraction were purchased from VWR international (West Chester, PA). Polyvinylidene difluoide (PVDF) syringe filters with a pore size of 0.2 μm were purchased from National Scientific Co. (Duluth, GA).

Soybean Materials. Dry food grade soybeans of 27 normal-lipoxygenase soybean samples were collected from local farmers or seed companies in the North Dakota−Minnesota region. Three lipoxygenase-free soybean samples, including two L-stars and one IA2032, were obtained from WhiteWave (Boulder, CO) and Stonebridge Ltd. (Cedar Falls, IA), respectively. The source information of these soybean samples is listed in **Table 1**. With the exception of the two black soybeans as indicated, all other cultivars were yellow soybeans. Broken seeds, damaged seeds, and foreign materials were removed from the samples. Whole soybeans were coarsely ground by a Straub Grinding Mill (model 4E, Straub Corporation, Philadelphia, PA) and then were finely ground to powder with a U/D Cyclone Sample Mill (model 3010-030, UDY Corp., Fort Collins, CO) and to pass through a 60-mesh sieve. The moisture content was determined by drying the soybean flour after 24 h at 105 °C in an air oven until a constant weight was obtained (*7*). The phenolic contents and antioxidants activities were expressed on a dry weight basis.

Extraction of Total Phenolics. Extraction procedures of our earlier studies were followed (*4*). Briefly, yellow soybean flours (0.5 g in triplicate) were extracted with acetone/water (50:50, v/v), while black soybean flour (0.5 g in triplicate) was extracted with acetone/water/acetic acid (70:29.5:0.5, v/v/v). The extracts were stored at 4 °C in the dark for use.

Table 1. Information of North Dakota−Minnesota Soybeans and Reference Soybeans[a]

code	varieties/cultivars	harvested year	source/provider	location
#1	Prosoy	2006	Ted Helms, NDSU	Fargo, ND
#2	Traill	2006		Fargo, ND
#3	Norpro	2006		Fargo, ND
#4	Jim	2006		Fargo, ND
#5	Pembina	2006		Fargo, ND
#6	Barnes (7129−7136)	2006		Fargo, ND
#7	LaMoure (16465−16472)	2006		Fargo, ND
#8	ND01-3906 (16025−16032)	2006		Fargo, ND
#9	Walsh (15185−15192)	2006		Fargo, ND
#10	Prosoy	2006	Sinner Brother & Bresnahan	Casselton, ND
#11	Traill	2006		Casselton, ND
#12	Norpro	2006		Casselton, ND
#13	S0880	2006		Casselton, ND
#14	91M10	2006		Casselton, ND
#15	Atwood	2006		Casselton, ND
#16	Proto 05	2005		Casselton, ND
#17	Proto 06	2006		Casselton, ND
#18	Black soybean	2006		Casselton, ND
#30	Black soybean	2005		Casselton, ND
#19	Tofooey	2006	Carl Peterson	Proser, ND
#20	Korada	2006		Proser, ND
#22	Prosoy	2006	John Buchholz	Durbin, ND
#21	Vinton 81	2005		Mountain Lake, MN
#28	2300	2005	Blue Stem	Mountain Lake, MN
#23	5389	2006	Brushvale Seed Inc.	Breckenridge, MN
#24	51C10	2006		Breckenridge, MN
#25	90T60	2006		Breckenridge, MN
#26	L-star (dehulled)	2005	WhiteWave Soyfood Co.	Boulder, CO
#27	L-star (dehulled)	2006		Boulder, CO
#29	IA2032	2005	Stonebridge Ltd.	Cedar Falls, IA

[a] Dried mature seeds were from 2006 crops if not otherwise stated.

Determination of Total Phenolic Content (TPC). The TPC was determined by a Folin−Ciocalteu assay (*8*) with slight modifications (*4*) using GA as the standard. The TPC was expressed as milligrams GA equivalents per gram soybean (mg GAE/g) on a dry weight basis through the calibration curve of GA. 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 (*4*). The TFCs were expressed as milligrams catechin equivalents per gram soybean (mg CAE/g) on a dry weight basis using the calibration curve of (+)-catechin. The linearity range of the calibration curve was 10−1000 μg/mL ($r = 0.99$).

DPPH Free Radical Scavenging Activity Assay. The DPPH free radical scavenging capacity of soybeans was evaluated according to our previous communication (*4*). The DPPH values were expressed as micromoles of Trolox equivalents per gram soybean (μmol TE/g) on a dry weight basis using the calibration curve of Trolox. The linearity range of the calibration cure was 20−1000 μM ($r = 0.99$).

Ferric Reducing Antioxidant Power (FRAP) Assay. The FRAP was performed as described previously (*4*). The FRAP value was expressed as millimoles of Fe^{2+} equivalent per 100 g soybean (mmol FE/100 g) on a dry weight basis 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) Assay. The hydrophilic ORAC 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 of Prior et al. (*9*). The ORAC values were expressed as micromoles of Trolox equivalent per gram soybean (μmol TE/g) on a dry weight basis using the calibration curve of Trolox. The linearity range of the calibration curve was 5.0−50 μM ($r = 0.99$).

HPLC Analysis of Free Phenolic Acid Content. *Extraction of Free Phenolic Acids.* The extraction of free phenolic acids was performed according to a reported method (*10*) with slight modifications. Briefly, the soybean flours (0.5 g in triplicate) were accurately weighed into a set of 15 mL of VWR centrifuge tubes. Six milliliters of methanol/water/acidic acid/butylated hydroxytoluene (BHT) (85:15: 0.5: 0.2, v/v/v/w) extraction solvent was added to each tube. The tubes were capped, and the mixtures were shaken at 300 rpm at room temperature on an orbital shaker for 4 h. The extracts were filtered through #1 Whatman paper. An additional volume of 4 mL of the extraction solvents was added into the residues. The above-mentioned extraction procedures were repeated. The two time extracts were combined and concentrated at 45 °C under vacuum to remove solvents. The residue was dissolved in 5 mL of water and freeze-dried. The freeze-dried extracts (10 mg) were dissolved in 2.5 mL of 25% methanol. The methanol solution was filtered through a 0.2 μm PVDF syringe filter and analyzed for free phenolic acid content by HPLC.

HPLC Analysis of Phenolic Acids. The quantitative analysis of free phenolic acids was performed by HPLC according to Robbins and Bean (*11*) with slight modifications as follows: (i) The column temperature was increased from 25 to 40 °C for improving reproducibility and resolution; (ii) 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 set at 270 nm was used; (iii) instead of C_{18} Luna column, a Zorbax Stablebond Analytical SB-C_{18} column (4.6 mm × 250 mm, 5 μm, Agilent Technologies, Rising Sun, MD) was used for improving resolution. 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 additional 15 min, and the gradient was increased to 100% B at 66 min. The solvent gradient was held at 100% B for an additional 10 min to clean up the column.

Identification and Quantification of Phenolic Acids. For the identification of sample peaks, 1 mg/mL stock solution of each individual compound was prepared and diluted to 100 μg/mL, the diluted working solutions were injected into HPLC, and spiking methods and external standard methods were used by comparing increasing peak areas and retention times. In addition, to confirm the identities of compound peaks through their UV spectrum information, individual phenolic acid and phenolic acid mixtures as well as several typical samples were selected to perform analysis on another HPLC (HP 1090, Hewlett-Packard, Waldbronn, Germany), which was equipped with UV-PDA detector.

All identified phenolic acids were quantified with external standards using HPLC analysis as described previously. To prepare the 1 mg/mL stock solution of standard mixture, 10 mg of each phenolic acid compound as well as (+)-catechin was mixed together and dissolved in 10 mL of 25% methanol. The stock solution was diluted into nine series 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 plotted peak areas against concentrations of nine series standard mixture working solutions. The phenolic acid contents were expressed as micrograms phenolic acid per gram soybean (μg/g) on a dry weight basis.

HPLC Analysis of Isoflavone Content. *Extraction of Isoflavones.* Isoflavones were extracted by modifying the methods of Murphy et al. (*12*) and Hou and Chang (*13*). Briefly, the soybean flours (1.0 g ± 0.01 in duplicate) were accurately weighed into a set of 15 mL of screw-top VWR centrifuge tubes. Five milliliters of acetonitrile, 4.5 mL of distilled water, 0.25 mL of internal standard THB (0.1 mg/mL), and 0.25 mL of internal standard HFL (0.1 mg/mL) were added to each tube. The HFL was used as an internal standard for the first time in our laboratory. The tubes were capped, and the mixtures were shaken at 250 rpm at room temperature on an orbital shaker for 2 h. Then, the slurry was centrifuged by an Allegra 21R Centrifuge (Beckman Coulter Ltd., Palo Alto, CA) at 5500 rpm for 20 min. The supernatant was filtered through Whatman #42 filter paper into a 125 mL flask and evaporated to dryness on a rotary evaporator at 34 °C. The residues in the flask were dissolved in 5 mL of 80% methanol and kept in a freezer (−20 °C) for less than 12 h before analysis. An aliquot of sample solution was filtered through a 0.2 μm PTFE syringe filter prior to HPLC assay.

HPLC Analysis of Isoflavones. The quantitative analysis of soybean isoflavones was performed by HPLC according to Hou and Chang (*13*) with a slight modification by using two internal standards. The same Waters Associates chromatography system as used for phenolic acids analysis was used for quantitative analysis of isoflavones, and the UV detector set at 262 nm was used. A YMC-Pack ODS-AM-303 C_{18} reversed phase column (4.6 mm × 250 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. 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, then increased to 29% for 31 min at the flow rate up to 1.5 mL/min, and then to 35% for 8 min at the same flow rate of 1.5 mL/min. Then, the gradient increased to 50% of solvent B in 2 min and kept 50% of solvent B for 10 min at the flow rate of 1.5 mL/min, then recycled back to 15% B at the flow rate of 1.0 mL/min in 2 min, and then, the column was equilibrated with initial solvent for 2 min prior to running the next sample.

Identification and Quantification of Isoflavones. Three aglycones, three 7-*O*-β-glucosides, two 6″-*O*-acetylglucosides (acetyldaidzin and acetylgenistin), and one 6″-*O*-malonylglucoside (malonylgenistin) were commercially available and directly used to identify the sample peaks by comparing their retention times and HPLC profiles to those of standard mixture. The peak identification for noncommercially available isoflavones was confirmed by comparing the chromatograms of several identical samples performed by a well-established isoflavone analytical laboratory (Dr. Pat Murphy at Iowa State University, Ames, IA). In addition, a spiking method was also used for peak identification of some samples.

