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Cellular Antioxidant Activity of Common Vegetables

Wei Song,[†] Christopher M. Derito,[†] M. Keshu Liu,[†] Xiangjiu He,[†] Mei Dong,[†] and Rui Hai Liu*,[†,‡]

[†]Department of Food Science and [‡]Institute of Comparative and Environmental Toxicology, Cornell University, Ithaca, New York 14853-7201

The measurement of antioxidant activity using biologically relevant assays is important to screen fruits, vegetables, natural products, and dietary supplements for potential health benefits. The cellular antioxidant activity (CAA) assay quantifies antioxidant activity using a cell culture model and was developed to meet the need for a more biologically representative method than the popular chemistry antioxidant capacity measures. The objective of the study was to determine the CAA, total phenolic contents, and oxygen radical absorbance capacity (ORAC) values of 27 vegetables commonly consumed in the United States. Beets, broccoli, and red pepper had the highest CAA values, whereas cucumber had the lowest. CAA values were significantly correlated to total phenolic content. Potatoes were found to be the largest contributors of vegetable phenolics and CAA to the American diet. Increased fruit and vegetable consumption is an effective strategy to increase antioxidant intake and decrease oxidative stress and may lead to reduced risk of developing chronic diseases, such as cancer and cardiovascular disease.

KEYWORDS: Vegetables; antioxidant; antioxidant activity; flavonoids; cancer; free radicals; cellular antioxidant activity

INTRODUCTION

Free radicals are reactive molecules with unpaired electrons that are able to exist independently. Endogenous metabolic processes, especially in chronic inflammations, are one of the important sources of free radicals (*1*). Free radicals can react with and damage all types of biomolecules − lipids, proteins, carbohydrates, and DNA (*2*). If damaged DNA is left unrepaired and the mutated cell gains the ability to survive and divide aberrantly, it may become cancerous. Thus, an increase in the consumption of dietary antioxidants, which can scavenge free radicals, may be a strategy to prevent free radical-induced damage to biomolecules of lipids, proteins, and DNA, including LDL oxidation and cancer cell initiation, an important beginning stage of carcinogenesis.

Doll and Peto (*3*) proposed that diet is responsible for about one-third of cancer incidence. Several studies have linked the consumption of fruits and vegetables to a reduced risk of cancer (*4*−*7*). Higher fruit intake in childhood has also been related to lower adult cancer risk (*8*). Fruits and vegetables are rich in bioactive compounds such as flavonoids, phenolic acids, stilbenes, coumarins, and tannins (*9*). The combined phytochemicals in plant foods have a wide variety of mechanisms of action, including antioxidant activity and quenching free radicals, regulation of cell cycle, effects on oncogene and tumor suppressor gene expression, apoptosis, detoxifying enzyme activity, immunity, metabolism, and infection (*9*). In a study that evaluated the effect of antioxidant activity on gastric cancer risk, antioxidant activity obtained from fruit and vegetable consumption was inversely associated with risk of gastric cancer (*10*). The latest report by the Economic Research Service described that U.S. fruit and vegetable consumption increased between 1970 and 2005, but that Americans are still not eating enough of these plant foods for optimum health (*11*). The 2005 Dietary Guidelines for Americans (*12*) recommends each person eats 2 cups (four servings) of fruit and 2.5 cups (five servings) of vegetables, based on a 2000 kcal diet, but the study found that in 2005, the average intake of fruits was only 0.9 cup and vegetable intake was 1.7 cups per day (*11*).

Due to the potential of antioxidants to decrease the risk of developing chronic diseases including cancer, cardiovascular disease, diabetes, Alzheimer's disease, cataracts, and age-related functional decline, it is important to be able to measure antioxidant activity using biologically relevant assays. The cellular antioxidant activity (CAA) assay was developed to measure the antioxidant activity of antioxidants, dietary supplements, and foods in cell culture (*13*) in response to a need for a more biologically representative method than the chemistry antioxidant activity assays commonly used to screen antioxidant materials for potential biological activity (*14*). The CAA assay utilizes 2′,7′-dichlorofluorescin diacetate (DCFH-DA) as a probe in cultured human HepG2 liver cancer cells (*13*). Nonpolar DCFH-DA is taken up by HepG2 cells by passive diffusion and deacetylated by cellular esterases to form polar 2′,7′-dichlorofluorescin (DCFH), which is trapped within the cells. Peroxyl radicals generated from 2,2′-azobis(2-amidinopropane) (ABAP) lead to the oxidation of DCFH to form a fluorescent compound dichlorofluorescein (DCF). The level of fluorescence formed within the cells is

*Address correspondence to this author at the Department of Food Science, Stocking Hall, Cornell University, Ithaca, NY 14853-7201 [telephone (607) 255-6235; fax (607) 254-4868; e-mail RL23@cornell.edu].

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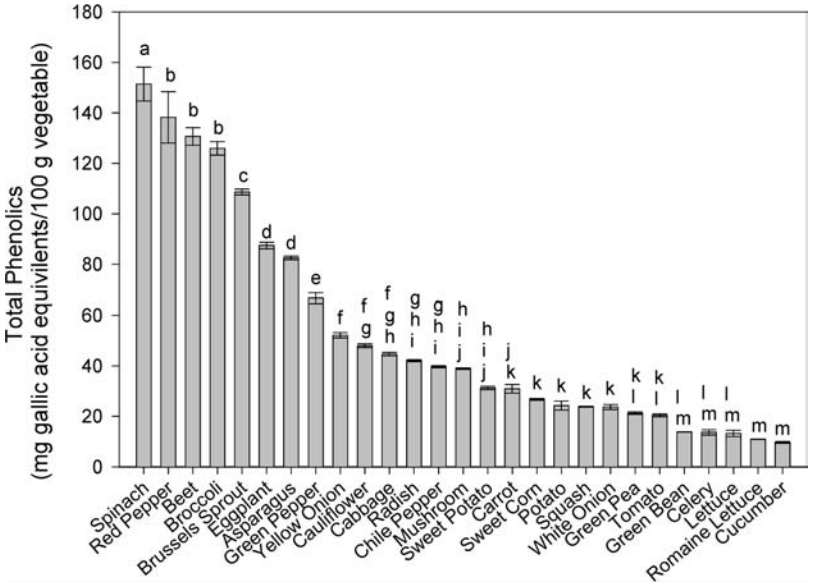

Figure 1. Total phenolic content of selected vegetables (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($p < 0.05$).

proportional to the level of oxidation. Pure phytochemical compounds, antioxidants, and fruit extracts quench peroxyl radicals and inhibit the generation of fluorescent DCF. The decrease in cellular fluorescence compared to the control cells indicates the antioxidant capacity of the compounds (*13*, *15*, *16*).

