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Cellular Antioxidant Activity of Common Fruits

KELLY L. WOLFE,[†,§] XINMEI KANG,[†,§,#] XIANGJIU HE,[§] MEI DONG,[§] QINGYUAN ZHANG,[#] AND RUI HAI LIU*[,§,⊥]

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

Measurement of antioxidant activity using biologically relevant assays is important in the screening of fruits for potential health benefits. The cellular antioxidant activity (CAA) assay quantifies antioxidant activity in cell culture 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 cellular antioxidant activity, total phenolic contents, and oxygen radical absorbance capacity (ORAC) values of 25 fruits commonly consumed in the United States. Pomegranate and berries (wild blueberry, blackberry, raspberry, and blueberry) had the highest CAA values, whereas banana and melons had the lowest. Apples were found to be the largest contributors of fruit phenolics to the American diet, and apple and strawberries were the biggest suppliers of cellular antioxidant activity. Increasing fruit consumption is a logical strategy to increase antioxidant intake and decrease oxidative stress and may lead to reduced risk of cancer.

KEYWORDS: Fruits; 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 important sources of free radicals (*1*), which 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 antioxidants, which can scavenge free radicals, may be a strategy to prevent 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 associations have been made between fruit and vegetable intake and a reduced risk of cancer (*4*−*10*). Higher fruit intake in childhood has also been related to lower adult cancer risk (*11*). Fruits are rich in bioactive phenolic compounds such as flavonoids, phenolic acids, stilbenes, coumarins, and tannins. The combined phytochemicals in plant foods have a variety of mechanisms of action, including effects on antioxidant activity and free radicals, cell cycle, oncogene and tumor suppressor gene expression, apoptosis, detoxifying enzyme activity, immunity, metabolism, and infection (*12*). 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 (*13*). The latest report by the Economic Research Service states 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 (*14*). The 2005 Dietary Guidelines for Americans (*15*) recommend 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 cups and vegetable intake was 1.7 cups per day (*14*).

Due to the potential of antioxidants to decrease the risk of developing cancer and other chronic diseases, 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 (*16*). The CAA assay utilizes a 2′,7′-dichlorofluorescin (DCFH) probe in cultured human HepG2 liver cancer cells, which fluoresces when oxidized by peroxyl radicals to 2′,7′-dichlorofluorescein. It was developed 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 (*17*). The antioxidant activity of fruits has been surveyed using the oxygen radical absorbance capacity (ORAC) assay (*18, 19*), inhibition of cupric ion-induced oxidation of lipoproteins (*20*), total oxyradical scavenging

* 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].

† These authors equally contributed to the manuscript.

§ Department of Food Science, Cornell University.

Harbin Medical University.

⊥ Institute of Comparative and Environmental Toxicology, Cornell University.

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capacity (TOSC) assay (*21*), ferric reducing/antioxidant power (FRAP) assay (*19, 22, 23*), Trolox equivalent antioxidant capacity (TEAC) assay (*19, 23*), and total radical-trapping antioxidant parameter (TRAP) assay (*23*). The antioxidant activities of a wide variety of fruits in a cell-based model have not been measured.

The objective of this study was to determine the cellular antioxidant activity of 25 commonly consumed fruits using the CAA assay. The total phenolic contents and ORAC values of the fruits were also measured to determine if they could be used to predict CAA values. The antioxidant quality of the fruits in the CAA assay and their individual contributions to the antioxidant activity of fruits in the American diet were calculated.

MATERIALS AND METHODS

Chemicals. 2′,7′-Dichlorofluorescin diacetate (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). Dimethyl sulfoxide was obtained from Fisher Scientific (Pittsburgh, PA), and 2,2′-azobis (2-amidinopropane) dihydrochloride (ABAP) was purchased from Wako Chemicals USA, Inc. (Richmond, VA). Sodium carbonate, methanol, acetone, and potassium phosphate were bought from Mallinckrodt Baker, Inc. (Phillipsburg, NJ), and gallic acid was from ICN Biomedical Inc. (Costa Mesa, CA). HepG2 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 Fruit Extracts. Apples were purchased from Cornell Orchards (Cornell University, Ithaca, NY), and wild blueberries were obtained from the Wild Blueberry Association of North America (Damariscotta, ME). All other fruits were purchased at a local supermarket (Ithaca, NY). Fruit phytochemical extracts were prepared from the edible portions of fruits using a modified method, as reported previously (*21*). Briefly, in triplicate, fresh fruit samples were blended for 5 min in chilled 80% acetone (1:2, w/v) using a Waring blender. Samples were then homogenized with a Polytron homogenizer for 3 min. The homogenates were filtered through Whatman no. 1 paper, and the filtrates were evaporated to dryness under vacuum at 45 °C. The samples 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.

Preparation of Solutions. A 200 mM stock solution of DCFH-DA in methanol was prepared, aliquoted, and stored at −20 °C. A 200 mM ABAP stock solution in water was prepared, and aliquots were stored at −40 °C. 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 growth 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 (*16*). Cells used in this study were between passages 12 and 32.

Cytotoxicity. The cytotoxicity of fruits toward HepG2 cells was measured, as described previously (*16, 24*). The median cytotoxic concentration (CC_{50}) was calculated for each fruit.