The internal standard, THB, was synthesized and purified in our laboratory according to procedures described by Murphy et al. (*12*) and Hou and Chang (*13*). The quantification of isoflavones was performed by calibrating the peak area obtained from HPLC analyses. The contents of three aglycones, three 7-*O*-glucosides, two 6″-*O*-acetylglucosides (acetyldaidzin and acetylgenistin), and one 6″-*O*-malonylglucoside (malonylgenistin) in the samples were directly quantified through their external−internal calibration curves. The calibration curves were obtained for each of nine external standards by plotting response factors (RFs) of each standard against concentration. The RFs are the ratios of the peak area of the external standards to the peak area of the internal standards. For the other isoflavones without commercial standards, concentrations were calculated from the standard curves that were adjusted appropriately from the standard curves of respective form of isoflavones based on the differences in molecular weight and molar extinction coefficients of the compounds. Isoflavone contents were expressed as micrograms isoflavone per gram soybean (μg/g) on a dry weight basis.

HPLC Analysis of Anthocyanin Content. *HPLC Conditions for Anthocyanins Analysis.* The free phenolic acid extracts were also used for anthocyanin analysis, and 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). 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 comparison of their retention times with those of standards, a blueberry reference sample, and literature (*14*). The stock solution of anthocyanins mixture was prepared by dissolving standards (unimolar mixture of 3-*O*-β-glucosides of delphinidin, cyanidin, petunidin, pelargonidin,

Table 2. TPC, TFC, and Moisture Contents of Soybeans[a]

code	varieties/cultivars	TPC (mg GAE/g)	TFC (mg CAE/g)	moisture (%)
#1	Prosoy	2.33 ± 0.09 khij	0.31 ± 0.02 ljkhi	4.61 ± 0.2
#2	Traill	2.43 ± 0.09 hig	0.29 ± 0.02 ljkhim	4.28 ± 0.2
#3	Norpro	2.31 ± 0.10 khilj	0.21 ± 0.01 on	4.63 ± 0.4
#4	Jim	2.40 ± 0.19 hig	0.25 ± 0.02 lknm	4.26 ± 0.0
#5	Pembina	2.73 ± 0.14 d	0.25 ± 0.03 lnm	4.82 ± 0.2
#6	Barnes	2.30 ± 0.12 kilj	0.34 ± 0.02 ghi	4.39 ± 0.1
#7	LaMoure	2.36 ± 0.12 khigj	0.31 ± 0.02 ljkhi	4.45 ± 0.0
#8	ND01-3906	2.18 ± 0.06 kml	0.25 ± 0.02 lknm	4.13 ± 0.2
#9	Walsh	2.19 ± 0.06 kmlj	0.24 ± 0.01 onm	4.45 ± 0.1
#10	Prosoy	2.45 ± 0.05 higf	0.40 ± 0.02 gf	5.82 ± 0.0
#11	Traill	2.63 ± 0.06 edf	0.39 ± 0.01 gf	6.01 ± 0.1
#12	Norpro	2.50 ± 0.04 hgf	0.32 ± 0.02 jkhi	6.36 ± 0.3
#13	S0880	2.90 ± 0.16 c	0.58 ± 0.03 c	6.28 ± 0.1
#14	91M10	2.48 ± 0.03 higf	0.27 ± 0.01 ljknm	5.39 ± 0.2
#15	Atwood	2.53 ± 0.06 egf	0.40 ± 0.02 gf	4.88 ± 0.1
#16	Proto (2005)	2.39 ± 0.08 hig	0.35 ± 0.02 gh	5.91 ± 0.3
#17	Proto (2006)	2.45 ± 0.07 higf	0.52 ± 0.02 d	6.44 ± 0.2
#19	Tofooey	2.50 ± 0.09 hgf	0.45 ± 0.02 ef	7.46 ± 0.2
#20	Korada	2.68 ± 0.06 ed	0.59 ± 0.04 c	9.23 ± 0.1
#21	Vinton 81 (2005)	2.37 ± 0.04 higj	0.31 ± 0.07 ljkhi	2.44 ± 0.2
#22	Prosoy	2.36 ± 0.11 khigj	0.32 ± 0.01 jhi	3.47 ± 0.1
#23	5389	2.07 ± 0.09 m	0.28 ± 0.02 ljknim	8.01 ± 0.0
#24	51C10	2.44 ± 0.05 hig	0.24 ± 0.01 onm	6.29 ± 0.1
#25	90T60	2.13 ± 0.13 mL	0.32 ± 0.02 jhi	7.69 ± 0.2
#26	L-star (2005)	2.12 ± 0.08 m	0.18 ± 0.01 o	3.64 ± 0.1
#27	L-star (2006)	2.32 ± 0.14 khij	0.32 ± 0.01 jhi	4.33 ± 0.1
#28	2300	2.34 ± 0.11khij	0.44 ± 0.00 ef	2.01 ± 0.1
#29	IA2032	2.54 ± 0.11 egf	0.47 ± 0.00 ed	3.93 ± 0.3
#30	Black soybean (2005)	8.75 ± 0.09 b	5.95 ± 0.15 a	2.68 ± 0.2
#18	Black soybean (2006)	9.01 ± 0.06 a	5.34 ± 0.02 b	7.33 ± 0.3

[a] Data are expressed as means ± standard deviations ($n = 3$) on a dry weight basis. Values marked by the same letter within each column are not significantly different ($p < 0.05$).

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 plotted peak areas against concentrations by duplicate injection of the six series diluted working solutions of standard mixture. Anthocyanin contents were expressed as micrograms anthocyanin per gram soybean (μg/g) on a dry weight basis.

Statistical Analysis. The data were expressed as means ± standard deviations of triplicate analyses. 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. A Pearson correlation test was conducted to determine the correlation between variables.

RESULTS AND DISCUSSION

Total Phenolic Composition Contents. TPCs (expressed in mg GAE/g) and TFCs (expressed in mg CAE/g) of all 30 soybean samples are presented in **Table 2**. TPCs of yellow soybean cultivars ranged from 2.07 mg GAE/g in cultivar 5389 to 2.90 mg GAE/g in cultivar S0880. TPCs of black soybean cultivars ranged from 8.75 to 9.01 mg GAE/g. TFCs of yellow soybean cultivars ranged from 0.18 mg CAE/g in cultivar L-star (2005) to 0.59 mg CAE/g in cultivar Korada. TFCs of black soybean cultivars ranged from 5.34 to 5.95 mg CAE/g.

Significant differences ($p < 0.05$) in TPC and TFC values were found between yellow soybeans and black soybeans and among many cultivars of yellow soybeans. Both TPCs and TFCs of the two black soybean samples were much higher than all yellow soybean cultivars, while both TPCs and TFCs of yellow soybean cultivar S0880 was the highest among all yellow soybean cultivars. The lowest TPC (2.07 mg GAE/g) was detected in cultivar 5389, and the lowest TFC (0.18 mg CAE/g) was detected in cultivar L-star (2005). Significant differences in both TPC and TFC existed among the three sources (grown in three different locations) of the cultivar Prosoy and between the two sources of cultivar Traill, as well as the two sources of cultivar Norpro, which were grown in two different locations. In addition, significant differences in both TPC and TFC were also found in the samples of the same cultivar but from two cropping years for yellow soybean Proto, L-star, and black soybeans.

The average TPC value (8.8 mg GAE/g) of the two black soybeans in our current report was within the range of that in the four black soybeans grown in Taiwan, which was approximately 5−9 mg GAE/g (*15*) but higher than that (6.2 mg GAE/g) of our previous report (*4*). The average TPC value (2.4 mg GAE/g) of 28 yellow soybeans was close to that (2.62 mg GAE/g) of our previous report on yellow soybean (*4*). TFCs in soybeans had not been reported previously except in our previous report (*4*). The average TFC value (0.34 mg CAE/g) of 28 yellow soybeans in our current report was lower than that (0.5 mg CAE/g) in our previous report. The differences between current results and previous reports may be attributed to the differences in the sources of the samples.

Antioxidant Capacities. Antioxidant activity determination is reaction mechanism-dependent. In the reaction to remove reactive oxygen species, the ORAC method utilizes the hydrogen transfer mechanism, whereas the DPPH utilizes the single electron transfer mechanism (*9*). The specificity and sensitivity of one method do not lead to 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 soybean samples. Antioxidant capacities of soybeans, including DPPH, FRAP, and ORAC, are presented **Table 3**. DPPH values of yellow soybean cultivars ranged from 0 μmol TE/g in cultivars LaMoure, 5389, and 51C10 to 1.16 μmol TE/g in cultivar IA 2032. DPPH values of the black soybeans ranged from 16.39 to 17.86 μmol TE/g. FRAP values of the yellow soybean cultivars ranged

Table 3. Antioxidant Capacities of Soybeans[a]