The antioxidant activity of vegetables has been surveyed using the oxygen radical absorbance capacity (ORAC) assay, the total oxyradical scavenging capacity (TOSC) assay, the ferric reducing/antioxidant power (FRAP) assay, the Trolox equivalent antioxidant capacity (TEAC) assay, and the total radical-trapping antioxidant parameter (TRAP) assay (*17*−*21*). The CAAs of a wide variety of fruits have been reported (*13*, *16*), but the CAAs of vegetables have not been measured.

The objective of this study was to determine the cellular antioxidant activity of 27 commonly consumed vegetables in the United States using the CAA assay. The total phenolic content and ORAC values of the vegetables were also measured to determine if they could be used to predict CAA values. The antioxidant quality of the vegetables in the CAA assay and their individual contributions to the antioxidant activity of vegetables in the American diet were also calculated.

MATERIALS AND METHODS

Reagents. DCFH-DA, fluorescein disodium salt, 6-hydroxy-2,5,7,8-tetramethylchoman-2-carboxylic acid (Trolox), Folin−Ciocalteu reagent, and quercetin dehydrate were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). ABAP was purchased from Wako Chemicals USA, Inc. (Richmond, VA). Dimethyl sulfoxide was obtained from Fisher Scientific (Pittsburgh, PA), and gallic acid was purchased from ICN Biomedical Inc. (Costa Mesa, CA). Phosphate-buffered saline (PBS), sodium carbonate, methanol, acetone, and potassium phosphate were purchased from Mallinckrodt Baker, Inc. (Phillipsburg, NJ). HepG2 human liver cancer cells were obtained from the American Type Culture Collection (ATCC) (Rockville, MD). Williams' Medium E (WME) and Hanks' Balanced Salt Solution (HBSS) were purchased from Gibco Life Technologies (Grand Island, NY). Fetal bovine serum (FBS) was obtained from Atlanta Biologicals (Lawrenceville, GA).

Preparation of Vegetable Extracts. Vegetables were purchased from a local supermarket (Ithaca, NY). Vegetable phytochemical extracts were prepared from the edible portions of vegetables using a modified method, as reported previously (*18*, *22*, *23*). Briefly, in triplicate, fresh vegetable samples were blended in a Waring blender using chilled 80% acetone (1:2, w/v) for 5 min. Samples were then homogenized with a Polytron homogenizer for 3 min. The vegetable slurries were filtered (Whatman no. 1) and washed twice with 10 mL of the acetone solution, and the filtrates were evaporated to dryness using a rotary evaporator at 45 °C. The extracts were reconstituted in 70% methanol and stored at −40 °C. Before use, the methanol was evaporated under a stream of nitrogen, and the extracts were reconstituted in water. Control extracts were prepared using the same extraction solvents and procedures without vegetables.

Preparation of Solutions. A 200 mM stock solution of DCFH-DA in methanol was prepared, aliquoted, and stored at −20 °C until use. A 200 mM ABAP stock solution in water was prepared, aliquoted, and stored at −40 °C until use. Quercetin solutions were prepared in dimethyl sulfoxide before further dilution in treatment medium (WME with 2 mM L-glutamine and 10 mM Hepes).

Cell Culture. HepG2 cells were grown in Complete Medium (WME supplemented with 5% FBS, 10 mM Hepes, 2 mM L-glutamine, 5 μg/mL insulin, 0.05 μg/mL hydrocortisone, 50 units/mL penicillin, 50 μg/mL streptomycin, and 100 μg/mL gentamycin) and were maintained at 37 °C and 5% CO_2 as described previously (*24*, *25*). Cells used in this study were between passages 12 and 32.

Cytotoxicity. The cytotoxicity of each vegetable toward HepG2 cells was measured, as described previously (*26*, *27*). The median cytotoxic concentration (CC_{50}) was calculated for each vegetable.

CAA of Vegetable Extracts. The CAA of vegetable extracts was determined using the protocol described previously by our laboratory (*13*, *16*). Briefly, HepG2 cells were seeded at a density of 6×10^4/well on a 96-well microplate in 100 μL of Complete Medium/well. Twenty-four hours after seeding, the growth medium was removed, and the wells were washed with 100 μL of PBS. Wells were then treated with 100 μL of treatment medium containing solvent control, control extracts, or tested vegetable extracts plus 25 μM DCFH-DA for 1 h. When a PBS wash was utilized, wells were washed with 100 μL of PBS. Then 600 μM ABAP was applied to the cells in 100 μL of oxidant treatment medium (HBSS with 10 mM Hepes), and the 96-well microplate was placed into a Fluoroskan Ascent FL plate reader at 37 °C. Emission at 538 nm was measured after excitation at 485 nm every 5 min for 1 h.

Quantification of CAA. After blank subtraction and subtraction of the initial fluorescence values, the area under the curve for fluorescence versus time was integrated to calculate the CAA value at each concentration of vegetable as (*13*, *16*)

$$\text{CAA unit} = 1 - \left(\int \text{SA} / \int \text{CA} \right)$$

where $\int$SA is the integrated area under the sample fluorescence versus time curve and $\int$CA is the integrated area from the control curve. The median effective dose (EC_{50}) was determined for the vegetable extracts from the median effect plot of $\log(f_a/f_u)$ versus log(dose), where f_a is the fraction