Cellular Antioxidant Activity (CAA) of Fruit Extracts. The CAA assay protocol was described previously (*16*). Briefly, HepG2 cells were seeded at a density of 6×10^4/well on a 96-well microplate in 100 μL of growth medium/well. Twenty-four hours after seeding, the growth medium was removed, and the wells were washed with PBS. Wells were treated in triplicate for 1 h with 100 μL of treatment medium containing tested fruit extracts plus 25 μM DCFH-DA. 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 HBSS, and the 96-well microplate was placed into a Fluoroskan Ascent FL plate reader (ThermoLabsystems, Franklin, MA) 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 initial fluorescence values, the area under the curve for fluorescence versus time was integrated to calculate the CAA value at each concentration of fruit as (*16*)

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

where ∫SA is the integrated area under the sample fluorescence versus time curve and ∫CA is the integrated area from the control curve. The median effective dose (EC_{50}) was determined for the fruits from the median effect plot of $\log(f_a/f_u)$ versus log(dose), where f_a is the fraction 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, expressed as micromoles of quercetin equivalents (QE) per 100 g of fruit, using the mean EC_{50} value for quercetin from five separate experiments.

Determination of Total Phenolic Content. The total phenolic contents of the fruits were measured using a modified colorimetric Folin−Ciocalteu method (*16, 25*). Volumes of 0.5 mL of deionized water and 0.125 mL of diluted fruit 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 fruit ± SD for triplicate fruit extracts.

Measurement of Oxygen Radical Scavenging Capacity (ORAC). The peroxyl radical scavenging efficacy of selected fruits was measured using the ORAC assay (*26*). Briefly, 20 μL of blank, Trolox standard, or fruit 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. The triplicate samples were distributed throughout the microplate and were not placed side-by-side, to avoid any effects on readings due to location. In addition, no outside wells were used, as use of those wells results in greater variation. 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 fruit ± 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 Fisher'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. 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. Determination of differences between cellular antioxidant quality for each fruit 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 fruits with activity in both the no PBS wash and PBS wash protocols. Normalization was necessary

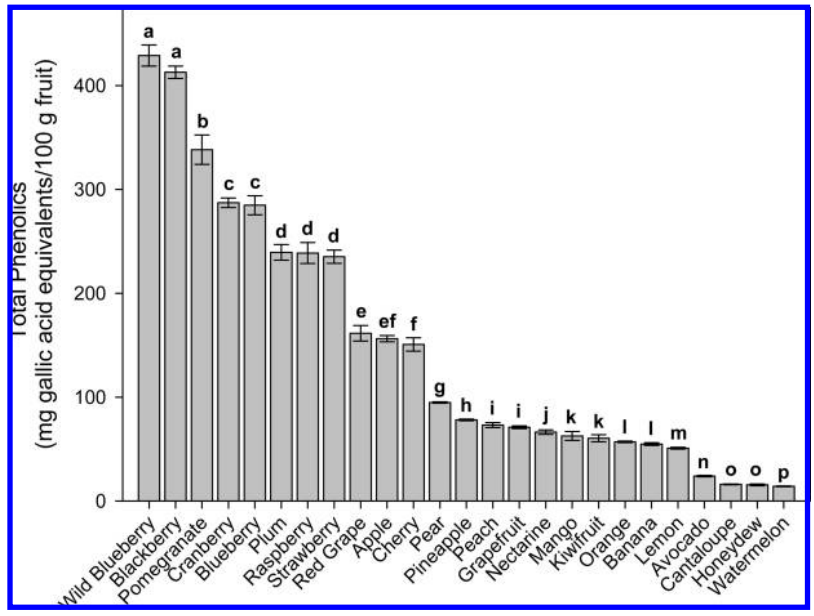

Figure 1. Total phenolic content of selected fruits (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($p < 0.05$).

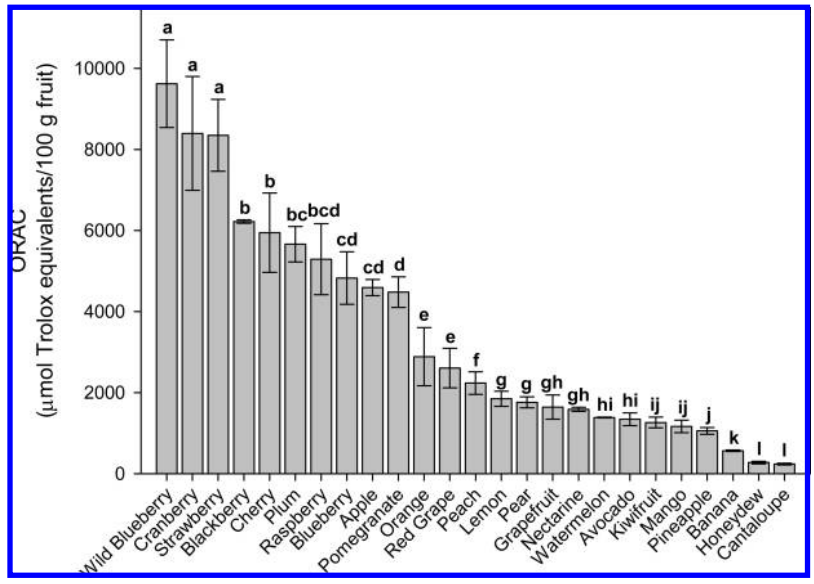

Figure 2. ORAC values of selected fruits (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($p < 0.05$).

because the two values could not be compared directly. For those fruits 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 fruit 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 *p* value < 0.05.

