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Phenolic Substance Characterization and Chemical and Cell-Based Antioxidant Activities of 11 Lentils Grown in the Northern United States

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Chemical and cellular antioxidant activities and phenolic profiles of 11 lentil cultivars grown in the cool northern parts of the United States were investigated. Individual phenolic compounds, including phenolic acids, flavan-3-ols, flavones, and anthocyanins, were further quantitatively investigated by HPLC. Cellular antioxidant activities (CAA) and peroxyl radical scavenging capacity (PRSC) were evaluated by fluorescence microplate reader. Cultivar Morton exhibited the highest individual flavan-3-ols (catechin and epicatechin) and total flavonoids, as well as the highest antioxidant properties (PRSC and CAA) among all lentils tested. Five phenolic acids of the benzoic types and their derivates (gallic, protocatechuic, 2,3,4-trihydroxybenzoic, *p*-hydroxybenzoic acid, and protocatechualdehyde) and four phenolic acids of the cinnamic type (chlorogenic, *p*-coumaric, *m*-coumaric, and sinapic acid) were detected in all lentil cultivars. Two flavan-3-ols [(+)-catechin and (−)-epicatechin] and one flavone (luteolin) were detected in all lentil cultivars. Among all phenolic compounds detected, sinapic acid was the predominant phenolic acid, and (+)-catechin and (−)-epicatechin were the predominant flavonoids. These results showed that different phenotype lentils possessed considerable variations in their individual phenolic compounds, as well as chemical and cellular antioxidant activities. Caffeic acid, catechin, epicatechin, and total flavonoids significantly ($p < 0.05$) correlated with peroxyl radical scavenging assay. Cellular antioxidant assay significantly correlated with chemical antioxidant assay ORAC. The results from this study could be very interesting for breeding programs to improve lentils for use as functional foods.

KEYWORDS: Lentils; phenolic acid; anthocyanin; flavan-3-ol; flavone; peroxyl radical; cellular antioxidant activity; HPLC

INTRODUCTION

Lentils are one of the oldest crops cultivated by humans, domesticated around 8000 BC in the Fertile Crescent (*1*) of the Mideast. They have been in constant use in different societies since then, and their consumption has been widespread in developed and developing countries alike (*2*). The major lentil-producing countries are India, Canada, Turkey, the United States, Nepal, Australia, Syria, China, Bangladesh, and Iran. Lentil is an important rotation crop in the cereal-based production systems in the U.S. Pacific Northwest. Initially, production of lentils in the United States concentrated in eastern Washington and northern Idaho. Production of lentils has expanded to other northern states including Montana and North Dakota (*2*). The U.S. production area has increased significantly in recent years (*3*).

Generally, lentils are canned or dry-packaged, whole or split, for retail sale or processed into flour. They are used in soups, stews, salads, snack foods, and vegetarian dishes. Lentils may be used as a meat extender or substitute because of the high protein content and quality (*4*). Flour made from lentils is gluten free and may be added to cereal flour to make bread, cakes, and baby foods (*5*). Beyond their nutrition functions, lentils have several potential health-promoting effects, such as reducing cholesterol (*6*), managing blood-sugar disorders (*7*, *8*), reducing blood lipids (*9*), and reducing the risk of cardiovascular diseases (*10*) and cancer (*11*).

It has been widely accepted that significant health-promoting effects of plant foods are related to high contents of phenolic components. Among lentils and other cool season food legumes, we have found that significant correlations exist between potent antioxidant capacities and high contents of phenolic substances (*12*, *13*). Phenolic compounds are a complex group, which can be divided into various classes, such as phenolic acids, flavan-3-ols, flavonols, flavones, isoflavones, lignans, lignins, procyanidins, and anthocyanins. Currently, we do not know enough about the quality and quantity of the health-promoting components in lentils. On the other hand, a variety of lentils exist with seed coat colors that range from yellow to red-orange to green, tan, brown, and black, which may be solid or speckled in color, whereas the cotyledon is yellow, red, or green. The diversity of phenotypes of

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Figure 1. Morphology and appearance of 11 lentil cultivars.

lentil varieties indicates that complex phytochemical profiles and bioactivities may exist between lentil varieties. However, very little information is available in the literature regarding phenolic compounds in lentils (*14−16*). There is no chemical information about lentils grown in the United States except that of our previous publications (*12*, *13*) and one Beluga black lentil grown in Idaho (*5*). It is important to discover and quantify functional phenolic components in various varieties of lentils in the United States.

Most recently, a cell-based antioxidant activity assay, cellular antioxidant activity (CAA) assay, was developed. It was believed that the CAA assay is a more biologically relevant antioxidant assay method than the popular chemistry assays (such as DPPH and ORAC) because it accounts for some aspects of uptake, metabolism, and location of antioxidant components within cells (*17*). Therefore, the present study was undertaken to investigate phenolic profiles as well as chemical antioxidant and CAA of lentils grown in the northern part of the United States and further explore the correlations between CAA and previously reported antioxidant activities (DPPH, FRAP, and ORAC) based on chemical assays (*12*) and the correlations between phenolic compounds and antioxidant assays.

MATERIALS AND METHODS

Chemicals and Reagents. Sixteen phenolic acids, three aldehydes, five flavan-3-ols [(+)-catechin, (−)-epicatechin, epigallocatechin, epicatechin-gallate, epigallatecatechin-gallate] and six flavonols or flavones (myricetin, luteolin, quercetin, apigenin, kaempferol, quercetin-3-rutinoside), trifluoroacetic acid (TFA), dimethyl sulfoxide (DMSO), and 2′,7′-dichlorofluorescin diacetate (DCFH-DA) were purchased from Sigma-Aldrich Inc. (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 or ABAP) 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 was 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. (BioWhittaker, 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. (Cellgro, Herndon, VA). Sterilized cell culture materials, such as T-25 and T-75 flasks, syringe filter, 15 and 50 mL tubes, 24- and 96-well plates, and pipets, were purchased from Corning Inc. (Corning, NY).

Lentil Materials. Dry lentil seeds (as shown in **Figure 1**) were provided by seed companies and local farms in the states of North Dakota, Idaho, and Washington. Characteristics of these collected lentil materials are summarized in **Table 1**. Broken seeds, damaged seeds, and foreign materials were removed from the samples. Moisture content was determined by drying the sample in an air oven at 110 °C until a constant weight was obtained (*18*). All calculation for determination of phenolic contents is on a dry weight basis.