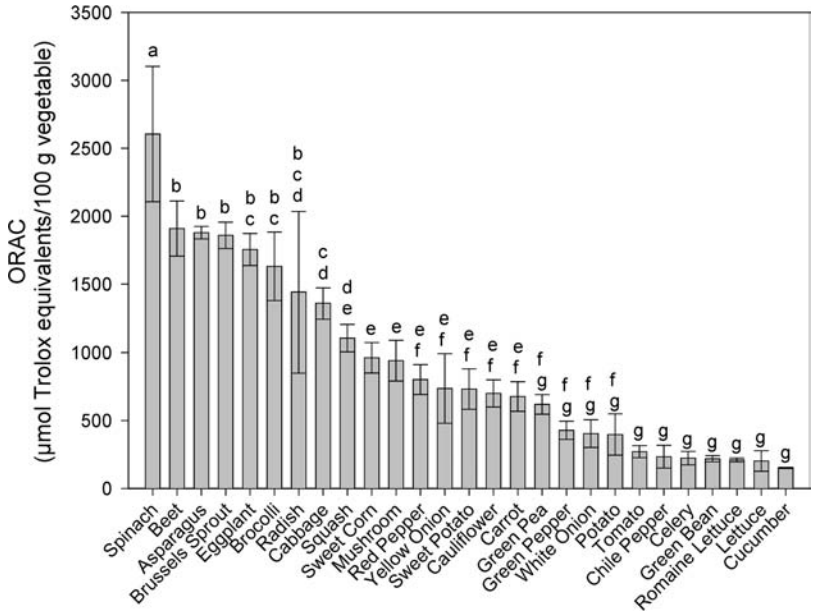

Figure 2. ORAC values of selected vegetables (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($p < 0.05$).

Table 1. Cellular Antioxidant Activities of Selected Vegetables Expressed as EC_{50} and CAA Values (Mean ± SD, $n = 3$)

	no PBS wash		PBS wash		cytotoxicity
vegetable	EC_{50} (mg/mL)	CAA (μmol of QE/100 g)	EC_{50} (mg/mL)	CAA (μmol of QE/100 g)	CC_{50} (mg/mL)
beet	19.3 ± 4.1	41.9 ± 6.2	134 ± 10	4.78 ± 0.38	>150
red pepper	19.2 ± 0.9	41.4 ± 1.8	138 ± 6	4.64 ± 0.19	>150
eggplant	21.0 ± 1.3	37.9 ± 2.4	148 ± 16	4.35 ± 0.48	>150
Brussels sprout	22.7 ± 2.3	35.3 ± 3.6	160 ± 34	4.15 ± 0.98	>150
broccoli	26.3 ± 2.7	30.4 ± 3.0	115 ± 15	5.61 ± 0.68	>150
cabbage	71.0 ± 8.1	21.0 ± 2.4	221 ± 20	2.90 ± 0.25	>150
mushroom	52.7 ± 1.6	15.1 ± 0.4	182 ± 5	3.52 ± 0.11	>150
asparagus	63.1 ± 1.5	12.6 ± 0.3	148 ± 15	4.35 ± 0.45	>150
green pepper	64.9 ± 6.8	12.3 ± 1.4	230 ± 16	2.79 ± 0.20	>150
cauliflower	38.2 ± 4.5	11.3 ± 1.3	726 ± 53	0.88 ± 0.06	>150
spinach	79.5 ± 4.6	10.0 ± 0.6	281 ± 59	2.90 ± 0.54	>150
carrot	81.5 ± 3.4	9.77 ± 0.40	126 ± 145	5.13 ± 0.58	>150
chili pepper	90.8 ± 7.2	8.80 ± 0.71	356 ± 40	1.81 ± 0.21	>150
sweet potato	93.2 ± 7.0	8.56 ± 0.64	362 ± 34	1.78 ± 0.16	>150
radish	108 ± 2	7.35 ± 0.13	555 ± 70	1.16 ± 0.15	>150
yellow onion	125 ± 2	6.40 ± 0.12	219 ± 2	2.92 ± 0.03	>150
lettuce	157 ± 10	5.07 ± 0.33	231 ± 6	2.77 ± 0.07	>150
potato	169 ± 22	4.76 ± 0.63	460 ± 7	1.39 ± 0.02	>150
white onion	234 ± 21	3.42 ± 0.32	492 ± 47	1.31 ± 0.12	>150
squash	239 ± 15	3.33 ± 0.21	477 ± 47	1.35 ± 0.13	>150
celery	262 ± 8	3.03 ± 0.09	465 ± 14	1.71 ± 0.05	>150
sweet corn	173 ± 18	4.62 ± 0.50	359 ± 65	1.82 ± 0.37	>150
romaine lettuce	331 ± 6	2.40 ± 0.05	800 ± 88	0.80 ± 0.09	>150
green pea	396 ± 25	2.01 ± 0.12	558 ± 41	1.15 ± 0.09	>150
green bean	522 ± 27	1.53 ± 0.08	nq[a]		>150
tomato	nq		690 ± 2	0.93 ± 0.01	>150
cucumber	1265 ± 39	0.63 ± 0.02	nq		>150

[a] nq, EC_{50} is not quantifiable due to low activity.

affected (CAA unit) and f_u is the fraction unaffected (1 − CAA unit) by the treatment. The EC_{50} values were stated as mean ± SD for triplicate sets of data obtained from the same experiment. EC_{50} values were converted to CAA values, which are expressed as micromoles of quercetin equivalents (QE) per 100 g of fresh vegetable, using the mean EC_{50} value for quercetin from five separate experiments.

Determination of Total Phenolic Content. The total phenolic contents of the vegetables were measured using the Folin−Ciocalteu colorimetric method (*28*), as modified by our laboratory (*29*, *30*). Volumes of 0.5 mL of deionized water and 0.125 mL of diluted vegetable extracts were added to a test tube. Folin−Ciocalteu reagent (0.125 mL) was added to the solution and allowed to react for 6 min. Then, 1.25 mL of 7% sodium carbonate solution was aliquoted into the test tubes, and the mixture was diluted to 3 mL with deionized water. The color was developed for 90 min, and the absorbance was read at 760 nm using a MRX II Dynex spectrophotometer (Dynex Technologies, Inc., Chantilly, VA). The measurement was compared to a standard curve of gallic acid concentrations and expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh vegetable ± SD for triplicate vegetable extracts.

Measurement of Total Antioxidant Activity. The total antioxidant activity of selected vegetables was measured using the oxygen radical scavenging capacity (ORAC) assay (*31*) as modified in our laboratory (*15*).