RESULTS

Total Phenolic Content. The total phenolic content of selected fruits (**Figure 1**) was determined from their extracts using the Folin−Ciocalteu method. Among the fruits, wild blueberry and blackberry had the highest total phenolic contents (429 ± 10 and 412 ± 6 mg of GAE/100 g, respectively), followed by pomegranate (338 ± 14 mg of GAE/100 g); cranberry and blueberry (287 ± 5 and 285 ± 9 mg of GAE/100 g, respectively); plum, raspberry, and strawberry (239 ± 7, 239 ± 10, and 235 ± 6 mg of GAE/100 g, respectively); and red grape and apple (161 ± 7 and 156 ± 3 mg of GAE/100 g, respectively). The total phenolic content of cherry (151 ± 6 mg of GAE/100 g) was not significantly different from that of apple. The remaining fruits in order of total phenolic content were pear (94.8 ± 0.7 mg GAE/100 g) > pineapple (78.1 ± 0.8 mg of GAE/100 g) > peach (73.1 ± 2.4 mg of GAE/100 g) = grapefruit (71.0 ± 1.3 mg of GAE/100 g) > nectarine (66.3 ± 2.1 mg of GAE/100 g) > mango (62.6 ± 4.2 mg of GAE/100 g) = kiwifruit (60.4 ± 3.3 mg of GAE/100 g) > orange (56.9 ± 0.8 mg of GAE/100 g) = banana (54.8 ± 1.3 mg of GAE/100 g) > lemon (50.8 ± 0.9 mg of GAE/100 g) > avocado (23.9 ± 0.7 mg of GAE/100 g) > cantaloupe (16.0 ± 0.4 mg of GAE/100 g) = honeydew (15.5 ± 0.9 mg of GAE/100 g) > watermelon (14.1 ± 0.3 mg of GAE/100 g).

ORAC. The antioxidant activities of the selected fruits (**Figure 2**) were evaluated using the ORAC assay. Wild blueberry, cranberry, and strawberry had the greatest peroxyl radical scavenging ability in this method, with ORAC values of 9621 ± 1080, 8394 ± 1405, and 8348 ± 888 μmol of TE/100 g of fruit, respectively. The next highest ORAC values were obtained from blackberry (6221 ± 43 μmol of TE/100 g), cherry (5945 ± 978 μmol of TE/100 g), plum (5661 ± 440 μmol of TE/100 g), and raspberry (5292 ± 877 μmol of TE/100 g of

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

	no PBS wash		PBS wash		cytotoxicity
fruit	EC_{50}[b] (mg/mL)	CAA (μmol of QE/100 g)	EC_{50}[b] (mg/mL)	CAA (μmol of QE/100 g)	CC_{50}[c] (mg/mL)
wild blueberry	2.53 ± 0.10	292 ± 11	6.77 ± 1.05	74.1 ± 12.5	>150
pomegranate[a]	2.95 ± 0.11	250 ± 10	3.03 ± 0.07	163 ± 3.6	>150
blackberry[a]	3.19 ± 0.15	232 ± 11	3.21 ± 0.14	154 ± 6.8	>150
strawberry	5.46 ± 0.66	136 ± 18	11.8 ± 0.9	42.2 ± 3.3	>150
blueberry	5.95 ± 1.33	128 ± 30	27.0 ± 6.2	19.0 ± 4.7	>150
raspberry	6.52 ± 0.60	114 ± 11	14.2 ± 0.9	35.0 ± 2.3	>150
cranberry[a]	15.6 ± 2.3	47.9 ± 6.5	14.7 ± 0.8	33.6 ± 2.0	>150
plum	22.9 ± 5.5	33.5 ± 8.6	38.3 ± 0.3	12.9 ± 0.1	>150
cherry	27.3 ± 3.9	27.4 ± 4.1	73.0 ± 7.7	6.81 ± 0.8	>150
apple[a]	34.4 ± 6.0	21.9 ± 4.0	29.0 ± 3.4	17.2 ± 2.0	>150
red grape[a]	45.3 ± 1.4	16.3 ± 0.5	39.6 ± 5.5	12.6 ± 1.8	>150
kiwifruit	46.4 ± 7.2	16.1 ± 2.6	108 ± 8	4.58 ± 0.31	76.1 ± 4.6
mango	48.5 ± 4.6	15.3 ± 1.5	78.0 ± 2.6	6.33 ± 0.21	>150
pineapple	49.8 ± 2.6	14.8 ± 0.8	NQ		>150
orange	54.0 ± 2.8	13.7 ± 0.7	NQ		68.5 ± 14.9
lemon	60.3 ± 4.9	12.3 ± 1.0	134 ± 6	3.68 ± 0.16	ND
grapefruit	63.4 ± 3.2	11.6 ± 0.6	NQ		63.9 ± 4.3
peach[a]	78.2 ± 6.4	9.47 ± 0.82	81.7 ± 21.7	6.31 ± 1.53	>150
pear[a]	101 ± 10	7.35 ± 0.67	96.5 ± 7.2	5.13 ± 0.40	>150
nectarine	108 ± 13	6.91 ± 0.89	NQ		>150
honeydew	183 ± 12	4.03 ± 0.28	NQ		>150
avocado	207 ± 17	3.58 ± 0.29	NQ		24.3 ± 0.1
cantaloupe	209 ± 21	3.54 ± 0.35	NQ		>150
banana	235 ± 16	3.15 ± 0.21	NQ		>150
watermelon	NQ		NQ		>150