Extraction of Total Phenolic Compositions. The lentil samples were ground to flour with an IKA all basic mill (IKA Works Inc., Wilmington, NC) and to pass through a 60-mesh sieve. Extraction procedures were based on our earlier paper (*19*). Briefly, lentil flours (0.5 g in triplicate for each sample) were extracted twice with a total 10 mL of acetone/water/acetic acid (70:29.5:0.5, v/v/v). The extracts of total phenolics were used for the determination of antioxidant activities.

Cellular Antioxidant Activity Assay. Human gastric adenocarcinoma cells (cell line AGS, CRL-1739) were grown in complete growth medium F-12K (Mediatech, Inc., Herndon, VA) supplemented with 10% FBS and 1% penicillin−streptomycin (v/v) and 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. (*20*) and Wolfe and Liu (*17*). Briefly, instead of human liver cancer cell line HepG2, human gastric adenocarcinoma cell line AGS was used for the CAA assay due to the rapid proliferation properties of the gastric cell line. AGS cells were seeded at a density of 6×10^4 cells/well on a 96-well microplate in 100 μL of complete growth medium. The outside wells of the

Table 1. Characteristics of Collected Lentil Samples[a]

code	variety/cultivar	phenotype of seed coat/cotyledon	1000 seed weight (g)	seed size	source/provider	original location
22	Merrit (ND)	green/yellow	67	large	Agricare United	Ray, ND
23	CDC Richlea	green/yellow	56	medium	Agricare United	Ray, ND
24	Red Chief	light tan/ red	55	medium	Spokane Seed Co.	Spokane, WA
25	Morton	green/red	30	small	Spokane Seed Co.	Spokane, WA
26	Pardina	grayish brown/yellow	36	small	USDPLS[b]	Craigmont, ID
27	Richlea	green/yellow	48	medium	USDPLS	Craigmont, ID
28	Regular	light brown/yellow	58	medium	USDPLS	Craigmont, ID
29	Crimson	reddish brown/red	36	small	Columbia Grain International	Clarkson, WA
30	Merrit (WA)	green/yellow	62	large	Columbia Grain International	Clarkson, WA
31	Brewer	green/yellow	57	medium	Columbia Grain International	Clarkson, WA
44	French Green	greenish brown/yellow	32	small	NDDPLS[c]	Bismarck, ND

[a] Dried mature seeds were 2004 crops if not otherwise stated. [b] U.S. Dry Pea and Lentil Association. [c] North Dakota Dry Pea and Lentil Association.

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 in inner wells were treated with 20 μL of various concentrations of lentil extracts in 180 μL of prewarmed treatment medium [Eagle's minimum essential medium (EMEM), phenol free, vitamin E free, antibiotics 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 each well, and the microplate was placed into a BMG Fluostar Optima microplate reader (BMG Labtech GmbH, Offenburg, Germany). The plate was incubated in the BMG plate reader for a minimum of 10 min to ensure each well was at 37 °C. Just prior to the assay, 25 mg of AAPH dry solid was dissolved into 5 mL of HBSS (prewarmed at 37 °C in a water bath) in a 15 mL of tube. Immediately after dissolving, 20 μL of AAPH solution was dispensed through the pump and autoinjector of the plate reader into appropriate wells according to a balanced layout. The plate reader was programmed to record the fluorescence of dichlorofluorescein (DCF) on each cycle. Kinetic reading was 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 curve (AUC) was calculated as $AUC = [R_1/2 + sum(R_2{:}R_{n-1}) + R_n/2] \times CT$, where R_1 is the fluorescence reading at the initiation of the reaction and R_n is the last measurement and CT is 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 $= 100 - (\text{net } AUC_{sample}/\text{net } AUC_{control}) \times 100$. A portion of hydrophilic total phenolic extracts was freeze-dried because cell-based assays must be performed under solvent-free conditions to eliminate solvent effects. The freeze-dried extract (10 mg) was dissolved in cell culture medium as the stock sample solution. The final concentrations of samples (0.312, 0.625, 1.25, 2.5, and 5 mg/mL) were prepared by diluting the stock solution with the medium. 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 software CurveExpert (version 1.3).

Peroxyl Radical Scavenging Capacity (PRSC). The PRSC assay, similar to CAA but in a cell-free system, was performed 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. An aliquot (400 μL) of 1 mM DCFH-DA solution was divided into a 0.5 mL Eppendorf tube and stored at −20 °C until use. Just prior to use, an aliquot was added into 3.6 mL of 1.0 mM KOH in a 15 mL Falcon tube, hydrolyzed for 3−5 min to remove the diacetate (DA) moiety. The resulting DCFH was diluted into a 10 μM working solution with prewarmed PBS buffer at 37 °C. Prepared just before the start of the assay, 27.2 mg of AAPH solid was dissolved into 5 mL of warmed HBSS (37 °C). Immediately after dissolving, 20 μL of AAPH solution was dispensed through the pump and autoinjector 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 DCF in each cycle. Kinetic reading was 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 was recorded by the software BMG OPTIMA running on a PC. The area under the average fluorescence-reaction time kinetic curve (AUC) for both control and samples was integrated and used as the basis for calculating 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 methods are similar to the ORAC assay according to our previously published paper (*19*) by calibration through Trolox standard curves. PRSC was expressed as micromoles of Trolox equivalent (μmol of TE/g).

HPLC Analysis of Free Phenolic Acids Content. *Extraction of Free Phenolic Acids.* The extraction of free phenolic acids was performed by modifying the method of Luthria and Pastor-Corrales (*22*). Briefly, the lentil samples (0.5 g in triplicate) were extracted twice at room temperature with a total of 10 mL (5 mL each time) of methanol/water/acetic acid/BHT (85:15:0.5:0.2, v/v/v/w) by shaking extraction tubes on an orbital shaker at 300 rpm for 4 h. The extracts were concentrated at 45 °C under vacuum. The dry residue was dissolved in 5 mL of water and then was 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.

HPLC Analysis of Phenolic Acids. The quantitative analysis of free phenolic acids was performed by HPLC according to the method of Robbins and Bean (*23*) with slight modifications (*24*). A Waters Associates (Milford, MA) chromatography system was equipped with a model 720 system controller, model 6000A solvent delivery system, model 7125 loading sample injector, and model 418 LC UV detector (270 nm). A 4.6 mm × 250 mm, 5 μm, Zorbax Stablebond Analytical SB-C_{18} column (Agilent Technologies, Rising Sun, MD) was used for separation at 40 °C, which was maintained with a column heater. Elution was performed using mobile phase A (0.1% TFA aqueous solution) and mobile phase B (methanol), and the flow rate was set to 0.7 mL/min. The solvent gradient in volumetric ratios was as follows: 5−30% B over 50 min. The solvent gradient was held at 30% B for an additional 15 min and increased to 100% B at 66 min. The solvent gradient was held at 100% B for an additional 10 min to clean up the column.