code	varieties/cultivars	DPPH (μmol TE/g)	FRAP (mmol FE/100 g)	ORAC (μmol TE/g)
#1	Prosoy	0.78 ± 0.06 e	1.06 ± 0.02 hkfgji	55.1 ± 4.7 fhg
#2	Traill	0.99 ± 0.03 d	1.26 ± 0.04 dc	55.5 ± 2.3 fhg
#3	Norpro	0.62 ± 0.06 fg	1.07 ± 0.03 hkfgji	51.1 ± 3.8 ih
#4	Jim	0.58 ± 0.03 g	1.10 ± 0.05 hfgjei	49.9 ± 1.5 ih
#5	Pembina	0.72 ± 0.03 fe	1.34 ± 0.06 c	72.8 ± 4.9 d
#6	Barnes	0.71 ± 0.06 fe	1.21 ± 0.02 dfce	46.8 ± 1.2 ij
#7	LaMoure	ND	1.02 ± 0.01 kjli	49.6 ± 3.0 ih
#8	ND01-3906	0.29 ± 0.02 kj	0.85 ± 0.05 nm	41.1 ± 3.4 kj
#9	Walsh	0.43 ± 0.01 h	0.90 ± 0.01 nml	47.7 ± 4.7 i
#10	Prosoy	0.55 ± 0.03 g	1.09 ± 0.03 hkfgji	49.3 ± 3.5 ih
#11	Traill	0.41 ± 0.02 ih	1.20 ± 0.01 dfce	53.3 ± 3.9 ihg
#12	Norpro	0.32 ± 0.02 ij	1.01 ± 0.01 kjli	59.2 ± 2.5 feg
#13	S0880	0.66 ± 0.04 fg	1.07 ± 0.01 hkfgji	91.3 ± 3.5 c
#14	91M10	0.73 ± 0.04 fe	1.03 ± 0.04 hkjli	63.5 ± 4.9 e
#15	Atwood	1.10 ± 0.08 c	1.17 ± 0.04 hdfge	53.3 ± 3.9 ihg
#16	Proto (2005)	0.57 ± 0.04 g	0.98 ± 0.02 kmjl	40.6 ± 2.4 kj
#17	Proto (2006)	0.71 ± 0.01 fe	1.12 ± 0.02 hdfgjei	27.4 ± 4.2 m
#19	Tofooey	0.41 ± 0.04 ih	1.02 ± 0.02 hkjli	61.5 ± 1.9 fe
#20	Korada	0.36 ± 0.05 ihj	0.99 ± 0.04 kjl	62.4 ± 4.9 e
#21	Vinton 81 (2005)	0.69 ± 0.02 fe	1.05 ± 0.01 hkgjli	55.7 ± 2.0 fhg
#22	Prosoy	0.21 ± 0.01 k	1.09 ± 0.05 hkfgji	50.6 ± 2.1 ih
#23	5389	ND	1.16 ± 0.02 hdfgei	21.2 ± 2.2 n
#24	51C10	ND	0.83 ± 0.01 n	39.0 ± 2.3 kl
#25	90T60	0.70 ± 0.04 fe	1.18 ± 0.04 dfge	22.2 ± 1.2 nm
#26	L-star (2005)	0.59 ± 0.05 g	0.95 ± 0.04 knml	36.6 ± 1.9 kl
#27	L-star (2006)	0.93 ± 0.07 d	1.24 ± 0.04 dce	33.9 ± 1.8 L
#28	2300	0.95 ± 0.07 d	1.07 ± 0.05 hkfgji	48.7 ± 4.2 ih
#29	IA2032	1.16 ± 0.16 c	1.23 ± 0.03 dce	53.2 ± 3.1 ihg
#30	Black soybean (2005)	16.39 ± 0.11 b	14.01 ± 0.32 a	111.6 ± 7.5 b
#18	Black soybean (2006)	17.86 ± 0.08 a	13.05 ± 0.22 b	119.3 ± 5.4 a

[a] Data are expressed as means ± standard deviations ($n = 3$) on a dry weight basis. Values marked by the same letter within each column are not significantly different ($p < 0.05$). ND, not detected.

from 0.83 mmol FE/100 g in cultivar 51C10 to 1.34 mmol FE/100 g in cultivar Pembina. FRAP values of the black soybeans ranged from 13.05 to 14.01 μmol TE/g. ORAC values of the yellow soybean cultivars ranged from 21.2 μmol TE/g in cultivar 5389 to 91.3 μmol TE/g in cultivar S0880. ORAC values of the black soybeans ranged from 111.6 to 119.3 μmol TE/g.

Significant differences in the antioxidant capacities ($p < 0.05$) (DPPH, FRAP, and ORAC values) existed between yellow soybeans and black soybeans and among many cultivars of yellow soybeans. DPPH, FRAP, and ORAC values of the two black soybeans were significantly ($p < 0.05$) higher than all yellow soybean cultivars. Cultivar IA 2032 had the highest DPPH value (1.16 μmol TE/g), whereas Pembina and S0880 had the highest FRAP value (1.34 μmol TE/g) and ORAC value (91.3 μmol TE/g), respectively, among all yellow soybean cultivars. Significant ($p < 0.05$) differences in DPPH values existed among the three Prosoy soybeans, which were grown in three different locations. However, no significant differences in FRAP and ORAC values existed among these three Prosoy samples. Significant differences in DPPH values existed between the two samples of Traill, as well as between the two samples of Norpro. In addition, significant differences in all three antioxidant values were also found in the samples from the same cultivar but grown in two cropping years of the black soybeans. DPPH and ORAC values in the two crop years of Proto differed, whereas DPPH and FRAP values differed in the two crop years of L-star.

The average FRAP value (1.0 mmol FE/100 g in fresh weight, based on average 7% moisture content) was higher than the average values (0.82 mmol FE/100 g based on fresh weight) in soybeans as reported by Halvorsen et al. (*16*). The differences between our results and their studies may be attributed partly to the differences in the sources of the soybean materials. There were no literature values on DPPH and ORAC values in matured dry soybeans except in our previous studies on three soybeans (*4, 5*).

Table 4. Correlation between Total Phenolic Components and Antioxidant Capacities

samples	correlation coefficients (r)	TPC	TFC	DPPH	FRAP	ORAC
all soybeans ($N = 30$)	TPC		0.99[a]	0.99[a]	0.99[a]	0.81[a]
	TFC			0.98[a]	0.99[a]	0.77[a]
	DPPH				0.99[a]	0.76[a]
	FRAP					0.75[a]
	ORAC					
yellow soybeans ($N = 28$)	TPC		0.56[a]	0.18	0.25[b]	0.73[a]
	TFC			0.22[b]	0.15	0.32[b]
	DPPH				0.51[a]	0.17
	FRAP					0.07
	ORAC					

[a] Correlation is significant at the 0.0001 level (two-tailed). [b] Significant at the 0.05 level.

The correlation coefficients among total phenolics and antioxidant activities of soybean samples are summarized in **Table 4**. When we put all soybean data (including the two black soybean samples) together, significant ($p < 0.0001$) linear correlations existed between TPC and TFC ($r = 0.99$), TPC and antioxidant activity DPPH ($r = 0.99$), TPC and FRAP ($r = 0.99$), TPC and ORAC ($r = 0.81$), TFC and antioxidant activity DPPH ($r = 0.98$), TFC and FRAP ($r = 0.99$), TFC and ORAC ($r = 0.77$), and between antioxidant activities DPPH and FRAP ($r = 0.99$), DPPH and ORAC ($r = 0.76$), and FRAP and ORAC ($r = 0.76$). When correlation analysis was performed on 28 yellow soybeans without the inclusion of black soybean data, both correlation significance and correlation coefficients were decreased. However, some significant linear correlations still existed between TPC and TFC ($r = 0.56$, $p < 0.0001$), TPC and ORAC ($r = 0.73$, $p < 0.0001$), and DPPH and FRAP

Table 5. Phenolic Acid Contents (μg/g) of Soybeans[a]

code	varieties/cultivars	individual benzoic acid and their derivates							subtotal benzoics
		GA	PA	TBA	HBA	VA	VN	SA	
#1	Prosoy	26.5 ± 1.4 o	ND[b]	4.5 ± 1.1 bcd	ND	4.6 ± 0.1 ef	ND	3.0 ± 0.2 cdef	40.3 ± 0.1 m
#2	Traill	45.5 ± 1.6 jih	ND	ND	ND	2.8 ± 0.1 ijk	12.0 ± 0.9 a	1.6 ± 0.1 hijk	61.9 ± 0.9 gh
#3	Norpro	33.2 ± 2.9 mn	ND	ND	4.8 ± 4.1 g	3.7 ± 0.2 gh	ND	ND	41.7 ± 7.2 mL
#4	Jim	51.7 ± 2.0 gih	ND	4.6 ± 0.8 cd	ND	0.6 ± 0.3 n	ND	0.4 ± 0.0 L	57.1 ± 2.6 hi
#5	Pembina	78.5 ± 0.0 a	ND	8.0 ± 2.1 a	ND	2.7 ± 0.4 ijk	ND	3.7 ± 0.4 cd	92.9 ± 2.9 a
#6	Barnes	66.6 ± 0.6 b	ND	5.6 ± 0.7 bc	ND	3.1 ± 0.5 hij	ND	4.9 ± 0.5 b	80.3 ± 0.8 b
#7	LaMoure	66.9 ± 2.1 b	ND	4.3 ± 0.2 cd	ND	3.7 ± 0.3 gh	ND	2.6 ± 0.2 defgh	77.7 ± 1.8 bcd
#8	ND01-3906	65.1 ± 5.6 b	ND	4.4 ± 1.1 bcd	ND	2.2 ± 0.2 kl	ND	3.2 ± 0.1 cde	74.9 ± 4.5 bcd
#9	Walsh	62.7 ± 4.7 bcd	ND	4.6 ± 0.1 bcd	ND	4.2 ± 0.5 fg	ND	1.5 ± 0.0 ijkl	72.9 ± 4.2 cde
#10	Prosoy	38.3 ± 3.2 mL	ND	ND	ND	6.5 ± 0.2 b	ND	2.1 ± 0.2 efghi	46.9 ± 3.1 kl
#11	Traill	56.9 ± 0.1 defg	ND	3.5 ± 1.7 defg	10.6 ± 0.3 c	2.6 ± 0.2 jk	ND	3.9 ± 0.3 c	77.5 ± 1.1 bcd
#12	Norpro	60.8 ± 0.8 bcde	ND	3.8 ± 0.5 ed	ND	ND	ND	1.3 ± 0.1 ijkl	65.9 ± 1.4 fg
#13	S0880	64.4 ± 2.7 bc	ND	3.7 ± 0.0 efd	ND	ND	ND	3.0 ± 0.9 cdef	71.1 ± 1.8 def
#14	91M10	63.6 ± 3.9 bc	ND	5.6 ± 0.4 bc	14.3 ± 0.3 b	3.5 ± 0.4 hi	ND	6.9 ± 0.3 a	93.9 ± 3.9 a
#15	Atwood	52.7 ± 2.7 gfh	ND	6.2 ± 0.7 b	17.4 ± 0.4 a	7.7 ± 0.2 a	ND	5.9 ± 0.8 b	89.8 ± 3.6 a
#16	Proto (2005)	46.7 ± 0.9 jih	ND	2.2 ± 0.1 efg	16.1 ± 0.8 ab	1.3 ± 0.1 m	ND	1.1 ± 0.8 ijkl	67.4 ± 1.0 efg
#17	Proto (2006)	44.5 ± 2.9 jk	ND	2.4 ± 0.0 efg	11.9 ± 0.7 c	1.3 ± 0.0 mn	ND	0.7 ± 0.1 jkl	60.9 ± 2.2 gh
#19	Tofooey	33.6 ± 0.4 mn	5.8 ± 0.2 b	ND	5.9 ± 0.3 fg	4.8 ± 0.4 ef	ND	0.7 ± 0.0 jkl	50.9 ± 1.4 ijk
#20	Korada	37.4 ± 0.9 mL	ND	ND	10.8 ± 0.0 c	6.3 ± 0.7 bc	ND	1.9 ± 0.3 fghi	56.5 ± 1.9 hi
#21	Vinton 81 (2005)	52.4 ± 3.9 gfh	ND	1.9 ± 0.2 fg	10.2 ± 0.8 cd	ND	ND	1.7 ± 0.1 ghijk	66.3 ± 4.5 efg
#22	Prosoy	34.5 ± 2.8 mln	ND	ND	9.5 ± 0.7 cde	6.2 ± 0.1 bc	ND	3.7 ± 1.2 cd	53.8 ± 0.9 ij
#23	5389	40.1 ± 3.7 lk	ND	1.8 ± 0.3 fg	7.7 ± 0.4 ef	1.9 ± 0.1 lm	ND	2.3 ± 0.6 efghi	53.7 ± 5.1 ij
#24	51C10	33.5 ± 0.2 mn	ND	ND	15.2 ± 0.9 ab	7.2 ± 0.7 a	ND	1.1 ± 0.6 ijkl	57.0 ± 0.3 hi
#25	90T60	38.3 ± 0.9 mL	13.6 ± 0.0 a	ND	5.5 ± 0.4 fg	ND	3.5 ± 0.2 b	1.9 ± 1.1 fghij	62.8 ± 2.6 gh
#26	L-star (2005)	50.0 ± 1.4 jih	ND	4.8 ± 0.2 bcd	4.2 ± 0.0 g	ND	ND	1.9 ± 0.4 fghij	60.9 ± 0.8 gh
#27	L-star (2006)	58.3 ± 4.1 cdef	ND	4.4 ± 0.2 cd	7.9 ± 0.5 def	5.2 ± 0.1 de	ND	2.3 ± 0.6 efghi	78.2 ± 2.9 bc
#28	2300	56.4 ± 5.2 efg	ND	3.6 ± 0.4 def	5.3 ± 0.5 fg	4.7 ± 0.3 ef	1.7 ± 0.1 c	0.6 ± 0.1 kl	72.3 ± 4.8 cdef
#29	IA2032	61.6 ± 3.1 bcde	ND	4.7 ± 0.1 bcd	ND	5.6 ± 0.4 cd	ND	2.9 ± 0.2 cdef	74.9 ± 2.8 bcd
#30	Black soybean (2005)	49.4 ± 0.9 jih	5.2 ± 0.6 b	4.9 ± 0.1 bcd	ND	ND	3.2 ± 0.2 b	3.6 ± 0.2 cd	66.3 ± 0.9 efg
#18	Black soybean (2006)	29.6 ± 1.8 on	5.4 ± 0.0 b	2.1 ± 0.0 efg	5.8 ± 0.2 fg	2.8 ± 0.0 ijk	ND	2.8 ± 0.6 cdefg	48.5 ± 1.5 jk