[a] EC_{50} values obtained from the no PBS wash and PBS wash protocols are not significantly different ($p > 0.05$). [b] NQ, EC_{50} values are not quantifiable due to low activity. [c] ND, CC_{50} values are not quantifiable due to lack of dose−response.

fruit), which were similar ($p > 0.05$). The other fruits had ORAC values of 4826 ± 649 μmol of TE/100 g (blueberry), 4592 ± 201 μmol of TE/100 g (apple), 4479 ± 378 μmol of TE/100 g (pomegranate), 2887 ± 717 μmol of TE/100 g (orange), 2605 ± 487 μmol of TE/100 g (red grape), 2235 ± 278 μmol of TE/100 g (peach), 1848 ± 186 μmol of TE/100 g (lemon), 1759 ± 136 μmol of TE/100 g (pear), 1640 ± 299 μmol of TE/100 g (grapefruit), 1586 ± 51 μmol of TE/100 g (nectarine), 1385 ± 11 μmol of TE/100 g (watermelon), 1343 ± 158 μmol of TE/100 g (avocado), 1262 ± 132 μmol of TE/100 g (kiwifruit), 1164 ± 155 μmol of TE/100 g (mango), 1055 ± 84 μmol of TE/100 g (pineapple), and 565 ± 18 μmol of TE/100 g (banana). Cantaloupe and honeydew melon had the lowest antioxidant capacity in the ORAC assay (237 ± 22 and 274 ± 31 μmol of TE/100 g of fruit, respectively). With a few exceptions, our ORAC data for fruits correspond well with those reported by the U.S. Department of Agriculture (*27*): only strawberry, cherry, red grape, and watermelon tested in our study had higher ORAC values.

CAA. The cellular antioxidant activities of selected fruits were measured using the CAA assay. The EC_{50} and CAA values for the fruits, along with their median cytotoxicity doses, are listed in **Table 1**. The cellular antioxidant activities were measured using two protocols, as described previously (*16*): in the PBS wash protocol, the HepG2 cells were washed with PBS between fruit extract and ABAP treatments; in the no PBS wash protocol, the cells were not washed between treatments. Both protocols were used because the difference between them provides some insight into how the antioxidants interact with the cells. In most cases, the EC_{50} values were significantly lower, and efficacy was higher, in the no PBS wash protocol compared to the PBS wash protocol for each fruit. However, there were no significant differences between the EC_{50} values obtained from the two protocols for pomegranate, blackberry, cranberry, apple, red grape, peach, and pear.

The CAA values for the fruits in the no PBS wash protocol are shown in **Figure 3** and **Table 1**. Wild blueberry had the highest CAA value (292 ± 11 μmol of QE/100 g of fruit), followed by pomegranate and blackberry, which had similar CAA values ($p > 0.05$). Strawberry, blueberry, and raspberry were next and were not significantly different from each other ($p > 0.05$). These were followed by cranberry, plum, cherry, mango, apple, red grape, kiwifruit, pineapple, orange, lemon, grapefruit, peach, pear, nectarine, and honeydew. Banana, cantaloupe, and avocado had the lowest CAA values, among the fruits. Watermelon was the only fruit tested that did not have quantifiable activity.

In the PBS wash protocol, pomegranate and blackberry had the greatest cellular antioxidant activity, with CAA values of 163 ± 4 and 154 ± 7 μmol of QE/100 g of fruit, respectively (**Figure 4**; **Table 1**). Wild blueberry ranked second for efficacy, and strawberry and raspberry were third. In declining order of cellular antioxidant activity, the remaining fruits were cranberry, blueberry, apple, plum, red grape, cherry, mango, peach, pear, and kiwifruit. Lemon had the lowest CAA value (3.68 ± 0.211 μmol of QE/100 g of fruit). Pineapple, orange, peach, nectarine, honeydew, avocado, cantaloupe, banana, and watermelon all had very low activities that could not be quantified in the PBS wash protocol.

Correlation Analyses. Using regression analyses, the relationships between total phenolic content, ORAC value, and CAA value for the fruits were determined. Total phenolics were significantly correlated to ORAC values ($R^2 = 0.761$, $p < 0.05$) and CAA values from the no PBS wash protocol ($R^2 = 0.811$, $p < 0.05$) and PBS wash protocols ($R^2 = 0.793$, $p < 0.05$). ORAC values for fruits were also significantly positively related to CAA values, although the correlation coefficients were lower ($R^2 = 0.678$, $p < 0.05$ for no PBS wash protocol; $R^2 = 0.522$, $p < 0.05$ for PBS wash protocol).