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

using HPLC analysis as described previously in our research (*24*). Standard curves of phenolic acids were plotted with peak areas against concentrations of nine series of working solutions by duplicate injection. Sample concentrations were adjusted to fit within the linear ranges of authentic phenolic acids prior to HPLC analyses.

HPLC Analysis of Anthocyanin Content. The free phenolic acid extracts were also used for anthocyanin analysis, using an HP 1090 HPLC (Hewlett-Packard) equipped with filter photometric detector with a YMC Pack ODS-AM column (4.6 × 250 mm, S-50 μm, 120A) according to our recent publication (*24*). 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 the literature (*25*). The stock solution of anthocyanins was prepared by dissolving standards (unimolar mixture, total 2 μmol, one-third micromole for each compound) in methanol to give a concentration of 1.0 mg/mL. Mass concentrations of individual anthocyanins were calculated according to their molecular weight. A portion of the stock solution was then diluted using methanol to the following series of dilutions: 1 in 5, 10, 20, 40, 80, and 160. Standard curves of anthocyanins were plotted with peak areas against concentrations of six series of diluted working solutions by duplicate injection.

HPLC Analysis of Flavan-3-ol and Flavone Content. *Extraction of Flavonols.* The ground lentils (0.5 g in triplicate) were extracted at room temperature with 10 mL of extraction solvent (70% acetone/29.5% water/0.5% acetic acid, v/v/v) by shaking extraction tubes on an orbital shaker at 250 rpm for 3 h. The slurry was centrifuged by an Allegra 21R centrifuge (Beckman Coulter Ltd., Palo Alto, CA) at 5500 rpm for 20 min. Five milliliters of the supernatant was evaporated to dryness on a rotary evaporator at 35 °C. The residues in the flask were dissolved in 2 mL of 80% methanol and kept in a freezer (−20 °C) for < 12 h before analysis. An aliquot of the sample solution was filtered through a 0.2 μm PTFE syringe filter prior to HPLC assay.

HPLC Analysis of Flavonols. The quantitative analysis of flavonols was performed according to the methodology of isoflavone analysis developed by Murphy et al. (*26*) with a slight modification (*24*). The same Waters Associates chromatography system as used for phenolic acid analysis was used for the analysis of flavonols with UV 262 nm detection. A YMC-Pack ODS-AM-303 C_{18} reversed phase column (250 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. A linear gradient mobile phase consisted of solvent A (0.1% acetic acid in water) and solvent B (0.1% acetic acid in acetonitrile). After injection of 20 μL of sample, the system was eluted with 15% of solvent B for 5 min at the flow rate of 1.0 mL/min, then increased to 29% for 31 min at the flow rate up to 1.5 mL/min, and then to 35% for 8 min at the same flow rate of 1.5 mL/min. Then the gradient was increased to 50% of solvent B in 2 min, kept at 50% of solvent B for 10 min at the flow rate of 1.5 mL/min, and then recycled back to 15% B at the flow rate of 1.0 mL/min in 2 min; the column was equilibrated with initial solvent for 2 min prior to running the next sample.

Identification and Quantification of Flavan-3-ol and Flavonols. Flavan-3-ols, such as catechin and epicatechin, have chiral isomers. According to literature investigations, the majority of flavan-3-ols in lentils existed as (+)-catechin and (−)-epicatechin (*15*, *16*). Therefore, five commercially available flavan-3-ols and nine flavonols or flavones were selected and directly used to identify the sample peaks by comparing their retention times and HPLC profiles to a standard mixture. In addition, a spiking method was used for peak identification of some samples.

The quantification of flavan-3-ols and flavonols was performed by calibrating the peak areas obtained from HPLC analyses. External calibration curves were obtained for each of six external standards by plotting the peak area of each standard against concentration. For the other flavonols without commercial standards, concentrations were calculated from the standard curves that were adjusted appropriately from the standard curves of respective forms of flavonols on the basis of the differences of molecular weight and molar extinction coefficients of compounds. Sample concentrations were adjusted to fit within linear ranges of authentic flavan-3-ols or flavonols prior to HPLC analyses. Flavonol concentrations were expressed as micrograms of flavonol per gram of dry lentil.

Statistical Analysis. 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 between group means at $p < 0.05$. A Pearson correlation test was conducted to determine the correlation between variables. Several antioxidant markers and total phenolic, total condensed tannins, and total flavonoids used for correlations with the individual phenolic compounds were taken from one of our recent publications (*12*).

RESULTS AND DISCUSSION

Diversity of Lentils. Natural phenolics exert their beneficial health effects mainly through their antioxidant activity (*27*). These compounds are capable of removing free radicals, chelating metal catalysts, activating antioxidant enzymes, and inhibiting oxidases (*28*). Total phenolic compounds contribute to the overall antioxidant activities of plant foods. Procyanidins (condensed tannins) are a class of phenolic compounds widely distributed in the plant kingdom. Procyanidins are the predominant phenolic compounds in legume seeds. They were widely found in lentils (*15*, *16*, *29*−*31*). The seed coat color can be clear, green, tan, gray, brown, or black, whereas the cotyledon is yellow, red, or green (as shown in **Figure 1**). The color differences of seed coats of lentils indicate that diverse compounds of pigments exist in lentils. Previously, we found that the highest concentrations of total phenolic content (TPC) and condensed tannin content (CTC) existed in Morton lentil, which is a winter-hardy cultivar, whereas the lowest concentrations of TPC and CTC were found in CDC Richlea, Brewer, and Merrit (grown in Clarkson, WA) (*12*). To better understand the role of phenolic composition in acting as antioxidants on food stability and human health, the following research was performed to analyze the profiles of individual phenolic compounds and to determine the potential interactions between individual phenolic compounds and antioxidant activities.

Phenolic Acid Compositions of Lentils. The free phenolic acid contents of lentils are presented in **Table 2**. Five phenolic acids of the benzoic type and their derivates (gallic, protocatechuic, 2,3,4-trihydroxybenzoic, *p*-hydroxybenzoic acid, and protocatechualdehyde) and four phenolic acids of the cinnamic type (chlorogenic, *p*-coumaric, *m*-coumaric and sinapic acid) were detected in all lentil cultivars. In addition, vanillic acid was detected in Merrit, CDC Richlea, Red Chief, Morton, Pardina, and Richlea. Caffeic acid was detected in Red Chief, Morton, Pardina, and Richlea. Among all of these detected compounds, gallic, chlorogenic, and sinapic acid were the major phenolic acids in all lentil cultivars. Gallic acid ranged from 90.9 μg/g in Pardina to 136.8 μg/g in Brewer. Chlorogenic acid ranged from 155.7 μg/g in CDC Richlea to 213.2 μg/g in French Green. Sinapic acid ranged from 1099.7 μg/g in Red Chief to 2217.4 μg/g in CDC Richlea. From a quantitative point of view, among these three major phenolic acids, sinapic acid was the predominant phenolic acid and was approximately 10-fold higher than the other two phenolic acids.

