Antioxidant Capacity of Tea and Common Vegetables

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Previously, some fruits were shown to contain high antioxidant activities. In this paper, we report the antioxidant activities of 22 common vegetables, one green tea, and one black tea measured using the automated oxygen radical absorbance capacity assay with three different reactive species: a peroxyl radical generator, a hydroxyl radical generator, and Cu^{2+} , a transition metal. Based on the fresh weight of the vegetable, garlic had the highest antioxidant activity (μ mol of Trolox equiv/g) against peroxyl radicals (19.4) followed by kale (17.7), spinach (12.6), Brussels sprouts, alfalfa sprouts, broccoli flowers, beets, red bell pepper, onion, corn, eggplant (9.8–3.9), cauliflower, potato, sweet potato, cabbage, leaf lettuce, string bean, carrot, yellow squash, iceberg lettuce, celery, and cucumber (3.8–0.5); kale had the highest antioxidant activity against hydroxyl radicals followed by Brussels sprouts, alfalfa sprouts, beets, spinach, broccoli flowers, and the others. The green and black teas had much higher antioxidant activities against peroxyl radicals than all these vegetables. However, the tea also showed a prooxidant activity in the presence of Cu²⁺, which was not found with any of the vegetables studied.

Keywords: Antioxidant; free radical; tea; vegetable

INTRODUCTION

Consumption of fruits and vegetables has been associated with lower incidence and lower mortality rates of cancer in several human cohort and case-control studies for all common cancer sites (Ames et al., 1993; Doll, 1990; Dragsted et al., 1993; Willett, 1994a). The antitumorigenic effects of vegetables were also found in experiments using cells (Maeda et al., 1992) and animals (Belman, 1983; Bingham, 1990; Bresnick et al., 1990; Maltzman et al., 1989; Stoewsand et al., 1988; Stoewsand et al., 1989; Wattenberg and Coccia, 1991). There is a highly significant negative association between intake of total fruits and vegetables and cardioand cerebrovascular disease mortality (Acheson and Williams, 1983; Armstrong et al., 1975; Burr and Sweetnam, 1982; Phillips et al., 1978; Verlangieri et al., 1985). Vegetarians and nonvegetarians with a high intake of fruits and vegetables also have reduced blood pressure (Ascherio et al., 1992; Sacks and Kass, 1988).

The protection that fruits and vegetables provide against diseases, including cancer and cardio- and cerebrovascular diseases, has been attributed to the various antioxidants, especially antioxidant vitamins, including ascorbic acid and α -tocopherol, contained in these fruits and vegetables (Ames, 1983; Gey, 1990; Gey et al., 1991; Riemersma et al., 1989; Stähelin et al., 1991a,b; Steinberg et al., 1989, 1991; Willett, 1994b). However, the majority of the antioxidant activity of a fruit or vegetable may be from compounds other than vitamin C, vitamin E, or β -carotene. For example, some flavonoids that are often found in the human diet have antioxidant activities (Bors and Saran, 1987; Bors et al., 1990; Hanasaki et al., 1994). Our laboratory has already reported that some common fruits have high antioxidant activities which cannot be accounted for by their vitamin C content (Wang et al., 1996). We also found that some flavonoids had much stronger antioxidant activities against peroxyl radicals than vitamin E, vitamin C, and glutathione (Cao et al., in press). The objective of this study was to determine the antioxidant capacities of 22 common vegetables, one green tea, and one black tea by using the oxygen radical absorbance capacity (ORAC) assay (Cao et al., 1993, 1995). Three different reactive species were used in the ORAC assay: (i) 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), a peroxyl radical (ROO[•]) generator, (ii) Cu²⁺⁻ H₂O₂, mainly a hydroxyl radical (OH[•]) generator, and (iii) Cu²⁺, a transition metal.

MATERIALS AND METHODS

Chemicals. β -Phycoerythrin (β -PE) from *Porphydium cruentum* was purchased from Sigma (St. Louis, MO). The β -PE that was used in these experiments usually lost more than 90% of its fluorescence within 30 min in the presence of 4 mmol/L AAPH. AAPH was purchased from Wako Chemicals USA Inc. (Richmond, VA). 6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) was obtained from Aldrich (Milwaukee, WI).