code	varieties/cultivars	individual cinnamic acid and their derivates							subtotal cinnamics	total phenolic acids
		CFA	CLA	PCA + SD	MCA + FA	SPA	OCA	TCA		
#1	Prosoy	69.2 ± 4.8 jk	444.5 ± 33.4 hij	12.6 ± 1.1 fg	ND	16.3 ± 0.4 ef	5.0 ± 0.1 cd	286.1 ± 24.2 cde	833.7 ± 64.0 hijk	873.9 ± 63.9 hi
#2	Traill	91.0 ± 4.2 defg	555.4 ± 41.0 de	13.0 ± 0.1 f	4.3 ± 0.3 b	7.3 ± 0.1 ij	4.6 ± 0.3 def	228.5 ± 14.4 hij	904.1 ± 60.3 def	965.9 ± 61.1 efg
#3	Norpro	91.8 ± 1.6 defg	449.3 ± 12.1 hi	16.6 ± 0.3 bc	ND	5.3 ± 0.8 j	2.9 ± 0.2 gh	237.5 ± 1.5 ghi	803.4 ± 10.8 ijkl	845.1 ± 3.6 ijk
#4	Jim	82.2 ± 3.7 fghi	394.7 ± 22.8 kl	12.8 ± 0.3 f	0.7 ± 0.5 fg	17.5 ± 0.5 de	3.5 ± 0.2 g	264.4 ± 13.3 efg	775.4 ± 38.7 klm	832.5 ± 36.1 ijk
#5	Pembina	98.9 ± 7.9 d	452.6 ± 13.6 hi	8.9 ± 0.6 ij	0.4 ± 0.1 fg	20.5 ± 2.8 cd	3.3 ± 0.4 gh	292.2 ± 10.9 cde	876.8 ± 34.1 fghi	969.7 ± 36.9 efg
#6	Barnes	95.3 ± 9.1 de	513.6 ± 11.1 fg	12.5 ± 0.5 fg	0.5 ± 0.1 fg	7.4 ± 0.2 ij	3.2 ± 0.5 gh	285.4 ± 0.6 cde	917.9 ± 1.4 def	998.1 ± 2.2 cde
#7	LaMoure	92.2 ± 3.1 def	361.6 ± 18.9 lm	18.1 ± 0.9 a	1.3 ± 0.1 ef	21.6 ± 2.4 b	0.8 ± 0.0 no	293.2 ± 16.3 cde	788.7 ± 36.9 jklm	866.4 ± 35.2 hij
#8	ND01-3906	109.2 ± 1.4 c	350.2 ± 16.2 m	13.7 ± 0.3 ef	1.8 ± 0.0 e	18.0 ± 2.5 de	0.8 ± 0.0 o	246.3 ± 1.7 fgh	739.9 ± 19.1 mno	814.8 ± 23.6 ijk
#9	Walsh	94.7 ± 2.0 de	345.7 ± 5.7 m	17.1 ± 1.4 abc	22.6 ± 0.9 a	11.1 ± 0.1 gh	28.5 ± 0.3 a	238.7 ± 5.7 gh	758.3 ± 0.8 lmn	831.2 ± 5.0 ijk
#10	Prosoy	108.1 ± 7.3 c	334.7 ± 1.9 mn	13.4 ± 0.1 ef	ND	ND	2.1 ± 0.0 jk	240.5 ± 5.1 fgh	698.7 ± 3.9 nop	745.6 ± 7.1 mL
#11	Traill	86.4 ± 1.5 efgh	421.6 ± 1.4 ijk	14.7 ± 1.1 de	0.2 ± 0.2 g	21.7 ± 2.1 c	3.1 ± 0.1 gh	291.5 ± 21.3 cde	839.2 ± 20.7 ghij	916.6 ± 21.8 gh
#12	Norpro	ND	575.4 ± 11.4 d	17.8 ± 1.4 ab	0.6 ± 0.1 fg	13.7 ± 2.1 fg	5.1 ± 0.3 c	308.2 ± 19.7 c	920.8 ± 12.1 def	986.7 ± 13.5 def
#13	S0880	ND	678.3 ± 1.9 bc	17.7 ± 0.1 ab	0.6 ± 0.2 fg	7.9 ± 0.1 ij	1.4 ± 0.1 lm	459.1 ± 28.5 a	1164.9 ± 26.3 a	1236.1 ± 28.1 a
#14	91M10	81.9 ± 5.3 fghi	452.9 ± 15.7 hi	16.9 ± 0.7 abc	2.7 ± 0.2 cd	29.8 ± 1.5 b	8.3 ± 0.1 b	394.6 ± 10.2 b	987.0 ± 19.6 bc	1080.9 ± 15.7 b
#15	Atwood	88.8 ± 3.8 defg	407.6 ± 1.7 jk	13.2 ± 0.8 f	2.1 ± 0.4 de	27.7 ± 0.4 b	4.6 ± 0.4 cde	283.6 ± 1.8 cde	827.6 ± 1.9 hijk	917.4 ± 1.7 gh
#16	Proto (2005)	81.5 ± 3.4 ghi	591.4 ± 18.2 d	10.0 ± 0.3 hi	ND	9.4 ± 0.2 hi	4.3 ± 0.1 ef	201.1 ± 12.7 jk	897.6 ± 9.2 efg	964.9 ± 10.2 efg
#17	Proto (2006)	91.1 ± 1.9 defg	659.1 ± 1.7 c	11.3 ± 0.2 gh	0.1 ± 0.1 g	5.8 ± 0.2 j	4.3 ± 0.2 ef	193.4 ± 9.8 k	965.1 ± 10.6 cd	1025.9 ± 12.8 bcde
#19	Tofooey	70.1 ± 6.1 jk	676.0 ± 0.2 bc	8.9 ± 0.2 ij	1.9 ± 0.7 de	ND	3.1 ± 0.2 gh	269.3 ± 14.8 def	1029.4 ± 21.0 b	1080.3 ± 22.4 b
#20	Korada	72.4 ± 3.2 ijk	767.9 ± 3.8 a	7.1 ± 0.1 kl	0.6 ± 0.4 fg	16.7 ± 1.1 ef	4.1 ± 0.4 f	293.4 ± 3.8 cde	1162.2 ± 2.7 a	1218.7 ± 4.6 a
#21	Vinton 81 (2005)	62.5 ± 4.3 k	429.5 ± 38.6 ijk	6.4 ± 0.1 mL	ND	21.9 ± 0.1 c	1.3 ± 0.0 lmn	283.4 ± 19.3 cde	804.9 ± 62.1 ijkl	871.2 ± 66.6 hi
#22	Prosoy	92.1 ± 1.9 def	282.2 ± 8.7 o	12.7 ± 0.3 f	ND	71.2 ± 0.1 a	2.3 ± 0.2 j	290.3 ± 21.1 cde	750.8 ± 14.7 lmno	804.6 ± 15.7 jkl
#23	5389	114.3 ± 6.5 c	305.2 ± 0.6 no	12.9 ± 0.2 f	ND	29.7 ± 0.9 b	1.7 ± 0.0 kl	226.0 ± 7.5 hij	689.8 ± 2.4 op	743.5 ± 7.4 mL
#24	51C10	90.9 ± 2.8 defg	522.0 ± 23.1 ef	6.5 ± 0.2 mL	ND	12.8 ± 0.2 fg	3.2 ± 0.2 gh	299.2 ± 10.3 cd	934.6 ± 16.6 cde	991.6 ± 16.5 cde
#25	90T60	126.8 ± 4.5 b	565.5 ± 11.0 d	8.3 ± 0.5 jk	3.0 ± 0.1 c	13.9 ± 0.9 fg	1.1 ± 0.3 mno	145.5 ± 7.8 L	864.3 ± 3.1 fghi	927.1 ± 5.6 fgh
#26	L-star (2005)	72.1 ± 3.8 ijk	507.1 ± 23.1 fg	7.7 ± 0.3 jkl	ND	13.7 ± 1.3 fg	1.1 ± 0.1 mno	131.2 ± 1.1 L	732.9 ± 21.4 mno	793.8 ± 20.6 klm
#27	L-star (2006)	76.9 ± 0.3 hij	641.3 ± 3.5 c	10.1 ± 0.4 hi	ND	23.3 ± 2.9 c	2.9 ± 0.3 hi	209.7 ± 8.1 ijk	964.1 ± 9.6 cd	1042.3 ± 12.5 bcd
#28	2300	68.5 ± 1.2 jk	321.6 ± 5.3 mn	7.1 ± 0.4 kl	ND	20.7 ± 1.3 cd	1.9 ± 0.2 jk	241.4 ± 9.0 fgh	661.2 ± 17.4 p	733.4 ± 22.2 m
#29	IA2032	65.8 ± 3.4 k	587.3 ± 12.7 d	5.6 ± 0.2 m	ND	22.3 ± 0.2 c	2.4 ± 0.2 ij	249.1 ± 4.9 fgh	932.5 ± 20.8 cde	1007.4 ± 23.6 cde
#30	Black soybean (2005)	138.3 ± 2.8 a	700.9 ± 2.8 b	15.8 ± 0.6 cd	1.8 ± 0.2 e	ND	2.3 ± 0.0 j	129.8 ± 4.0 L	988.9 ± 10.3 bc	1055.2 ± 11.2 bc
#18	Black soybean (2006)	67.7 ± 6.0 jk	480.9 ± 37.2 gh	9.8 ± 0.9 i	ND	ND	4.28 ± 0.01ef	199.6 ± 1.5 jk	762.4 ± 40.7 lm	810.9 ± 42.2 ijk