Significant differences ($p < 0.05$) in individual phenolic acids and subtotal and total phenolic acid contents were found among most lentil cultivars. Significant differences ($p < 0.05$) in gallic, 2,3,4-trihydroxybenzoic, *p*-hydroxybenzoic, chlorogenic, and sinapic acid, subtotal cinnamics, and total phenolic acid contents were also found between two Merrit cultivars, which were grown in different locations. Among all lentils, Regular possessed the highest content in protocatechuic acid (37.7 μg/g), 2,3,4-trihydroxybenzoic acid (29.3 μg/g), *p*-hydroxybenzoic acid (44.9 μg/g), protocatechualdehyde (12.1 μg/g), and subtotal benzoics (248.1 μg/g). Merrit (grown in Clarkson, WA) and Brewer possessed the highest content in gallic acid (136.1 μg/g). Red Chief possessed the highest

Table 2. Free Phenolic Acids Content (Micrograms per Gram) of Lentils[a]

		individual benzoic acid derivates						
code	variety/cultivar	GA	PA	TBA	PCD	HBA	VA	subtotal benzoics
22	Merrit (ND)	123.1 ± 4.29ab	27.33 ± 1.12c	26.89 ± 0.54ab	3.93 ± 0.09e	30.15 ± 2.23ef	3.22 ± 0.07a	214.6 ± 6.98bc
23	CDC Richlea	98.26 ± 8.65cd	21.35 ± 2.05d	23.53 ± 0.94c	3.69 ± 0.12e	22.27 ± 1.51g	0.59 ± 0.12c	169.7 ± 9.73e
24	Red Chief	126.3 ± 0.56a	22.91 ± 2.34d	27.47 ± 0.33a	10.05 ± 0.59b	38.34 ± 1.98b	2.98 ± 0.17a	228.1 ± 3.98ab
25	Morton	102.8 ± 1.35cd	28.53 ± 1.94c	29.16 ± 1.17a	5.82 ± 0.11d	37.05 ± 1.14bc	1.19 ± 0.32b	204.5 ± 3.81cd
26	Pardina	90.85 ± 1.69d	26.59 ± 1.39c	24.31 ± 1.63bc	7.64 ± 0.99c	33.79 ± 1.19cd	0.79 ± 0.22c	183.9 ± 3.55de
27	Richlea	106.2 ± 4.84c	32.78 ± 0.19b	23.86 ± 0.36c	5.68 ± 0.29d	15.79 ± 1.79h	3.15 ± 0.10a	187.4 ± 2.11de
28	Regular	124.1 ± 3.21ab	37.72 ± 0.93a	29.26 ± 2.37a	12.14 ± 0.56a	44.94 ± 3.38a	ND[b]	248.1 ± 7.99a
29	Crimson	110.1 ± 4.99bc	21.15 ± 1.74d	18.19 ± 1.91d	5.99 ± 0.98d	16.83 ± 1.19h	ND	172.2 ± 7.99e
30	Merrit (WA)	136.0 ± 6.53a	27.35 ± 1.58c	24.76 ± 1.17bc	4.52 ± 0.05e	28.10 ± 0.74f	ND	220.8 ± 6.21bc
31	Brewer	136.8 ± 20.37a	29.47 ± 2.84c	23.02 ± 1.65c	8.14 ± 0.82c	33.31 ± 3.43ed	ND	230.7 ± 28.87ab
44	French Green	127.6 ± 8.36a	20.28 ± 1.05d	16.96 ± 1.51d	7.98 ± 0.49c	16.24 ± 1.06h	ND	189.0 ± 10.28de

		individual cinnamic acid derivates						
code	variety/cultivar	CFA	CLA	PCA+SD	MCA+FA	SPA	subtotal cinnamics	total phenolic acids
22	Merrit (ND)	ND[b]	170.9 ± 8.55dce	5.85 ± 0.47e	2.72 ± 0.25cd	1527.9 ± 42.23b	1707.4 ± 49.49b	1921.9 ± 54.33bc
23	CDC Richlea	ND	155.7 ± 14.69e	4.67 ± 0.33f	3.97 ± 0.59b	2217.4 ± 196.1a	2381.7 ± 207.7a	2551.4 ± 217.4a
24	Red Chief	10.57 ± 0.98a	191.1 ± 17.49abc	11.89 ± 0.55a	2.37 ± 0.20cd	1099.7 ± 62.34d	1315.6 ± 66.49c	1543.7 ± 64.74d
25	Morton	10.45 ± 0.18a	202.2 ± 14.62ab	9.22 ± 0.55b	2.84 ± 0.24c	1552.2 ± 31.22b	1776.9 ± 45.52b	1981.4 ± 47.99b
26	Pardina	10.55 ± 0.83a	159.4 ± 12.33de	9.07 ± 0.40b	4.46 ± 0.24a	1322.4 ± 57.96c	1505.9 ± 67.88c	1689.9 ± 70.95d
27	Richlea	7.09 ± 0.12b	173.2 ± 8.37dce	6.90 ± 0.06c	1.19 ± 0.06e	1667.1 ± 144.5b	1855.5 ± 152.9b	2042.9 ± 155.1b
28	Regular	ND	187.4 ± 16.49abcd	7.18 ± 0.36c	2.32 ± 0.15cd	1290.2 ± 106.7c	1487.1 ± 109.5c	1735.2 ± 114.7cd
29	Crimson	ND	182.7 ± 16.90bcde	6.78 ± 0.24cd	1.35 ± 0.09e	1603.1 ± 155.6b	1793.9 ± 168.3b	1966.2 ± 174.5b
30	Merrit (WA)	ND	195.4 ± 2.56abc	5.52 ± 0.43e	2.61 ± 0.28cd	1141.0 ± 71.76cd	1344.5 ± 73.38c	1566.3 ± 79.02d
31	Brewer	ND	184.9 ± 19.89bcd	6.04 ± 0.59de	2.20 ± 0.36d	1242.0 ± 20.29cd	1435.2 ± 40.78c	1665.9 ± 68.24d
44	French Green	ND	213.2 ± 16.03a	5.68 ± 0.59e	3.75 ± 0.20b	1672.1 ± 69.48b	1894.7 ± 61.56b	2083.8 ± 69.18b