Tea and Vegetables. Twenty-two vegetables were purchased on three separate occasions from local supermarkets. The 22 vegetables were garlic, kale, spinach, Brussels sprouts, alfalfa sprouts, broccoli flowers, beets, red bell pepper, onion, corn, eggplant, cauliflower, potato, sweet potato, cabbage, leaf lettuce, string bean, carrot, yellow squash, iceberg lettuce, celery, and cucumber. The green tea used in the study was Chin Chu oriental blend tea. The black tea (all black teas are fermented teas) was a dried powder and provided by Tea Trade Health Research Association.

Sample Preparation. The black tea was completely dissolved in deionized water (5 mg/mL) and used for ORAC assay directly after suitable dilution with phosphate buffer (75 mM, pH 7.0). The green tea was brewed for 30 min in deionized water (1:60, w/v, 95–100 °C). The edible portion of a vegetable was weighed and then homogenized by using a blender after adding deionized water (1:2, w/v). The brewed green tea and vegetable homogenate were then centrifuged

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Table 1. Total Antioxidant Capacity of Tea and Common Vegetables^a

	drv matter	ORAC _{ROO} . ^b		$ORAC_{OH}$		$ORAC_{Cu}^{c}$		antioxidant
item	(%)	WM basis	DM basis	WM basis	DM basis	WM basis	DM basis	\mathbf{score}^d
green tea			814 ± 30		35.8 ± 6.0		$\textbf{-41.9} \pm \textbf{7.1}$	
black tea			927		NM^e		NM	
garlic	42.9 ± 2.7	19.4 ± 3.1	46 ± 9	1.1 ± 0.4	2.7 ± 0.9	2.7 ± 0.41	6.4 ± 1.1	23.2
kale	10.4 ± 1.7	17.7 ± 0.6	179 ± 32	6.2 ± 0.3	61.3 ± 7.5	0.2 ± 0.03	2.3 ± 0.5	24.1
spinach	9.8 ± 0.6	12.6 ± 0.3	129 ± 6	2.8 ± 0.4	29.6 ± 6.1	1.6 ± 0.19	16.0 ± 1.9	17.0
B russels sprouts	14.0 ± 0.5	9.8 ± 1.8	70 ± 10	5.4 ± 0.8	38.5 ± 4.7	0.6 ± 0.09	4.3 ± 0.9	15.8
alfalfa sprouts	8.0 ± 0.2	9.3 ± 0.7	117 ± 12	4.6 ± 0.5	58.1 ± 6.9	0.6 ± 0.05	7.0 ± 0.7	14.5
broccoli flowers	15.1 ± 0.3	8.9 ± 1.0	59 ± 5	2.4 ± 0.3	15.6 ± 1.8	1.6 ± 0.09	10.5 ± 0.4	12.9
beets	12.0 ± 2.7	8.4 ± 0.2	81 ± 28	3.1 ± 0.1	36.0 ± 7.7	0.2 ± 0.03	2.2 ± 0.7	11.7
red bell pepper	9.8 ± 0.5	7.1 ± 0.5	74 ± 9	0.6 ± 0.1	6.2 ± 0.9	0.4 ± 0.08	3.7 ± 0.7	8.1
onion	11.2 ± 0.7	4.5 ± 0.5	40 ± 2	0.5 ± 0.1	4.1 ± 0.9	0.6 ± 0.17	5.4 ± 1.4	5.6
corn	18.6 ± 2.4	4.0 ± 0.5	22 ± 4	2.2 ± 0.2	11.7 ± 0.5	1.0 ± 0.15	5.2 ± 0.7	7.2
eggplant	5.3 ± 1.1	3.9 ± 0.3	80 ± 22	1.1 ± 0.1	22.4 ± 3.5	0.1 ± 0.03	1.3 ± 0.2	5.1
cauliflower	8.3 ± 0.9	3.8 ± 1.0	46 ± 11	1.1 ± 0.1	13.6 ± 2.3	0.2 ± 0.07	2.7 ± 0.6	5.1
potato	22.7 ± 2.1	3.1 ± 1.0	15 ± 5	1.0 ± 0.2	4.4 ± 1.2	0.5 ± 0.11	2.3 ± 0.5	4.6
sweet potato	21.8 ± 1.7	3.0 ± 0.3	14 ± 2	1.0 ± 0.1	4.4 ± 0.3	0.3 ± 0.03	1.2 ± 0.2	4.3
cabbage	9.5 ± 0.7	3.0 ± 0.3	32 ± 2	1.5 ± 0.1	15.8 ± 0.5	0.3 ± 0.02	3.4 ± 0.4	4.8
leaf lettuce	5.4 ± 0.5	2.6 ± 0.2	49 ± 7	1.4 ± 0.2	25.0 ± 1.4	0.1 ± 0.03	1.5 ± 0.4	4.1
string bean	7.4 ± 1.5	2.0 ± 0.5	30 ± 8	1.7 ± 0.2	24.2 ± 3.3	0.2 ± 0.04	2.3 ± 0.6	3.9
carrot	7.7 ± 0.6	2.1 ± 0.7	26 ± 8	0.8 ± 0.1	10.3 ± 0.4	0.5 ± 0.06	7.2 ± 1.0	3.4
yellow squash	12.0 ± 3.1	1.5 ± 0.3	17 ± 3	1.1 ± 0.2	12.5 ± 1.5	0.2 ± 0.02	1.7 ± 0.2	2.8
iceberg lettuce	3.7 ± 1.2	1.2 ± 0.2	39 ± 12	0.7 ± 0.1	23.2 ± 6.9	0.4 ± 0.08	11.9 ± 3.2	2.3
celery	5.0 ± 0.4	0.6 ± 0.1	13 ± 2	0.3 ± 0.1	6.0 ± 1.0	0.2 ± 0.09	4.3 ± 2.0	1.1
cucumber	3.5 ± 0.2	0.5 ± 0.1	15 ± 2	0.3 ± 0.1	7.1 ± 1.4	0.3 ± 0.02	9.2 ± 0.8	1.1