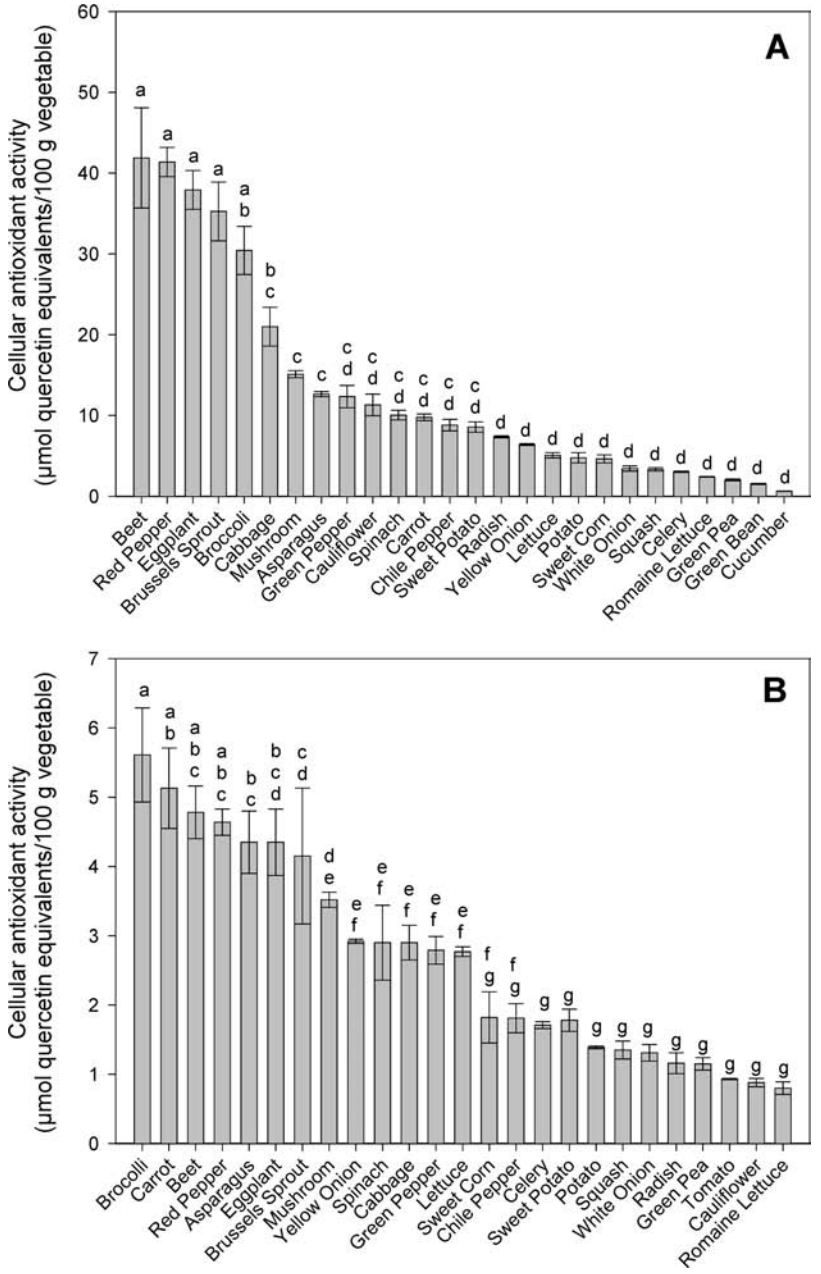

Figure 3. CAA values of selected vegetables in the (**A**) no PBS wash protocol and (**B**) PBS wash protocol (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($p < 0.05$).

Briefly, 20 μL of blank, Trolox standard, or vegetable extracts in 75 mM potassium phosphate buffer, pH 7.4 (working buffer), was added to triplicate wells in a black, clear-bottom, 96-well microplate. A volume of 200 μL of 0.96 μM fluorescein (in working buffer) was added to each well and incubated at 37 °C for 20 min, with intermittent shaking, before the addition of 20 μL of freshly prepared 119 mM ABAP in working buffer using a 12-channel pipetter. The microplate was immediately inserted into a Fluoroskan Ascent FL plate reader (ThermoLabsystems) at 37 °C. The decay of fluorescence at 538 nm was measured with excitation at 485 nm every 4.5 min for 2.5 h. The areas under the fluorescence versus time curve for the samples minus the area under the curve for the blank were calculated and compared to a standard curve of the areas under the curve for 6.25, 12.5, 25, and 50 μM Trolox standards minus the area under the curve for blank. ORAC values were expressed as mean micromoles of Trolox equivalents (TE) per 100 g of vegetable ± SD for triplicate data from one experiment.

Statistical Analyses. All results are presented as mean ± SD, and statistical analyses were performed using Minitab 15 (Minitab Inc., State College, PA). Differences between means were detected by ANOVA, followed by multiple comparisons using Tukey's least significant difference test. ANOVA was performed on log-transformed total phenolic, ORAC, and CAA values because the assumptions of normally distributed residuals and equal variances were not met by the untransformed data. Correlations were determined using linear regression on log-transformed data. The differences between mean EC_{50} values for CAA, comparing the results from the no PBS wash and PBS wash protocols, were evaluated using a two-tailed paired Student's t test. The determination of the differences between cellular antioxidant quality for each vegetable was performed using a paired Student's t test on normalized [(antioxidant quality − mean antioxidant quality)/standard deviation for antioxidant qualities in protocol] values for those vegetables with activity in both the no PBS wash and PBS wash protocols. Normalization was necessary because the two values could not be compared directly. For those vegetables with no activity in the PBS wash protocol, the difference between the cellular antioxidant quality in the no PBS wash protocol and zero was determined using a one-way Student's t test. Interaction between the vegetable and the protocol in cellular antioxidant quality was assessed by two-way ANOVA of the normalized antioxidant qualities. Results were considered to be significant when the p value was < 0.05.