[a] Data are expressed as mean ± standard deviation ($n = 3$) on a dry weight basis. Values marked by the same letter within columns are not significantly different ($p < 0.05$). Phenolic acids: GA, gallic acid; PA, protocatechuic acid; TBA, 2,3,4-trihydroxybenzoic acid; PCD, protocatechualdehyde; HBA, *p*-hydroxybenzoic acid; VA, vanillic acid; CFA, caffeic acid; CLA, chlorogenic acid; PCA+SD, *p*-coumaric acid + syringaldehyde; MCA+FA, *m*-coumaric acid + ferulic acid; SPA, sinapic acid. [b] ND, not detectable. Limit of detection (LOD) and limit of quantitation (LOQ) of GA, PA, TBA, PCD, HBA, VA, CFA, and PCA+SD were 0.01 and 0.1 μg/mL, respectively. LOD and LOQ of CLA and SPA were 0.1 and 1.0 μg/mL, respectively. LOD and LOQ of MCA+FA were 0.002 and 0.01 μg/mL, respectively.

content in caffeic acid (10.6 μg/g) and *p*-coumaric acid (11.9 μg/g). French Green possessed the highest content in chlorogenic acid (213.2 μg/g). Pardina possessed the highest content in *m*-coumaric acid (4.5 μg/g). CDC Richlea possessed the highest content in sinapic acid (2217.4 μg/g), subtotal cinnamics (2381.7 μg/g), and total phenolic acids (2551.4 μg/g), whereas Red Chief possessed the lowest content in sinapic acid (1099.7 μg/g), subtotal cinnamics (1315.6 μg/g), and total phenolic acids (1543.7 μg/g). In summary, phenolic acid profiles in various cultivars exhibited substantial variability, which may be influenced by complex factors, which included phenotype, crop location, weather conditions, and environmental stress as well as postharvest environments.

Phenolic acid contents in lentils grown in Spain have been reported in several earlier studies (*14*, *15*, *31*, *32*). However, quantification of phenolic acid content was based on either separated parts (seed coats, cotyledon) or germinated or enzyme-treated lentils. In our current investigation, the phenolic acid profiles and contents of 11 lentil cultivars grown in the northern part of the United States were different from those of Spanish lentils (*14*, *32*), in which fewer phenolic acids (protocatechuic, *p*-hydroxybenzoic, *p*-coumaric, ferulic acid) with lower content (ranging from 0.1 to 7.5 μg/g) were detected. However, the individual phenolic acids of raw lentils in another paper (*31*) on a Spanish lentil were higher than the respective contents in the lentil of our current investigation. However, fewer phenolic acids and lower content of total phenolic acids were detected in these Spanish lentils. In addition, three major phenolic acids (gallic, chlorogenic, and sinapic acid) detected in the current lentil samples were not reported in the Spanish lentils. The discrepancies may be attributed to the differences of sample sources or extraction methods.

Anthocyanin Compositions of Lentils. From the appearance point of view, the seed coat color of collected lentil samples (**Table 1**) exhibited green, tan, brown, or red color, whereas the cotyledon exhibited yellow or red color. The color differences between lentils indicated that some special pigments (may be anthocyanins) existed in lentils. However, anthocyanins in seed lentils have not been widely investigated so far. Only one investigation was performed on Beluga black lentil (*5*), in which one anthocyanin was isolated and identified.

The anthocyanin contents of 11 lentils grown in the northern part of the United States were investigated. Delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, pelargonidin-3-glucoside, and malvidin-3-glucoside were not detected in all lentils determined under 540 nm by HPLC. However, one unknown peak (not standard compound peaks) was detected in Pardina and French Green by HPLC with visible detection at 540 nm. When the HPLC detector was switched to UV 262 nm, the peak still existed at the same retention time, but peak height became lower as compared to visible detection. This is a typical characteristic of anthocyanin. Therefore, the peak from Pardina and French was qualitatively considered as unknown anthocyanin. However, due to a lack of commercial compounds, characterization and quantification of this anthocyanin are difficult. Therefore, the content of this unidentified anthocyanin was expressed as delphinidin-3-glucoside equivalent (μg/g). From a quantitative point of view, French Green (665.6 μg/g) exhibited much higher anthocyanin content than Pardina (157.3 μg/g).

Flavan-3-ol and Flavone Compositions of Lentils. Chemical profiles of flavan-3-ol and flavone in the Spanish lentils have been investigated in several earlier papers (*15*, *32*, *33*). However, no systematic study had been performed on flavan-3-ol and

Table 3. Flavan-3-ol, Flavone, and Anthocyanin Contents (Micrograms per Gram) of Lentils[a]

		flavan-3-ol		flavone	anthocyanin		
code	variety/cultivar	(+)-catechin	(−)-epicatechin	luteolin	delphinidin-3-glucoside	unknown[c]	total flavonoids[d]
22	Merrit (ND)	1090.6 ± 78.5cd	3793.2 ± 125.7bc	27.80 ± 1.10ef	ND[b]	ND	4911.6 ± 48.28c
23	CDC Richlea	1105.8 ± 90.1cd	4557.9 ± 95.9a	21.80 ± 0.77g	ND	ND	5685.6 ± 6.66b
24	Red Chief	1198.9 ± 27.5bc	2773.0 ± 65.6d	29.85 ± 0.28e	ND	ND	4001.8 ± 93.32de
25	Morton	1898.9 ± 4.7a	4946.7 ± 408.9a	25.15 ± 0.34f	ND	ND	6870.8 ± 414.0a
26	Pardina	931.8 ± 52.4ef	3373.7 ± 74.1c	18.22 ± 0.59h	ND	157.3 ± 0.40	4323.7 ± 22.27d
27	Richlea	1312.2 ± 42.9b	3705.0 ± 44.5bc	30.68 ± 1.21e	ND	ND	5047.8 ± 2.79c
28	Regular	876.6 ± 37.3f	2606.9 ± 61.9d	40.56 ± 0.62c	ND	ND	3524.1 ± 25.29e
29	Crimson	266.9 ± 3.4g	2535.1 ± 38.0d	38.83 ± 0.96cd	ND	ND	2840.8 ± 40.45f
30	Merrit (WA)	1020.6 ± 81.9ed	2664.2 ± 45.4d	49.64 ± 1.76b	ND	ND	3734.4 ± 129.1e
31	Brewer	863.3 ± 83.8f	3911.6 ± 345.6b	36.81 ± 2.38d	ND	ND	4811.6 ± 527.0c
44	French Green	1186.2 ± 8.2bc	3604.9 ± 165.0bc	77.13 ± 2.57a	ND	665.6 ± 9.11	4868.2 ± 170.7c