Cellular Antioxidant Quality. The cellular antioxidant quality of the phytochemical extracts was determined for the fruits from their CAA values and total phenolic contents (**Table 2**). This is a measurement of the cellular antioxidant activity, in quercetin equivalents, per 100 μmol of phenolic compounds

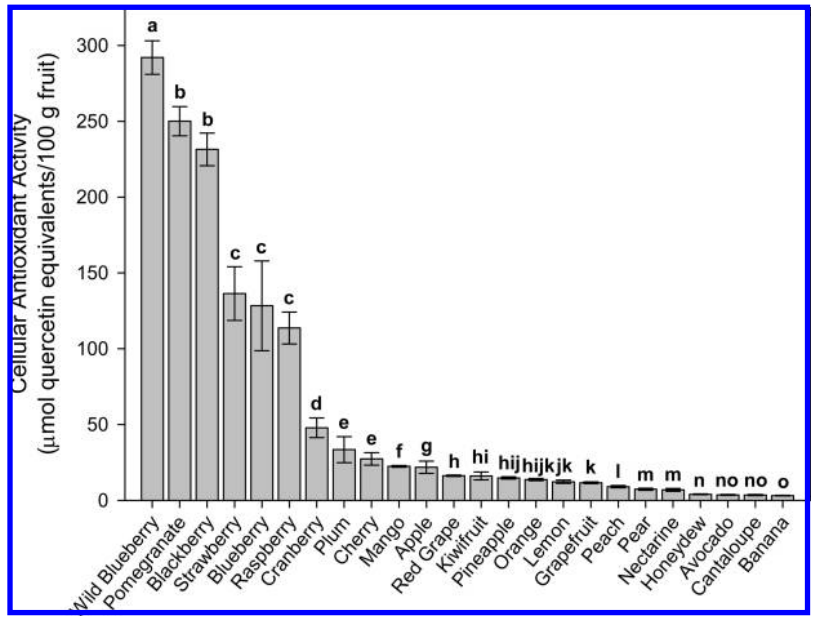

Figure 3. CAA values of selected fruits in the no PBS wash protocol (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($p < 0.05$).

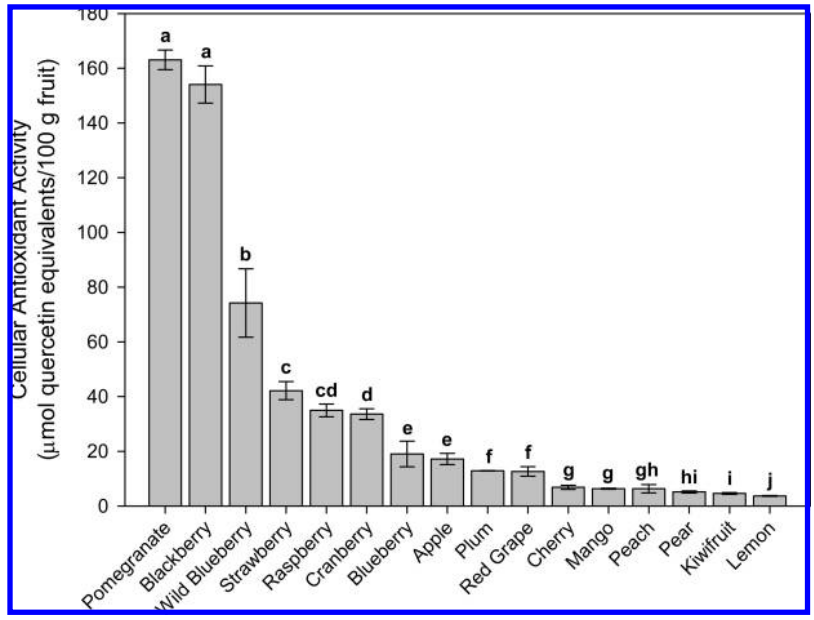

Figure 4. CAA values of selected fruits with quantifiable activity in the PBS wash protocol (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($p < 0.05$).

present in the fruit and was described previously (*16*). The cellular antioxidant quality from the fruits in the no PBS protocol ranged from 1.0 ± 0.1 μmol of QE/100 μmol of phenolics (banana) to 12.6 ± 0.5 μmol of QE/100 μmol of phenolics (pomegranate). Pomegranate was followed by wild blueberry, strawberry, blackberry, raspberry, blueberry, kiwifruit, honeydew, mango, lemon, orange, cantaloupe, pineapple, cherry, cranberry, grapefruit, avocado, apple, plum, peach, nectarine, red grape, and pear. The range of antioxidant qualities in the PBS wash protocol was from 0.8 ± 0.1 μmol of QE/100 μmol of phenolics (cherry) to 8.2 ± 0.2 μmol of QE/100 μmol of phenolics (pomegranate). The remaining fruits, in order of highest to lowest cellular antioxidant quality, were blackberry, strawberry, wild blueberry, raspberry, cranberry, apple, mango, peach, red grape, kiwifruit, lemon, blueberry, pear, and plum. There was a significant interaction between the protocol and fruits ($p < 0.05$). Because the antioxidant qualities of each fruit obtained from the no PBS wash and PBS wash protocols could not be compared directly, the values were normalized. After normalization, it was found that, relative to the other fruits, the antioxidant qualities of pomegranate, blackberry, cranberry, apple, peach, red grape, and pear were significantly lower in the no PBS wash protocol than in the PBS protocol, whereas the antioxidant qualities of wild blueberry, raspberry, and blueberry were higher in the no PBS wash protocol ($p < 0.05$). There was no difference between normalized antioxidant qualities from the two protocols for strawberry, kiwifruit, honeydew, mango, lemon, cantaloupe, pineapple, cherry, plum, and nectarine ($p > 0.05$).