Table 2. Cellular Antioxidant Quality of Vegetable Phenolics in the Cellular Antioxidant Activity Assay (Mean ± SD, $n = 3$)

	cellular antioxidant quality[a] (μmol of QE/100 μmol of phenolics)	
vegetable	no PBS wash	PBS wash
cabbage	7.44 ± 0.98 a	1.11 ± 0.1 efg
eggplant	7.38 ± 0.47 b	0.85 ± 0.09 fghij
mushroom	6.6 ± 0.19 c	1.54 ± 0.05 d
lettuce	6.52 ± 0.42 c	3.56 ± 0.09 a
Brussels sprout	5.52 ± 0.57 d	0.65 ± 0.16 hijkl
carrot	5.38 ± 0.22 d	2.82 ± 0.32 b
beet	5.45 ± 0.81 d	0.62 ± 0.05 ijkl
red pepper	5.09 ± 0.22 de	0.57 ± 0.02 ijk
sweet potato	4.66 ± 0.35 e	0.97 ± 0.9 efgh
broccoli	4.11 ± 0.4 f	0.76 ± 0.09 hijk
cauliflower	4.01 ± 0.47 f	0.31 ± 0.02 ghijk
celery	3.78 ± 0.11 fg	2.13 ± 0.06 c
chili pepper	3.77 ± 0.31 fg	0.78 ± 0.09 ghijk
romaine	3.74 ± 0.07 fgh	1.25 ± 0.15 de
potato	3.34 ± 0.45 ghi	0.97 ± 0.01 efgh
green pepper	3.15 ± 0.35 hij	0.71 ± 0.05 jkl
radish	2.97 ± 0.05 ijk	0.47 ± 0.06 kl
asparagus	2.6 ± 0.07 jkl	0.90 ± 0.09 fghij
white onion	2.45 ± 0.23 klm	0.94 ± 0.09 efghi
squash	2.37 ± 0.15 lm	0.96 ± 0.09 efgh
yellow onion	2.08 ± 0.04 lm	0.95 ± 0.01 efghi
green bean	1.87 ± 0.1 mn	nq
green pea	1.61 ± 0.1 no	0.92 ± 0.07 efghi
sweet corn	1.59 ± 0.12 no	1.16 ± 0.24 ef
spinach	1.13 ± 0.07 o	0.33 ± 0.06l
cucumber	1.1 ± 0.03 o	nq
tomato	nq	0.77 ± 0.01 ghijk

[a] Values in each column with no letters in common are significantly different ($p < 0.05$).

RESULTS

Total Phenolic Content. The total phenolic content of selected vegetables (**Figure 1**) was determined from their extracts using the Folin−Ciocalteu method. Of the vegetables tested, spinach had the highest total phenolic content (151 ± 7 mg of GAE/100 g), followed by red pepper, beet, and broccoli (138 ± 10, 131 ± 3, and 126 ± 3 mg of GAE/100 g, respectively), Brussels sprout (109 ± 1 mg of GAE/100 g), eggplant and asparagus (87.4 ± 1.3 and 82.5 ± 0.7 mg of GAE/100 g, respectively), and green pepper (66.7 ± 2.1 mg of GAE/100 g). There was no significant difference between yellow onion (52.1 ± 1.0 mg of GAE/100 g), cauliflower (48.0 ± 0.7 mg of GAE/100 g), and cabbage (44.6 ± 0.7 mg of GAE/100 g). The total phenolic contents of radish (42.1 ± 0.4 mg of GAE/100 g), chili pepper (39.7 ± 0.4 mg of GAE/100 g), mushroom (38.9 ± 0.3 mg of GAE/100 g), and sweet potato (31.3 ± 0.6 mg of GAE/100 g) were also not significantly different from each other. The remaining vegetables in order of total phenolic content were carrot (30.9 ± 1.7 mg of GAE/100 g), sweet corn (26.7 ± 0.4 mg of GAE/100 g), potato (24.2 ± 1.8 mg of GAE/100 g), squash (23.8 ± 0.2 mg of GAE/100 g), white onion (23.7 ± 1.0 mg of GAE/100 g), green pea (21.3 ± 0.5 mg of GAE/100 g), tomato (20.4 ± 0.6 mg of GAE/100 g), green bean (13.9 ± 0.1 mg of GAE/100 g), celery (13.6 ± 1.0 mg of GAE/100 g), lettuce (13.2 ± 1.3 mg of GAE/100 g), romaine lettuce (10.9 ± 0.1 mg of GAE/100 g), and cucumber (9.7 ± 0.4 mg of GAE/100 g).

Total Antioxidant Activity. The total antioxidant activities of the selected vegetables (**Figure 2**) were evaluated using the ORAC assay. Spinach had the greatest peroxyl radical scavenging ability in this method, with an ORAC value of 2605 ± 498 μmol of TE/100 g of vegetable. The next highest ORAC values were obtained from beet (1909 ± 203 μmol of TE/100 g), asparagus (1879 ± 46 μmol of TE/100 g), Brussels sprout (1859 ± 97 μmol of TE/100 g), eggplant (1755 ± 118 μmol of TE/100 g), broccoli (1631 ± 252 μmol of TE/100 g), and radish (1442 ± 593 μmol of TE/100 g of vegetable), which were similar ($p > 0.05$), followed by cabbage (1359 ± 113 μmol of TE/100 g), squash (1107 ± 101 μmol of TE/100 g), sweet corn (962 ± 112 μmol of TE/100 g), mushroom (941 ± 150 μmol of TE/100 g), yellow onion (736 ± 256 μmol of TE/100 g), sweet potato (732 ± 149 μmol of TE/100 g), and cauliflower (700 ± 100 μmol of TE/100 g). The other vegetables had ORAC values of 619 ± 72 μmol of TE/100 g (green pea), 404 ± 102 (white onion), 397 ± 152 (potato), 271 ± 44 (tomato), 234 ± 84 (chili pepper), 223 ± 50 (celery), and 202 ± 76 (lettuce). With a few exceptions, our ORAC data for vegetables correspond well to those reported by the USDA (*32*): only cabbage and squash tested in our study had higher ORAC values.