[a] Data are expressed as mean ± standard deviation ($n = 3$) on a dry weight basis. Values marked by the same letter within columns are not significantly different ($p < 0.05$). [b] ND, not detectable. [c] Compound was not identified; content of compound was expressed as delphinidin-3-glucose equivalent (μg/g). [d] Total flavonoids = sum of individual flavonoid compound content (anthocyanin was not involved in this calculation). LOD and LOQ of both catechin and epicatechin were 1 and 5 μg/mL, respectively. LOD and LOQ of luteolin were 0.1 and 0.6 μg/mL, respectively. LOD and LOQ of delphinidin-3-glucoside were 0.01 and 0.05 μg/mL, respectively.

flavone profiles of the U.S. lentils. The flavan-3-ol and flavone contents of the 11 lentil cultivars grown in the northern part of the United States are presented in **Table 3**. Two major flavan-3-ols [(+)-catechin and (−)-epicatechin] and one flavone (luteolin) were detected in all lentil cultivars. Among the three compounds detected, (+)-catechin and (−)-epicatechin are the major flavonoid compositions in all lentil cultivars. The content of (+)-catechin ranged from 266.9 μg/g in Crimson to 1898.9 μg/g in Morton. The content of (−)-epicatechin ranged from 2535.1 μg/g in Crimson to 4946.7 μg/g in Morton, whereas the content of luteolin ranged from 21.8 μg/g in CDC Richlea to 77.1 μg/g in French Green.

Significant differences ($p < 0.05$) in the contents of individual flavonol and total flavonoids existed among most lentil cultivars. Significant differences ($p < 0.05$) in the contents of individual flavonol and total flavonoids also existed between the two Merrit cultivars, which were grown in different locations. As compared to the other cultivars, Morton possessed the highest content in (+)-catechin (1898.9 μg/g), (−)-epicatechin (4946.7 μg/g), and total flavonoids (6870.8 μg/g); French Green possessed the highest content in luteolin (77.1 μg/g), whereas Crimson possessed the lowest content in (+)-catechin (266.9 μg/g), (−)-epicatechin (2535.1 μg/g), and total flavonoids (2840.8 μg/g). CDC Richlea possessed the lowest content in luteolin (21.8 μg/g). Similar to the phenolic acids, the flavonol profiles in various cultivars also exhibited large variability. The variability may be influenced by similar complex factors as listed in the previous sections.

The results from 11 lentil cultivars grown in the northern United States verified previous findings (*15*, *32*, *33*) about the existence of (+)-catechin, (−)-epicatechin, and luteolin in lentils. However, several flavonol glycosides detected in Spanish lentils, such as kaempferol glycoside, apigenin glycoside, quercetin glycoside, and myricetin glycoside, were not detected in our lentil samples. From a quantitative point of view, flavonol contents in Pardina lentil grown in the United States are higher than those of the Pardina lentils grown in Spain as reported by Duenas et al. (*32*). The differences may be partly attributed to the differences of sample sources.

Peroxyl Radical Scavenging and Cellular Antioxidant Capacities of Lentils. DCFH-DA was chosen as fluorescence probe for current assays due to the stability of DCFH-DA under oxidation conditions, whereas DCFH-DA can be hydrolyzed under alkaline condition to remove the DA moiety (*21*). In addition, DCFH-DA is a cell-permeable probe, which was widely used as a probe in cell-based bioassays. DCFH-DA can diffuse through cell membranes into intracellular compartments where cellular esterase can cleave the diacetate moiety to form the more polar DCFH according to proposed principle of CAA assay by Wolfe and Liu (*17*). Thermal degradation of 2,2′-azobis(amidinopropane) produces peroxyl radicals (ROO•), which oxidizes nonfluorescent dichlorofluoresein (DCFH) to fluorescent dichlorofluorescein (DCF). The degree of inhibition of DCFH oxidation, by antioxidants that scavenge peroxyl radicals, was used as the basis for calculating antioxidant activity.

The kinetics of DCFH oxidation by peroxyl radicals recorded as fluorescence generation is shown in **Figure 2A** for the standard compound Trolox in PRSC assay. The results indicated that peroxyl radicals generated from thermal degradation of AAPH oxidize DCFH into fluorescent products over time and that antioxidant Tolox could scavenge peroxyl radicals and inhibit the oxidation reaction in a dose-dependent manner (**Figure 2B**). The inhibitory effects of sample lentils against peroxyl radical induced DCFH oxidation in cell-free antioxidant systems are summarized in **Table 4**. Significant differences ($p < 0.05$) in PRSC values were found among most lentil cultivars. Significant different ($p < 0.05$) PRSC values were also found between two Merrit cultivars, which were grown in two different locations. Among 11 lentils tested, Morton lentil exhibited the strongest peroxyl radical scavenging capacity, whereas Merrit lentil (grown in Clarkson, WA) exhibited the lowest peroxyl radical scavenging capacity. To the best of our knowledge, the peroxyl radical scavenging capacity of lentils had not been investigated previously.

For the purpose of comparison, a more physiologically relevant antioxidant assay based on an in vitro cell cultivation system was performed to investigate the peroxyl radical scavenging capacities of lentils. We used gastric tumor cells instead of liver tumor cells because we found gastric cells grow much faster than the latter. Cells with 80% confluence in a T-75 flask were enough for three CAA assays performed in a 96-well plate. Choosing this cell line can save time and obtain reproducible data. The inhibiting kinetic of lentil against cellular oxidative stress in AGS cells by peroxyl radicals generated from AAPH is shown in **Figure 2C**. Lentils exhibited peroxyl radical scavenging activity in cell culture system in a dose-dependent manner (**Figure 2D**). To calculate the EC_{50} value, the dose−response curve from the ratio of the area under the curve of the sample to that of the control and the median effect curve was plotted for each lentil sample. The EC_{50} values for CAA of lentil samples are presented in **Table 4**. Among all lentils tested, Morton lentil was found to be the most effective peroxyl radical scavenger indicated by its lowest EC_{50} value,