[a] Data are expressed as means ± standard deviations ($n = 3$) on a dry weight basis. Values marked by the same letter within column are not significantly different ($p < 0.05$). Phenolic acids: PCA + SD, *p*-coumaric acid + syringaldehyde; and MCA + FA, *m*-coumaric acid + ferulic acid. [b] ND, not detectable.

($r = 0.51$, $p < 0.0001$). These correlation results indicated that antioxidant assay methods were well-correlated and meanwhile indicated that TPCs correlated with ORAC of yellow soybeans.

Free Phenolic Acid Contents. The free phenolic acid contents of soybeans are presented in **Table 5**. Among the seven benzoic phenolic acids detected, GA was the highest in all tested soybean cultivars with contents ranging from 26.5 to 78.5 μg/

g. VA, TBA, and SA were detected in most soybean cultivars with contents less than 10 μg/g. *p*-Hydroxybenzoic acid (HBA) was detected in half of all soybean cultivars with the content ranging from 4.2−17.4 μg/g, while each of the PA and VN was detected in low contents in only four cultivars.

Because of instrumental limitation of current HPLC and performance of the column, PCA and SD as well as MCA and FA did not get 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 the integrated peak area contributed by these two compounds against concentration (PCA + SD in 1:1 mass ratio). MCA and FA were estimated as one compound (MCA + FA) in a similar manner. Among the seven cinnamic phenolic acids detected, CLA, PCA + SD, OCA, and TCA existed in all soybean cultivars with the CLA content ranging from 282.2 μg/g in cultivar Prosoy (Durbin, ND) to 767.9 μg/g in cultivar Korada (2006, Proser, ND), PCA + SD ranging from 5.6 in cultivar IA 2032 to 18.1 μg/g in cultivar LaMoure, OCA ranging from 0.8 in cultivar LaMoure to 28.5 μg/g in cultivar Walsh, and TCA ranging from 129.8 μg/g in black soybean (2005) to 459.1 μg/g in cultivar S0880 (Casselton, ND), respectively. CFA, MCA + FA, and SPA were detected in most soybean cultivars with contents ranging from 62.5 to 138.3, from 0.1 to 22.6, and from 5.8 to 71.2 μg/g, respectively. The total free phenolic acid content ranged from 733.4 μg/g in cultivar 2300 (Proser, ND) to 1236.1 μg/g in cultivar S0880 (Casselton, ND). CLA, TCA, CFA, and GA were the predominant phenolic acids in soybeans. Total free phenolic acids in soybeans were mostly contributed by the cinnamic type of phenolic acid, such as CLA and TCA.

Significant differences ($p < 0.05$) in individual phenolic acid, subtotal phenolic acids, and total free phenolic acids were found between yellow soybeans and black soybeans and among some cultivars of yellow soybeans. The total free phenolic acid content and subtotal cinnamic acid content of yellow soybean cultivar S0880 and Korada (2006, Proser, ND) were significantly ($p < 0.05$) higher than that of other soybean cultivars. Significant differences in GA, CFA, CLA, and total phenolic acid contents existed among the three Prosoy samples grown in three different locations. Significant differences in total phenolic acid content existed between two the samples of Traill, as well as the two samples of Norpro. In addition, significant differences in total phenolic acid content were found in the samples from the same cultivars grown in two continued cropping years for yellow soybean Proto, L-star, and black soybeans.

To the best of our knowledge, investigations on individual phenolic acid composition in dry soybean seeds were absent in the recent two decades. There were only a few reports on phenolic acids in soybean in the 1970s and 1980s. Using gas−liquid chromatography separation technique, Maga and Lorenz (*17*) identified FA, SA, and VA as the major phenolic acids in soybean flours. Dabrowski and Sosulski (*18*) found HBA, CFA, PCA, FA, and SA in soybean flours and demonstrated that SA is the major phenolic acid present. Meanwhile, using HPLC, How and Morr (*19*) tentatively identified eight phenolic acids as gentistic, VA, CLA, SA, OCA, PCA, FA, and salicylic acids. The compounds OCA, PCA, and FA were predominant. However, because of the differences in extraction and pretreatment methods, the free phenolic acid profiles of current investigated Northern U.S.-grown soybeans are different from the soybeans reported in the literature (*18*), in which alkaline hydrolysis treatments were used in their study. These pretreatments caused the hydrolysis of CLA and the loss of the constituent aglycone, CFA (*18*). That was why CLA, CFA, and TCA were the predominant phenolic acids in the Northern grown soybeans, but they had not been detected by Dabrowski and Sosulski (*18*). In addition, the average content (931.7 μg/g) of the total phenolic acid in the current 30 soybean cultivars was higher than that (736 μg/g) reported by Dabrowski and Sosulski in 10 oilseed samples (*18*). The differences between our results and previous reports may be partially due to the sources of the samples.

Flavonol and Anthocyanin Contents. The flavonol and anthocyanin contents of the 30 soybeans are presented in **Table 6**. (+)-Catechin, one type of flavonol compound, was identified during HPLC phenolic acid analysis. Therefore, the quantification of (+)-catechin was simultaneously performed during phenolic acid quantification. (+)-Catechin contents in all soybean cultivars ranged from 44.35 to 64.08 μg/g. There were no significant differences among most soybean cultivars.

One yellow soybean cultivar Prosoy and two black soybeans were selected for anthocyanin quantification. There were no detectable anthocyanins in the selected Prosoy soybean. Therefore, other yellow soybeans were not analyzed for anthocyanins. Two anthocyanins (cyanidin-3-glucoside and peonidin-3-glucoside) were detected in black soybeans, and the dominant component was cyanidin-3-glucoside. These findings are in accordance with that of Yoshida et al. (*14*) who found that cyanidin-3-glucoside is the major anthocyanin in black soybeans.

Isoflavone Contents. Although several investigations had been performed on isoflavone profiles of U.S. soybean cultivars grown in different geographical zones, such as Ohio (*2, 20–22*), Iowa (*23, 24*), and South Dakota (*25*), there were no systematic investigations on the isoflavone profiles of the North Dakota−Minnesota soybeans to date. North Dakota is one of the highest latitude geographical zones in the United States for growing warm season legume soybeans. On the basis of the theory that stress environments induce the generation of the secondary metabolites (phytochemicals) in plants, we proposed a hypothesis that Northern grown (such as North Dakota and northern Minnesota) soybeans in relatively low temperature environments may have higher total isoflavone (TI) contents than those of soybeans grown in the warmer areas. Therefore, the isoflavone contents of 22 North Dakota soybeans and five Minnesota soybeans, as well as two Colorado soybeans and one Iowa soybean as references, were investigated and are presented in **Tables 7** and **8**. The results are presented in three ways. (i) Individual isoflavone contents (**Table 7**) were directly measured from HPLC chromatograms for all 12 forms. (ii) Subtotal isoflavone contents of aglycones (aglycone equivalents) for each of the three types of isoflavones (**Table 7**) were calculated by converting the malonylglucosides, acetylglucosides, and 7-*O*-*β*-glucosides weight into the aglycone weight using the respective molecular weight factors prior to summation. TI contents (**Table 7**) were the sum of the adjusted sums of total genistein + total daidzein + total glycitein according to Murphy et al. (*12*). Therefore, the TI values were not the simple addition of the mean individual values. (iii) The percentage content of subtotal individual aglycones (**Table 8**) was obtained by dividing subtotal individual aglycones contents by TI.