Table 3. Total Phenolic Content and ORAC Values of Selected Vegetables (Mean ± SD, $n = 3$)

vegetable	total phenolics (mg of GAE/100 g of vegetable)	ORAC (μmol of TE/100 g of vegetable)
spinach	151 ± 7	2605 ± 498
beet	131 ± 3	1909 ± 203
asparagus	82.5 ± 0.7	1879 ± 46
Brussels sprout	109 ± 1	1859 ± 97
eggplant	87.4 ± 1.3	1755 ± 118
broccoli	126 ± 3	1631 ± 252
radish	42.1 ± 0.4	1442 ± 593
cabbage	44.6 ± 0.7	1359 ± 113
squash	23.8 ± 0.2	1107 ± 101
sweet corn	26.7 ± 0.4	962 ± 112
mushroom	38.9 ± 0.3	941 ± 150
red pepper	138 ± 10	802 ± 110
yellow onion	52.1 ± 1.0	736 ± 256
sweet potato	31.3 ± 0.6	732 ± 149
cauliflower	48.0 ± 0.7	700 ± 100
carrot	30.9 ± 1.7	677 ± 109
green pea	21.3 ± 0.5	619 ± 72
green pepper	66.7 ± 2.1	404 ± 102
white onion	23.7 ± 1.0	404 ± 102
potato	24.2 ± 1.8	397 ± 152
tomato	20.6 ± 0.6	271 ± 44
chili pepper	39.7 ± 0.4	234 ± 84
celery	13.6 ± 1.0	223 ± 50
green bean	13.9 ± 0.1	219 ± 23
romaine lettuce	10.9 ± 0.1	210 ± 15
lettuce	13.2 ± 1.3	202 ± 76
cucumber	9.7 ± 0.4	152 ± 4

Cellular Antioxidant Activity. The CAAs of selected vegetables were measured using the CAA assay. The EC_{50} and CAA values for the vegetables, along with their median cytotoxicity doses, are listed in **Table 1**. The cellular antioxidant activities were measured using two protocols (PBS wash and no PBS wash), as described previously (*13*).

The CAA values for the vegetables in the no PBS wash protocol are shown in **Figure 3A** and **Table 1**. Beet, red pepper, eggplant, Brussels sprout, and broccoli had the highest CAA values (41.9 ± 6.2, 41.4 ± 1.8, 37.9 ± 2.4, 35.3 ± 3.6, and 30.4 ± 3.0 μmol of QE/100 g of vegetable, respectively), followed by cabbage (21.0 ± 2.4 μmol of QE/100 g), mushroom (15.1 ± 0.4 μmol of QE/100 g), and asparagus (12.6 ± 0.3 μmol of QE/100 g). These were followed by green pepper, cauliflower, spinach, carrot, chili pepper, sweet potato, radish, yellow onion, lettuce, potato, sweet corn, white onion, squash, celery, romaine lettuce, green pea, green bean, and cucumber. The CAA values for these 18 vegetables were not significantly different from each other. Tomato did not have quantifiable activity with the no PBS wash protocol.

In the PBS wash protocol, broccoli, carrot, beet, and red pepper had the greatest cellular antioxidant activity, with CAA

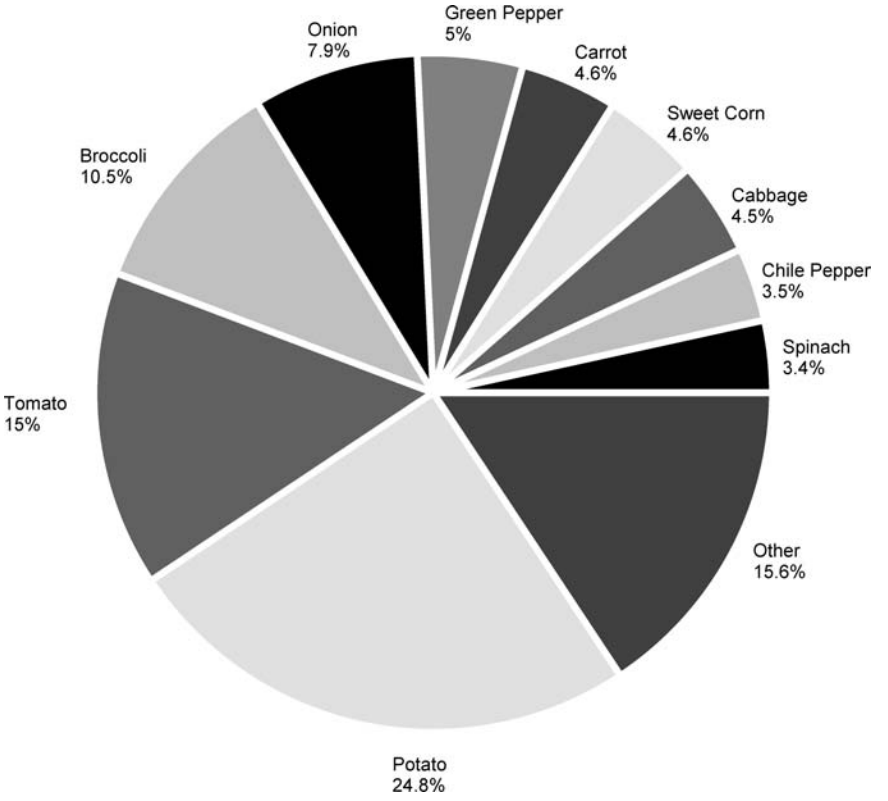

Figure 4. Contribution of total phenolics from selected vegetables as a percent of total phenolics from all vegetables consumed by Americans.

values of 5.61 ± 0.68, 5.13 ± 0.58, 4.78 ± 0.38, and 4.64 ± 0.19 μmol of QE/100 g of vegetable, respectively (**Figure 3B**; **Table 1**), followed by asparagus, eggplant, Brussels sprout, mushroom, yellow onion, spinach, cabbage, green pepper, lettuce, sweet corn, and chili pepper. The rest of the common vegetables (celery, sweet potato, potato, white onion, radish, green pea, tomato, cauliflower, and romaine lettuce) had lower CAA values. The CAA values of green bean and cucumber could not be quantified due to their low activities in the PBS wash protocol.

Correlation Analyses. Using regression analyses, the relationships between total phenolic content, ORAC value, and CAA values for the vegetables were determined. Total phenolics were significantly correlated to ORAC values ($R^2 = 0.603, p < 0.05$) and CAA values from the no PBS wash protocol ($R^2 = 0.645, p < 0.05$) and PBS wash protocols ($R^2 = 0.493, p < 0.05$). ORAC values for vegetables were not significantly positively related to CAA values ($R^2 = 0.343, p > 0.05$ for no PBS wash protocol; $R^2 = 0.293, p > 0.05$ for PBS wash protocol).