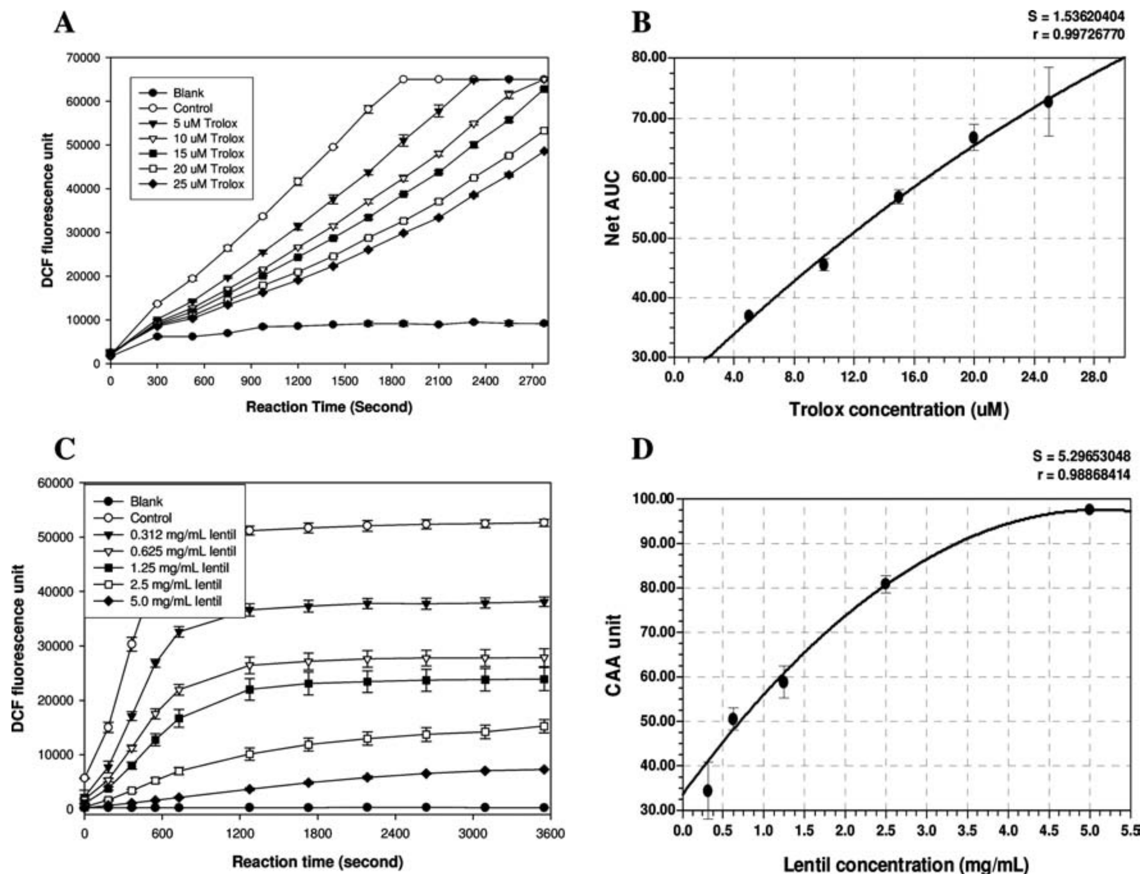

Figure 2. Typical time kinetics and dose−response curves for inhibition of peroxyl radical-induced DCFH oxidation in cell-free (PRSC) assay (**A**, **B**) and cell-based antioxidant (CAA) assay (**C**, **D**): (**A**) time kinetics of Trolox against DCFH oxidation in PRSC assay; (**B**) dose−response plots of Trolox against DCFH oxidation in PRSC assay; (**C**) time kinetics of lentil against DCFH oxidation in CAA assay; (**D**) dose−response plots of lentil against DCFH oxidation in CAA assay. Blank wells contained fluorescence probe without oxidant AAPH. Control wells contained with both fluorescence probe and oxidant AAPH but not samples. Net AUC = $AUC_{sample} - AUC_{blank}$. CAA unit was expressed as CAA unit = 100 − (net AUC_{sample}/net $AUC_{control}$) × 100.

Table 4. Inhibitory Effects of Lentils on Peroxyl Radical Induced DCFH Oxidation in Cell-free (PRSC) and Cell-Based Antioxidant (CAA) System[a]

		PRSC	cellular antioxidant assay	
code	variety/cultivar	Trolox equivalent (μmol of TE/g)	EC_{50} (mg/mL)	reciprocal value of CAA ($1/EC_{50}$)
22	Merrit (ND)	58.05 ± 3.42c	0.68 ± 0.05	1.47 ± 0.06
23	CDC Richlea	50.29 ± 3.47f	0.33 ± 0.03	3.03 ± 0.28
24	Red Chief	66.60 ± 3.00b	1.41 ± 0.05	0.71 ± 0.03
25	Morton	82.08 ± 0.69a	0.30 ± 0.02	3.31 ± 0.27
26	Pardina	64.72 ± 3.81b	1.06 ± 0.09	0.94 ± 0.07
27	Richlea	52.76 ± 4.31e	1.22 ± 0.11	0.82 ± 0.08
28	Regular	48.62 ± 1.16 g	0.69 ± 0.05	1.45 ± 0.04
29	Crimson	54.01 ± 1.68d	0.48 ± 0.03	2.08 ± 0.11
30	Merrit (WA)	38.92 ± 2.37 h	0.54 ± 0.05	1.85 ± 0.08
31	Brewer	54.75 ± 1.83d	0.65 ± 0.04	1.54 ± 0.06
44	French Green	65.18 ± 3.64b	0.96 ± 0.10	1.04 ± 0.13

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

followed by CDC Richlea, Crimson, Merrit (WA), Brewer, Merrit (ND), Regular, and French Green. Red Chief was found to be the weakest peroxyl radical scavenger as indicated by its highest EC_{50} value.

Correlations between Phenolic Compounds and Antioxidant Capacities. It was of interest to understand the correlations between these phenolic compounds and antioxidant activities as well as correlations between different antioxidant assays. The antioxidant activities (DPPH, FRAP, and ORAC values) and total phenolics (TPC and CTC) of lentils reported in our previous paper (*12*) were included along with the current antioxidant values (PRSC and CAA) and individual phenolic contents for linear regression analyses to investigate correlations between these variables. Because CAA was expressed as EC_{50} values, a lower value indicated a higher activity. Therefore, the reciprocal values of CAA ($1/EC_{50}$) were calculated and are listed in **Table 4**. These reciprocal values of CAA were used for correlation analysis. The linear correlation coefficients between phenolic compounds in 11 lentils and their antioxidant activities (DPPH, FRAP, ORAC, PRSC, and CAA) are summarized in **Table 5**. *m*-Coumaric acid + ferulic acid, sinapic acid, subtotal cinnamic acid, and total phenolic acid exhibited significant ($p < 0.05$) correlations with the DPPH values. TPC, CTC, protocatechualdehyde, caffeic acid, chlorogenic acid, and *p*-coumaric acid + syringaldehyde exhibited significant ($p < 0.05$ or 0.0001) correlations with the FRAP values. CTC, 2,3,4-trihydroxybenzoic acid, catechin, epicatechin, and total flavonoids exhibited significant ($p < 0.05$) correlations with the ORAC values. TPC, CTC, caffeic acid, *p*-coumaric acid + syringaldehyde, catechin, epicatechin, and total flavonoids exhibited significant ($p < 0.05$ or 0.0001) correlations with the PRSC values. Sinapic acid, subtotal cinnamic acid, total phenolic acid, epicatechin, and total flavonoid exhibited significant ($p < 0.05$) correlations with the reciprocal values of CAA. These results indicated that different phenolic substances had different degrees of contributions to the overall antioxidant activities. Furthermore, the mixture of phenolic