^{*a*} Data expressed as means \pm SEM of three samples purchased and analyzed independently, except for the black tea. ^{*b*} Data expressed as μ mol of Trolox equiv/g of wet matter (WM) or dry matter (DM). ^{*c*} Data expressed as $\times 10^3$ units/g of wet matter (WM) or dry matter (DM). ^{*d*} Antioxidant score = ORAC_{ROO'} + ORAC_{OH'} + ORAC_{Cu} (WM basis). ^{*e*} NM, not measured.

at 34000g for 30 min (4 °C). The supernatant (water soluble fraction) was recovered and used directly for the ORAC assay after suitable dilution with the phosphate buffer. The pulp (water insoluble fraction) was washed twice with deionized water and further extracted by using pure acetone (1:4, w/v) with shaking at room temperature for 30 min. Acetone has been used by our laboratory (Wang et al., 1996) and others (Daniel et al., 1989; Mass et al., 1991) to extract antioxidants from fruit pulp. The acetone extract was recovered after centrifugation (34000g, 10 min, 4 °C), and the sample was used for the ORAC assay after suitable dilution with phosphate buffer. The ORAC activity of a vegetable or the green tea was calculated by adding the ORAC activity from its water soluble fraction and its pulp fraction extracted with acetone. The dry matter of a vegetable was determined after drying the vegetable at 40 °C for 1 week.