Contribution of Fruits to Dietary Phenolics and Cellular Antioxidant Activity. The contribution of the selected fruits to the total phenolics and CAA in the United States from all fruits in the American diet was calculated from consumption data from the U.S. Department of Agriculture Food Availability

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

	cellular antioxidant quality[d] (μmol of QE/100 μmol of phenolics)	
fruit	no PBS wash	PBS wash
pomegranate[a]	12.6 ± 0.5 a	8.2 ± 0.2 a
wild blueberry[b]	11.6 ± 0.4 b	2.9 ± 0.5 c
strawberry	9.9 ± 1.3 c	3.0 ± 0.2 c
blackberry[a]	9.5 ± 0.4 d	6.3 ± 0.3 b
raspberry[b]	8.1 ± 0.8 d	2.5 ± 0.2 d
blueberry[b]	7.7 ± 1.8d	1.1 ± 0.3 gh
kiwifruit	4.5 ± 0.7 e	1.3 ± 0.1 g
honeydew[c]	4.4 ± 0.3 e	
mango	4.2 ± 0.4 e	1.7 ± 0.1 ef
lemon	4.1 ± 0.3 e	1.2 ± 0.1 gh
orange[c]	4.1 ± 0.2 e	
cantaloupe[c]	3.8 ± 0.4 e	
pineapple[c]	3.2 ± 0.2 f	
cherry	3.1 ± 0.5 fg	0.8 ± 0.1 i
cranberry[a]	2.8 ± 0.4 fg	2.0 ± 0.1 e
grapefruit[c]	2.8 ± 0.1 fg	
avocado[c]	2.5 ± 0.2 fgh	
apple[a]	2.4 ± 0.2 fgh	1.9 ± 0.2 e
plum	2.4 ± 0.6 ghi	0.9 ± 0.0 hi
peach[a]	2.2 ± 0.2 ghi	1.5 ± 0.4 fg
nectarine[c]	1.8 ± 0.2 hij	
red grape[a]	1.7 ± 0.1 hij	1.3 ± 0.2 g
pear[a]	1.3 ± 0.1 ij	0.9 ± 0.1 hi
banana[c]	1.0 ± 0.1 j	
watermelon		

[a] Normalized cellular antioxidant quality from no PBS wash protocol is significantly lower than normalized cellular antioxidant quality from PBS wash protocol ($p < 0.05$). [b] Normalized cellular antioxidant quality from no PBS wash protocol is significantly higher than normalized antioxidant quality from PBS wash protocol ($p < 0.05$). [c] Cellular antioxidant quality for no PBS wash protocol is significantly different from zero ($p < 0.05$). [d] Values in each column with no letters in common are significantly different ($p < 0.05$).

(Per Capita) Data for 2005 (*28*). Loss-adjusted food availability data for fresh, canned, frozen, dried, and juice were used, which are adjusted for nonedible fruit 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 fruits in the American diet are shown in **Figure 5**. Apples were the largest supplier of fruit phenolics to the population (33.1%), followed by orange (14.0%), grape (12.8%), and strawberry (9.8%). Plum, banana, pear, cranberry, pineapple, and peach rounded out the top 10. The contributions of the selected fruits to cellular antioxidant activity, as calculated from the no PBS wash protocol results (**Figure 6A**), were similar to the phenolic contributions, with strawberry (28.8%), apple (23.6%), orange (17.1%), and grape (6.5%) providing the most CAA. Plum, cranberry, blueberry, pineapple, pear, and peach were also top 10 contributors. From the PBS wash protocol data (**Figure 6B**), the most cellular antioxidant activity for fruits, by far, was supplied by apple at 45.6%, followed by strawberry (22.0%) and grape (12.5%). Most of the remaining activity from fruits was contributed by cranberry, plum, pear, peach, blackberry, blueberry, and raspberry.

DISCUSSION

The CAA assay is a valuable new tool for measuring the antioxidant activity of antioxidants, dietary supplements, and foods in cell culture (*16*). It is an improvement over the traditional chemistry antioxidant activity assays 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

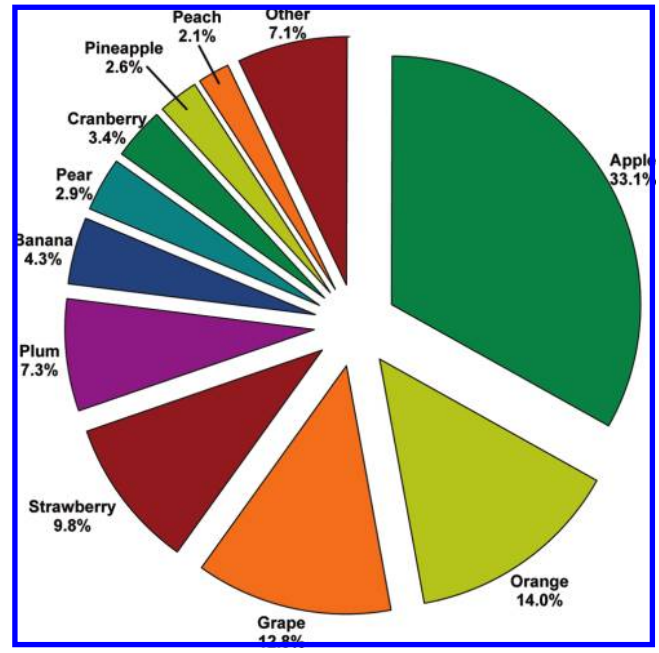

Figure 5. Contribution of total phenolics from selected fruits as a percent of total phenolics from all fruits consumed by Americans.

are important modulators of bioactivity (*29*), so it may better predict antioxidant behavior in biological systems. The assay utilizes HepG2 cells because they yield consistent results with lower coefficient of variation. Results obtained from other cell lines, including intestinal Caco-2 cells and RAW 264.7 cells, were similar to those found using HepG2 cells, but with much higher variation (data not shown). In addition, HepG2 cells are a better model choice to address metabolism issues.