Cellular Antioxidant Quality. The cellular antioxidant quality (**Table 2**) of the phytochemical extracts was determined for the vegetables from their CAA values and total phenolic contents (**Table 3**). This is a measurement of the cellular antioxidant activity, in quercetin equivalents, per 100 μmol of phenolic compounds present in the vegetable and was described previously (*13*). The cellular antioxidant quality from the vegetables in the no PBS protocol ranged from 1.1 ± 0.03 (cucumber) to 7.44 ± 0.98 (cabbage) μmol of QE/100 μmol of phenolics. Cabbage was followed by eggplant, mushroom, lettuce, Brussels sprout, carrot, beet, red pepper, sweet potato, broccoli, cauliflower, celery, chili pepper, romaine lettuce, potato, green pepper, radish, asparagus, white onion, squash, yellow onion, green bean, green pea, sweet corn, spinach, cucumber, and tomato. The range of antioxidant qualities in the PBS wash protocol was from 0.31 ± 0.02 (cauliflower) to 3.56 ± 0.09 (lettuce) μmol of QE/100 μmol of phenolics. The remaining vegetables, in order of highest to lowest cellular antioxidant quality in the PBS wash protocol, were carrot, celery, mushroom, romaine lettuce, sweet corn, cabbage, sweet potato, potato, squash, yellow onion, white onion, green pea, asparagus, eggplant, chili pepper, tomato, broccoli, green pepper, Brussels sprout, beet, red pepper, radish, and spinach.

Contribution of Vegetables to Dietary Phenolics and Cellular Antioxidant Activity. The contribution of the selected vegetables to the total phenolics and CAA in the United States from all vegetables in the American diet was calculated from consumption data from the U.S. Department of Agriculture Food Availability (Per Capita) Data for 2008 (*33*). Loss-adjusted food availability data for fresh, canned, frozen, dried, and juice were used, which are adjusted for nonedible vegetable parts and losses due to waste, spoilage, and other factors. The top 10 phenolic contributors expressed as a percentage of the total phenolic contribution from vegetables in the American diet are shown in **Figure 4**. Potatoes were the largest supplier of vegetable phenolics to the population (24.8%), followed by tomato (15.0%), broccoli (10.5%), onion (7.9%), and green pepper (5%). Carrot, sweet corn, cabbage, chili pepper, and spinach were also among the top 10 contributors. From the no PBS wash protocol data (**Figure 5A**), the greatest cellular antioxidant activity contributors were potato (26.8), broccoli (14.0%), carrot (8.1%), lettuce (6.7%), and cabbage (6.4%), followed by onion, green pepper, mushroom, sweet corn, and chili pepper. From the PBS wash protocol data (**Figure 5B**), the contribution of the selected vegetables to cellular antioxidant activity was similar to the phenolic contribution, with potato (23%), carrot (12.5%), tomato (11.3%), lettuce (10.8%), and broccoli (7.6%) providing the most CAA to the American diet and with onion, sweet corn, cabbage, green pepper, and mushroom rounding out the top 10 contributors.

DISCUSSION

The CAA assay was developed to provide a biologically relevant means of quantifying the antioxidant activity of pure