Automated ORAC Assay. The automated ORAC assay was carried out on a COBAS FARA II spectrofluorometric centrifugal analyzer (Roche Diagnostic System Inc., Branchburg, NJ) with fluorescent filters (ex, 540 nm; em, 565 nm). The procedure was based on a previous report of Cao and coworkers (Cao et al., 1993), as modified for the COBAS FARA II (Cao et al., 1995). Briefly, in the final assay mixture (0.4 mL total volume), β -PE (16.7 nM) was used as a target of free radical (or oxidant) attack, with either (i) AAPH (4 mM) as a peroxyl radical generator (ORAC_{ROO} assay), (ii) H₂O₂-Cu²⁺ $[H_2O_2, 0.3\%; Cu^{2+}$ (as CuSO₄), 9 μ M] as mainly a hydroxy radical generator (ORAC_{OH} assay), or (iii) Cu²⁺ (as CuSO₄) (18 μ M) as a transition metal oxidant (ORAC_{Cu} assay). Trolox was used as a control standard. A 0.1 mM stock solution was stable for at least 1 month at -80 °C. The analyzer was programmed to record the fluorescence of β -PE every 2 min after AAPH, H₂O₂-Cu²⁺, or Cu²⁺ was added. All fluorescent measurements were expressed relative to the initial reading. Final results were calculated using the differences of areas under the β -PE decay curves between the blank and a sample and are expressed as μ mol of Trolox equiv/g of tea or vegetables (Cao et al., 1993, 1995), except when Cu^{2+} alone (i.e., without $H_2O_2)$ was used as an oxidant in the assay. In the presence of Cu²⁻ alone, Trolox cannot be used as an antioxidant standard since Trolox may act as a prooxidant in the presence of Cu²⁺ (Cao and Cutler, 1993). Therefore, the result of the ORAC_{Cu} assay in this case was calculated using (area_{sample} - area_{blank})/area_{blank} and expressed as antioxidant units; 1 unit equals the antioxidant activity which increases the area under the β -PE decay curve by 100% in the $ORAC_{Cu}$ assay. A negative $ORAC_{Cu}$ value indicated a $Cu^{2+}\mbox{-initiated}$ prooxidant activity.

RESULTS

The antioxidant activities against peroxyl radicals (ORAC_{ROO} activity) of 22 common vegetables, one green tea, and one black tea are shown in Table 1. Based on the *fresh* or *wet* weight of a vegetable, garlic and kale were in the top quintile of $ORAC_{ROO}$ measured in the 22 vegetables. Spinach, Brussels sprouts, alfalfa sprouts, broccoli flowers, beets, red bell pepper, onion, corn, and eggplant had $ORAC_{ROO}$ values that fell in the middle three quintiles (3.9–12.6). Cauliflower, potato, sweet potato, cabbage, leaf lettuce, string beans, carrot, yellow squash, iceberg lettuce, celery, and cucumber were in the lowest quintile of ORAC_{ROO} activities of the vegetables measured. However, based on the dry weight of a vegetable, kale had the highest ORAC_{ROO} activity followed by spinach, alfalfa sprouts, beets, eggplant, red bell pepper, Brussels sprouts, broccoli flowers, leaf lettuce, garlic, cauliflower, onion, and iceberg lettuce. Cabbage, string beans, carrots, corn, yellow squash, cucumber, potato, sweet potato, and celery were in the lowest quintile (below 32.0) of ORAC_{ROO} activities expressed on a dry matter basis. Green and black teas had much higher ORAC_{ROO} activities than any of the vegetables studied (4.5-5-fold higher than kale and 60-70-fold higher than celery, based on the dry weight).

The antioxidant activities against hydroxyl radicals (ORAC_{OH} activity) of the vegetables and green tea are also shown in Table 1. Based on the *fresh* or *wet* weight of a vegetable, kale had the highest ORAC_{OH} activity followed by Brussels sprouts, alfalfa sprouts, beets, spinach, broccoli flowers, corn, string beans, cabbage, leaf lettuce, eggplant, cauliflower, yellow squash, garlic, potato, sweet potato, carrot, iceberg lettuce, red bell pepper, onion, celery, and cucumber. Based on the *dry* weight of a vegetable, kale also had the highest ORAC_{OH} activity followed by alfalfa sprouts, Brussels sprouts, beets, spinach, leaf lettuce, string bean, iceberg



Figure 1. Antioxidant/prooxidant activities of green tea, broccoli flower, garlic, and spinach as a function of their extract concentrations (% of the undiluted extracts). The positive $ORAC_{Cu}$ values indicate antioxidant activities, while the negative $ORAC_{Cu}$ values indicate prooxidant activities (see Materials and Methods).

lettuce, eggplant, cabbage, broccoli flower, cauliflower, yellow squash, corn, carrot, cucumber, red bell pepper, celery, potato, sweet potato, onion, and garlic. The $ORAC_{OH}$ activity of green tea, based on *dry* weight, was between that of beets and spinach.