Twenty-five common fruits consumed in the United States were evaluated for their antioxidant activity in the CAA assay. In general, the CAA values of the berries (wild blueberry, blackberry, strawberry, blueberry, raspberry, and cranberry) tended to be the highest (**Figures 3** and **4**). They also had among the most total phenolics (**Figure 1**) and the top ORAC values (**Figure 2**). The high antioxidant efficacy of berries in the CAA and ORAC assays is in agreement with that measured in other antioxidant activity assays (*20, 23*). Berries tend to be rich in anthocyanins, and fruits rich in those flavonoids have high activity in the TEAC, FRAP, and ORAC assays (*19*). Pomegranate had very high activity in the CAA assay, ranking first in the PBS wash protocol and second in the no PBS protocol. Pomegranate also had the highest activity among the fruits tested by Halvorsen et al. (*22*) using the FRAP assay. Despite having a very high total phenolic content, pomegranate did not rank highly in the ORAC assay. The melons had the lowest activities of all the fruits in the CAA assay. They had such low effectiveness using the PBS wash protocol that CAA values could not be quantified. The melons also had low total phenolic contents and low ORAC values. Melons ranked low among fruits in antioxidant activity in other studies (*20, 22, 23*), as well.

The CAA values for fruits were significantly positively related to total phenolic content when log-transformed data were analyzed ($p < 0.05$). The correlation coefficients for CAA values and total phenolics were $R^2 = 0.811$ for the no PBS wash protocol and $R^2 = 0.793$ for the PBS wash protocol. The log-transformed CAA and ORAC values were also significantly correlated ($R^2 = 0.678$ for the no PBS wash protocol; $R^2 = 0.522$ for the PBS wash protocol, $p < 0.05$). This is in contrast to a study involving broccoli extracts, in which prevention of

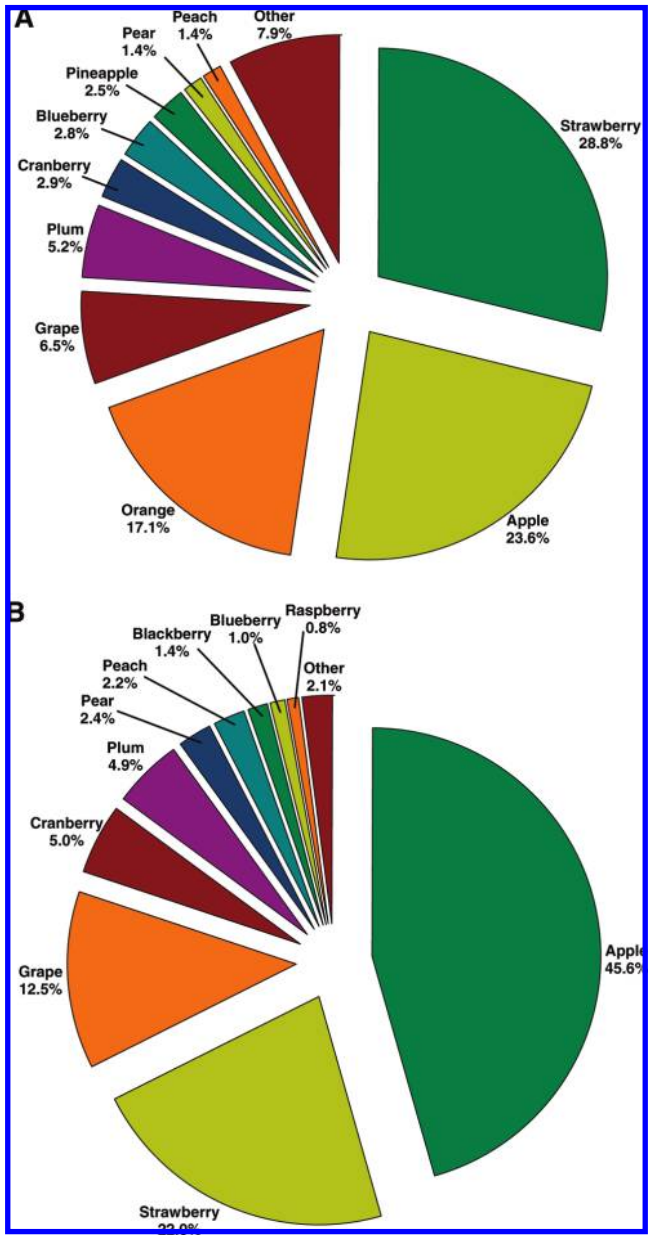

Figure 6. Contribution of (**A**) CAA from no PBS wash protocol and (**B**) CAA from PBS wash protocol from selected fruits as a percent of total cellular antioxidant activity from all fruits consumed by Americans.