Among the 12 individual isoflavone compounds, 10 isoflavones including three 7-*O*-glucosides (daidzin, glycitin, and genistin), three 6″-*O*-malonyl-*β*-glucosides (malonyldaidzin, malonylglycitin, and malonylgenistin), two 6″-*O*-acetyl-*β*-glucosides (acetyldaidzin and acetylglycitin), and two aglycones (daidzein and genistein) were detected in all tested soybean

Table 6. Flavonol and Anthocyanin Contents (μg/g) of Soybeans[a]

		flavonols	anthocyanins	
code	varieties/cultivars	(+)-catechin	cyanidin-3-glucose	peonidin-3-glucose
#1	Prosoy	53.84 ± 2.4 bcdef	ND[b]	ND
#2	Traill	59.23 ± 1.7 ab	NS[c]	NS
#3	Norpro	53.68 ± 1.5 bcdefg	NS	NS
#4	Jim	56.00 ± 4.7 bcde	NS	NS
#5	Pembina	56.14 ± 4.4 bcde	NS	NS
#6	Barnes	54.84 ± 3.1 bcdef	NS	NS
#7	LaMoure	53.06 ± 5.3 cdefg	NS	NS
#8	ND01-3906	57.69 ± 3.2 bcd	NS	NS
#9	Walsh	51.91 ± 0.9 defgh	NS	NS
#10	Prosoy	64.08 ± 1.4 a	NS	NS
#11	Traill	51.56 ± 0.3 defghi	NS	NS
#12	Norpro	51.44 ± 1.1 defghi	NS	NS
#13	S0880	46.68 ± 2.2 hijk	NS	NS
#14	91M10	52.99 ± 1.7 cdefg	NS	NS
#15	Atwood	54.71 ± 1.0 bcdef	NS	NS
#16	Proto (2005)	44.35 ± 3.2 k	NS	NS
#17	Proto (2006)	49.12 ± 1.7 fghijk	NS	NS
#19	Tofooey	52.89 ± 1.8 cdefgh	NS	NS
#20	Korada	50.61 ± 0.4 efghij	NS	NS
#21	Vinton 81 (2005)	46.68 ± 2.5 hijk	NS	NS
#22	Prosoy	45.58 ± 4.5 ijk	NS	NS
#23	5389	51.00 ± 0.5 efghij	NS	NS
#24	51C10	51.54 ± 0.1 defghi	NS	NS
#25	90T60	52.89 ± 4.3 cdefgh	NS	NS
#26	L-star (2005)	44.88 ± 1.4 jk	NS	NS
#27	L-star (2006)	48.74 ± 3.4 fghijk	NS	NS
#28	2300	50.84 ± 1.3 efghijk	NS	NS
#29	IA2032	45.56 ± 0.8 ijk	NS	NS
#30	Black soybean (2005)	58.29 ± 0.2 bc	43.62 ± 0.5 a	6.44 ± 0.6
#18	Black soybean (2006)	47.47 ± 2.5 ghijk	26.34 ± 0.1 b	ND

[a] Data are expressed as means ± standard deviations ($n = 3$) on a dry weight basis. Values marked by the same letter within each column are not significantly different ($p < 0.05$). [b] ND, not detectable. [c] NS, not sampled.

cultivars. While acetylgenistin was detected in most soybean cultivars, glycitein was detected in only three cultivars. Most isoflavones existed as glucosides. The highest proportion at more than 75% of the total was 6″-*O*-malonyl-β-glucosides (sum of the malonyldaidzin, malonylgenistin and malonylglycitin contents), followed by 7-*O*-β-glucosides at 20% (sum of the daidzin, genistin, and glycitin contents), whereas 6″-*O*-acetyl-β-glucosides and aglycones existed in only very small proportions, which were consistent with the literature reports (*2, 20, 23*).

Similar to the literature reports (*2, 25*), the subtotal individual genistein content was the highest (average 1283.8 μg/g, 69.2% of TI), followed by subtotal daidzein (average 456.1 μg/g, 24.6% of TI) and subtotal glycitein (116.3 μg/g, 6.3% of TI). Cultivar S0880 contained the highest contents of malonyldaidzin (1152.7 μg/g), acetylgenistin (42.7 μg/g), acetylglycitin (126.9 μg/g), subtotal individual daidzein (888.3 μg/g), subtotal individual genistein (1812.3 μg/g), and TI (2862.7 μg/g) among all tested cultivars. Cultivar 91M10 had the highest content of daidzin (531.9 μg/g), genistin (571.8 μg/g), and daidzein (31.6 μg/g). Cultivar 51C10 had the highest content of malonylgenistin (2924.5 μg/g). Cultivar Jim had the highest content of malonylglycitin (158.5 μg/g) and subtotal individual glycitein (185.8 μg/g). Cultivar 90T60 exhibited the lowest content daidzin (71.0 μg/g), malonylgenistin (1405.4 μg/g), subtotal individual genistein (818.5 μg/g), and TI (1181.9 μg/g). Among all 30 soybean materials tested, S0880, 51C10, Jim, Korada, Traill, and Pembina were the top six subtotal genistein-containing cultivars.

The TI content ranged from 1181.9 μg/g in cultivar 90T60 to 2862.7 μg/g in cultivar S0880 (**Table 7**), which was in the range (1116−2743 μg/g) of the South Dakota soybeans (*25*). Among all soybean samples, 30% of all cultivars (nine in total 30) exhibited high TI content in the range of 2000−3000 μg/g, 53.3% (16 in total 30) exhibited medium TI content in the range of 1500−2000 μg/g, while 16.6% (5 in total 30) exhibited low TI content in the range of 1000−1500 μg/g. The average TI value (1856.2 μg/g) of all 30 soybeans and the average value (1956.6 μg/g) of 22 North Dakota cultivars were close to the average value (1978.5 μg/g) of 210 South Dakota cultivars (*25*) but higher than the average value (1280 μg/g) of 22 U.S. food quality soybeans (*26*), the average value (1530 μg/g) of 14 U.S. commodity grade soybeans (*26*), the average value (1450 μg/g) of 18 Korean soybeans, and the average value (1180 μg/g) of seven Japanese soybeans (*23, 26*). These results were consistent with our hypothesis that soybeans grown in the Northern region might yield higher TI contents than the soybeans grown in the warmer areas. Literature reports had shown that low temperatures during seed development resulted in higher isoflavone contents (*27–29*).

From the genetic point of view, the ratios of 6″-*O*-malonyldaizin to daidzin (MDin/Din) and of 6″-*O*-malonylgenistin to genistin (MGin/Gin) in soybeans may be the characteristics of different genotypes (*23, 30, 31*). Therefore, the ratios of MDin/Din and MGin/Gin of current 30 soybeans were evaluated (**Table 8**). The ratios of MDin/Din for 28 yellow soybeans ranged from 1.3 in cultivar 91M10 to 6.9 in cultivar Tofooey. The ratios of MGin/Gin for 28 yellow soybeans ranged from 3.4 in cultivar L-star to 15.0 in cultivar 5389. The overall average ratios of 3.1 for MDin/Din and 6.7 for MGin/Gin were comparable to some Japanese varieties grown in Iowa, with ratios of 3−6, but were in marked contrast to some American varieties grown in Iowa, with ratios of 1−3 (*23*). However, Vinton 81 (2005) from Minnesota in our current investigation exhibited much higher ratios (2.7 and 6.2, respectively) than that (1.0 and 1.7, respectively) of Vinton 81 (1991) from Iowa

Table 7. Isoflavones Contents (μg/g) of Soybeans[a]

		7-*O*-β-glucosides			malonylglucosides			acetylglucosides		
code	varieties/cultivars	Din	Gin	Gly	MDin	MGin	MGly	ADin	AGin	AGly
#1	Prosoy	162.1 ± 6.2	232.9 ± 3.0	48.9 ± 1.8	654.4 ± 10.5	1938.5 ± 22.2	80.7 ± 1.6	85.2 ± 3.2	7.7 ± 1.2	17.1 ± 0.1
#2	Traill	97.5 ± 2.3	185.1 ± 7.7	28.3 ± 0.9	295.1 ± 11.6	1695.4 ± 15.7	109.7 ± 4.0	3.9 ± 0.3	7.9 ± 0.5	44.1 ± 2.1
#3	Norpro	155.5 ± 9.9	238.9 ± 13.6	35.5 ± 2.4	650.9 ± 13.7	2001.4 ± 30.6	122.5 ± 1.7	7.9 ± 0.1	8.8 ± 1.2	78.9 ± 4.6
#4	Jim	272.2 ± 26.8	478.8 ± 16.7	59.6 ± 5.6	745.9 ± 9.1	2618.6 ± 29.9	158.5 ± 9.1	15.6 ± 0.9	9.7 ± 0.0	108.2 ± 5.2
#5	Pembina	371.1 ± 34.9	538.6 ± 7.5	39.2 ± 3.4	648.2 ± 29.6	2248.2 ± 113.4	140.7 ± 8.4	10.2 ± 0.9	18.8 ± 1.5	94.6 ± 3.9
#6	Barnes	108.4 ± 8.9	238.1 ± 17.7	25.5 ± 1.1	385.9 ± 11.4	1591.8 ± 119.9	77.3 ± 6.6	70.9 ± 2.9	18.5 ± 2.5	15.3 ± 1.4
#7	LaMoure	135.9 ± 12.2	235.3 ± 25.5	41.6 ± 1.1	489.8 ± 6.2	1581.8 ± 52.4	101.7 ± 2.3	3.7 ± 0.4	30.9 ± 0.6	19.9 ± 1.5
#8	ND01−3906	214.7 ± 7.2	348.4 ± 21.6	54.1 ± 1.4	542.1 ± 21.0	2229.6 ± 3.0	85.1 ± 4.9	10.2 ± 0.2	1.3 ± 0.1	94.9 ± 0.8
#9	Walsh	148.6 ± 7.6	286.5 ± 3.7	48.1 ± 3.3	445.6 ± 27.0	2106.8 ± 203.7	89.9 ± 9.0	78.1 ± 10.9	0.9 ± 0.9	20.3 ± 3.5
#10	Prosoy	123.3 ± 4.6	226.4 ± 16.0	29.5 ± 1.5	640.7 ± 4.8	2061.9 ± 49.6	91.2 ± 5.5	29.6 ± 0.0	21.5 ± 1.7	116.4 ± 1.9
#11	Traill	262.1 ± 10.8	505.6 ± 6.5	57.9 ± 1.2	754.9 ± 10.8	2537.7 ± 108.0	78.5 ± 2.1	20.4 ± 1.2	9.6 ± 0.3	110.5 ± 1.7
#12	Norpro	313.2 ± 8.0	445.7 ± 15.2	47.2 ± 2.5	770.3 ± 22.9	2238.3 ± 62.1	76.3 ± 0.8	10.4 ± 0.5	37.0 ± 0.9	103.6 ± 2.1
#13	S0880	468.1 ± 2.4	539.3 ± 14.5	57.3 ± 1.5	1152.7 ± 44.5	2739.2 ± 205.8	96.5 ± 8.9	6.0 ± 0.2	42.7 ± 0.0	126.9 ± 10.4
#14	91M10	531.9 ± 6.5	571.8 ± 44.3	86.8 ± 6.4	710.2 ± 36.4	2015.0 ± 97.5	91.6 ± 6.2	20.6 ± 2.5	8.8 ± 0.2	111.8 ± 4.2
#15	Atwood	247.3 ± 3.9	361.7 ± 10.5	43.0 ± 3.7	640.3 ± 36.1	1907.4 ± 108.9	58.8 ± 4.3	5.4 ± 0.6	6.9 ± 0.1	82.0 ± 6.4
#16	Proto (2005)	190.6 ± 16.1	266.1 ± 3.7	81.9 ± 5.1	563.4 ± 11.9	1740.5 ± 31.9	86.6 ± 1.8	78.2 ± 0.7	4.3 ± 1.1	23.3 ± 1.8
#17	Proto (2006)	109.2 ± 8.5	186.4 ± 7.0	80.5 ± 8.1	547.8 ± 37.6	1763.4 ± 2.3	89.9 ± 3.4	76.8 ± 2.7	4.5 ± 3.6	21.1 ± 2.1
#19	Tofooey	140.9 ± 10.4	254.7 ± 7.6	60.0 ± 4.4	976.2 ± 64.6	2498.3 ± 72.2	54.4 ± 1.9	107.9 ± 7.1	ND	59.9 ± 0.1
#20	Korada	207.9 ± 5.9	344.2 ± 24.7	59.1 ± 1.5	832.9 ± 42.4	2786.9 ± 198.7	67.0 ± 6.7	11.6 ± 0.4	ND	134.7 ± 6.0
#21	Vinton 81 (2005)	226.3 ± 17.2	274.0 ± 20.0	38.5 ± 2.2	592.8 ± 39.8	1694.7 ± 78.3	50.1 ± 3.8	11.6 ± 1.1	14.6 ± 1.4	13.8 ± 0.5
#22	Prosoy	142.8 ± 1.4	306.3 ± 2.6	45.4 ± 0.0	913.9 ± 25.7	2161.0 ± 80.3	39.2 ± 2.2	86.8 ± 1.6	ND	20.5 ± 0.7
#23	5389	79.5 ± 7.5	130.8 ± 4.8	41.2 ± 2.5	288.2 ± 34.3	1963.0 ± 111.6	42.9 ± 1.2	55.4 ± 1.6	6.1 ± 0.6	67.9 ± 6.2
#24	51C10	189.4 ± 0.2	270.9 ± 6.4	63.7 ± 2.0	980.8 ± 29.9	2924.5 ± 113.0	101.5 ± 5.8	20.7 ± 0.4	24.6 ± 1.7	126.9 ± 0.0
#25	90T60	71.0 ± 4.9	117.4 ± 10.9	60.2 ± 2.3	396.9 ± 12.8	1405.5 ± 65.6	49.8 ± 3.6	65.0 ± 0.2	ND	19.3 ± 1.0
#26	L-star (2005)	216.6 ± 7.8	548.9 ± 12.3	10.2 ± 1.5	361.0 ± 12.4	1874.7 ± 114.3	11.3 ± 1.7	4.9 ± 0.1	26.0 ± 0.2	87.9 ± 2.3
#27	L-star (2006)	168.9 ± 12.1	240.2 ± 18.4	47.9 ± 0.7	549.2 ± 10.2	1684.2 ± 88.5	53.4 ± 4.9	3.1 ± 0.1	9.1 ± 0.2	77.1 ± 2.2
#28	2300	187.9 ± 8.8	309.8 ± 19.2	40.8 ± 4.0	551.8 ± 47.7	1884.4 ± 91.1	79.5 ± 7.9	19.4 ± 1.1	16.2 ± 0.6	111.2 ± 5.3
#29	IA2032	260.7 ± 13.2	333.3 ± 14.3	58.1 ± 4.7	712.1 ± 31.1	1980.5 ± 20.6	87.5 ± 3.0	88.3 ± 3.2	32.8 ± 1.7	34.7 ± 1.6
#30	2005 Black soybean	84.4 ± 2.8	116.9 ± 1.5	82.8 ± 4.0	331.5 ± 6.6	2115.6 ± 53.3	159.6 ± 3.6	37.8 ± 3.2	1.8 ± 0.2	10.7 ± 0.5
#18	2006 Black soybean	117.2 ± 3.4	125.9 ± 5.7	89.1 ± 2.52	628.8 ± 7.8	2129.3 ± 86.4	154.1 ± 2.3	101.9 ± 6.8	ND	22.5 ± 0.5
	average	195.1 ± 103.7	304.7 ± 130.9	50.6 ± 18.0	602.1 ± 204.8	2041.9 ± 370.8	86.5 ± 34.7	38.9 ± 35.0	12.5 ± 11.3	63.7 ± 42.4