Green tea showed a prooxidant activity (*negative* ORAC_{Cu} activity) in the presence of Cu²⁺ (without H₂O₂) (Table 1). This Cu²⁺-initiated prooxidant activity, however, was not found in any vegetables evaluated in this study. Based on the *fresh* or *wet* weight of the vegetable, garlic had the highest antioxidant activities against Cu²⁺ (ORAC_{Cu} activity) followed by broccoli flowers, spinach, and the others. However, spinach had the highest ORAC_{Cu} activity, if activity was based on the *dry* weight, followed by iceberg lettuce, broccoli flowers, and the others.

The 'antioxidant score' of a vegetable shown in Table 1 was calculated by simply adding ORAC_{ROO}. (umol of Trolox equiv), ORAC_{OH} (µmol of Trolox equiv), and $ORAC_{Cu}$ (10³ units), based on the wet weight of the vegetable. One nanomole of Trolox equivalent calculated from ORAC_{ROO} assay and 1 ORAC_{Cu} unit calculated from $ORAC_{Cu}$ assay represent a similar area difference under the β -PE decay curve between the blank and a sample, which was used in the ORAC quantification. Because $ORAC_{ROO}$ activity of a vegetable weights the score more heavily than ORAC_{OH} or ORAC_{Cu} activity of the vegetable in the scoring system, the 'antioxidant score' did not rank the vegetables in a significantly different order than what was observed with the ORAC_{ROO} assay. The 'antioxidant score' was not given for the teas since they are dry, not fresh.

The ORAC_{Cu} activities of tea and vegetables were determined using different extract concentrations, since the Cu²⁺-initiated prooxidant activity of some antioxidants is seen only at a relatively high concentration (Cao and Cutler, 1993). The results in Figure 1 show that in the presence of Cu²⁺ (without H₂O₂), tea acts as a prooxidant at all concentrations, and the *prooxidant* activity increased with increased tea concentration. However, of the tested vegetables including spinach,

garlic, and broccoli flowers, all act as antioxidants against Cu^{2+} , and their *antioxidant* activity increased as their concentration increased in the assay system.

Figure 2 presents the calculated $ORAC_{ROO}$ intake based upon a common measured size or serving. For many of the vegetables this common measured proportion represents a 1/2 cup serving size except for garlic (1 clove), onion (1 tablespoon), potato (1 potato), and lettuce (1 leaf). In Figure 2, the common serving size is presented in grams. Based upon this calculation, kale, beets, red bell pepper, Brussels sprouts, broccoli flowers, spinach, potatoes, and corn likely provide a significant amount of $ORAC_{ROO}$ in the diet if these vegetables are consumed on a regular basis. Frequency of consumption of the individual vegetables would be the other factor determining which vegetables contribute the most to the ORAC consumed in a common diet.

DISCUSSION

The ORAC assay developed recently by Cao and coworkers (Cao et al., 1993, 1995) provides a unique and novel way to evaluate the potential antioxidant activities of various compounds and biological samples. This method is superior to other similar methods for two reasons. First, the ORAC assay system uses an areaunder-curve (AUC) technique and thus combines both inhibition time and inhibition degree of free radical action by an antioxidant into a single quantity (Cao et al., 1995). Other similar methods (Ghiselli et al., 1994; Glazer, 1990; Miller et al., 1993; Wayner et al., 1985; Whitehead et al., 1992) use either the inhibition time at a fixed inhibition degree or the inhibition degree at a fixed time as the basis for quantitating the results. Second, different free radical generators or oxidants can be used in the ORAC assay. This is important because the measured antioxidant activity of a biological sample depends upon which free radical or oxidant is used in the assay (Cao et al., 1996a,b).