DCFH oxidation in HepG2 cells by broccoli extracts was not correlated to ORAC or total phenolics (*30*). From the results of our study, total phenolic content is likely a better predictor for the cellular antioxidant activity of fruits than ORAC value, despite the commonality of measuring peroxyl radical scavenging abilities in both of the antioxidant activity assays.

The EC_{50} values for CAA were similar in the no PBS wash and PBS protocols for pomegranate, blackberry, cranberry, apple, red grape, peach and pear, whereas the rest of the fruits showed lower activities and higher EC_{50} values in the PBS wash protocol. This is likely a reflection of the type and location of the fruit antioxidants in the HepG2 cells. The differences in solubility, molecular size, and polarity of the wide variety of compounds present in fruits and vegetables give each of them unique bioavailability and distribution at the cellular, organ, and tissue levels, allowing for bioactivity at many sites (*12*). Some phenolics, such as quercetin, epigallocatechin gallate, and luteolin, showed similar cellular antioxidant activity in both the no PBS wash protocol and the PBS wash protocol (*16*). Others, such as gallic acid, caffeic acid, and catechin, displayed a dramatic decrease in activity when a PBS wash was done between phytochemical and oxidant (ABAP) treatments, compared when no PBS was performed (*16*). Those phenolics that are better absorbed by the HepG2 cells or tightly bound to the cell membrane are more likely to be present to exert their radical scavenging activities after the cells are washed in the PBS wash protocol than those that are poorly absorbed or only loosely associated with the cell membrane and washed away easily. Thus, the difference in EC_{50} values (and CAA values) between the two protocols is likely a good indicator of the extent of uptake and cell membrane association of the antioxidant compounds present in the fruit extracts.

Cellular antioxidant quality is a measure of the cellular antioxidant activity provided by 100 μmol of phenolics found in the fruit, so it gives a 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. (*20*) to assess fruits. Pomegranate had the highest antioxidant quality in both the PBS wash and no PBS wash protocols (**Table 2**). Wild blueberry, strawberry, blackberry, and raspberry also ranked highly in both protocols. For all fruits in our study, the antioxidant quality was lower from the PBS wash protocol than from the no PBS protocol, even for those fruits with similar EC_{50} values in both protocols (**Tables 1** and **2**). This is due to the quercetin standard's aberrant behavior of having higher activity, and a lower EC_{50} value, in the PBS wash protocol than in the no PBS wash protocol, which was also seen previously (*16*). Because the cellular antioxidant quality values for each fruit in the two protocols were not comparable, the values were normalized. Wild blueberry, raspberry, and blueberry had lower cellular antioxidant quality in the PBS wash protocol than in the no PBS protocol, indicating that, relative to the other fruits, the phenolic antioxidants in these fruits are taken up less well by the cells or bound less tightly to the cell membrane. The normalized antioxidant qualities of pomegranate, blackberry, cranberry, apple, peach, red grape, and pear were higher in the PBS wash protocol, suggesting their phenolics were more closely associated with the cells than those from the other fruits.

The contribution of total phenolics from fruits in the American diet was estimated from our total phenolic measurements and per capita loss-adjusted food availability data for the United States (*28*). Apple was the largest contributor to total phenolics (**Figure 5**) of all fruits consumed by Americans. In comparison to the other fruits examined, apple had medium phenolic content, but the per capita consumption of apples is high (*28*). Other substantial contributors to phenolic intake were orange, grape, strawberry, and plum. The percent contribution of phenolics from orange and banana were 14.0 and 4.3%, respectively, because of high consumption, despite their comparatively low total phenolic contents (**Figure 1**). Our ranking of phenolic contribution from fruits differed greatly from that pubalished in 2001 by Vinson et al. (*20*), who placed banana at the top and included watermelon and cantaloupe in the top six. The differences in rankings can be explained by three major factors: juice consumption data were included in our study and not in the analysis by Vinson et al.; phenolics were measured using a catechin standard curve in the earlier study by Vinson et al., instead of the gallic acid standard curve we used; and consumption patterns may have changed.

Contribution of CAA activities from fruits in the American diet was also discussed. Strawberry, apple, orange, and grape

were the top providers of CAA from the no PBS wash protocol (**Figure 6A**), whereas apple, strawberry, grape, and cranberry were the highest contributors from the PBS wash protocol (**Figure 6B**). Strawberry ranked well because of its high activity, even though its consumption is not great. Banana did not even place in the top 10 contributors of fruit cellular antioxidant activity in the no PBS wash protocol due to its low CAA value. Orange and banana did not have any activity in the PBS wash protocol, so despite the high intake of oranges and bananas in the United States, they did not supply any PBS wash CAA to the population. Small increases in the consumption of berries, such as blueberry, blackberry, cranberry, and raspberry, would have a large impact on their percent contributions figures because of their very high phenolic content and cellular antioxidant activity.

This study shows the cellular antioxidant activity of 25 common fruits. Berries and pomegranate demonstrated the highest antioxidant activity, whereas melons had low activity. CAA values were significantly associated with total phenolic and ORAC values, although the correlation coefficients were much lower between CAA and ORAC values than between CAA and total phenolic values. Apples were the largest contributor of total phenolics and cellular antioxidant power to Americans. Antioxidant activity provided by fruits may be important in the prevention of cancer and other chronic diseases. Measuring the antioxidant activity of fruits 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. Further testing is needed to confirm the relationship between CAA values for fruits 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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