Table 5. Correlations between Phenolic Compounds and Antioxidant Assays[a]

correlation coefficient (r)	DPPH[b]	FRAP[b]	ORAC[b]	PRSC	1/CAA
TPC	−0.11	0.80**	0.07	0.64**	0.02
CTC	0.15	0.36*	0.44*	0.83**	0.19
gallic acid	−0.22	−0.01	−0.06	−0.34	−0.30
protocatechuic acid	−0.22	0.26	0.02	−0.19	−0.09
2,3,4-trihydroxybenzoic acid	−0.55*	0.08	0.47*	0.15	0.14
protocatechualdehyde	−0.01	0.41*	−0.21	0.12	−0.49*
p-hydroxybenzoic acid	−0.36*	0.27	0.19	0.19	−0.04
vanillic acid	−0.45*	−0.33	0.32	0.27	−0.28
caffeic acid	−0.28	0.41*	0.22	0.67**	−0.12
chlorogenic acid	−0.12	0.38*	0.06	0.22	−0.01
p-coumaric acid + syringaldehyde	−0.41	0.39*	0.07	0.59*	−0.27
m-coumaric acid + ferulic acid	0.47*	0.03	0.34	0.23	0.09
sinapic acid	0.44*	−0.45*	0.27	0.01	0.53*
subtotal benzoics	−0.41*	0.18	0.10	−0.13	−0.26
subtotal cinnamics	0.44*	−0.42*	0.29	0.04	0.53*
total phenolic acid	0.43*	−0.45*	0.36	−0.07	0.59*
(+)-catechin	−0.14	0.19	0.71*	0.55*	0.25
(−)-epicatechin	0.37	−0.09	0.51*	0.46*	0.56*
luteolin	0.22	0.19	−0.13	−0.12	−0.24
total flavonoids	0.23	−0.01	0.63*	0.53*	0.50*

[a] *, correlation is significant at the 0.05 level (two-tailed). **, correlation is significant at the 0.0001 level (two-tailed). $N = 11$. [b] The antioxidant data are from our previous publication (*12*). TPC stands for total phenolic acid and CTC for condensed tannins content. The full names of the antioxidant properties are given under Abbreviations Used.

substances in the extract may have synergistic effect, which is affected by varying test conditions. In addition, it is increasingly apparent that phenolics can display various biological activities (anticancer, prevention of cardiovascular diseases) through a number of molecular mechanisms and that not all of these activities could be directly related to the molecular function as antioxidants (*34*).

Correlations between different antioxidant assays were also tested. The reciprocal values of cellular antioxidant activities showed significant ($r = 0.37$, $p < 0.05$) linear correlation with ORAC. The PRSC values showed significant ($r = 0.37$, $p < 0.05$) linear correlation with FRAP. There were no significant linear correlations between any other two assays. Different antioxidant test methods utilize different reaction mechanisms. The ORAC involves chemical reactions for removing reactive oxygen species by utilizing the hydrogen transfer mechanism, whereas the DPPH utilizes the electron transfer mechanism (*35*). Each chemical antioxidant test has its strengths and weaknesses because each is based upon detecting antioxidant interference with well-known, but limited, chemical reactions (*36*). Cell-based testing systems in vitro eliminate many of the limitations of the chemical interaction based assays. The positive aspect is that the results are more relevant for biological systems (*17*). One of the negative aspects is that a singular value is no longer easily obtainable (*36*). To our knowledge, cell response to antioxidant exposure is quite complex, depending on exposure dose and time, properties of cell lines, and growth status of cells. As compared with chemical interference of conventional chemical antioxidant assays, more complex biological factors will affect cell-based antioxidant assay. For example, the level of basal reactive oxygen species (ROS) produced by the cell mitochondria influences cell proliferative signal transduction, some antioxidants can induce programmed cell death (apoptosis), and sample solubility in culture medium affects antioxidant assay. In addition, the properties of cell lines are one of the critical factors that affect cellular antioxidant determination. For example, some cell lines such as MCF-7 and LNCap contain estrogen receptor or androgen receptor. Many phenolic compounds (such as genistein) act as phytoestrogens when exposed to these cells. Due to the complex reasons described above, it is very difficult to interpret their cellular antioxidant effects. Under certain physiological situation, and certain exposure dose or time, antioxidants can be prooxidants. Therefore, the cell-based antioxidant assay has both positive and negative consequences. We must carefully choose a rational model to explain health-promoting effects of phytochemicals. Gastric cell line AGS was employed in our current preliminary cellular antioxidant assay instead of hepatocarcinoma cell line HepG2 due to the rapid proliferation properties of AGS. On the other hand, the use of HepG2 received the criticism that HepG2 has altered functional response (increased catalase mRNA expression) to oxidative stress and performed asymmetrical cell divisions (*36*). However, as a preliminary research, we do not know for sure the AGS cell line is the best choice for cellular antioxidant assay because this cell's physiological significance may suffer in the same manner as the HepG2 cell lines. Therefore, more comprehensive investigations should be undertaken to create a standardized cellular antioxidant assay method.

In summary, 11 lentil samples were analyzed for antioxidant properties and individual phenolic compositions of four major phenolic groups, including phenolic acids, anthocyanins, flavones, and flavan-3-ols. The different cultivars of lentils showed considerable variations in their individual phenolic compounds and chemical and cellular antioxidant activities. Cultivar Morton was found to have the highest individual flavan-3-ols (catechin and epicatechin) and total flavonoids as well as the highest antioxidant properties (PRSC and CAA). 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 lentils with high phenolic content and high antioxidant properties for use as ingredients for manufacturing functional foods or nutraceuticals for promoting consumer health.

ABBREVIATIONS USED

AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; AUC, area under curve; CAA, cellular antioxidant activity; DCFH-DA, 2′,7′-dichlorofluorescin diacetate; DMSO, dimethyl sulfoxide; DPPH, 2-diphenyl-1-picrylhydrazyl radical; FBS, fetal bovine serum; FRAP, ferric reducing antioxidant power; HBSS, Hanks' balanced salt solution; ORAC, oxygen radical absorbing capacity; CTC, condensed tannin content; PBS, phosphate-buffered saline; PRSC, peroxyl radical scavenging activity; TPC, total phenolic content.

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