Peroxyl radical (ROO[•]) is a common free radical found in the body and used in the antioxidant activity assays (Wayner et al., 1985; Glazer, 1990; Cao et al., 1993, 1995; Ghiselli et al., 1994). It is slightly less reactive than OH[•] and thus possesses an "extended " half-life of seconds instead of nanoseconds (Grisham, 1992). The total antioxidant capacity of some common fruits was thus determined by us using the ORAC_{ROO}• assay (Wang et al., 1996), which measures all *traditional* antioxidants including ascorbic acid, α -tocopherol, β -carotene, glutathione, bilirubin, uric acid, melatonin (Cao et al., 1993; Pieri et al., 1994), and flavonoids (Cao et al., in press).

In the current study, $Cu^{2+}-H_2O_2$ (a "OH•" generator) and Cu²⁺ alone were also used to assess the antioxidant activities of one green tea and 22 vegetables. Most of the "OH•" thought to be generated in vivo comes from metal-dependent reduction of H2O2, except during abnormal exposure to ionizing radiation. In vitro the metal can be titanium, copper, iron, or cobalt, but the best candidates for promoters of OH• formation in vivo seem to be iron and, to a smaller extent, copper. Cu^{2+} - H_2O_2 or Cu^{2+} alone is frequently used in inducing oxidative damage to protein and nucleic acids (Parthasarathy et al., 1989; Sato et al., 1992). The ORAC_{OH} assay with $Cu^{2+}-H_2O_2$ as a OH[•] generator measures compounds like mannitol, glucose, uric acid (at physiological concentrations), proteins, and transition metal chelators, but not compounds, such as ascorbic acid, that react directly with copper and produce reactive species.



Figure 2. Amount of ORAC_{ROO} activity consumed (µmol of Trolox equiv) (left *y* axis) per common serving or measured quantity (g) (right *y* axis). Common serving sizes were obtained from USDA Agriculture Handbook No. 8-11 (*Composition of Foods: Vegetables and Vegetable Products*).

The ORAC_{Cu} assay using copper alone measures not only the antioxidant activity (positive ORAC_{Cu} value) of a compound which can sequester transition metals but also the transition metal-initiated *prooxidant* activity (negative ORAC_{Cu} value) of a compound, such as ascorbic acid (Cao and Cutler, 1993) and some flavonoids (Cao et al., in press).

At this point, we do not have a good indication as to which radical generator provides the 'best' estimate of antioxidant activity of the vegetables. Perhaps, the formulation of an 'antioxidant score', which takes into account the antioxidant activities determined by the three different reactive species or oxidants, can give us some additional useful information. We calculated the 'antioxidant score' of each vegetable in this study by simply adding ORAC_{ROO}, ORAC_{OH}, and ORAC_{Cu} values, based on wet weight of the vegetable, since 1 nmol of Trolox equiv calculated from ORAC_{ROO} assay and 1 ORAC_{Cu} unit calculated from ORAC_{Cu} assay represent a similar area difference under the β -PE decay curve between the blank and a sample. The ORAC_{ROO} value of a vegetable weights the score more heavily than the $ORAC_{OH}$ or $ORAC_{Cu}$ value of the vegetable in the scoring system, which also seems reasonable because peroxyl radicals tend to be more prevalent in biological systems. However, the 'antioxidant score' did not rank the vegetables in a significantly different order than what was observed with the ORAC_{ROO} assay.

Our results demonstrated clearly that all vegetables tested in this study had antioxidant activities against not only peroxyl radicals but also hydroxyl radicals and transition metals (Cu^{2+}), although their ORAC_{ROO}, ORAC_{OH}, and ORAC_{Cu} activities vary considerably from one kind of vegetable to another. It is an important finding that these vegetables (and also fruits like strawberry, unpublished data) acted as antioxidants when a transition metal oxidant was used in the ORAC assay and the antioxidant activity also increased as their concentrations increased. The transition metalinitiated prooxidant actions of ascorbic acid (Beach and Giroux, 1992) and α -tocopherol (Iwatsuki et al., 1995; Maiorino et al., 1993; Yoshida et al., 1994) have been described. Using Cu²⁺-H₂O₂ in the ORAC assay, it was also found that ascorbic acid and Trolox (at a relatively high concentration), a water soluble α -tocopherol analogue, acted as prooxidants (Cao and Cutler, 1993). Therefore, in terms of the antioxidant quality in vitro, the natural antioxidant mixture contained in fruits or vegetables appears to be better than a single antioxidant or a simple antioxidant mixture of ascorbic acid, α -tocopherol, and β -carotene.