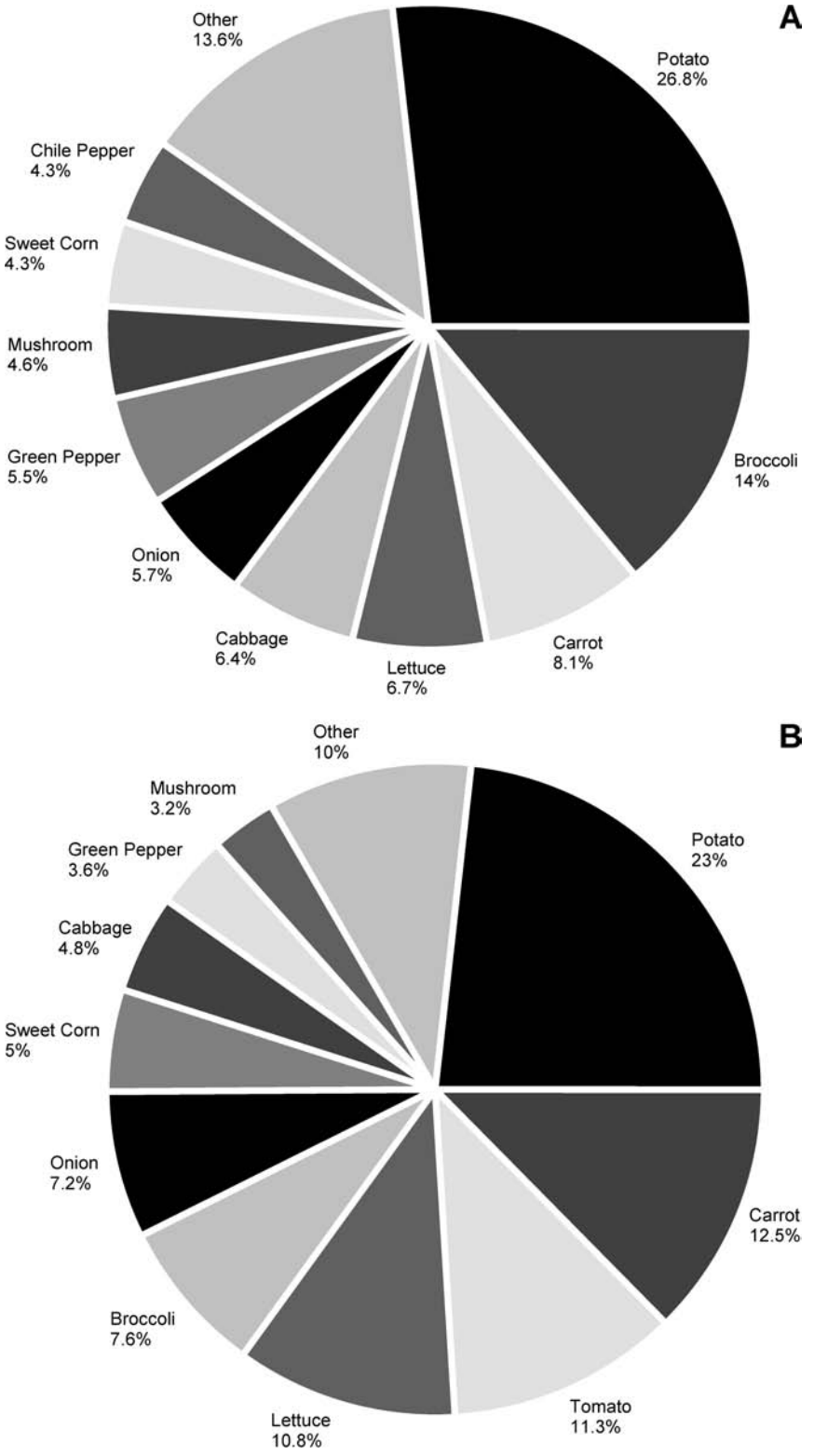

Figure 5. Contribution of CAA from the (**A**) no PBS wash protocol and (**B**) PBS wash protocol from selected vegetables as a percent of total CAA from all vegetables consumed by Americans.

compounds, dietary supplements, and foods in cell culture (*13*). The CAA assay is an improvement over the traditional chemistry-based antioxidant activity assays (ORAC, TOSC, FRAP, TEAC, TRAP) because it mimics some of the cellular processes that occur in vivo. The CAA assay takes into account some aspects of cell uptake, metabolism, and distribution of bioactive compounds, which are important modulators of bioactivity (*34*). Therefore, the CAA assay may provide a better prediction of antioxidant behavior in biological systems. A recent study in our laboratory demonstrated the robust nature of the CAA assay by using it to compare the antioxidant activities of 25 fruits consumed in the United States (*16*). In the present study, 27 vegetables were evaluated using both the CAA assay and the ORAC assay. The resulting data were then compared to the total phenolic content of each vegetable. Of the vegetables tested, beet, red pepper, and broccoli were consistently among the top five

when analyzed by the CAA and ORAC assays (**Figures 2** and **3**) and were also among the highest with respect to total phenolic content (**Figure 1**). These results are consistent with other studies that have reported high antioxidant activities of these three vegetables (*18*, *20*, *35*−*37*). Despite having the highest total phenolic content and ORAC value, spinach did not rank highly in CAA assay, ranking 10th in the PBS wash protocol and 11th in the no PBS wash protocol. Cucumber had the lowest phenolic content, and its CAA value could not be quantified in the PBS wash protocol.

The CAA values for vegetables were positively related to total phenolic content when log-transformed data were analyzed ($p < 0.05$). The correlation coefficients for CAA values and total phenolics for vegetables were $R^2 = 0.645$ ($p < 0.05$) for the no PBS wash protocol and $R^2 = 0.493$ ($p < 0.05$) for the PBS wash protocol. Compared with vegetable extracts, there is a more significant positive relationship between the CAA values and total phenolic content for fruits ($p < 0.05$) (*16*). Generally, the vegetables tested showed lower activities and higher EC_{50} values with the PBS wash protocol compared to the results from the no PBS wash protocol.

Cellular antioxidant quality is a measure of the cellular antioxidant activity provided by 100 μmol of phenolics found in the vegetable, so it gives a measure of the relative potency of the antioxidants present. An index of antioxidant quality, expressed as phenolic content/IC_{50} for inhibition of lipoprotein oxidation, has also been used by Vinson et al. (*38*) to assess fruits. For all vegetables examined in our study, the antioxidant quality was lower and the EC_{50} value was higher in the PBS wash protocol compared to results from the no PBS wash protocol (**Table 2**). This could be attributed to the quercetin standard's aberrant behavior of having higher activity and a lower EC_{50} value in the PBS wash protocol compared to the no PBS wash protocol (*13*, *16*).

The contribution of vegetables toward the total phenolics in the American diet was estimated from our total phenolic measurements and per capita loss-adjusted food availability data for the United States (*33*). Despite having low phenolic contents, potato and tomato were the largest contributors to total phenolics in the American diet (**Figure 4**). This is due to the high per capita consumption of these two vegetables. Other substantial contributors to phenolic intake were broccoli, onion, green pepper, carrot, sweet corn, cabbage, chili pepper, and spinach. Romaine lettuce and lettuce did not rank in the top 10 because of their low phenolic content, despite their high consumption.

Contribution of CAA activity from vegetables in the American diet was also discussed. Potato, broccoli, carrot, lettuce, cabbage, onion, green pepper, mushroom, sweet corn, and chili pepper were the top providers of CAA when assessed using the no PBS wash protocol (**Figure 5A**). Potato, carrot, tomato, and lettuce were the highest contributors when assessed using the PBS wash protocol (**Figure 5B**). Although its antioxidant activity is low, potato ranked high in contribution of total phenolics, CAA, and ORAC due to its high consumption in the American diet.

This study shows the cellular antioxidant activity of 27 common vegetables. Beet, broccoli, and red pepper demonstrated the highest cellular antioxidant activity, whereas cucumber had the lowest activity. CAA values were significantly correlated with total phenolic content ($p < 0.05$), whereas ORAC values for vegetables were not significantly positively related to CAA values. Potatoes were the largest contributor of total phenolics and CAA in the American diet. Antioxidant activity provided by vegetables may be important in the prevention of cancer and other chronic diseases. Measuring the antioxidant activity of vegetables in cell culture is an important step in screening for potential bioactivity and is more biologically representative than data obtained from chemistry antioxidant activity assays. Among the vegetables tested, some had significant biological effects in this assay, warranting further testing in more specific cellular models. Further testing is needed to confirm the relationship between CAA values for vegetables and their modulation of oxidative stress markers in vivo.

ABBREVIATIONS USED

ABAP, 2,2′-azobis(2-amidinopropane) dihydrochloride; CAA, cellular antioxidant activity; DCFH, 2′,7′-dichlorofluorescin; DCFH-DA, 2′,7′-dichlorofluorescin diacetate; FRAP, ferric reducing/antioxidant power; ORAC, oxygen radical absorbance capacity; QE, quercetin equivalents; TE, Trolox equivalents; TEAC, Trolox equivalent antioxidant capacity; TRAP, total radical-scavenging antioxidant parameter.

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