		aglycones			subtotal individuals[b]			total[c]
code	varieties/cultivars	Dein	Gein	Glein	T-Dein	T-Gein	T-Glein	isoflavones
#1	Prosoy	7.2 ± 0.2	14.2 ± 0.5	9.0 ± 0.1	484.5 ± 0.5 h	1174.6 ± 12.2 ijkl	93.4 ± 2.1 i	1752.4 ± 9.7 gh
#2	Traill	11.7 ± 0.6	25.8 ± 1.3	ND	222.7 ± 5.7 q	1010.8 ± 7.1 n	102.3 ± 1.9 h	1335.9 ± 13.7 m
#3	Norpro	7.6 ± 0.2	12.9 ± 0.6	ND	436.3 ± 13.2 ij	1210.4 ± 24.4 hij	134.1 ± 5.1 f	1780.8 ± 42.7 fgh
#4	Jim	9.3 ± 0.2	25.1 ± 0.7	ND	561.6 ± 13.9 fg	1694.9 ± 15.8 b	185.8 ± 4.5 a	2442.3 ± 17.4 bc
#5	Pembina	27.0 ± 3.2	54.5 ± 2.8	ND	587.2 ± 35.9 ef	1573.7 ± 61.4 c	155.4 ± 4.4 cd	2316.3 ± 84.9 cd
#6	Barnes	8.0 ± 0.5	15.9 ± 0.4	ND	308.8 ± 8.5 op	1005.0 ± 59.9 n	66.5 ± 3.6 k	1380.3 ± 64.6 m
#7	LaMoure	11.3 ± 0.5	29.3 ± 2.5	ND	346.7 ± 4.9 mn	1019.2 ± 39.1 n	92.5 ± 2.9 i	1458.4 ± 46.6 klm
#8	ND01-3906	12.2 ± 0.7	26.3 ± 0.0	ND	405.1 ± 26.7 ljk	1415.2 ± 17.9 de	135.2 ± 2.8 f	1995.6 ± 42.6 e
#9	Walsh	10.1 ± 0.3	24.7 ± 0.9	ND	371.5 ± 9.8 lm	1302.7 ± 73.4 fg	91.8 ± 4.4 i	1765.9 ± 72.6 gh
#10	Prosoy	7.6 ± 0.1	20.7 ± 0.2	ND	423.4 ± 0.3 ijk	1249.1 ± 36.6 ghi	135.4 ± 2.8 f	1807.9 ± 39.1 fg
#11	Traill	11.3 ± 0.1	29.0 ± 0.6	ND	564.6 ± 11.3 fg	1673.2 ± 61.1 b	143.3 ± 2.9 e	2381.1 ± 75.3 c
#12	Norpro	18.2 ± 1.6	28.9 ± 1.5	ND	604.9 ± 14.6 de	1495.3 ± 39.9 cd	131.3 ± 3.3 f	2231.5 ± 51.2 d
#13	S0880	15.9 ± 1.0	23.1 ± 0.8	ND	888.3 ± 19.9 a	1812.3 ± 115.5 a	162.1 ± 11.8 bc	2862.7 ± 147.2 a
#14	91M10	31.6 ± 1.3	47.9 ± 3.4	ND	727.1 ± 14.6 b	1460.7 ± 81.8 d	169.5 ± 4.9 b	2357.2 ± 101.3 cd
#15	Atwood	11.7 ± 0.7	18.5 ± 0.3	9.5 ± 0.7	544.3 ± 16.9 g	1260.2 ± 0.7 fghi	100.9 ± 3.6 h	1905.5 ± 21.2 f
#16	Proto (2005)	10.5 ± 0.2	19.5 ± 0.0	ND	455.3 ± 16.1 hi	1095.5 ± 19.6 klmn	112.1 ± 1.3 g	1662.9 ± 34.4 hij
#17	Proto (2006)	8.8 ± 0.3	15.7 ± 0.7	ND	395.3 ± 12.1 kl	1053.9 ± 2.9 mn	111.7 ± 2.1 g	1560.9 ± 17.1 jkl
#19	Tofooey	9.5 ± 0.5	15.3 ± 0.0	ND	649.3 ± 42.4 c	1476.6 ± 42.4 d	102.3 ± 1.7 h	2228.3 ± 83.1 d
#20	Korada	10.2 ± 0.7	20.5 ± 0.9	ND	565.1 ± 25.9 fg	1688.2 ± 119.9 b	152.0 ± 8.1 d	2405.3 ± 153.9 bc
#21	Vinton 81 (2005)	8.6 ± 0.1	15.6 ± 1.5	ND	457.1 ± 10.3 hi	1079.2 ± 52.8 lmn	59.9 ± 2.9 k	1590.3 ± 57.4 ij
#22	Prosoy	6.4 ± 0.1	13.1 ± 0.7	ND	604.1 ± 14.8 de	1330.9 ± 42.8 f	61.9 ± 0.8 k	1996.9 ± 56.8 e
#23	5389	5.9 ± 0.6	15.0 ± 0.9	ND	229.9 ± 19.9 q	1128.5 ± 51.2 jklm	89.0 ± 4.1 i	1447.4 ± 66.4 lm
#24	51C10	5.9 ± 0.1	17.4 ± 0.2	ND	629.4 ± 14.6 cd	1725.1 ± 62.2 ab	168.9 ± 4.3 b	2523.4 ± 81.2 b
#25	90T60	6.9 ± 0.4	12.6 ± 0.6	ND	287.2 ± 3.2 p	818.5 ± 40.4 o	76.3 ± 4.0 j	1181.9 ± 39.6 n
#26	L-star (2005)	9.9 ± 0.3	23.7 ± 1.7	ND	327.5 ± 10.7 no	1358.8 ± 50.3 ef	60.3 ± 3.2 k	1746.6 ± 64.2 gh
#27	L-star (2006)	8.6 ± 0.2	16.6 ± 0.7	ND	391.4 ± 12.8 kl	1049.8 ± 16.8 mn	104.0 ± 3.5 h	1545.1 ± 74.7 jkl
#28	2300	6.7 ± 1.0	14.4 ± 1.6	ND	410.3 ± 28.1 jk	1188.1 ± 47.2 jih	133.7 ± 3.4 h	1732.0 ± 75.8 ghi
#29	IA2032	14.6 ± 2.2	31.1 ± 2.2	ND	582.9 ± 23.4 ef	1290.4 ± 18.4 gh	104.2 ± 3.7	1977.6 ± 45.5 e
#30	2005 Black soybean	7.1 ± 0.0	11.4 ± 0.1	7.2 ± 0.0	247.3 ± 0.1 q	1188.3 ± 28.9 jih	151.5 ± 0.3	1587.1 ± 28.5 jk
#18	2006 Black soybean	8.1 ± 0.4	14.8 ± 0.2	ND	454.3 ± 10.2 hi	1203.3 ± 48.4 hij	152.2 ± 3.1 d	1809.8 ± 61.7 fg
	average (N = 30)	10.7 ± 5.7	21.9 ± 10.1	0.7 ± 2.4	456.1 ± 150.0	1283.8 ± 247.9	116.3 ± 35.9	1856.2 ± 399.6

[a] Data are expressed as means ± standard deviations (n = 3) on a dry weight basis. Values marked by the same letter within column are not significantly different (p < 0.05). Din, daidzin; Gin, genistin; Gly, glycitin; MDin, malonyldaidzin; MGin, malonylgenistin; MGly, malonylglycitin, ADin, acetyldaidzin; AGin, acetylgenistin; AGly, acetylglycitin; Dein, daidzein; Gein, genistein; Glein, glycitein. T-Dein, subtotal daidzein; T-Gein, subtotal genistein; and T-Glein, subtotal glycitein. [b] Subtotal individuals = moles of isoflavone × molecular weight of aglycone form isoflavone. [c] TIs = sum of subtotal individuals of aglycones.

as reported in the literature (*23*). Therefore, the location of production had an impact on the ratios of malonyl forms to glucoside forms of isoflavones. These results were incompatible with the viewpoint (*23*) that genetics played a significant role in soy isoflavone distribution. However, surprisingly, the ratios of two black soybean cultivars exhibited much higher ratios of

Table 8. Normalized Isoflavone Content and Ratios of Malonylglucosides to 7-*O*-β-Glucosides of Soybeans

code	varieties/Cultivars	TI (μg/g)	total daidzein (%)[a]	total genistein (%)[a]	total glycitein (%)[a]	malonyldaidzin/daidzin[b]	malonylgenistin/genistin[b]
#1	Prosoy	1752.4	27.6	67.0	5.3	4.0	8.3
#2	Traill	1335.9	16.7	75.7	7.7	3.0	8.9
#3	Norpro	1780.8	24.5	67.9	7.5	4.2	8.4
#4	Jim	2442.3	23.0	69.4	7.6	2.7	5.5
#5	Pembina	2316.3	25.5	67.9	6.7	1.8	4.2
#6	Barnes	1380.3	22.4	72.8	4.8	3.6	6.7
#7	LaMoure	1458.4	23.8	69.9	6.3	3.5	6.7
#8	ND01-3906	1995.6	20.7	72.4	6.9	2.9	6.4
#9	Walsh	1765.9	21.6	73.1	5.4	3.0	7.1
#10	Prosoy	1807.9	23.4	69.1	7.5	5.2	9.1
#11	Traill	2381.1	23.7	70.3	6.0	2.9	5.0
#12	Norpro	2231.5	27.1	67.0	5.9	2.5	5.0
#13	S0880	2862.7	31.0	63.3	5.7	2.5	5.1
#14	91M10	2357.2	30.8	61.9	7.2	1.3	3.5
#15	Atwood	1905.5	28.6	66.1	5.3	2.8	5.6
#16	Proto (2005)	1662.9	27.4	65.9	6.7	2.9	6.5
#17	Proto (2006)	1560.9	25.3	67.5	7.2	5.0	9.5
#19	Tofooey	2228.3	29.1	66.3	4.6	6.9	9.8
#20	Korada	2405.3	23.5	70.2	6.3	4.0	8.1
#21	Vinton 81 (2005)	1590.3	28.6	67.7	3.7	2.7	6.2
#22	Prosoy	1996.9	30.3	66.6	3.1	6.4	7.1
#23	5389	1447.4	15.9	77.9	6.2	3.6	15.0
#24	51C10	2523.4	24.9	68.4	6.7	5.2	10.8
#25	90T60	1181.9	24.3	69.3	6.5	5.6	11.9
#26	L-star (2005)	1746.6	18.8	77.8	3.5	1.7	3.4
#27	L-star (2006)	1545.1	25.3	67.9	6.7	3.3	7.0
#28	2300	1732.0	23.6	68.7	7.7	2.9	6.1
#29	IA2032	1977.6	29.5	65.3	5.3	2.7	5.9
#30	2005 Black soybean	1587.1	15.6	74.9	9.5	3.9	18.1
#18	2006 Black soybean	1809.8	25.1	66.5	8.4	5.4	16.9
	average ($N = 30$)	1856.2	24.6	69.2	6.3	3.1	6.7

[a] The percentage content of subtotal individual aglycones was normalized by dividing the subtotal individual aglycones content by the TI content. [b] The ratios equal the malonylglucoside content divided by the 7-*O*-β-glucoside content.

MGin/Gin than all yellow soybean cultivars (**Table 8**). The exact reasons remain unclear.

The influences of biological and environmental factors, such as genotypes, crop years, cropping locations, soil nutrition, storage period, and genotype × environment interactions on isoflavone content of soybeans had been observed previously (*2, 20–24*). Significant differences ($p < 0.05$) in subtotal individual isoflavones and TI content were found among most cultivars in our study. Cultivar S0880 possessed significantly ($p < 0.05$) higher subtotal individual daidzein, subtotal individual genistein, and TI content than did the other cultivars. Cultivar Jim possessed significantly ($p < 0.05$) higher subtotal individual glycitein than did the other cultivars. In the case of the same cultivars grown in the different locations, significant differences in subtotal individual daidzein, subtotal individual genistein, subtotal individual glycitein, and TI content existed among the three Prosoy samples, which were grown in three different locations in North Dakota (Fargo, Casselton, and Durbin) with the TI content ranging from 1752.4 to 1996.9 μg/g. Significant differences in subtotal individual daidzein, subtotal individual genistein, and TI content also were found between the two Traill samples and the two Norpro samples, which were grown in two different locations (Fargo and Casselton), respectively. In the case of the same cultivars grown in two continued cropping years, significant differences in TI content were found in Proto, L-star, and black soybean cultivars. Similar observations were made among Vinton 81 soybean (*23*), in which TI ranged from 1176 to 3309 μg/g for soybeans grown in different crop years, and ranged from 1176 to 1749 μg/g for soybeans grown in different locations. Crop year seemed to have a much greater influence on TI than planting location. Hoeck et al. (*24*) tested six soybean cultivars grown in eight locations in Iowa and showed that genotype, genotype−year, genotype−location, and genotype−year−location interactions all influenced isoflavone concentrations in soybeans. Similar observations were also made among Ohio soybean cultivars (*21, 22*).

Correlation Analyses of Antioxidant Properties and Major Phenolic Compounds. The linear correlation coefficients between major phenolic compounds in 28 yellow soybeans and their antioxidant activity ORAC values are presented in **Table 9**. Daidzin, genistin, malonyldaidzin, daidzein, genistein, subtotal daidzein, subtotal genistein, and TI exhibited significant correlations with the ORAC values at $p < 0.0001$. Malonylgenistin, malonylglycitin, acetylgenistin, and subtotal glycitein exhibited significant correlations with the ORAC values at $p < 0.05$. The other isoflavones, such as glycitin, acetyldaidzin, and acetylglycitin, exhibited insignificant ($p > 0.05$) correlations with the ORAC values. In the case of major phenolic acid components, TCA, subtotal cinnamic acids, and total phenolic acids exhibited significant correlations with the ORAC values at $p < 0.0001$. GA exhibited significant correlations with the ORAC values at $p < 0.05$. The other major phenolic acids, such as CFA and CLA, exhibited insignificant ($p > 0.05$) correlations with the ORAC values. In the case of major flavonol component, (+)-catechin did not exhibit significant ($p > 0.05$) correlations with the ORAC values. These correlation assays suggested that both isoflavones and phenolic acids contributed to the antioxidant activity ORAC values of yellow soybeans.

In summary, 30 soybean samples were analyzed for total phenolics content, individual phenolic compositions of four major phenolic groups, including phenolic acids, anthocyanins, flavonols, and isoflavones, and antioxidant properties. Yellow soybean cultivars S0880, Pembina, 91M10, Tofooey, Korada, and black soybeans had high TPC and/or high isoflavone contents and/or high antioxidant properties. Among all yellow soybean cultivars, S0880 had the highest phenolic substance

Table 9. Correlations between Antioxidant Activities and Major Phenolic Compounds in 28 Yellow Soybeans

types of phenolic	compounds	ORAC correlation coefficients (*r*)
isoflavones		
7-*O*-β-glucosides	daidzin	0.68[b]
	genistin	0.59[b]
	glycitin	0.03
malonylglucosides	malonyldaidzin	0.59[b]
	malonylgenistin	0.41[a]
	malonylglycitin	0.38[a]
acetylglucosides	acetyldaidzin	−0.23
	acetylgenistin	0.37[a]
	acetylglycitin	0.29
aglycones	daidzein	0.56[b]
	genistein	0.47[b]
subtotal aglycones	subtotal daizein	0.69[b]
	subtotal genistein	0.54[b]
	subtotal glycitein	0.39[b]
total	TI	0.62[b]
phenolic acids		
	GA	0.31[a]
subtotal benzoics	subtotal benzoic acids	0.29
cinnamic acids	CFA	−0.60[b]
	CLA	0.23
	TCA	0.76[b]
subtotal cinnamics	subtotal cinnamic acids	0.48[b]
total	total phenolic acids	0.49[b]
flavonols		
flavan-3-ols	(+)-catechin	0.06

[a] Correlation is significant at the 0.05 level (two-tailed). [b] Correlation is significant at the 0.0001 level (two-tailed); $N = 28$.

profiles and the highest ORAC values, which approached the high ORAC values of black soybeans. The variability in phenolic content among different phenotypes could be useful for breeders and farmers to select high phenolic cultivars to plant. The food industry may prefer soybeans with high phenolic content and high antioxidant properties for use as the ingredient for manufacturing functional foods or nutraceuticals for promoting consumer's health.

ABBREVIATION USED

TPC, total phenolic content; TFC, total flavonoid content; DPPH, 2-diphenyl-1-picryhydrazyl radical; FRAP, ferric reducing antioxidant power; ORAC, oxygen radical absorbing capacity; GA, gallic acid; PA, protocatechuic acid; TBA, 2,3,4-trihydroxybenzoic acid; HBA, *p*-hydroxybenzoic acid; VA, vanillic acid; CFA, caffeic acid; CLA, chlorogenic acid; VN, vanillin; SA, syringic 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; TI, total isoflavone.

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