The antioxidant capacity varies considerably from one kind of vegetable to another, similar to what we found in fruits (Wang et al., 1996). The $ORAC_{ROO}$ activities of kale and spinach were similar to that observed in strawberries (Wang et al., 1996) whether the data were based on *wet* or *dry* weight. For example, based on the wet weight of a fresh vegetable, the $ORAC_{ROO}$ activity (which measures all traditional antioxidants) for kale was about 2 times the activity measured in beet and broccoli flowers, 8-9 times the activity measured in carrots and string beans, and 29-35 times the activity measured in celery and cucumber. Based upon a common measured or serving size, kale, beets, red peppers, Brussels sprouts, broccoli flowers, spinach, potatoes, and corn likely provide the largest amount of ORAC_{ROO} consumed from vegetables (Figure 2), although frequency of consumption of the individual vegetables would be another factor determining which vegetables contribute the most to ORAC consumed in a common diet.

The green and black teas had much higher antioxidant activities against peroxyl radicals than all fruits and vegetables that we have examined. Their ORAC-ROO activity, based on dry weight, was 4.5-6.0 times the activity measured in kale and strawberry (Wang et al., 1996). The ORAC_{OH} activity of the green tea, based on the dry weight, was only 58% of that measured in kale. The ORAC_{OH} activity of the tea was actually compromised by the Cu²⁺ used in the assay, since the

 $ORAC_{Cu}$ activity of the tea was negative, indicating a prooxidant activity of the tea in the presence of Cu^{2+} . It seems clear that some tea components can absorb hydroxyl radicals and other reactive species produced from the reaction between Cu^{2+} and H_2O_2 . Some tea components apparently can produce reactive species through direct reactions among these tea components, Cu^{2+} , and O_2 , when $Cu^{2+}-H_2O_2$ is used as a reactive species generator in the ORAC assay. It is also possible that some tea components, such as some flavonoids (Cao et al., in press), can play these two opposite roles at the same time. However, the transition metal-initiated prooxidant actions of tea, ascorbic acid, and α -tocopherol may not be important in vivo, where transition metals will be largely sequestered, except perhaps in certain diseases involving metal overload. Recent experiments have already demonstrated the inhibition by tea and tea polyphenols of tumorigenesis in different animal models (Yang and Wang, 1993), although the effect of tea consumption on cancer risk in humans as revealed by epidemiologic studies is less clear (International Agency for Research on Cancer, 1991).

The antioxidant defense system of the body is composed of different antioxidant components. The antioxidant capacities of these antioxidant components depend upon which free radicals or oxidants are produced in the body. Some fruits and vegetables contain a group of natural antioxidants that have not only a high antioxidant activity but also a good antioxidant quality. Therefore, the supplementation of these natural antioxidants through a balanced diet containing enough fruits and vegetables could be much more effective and economical than the supplementation of an individual antioxidant, such as ascorbic acid or α -tocopherol, in protecting the body against various oxidative stresses.

In summary, the antioxidant activities of 22 common vegetables, one green tea, and one black tea were measured using the automated ORAC assay with three different reactive species: a peroxyl radical generator, a hydroxyl radical generator, and Cu²⁺, a transition metal. Based on the fresh weight of a vegetable, garlic had the highest antioxidant activity against peroxyl radicals followed by kale, spinach, Brussels sprouts, alfalfa sprouts, and others, while kale had the highest antioxidant activity against hydroxyl radicals followed by Brussels sprouts, alfalfa sprouts, beets, spinach, and the others. Kale also had the highest ORAC_{ROO} activity, when results were expressed on a dry weight basis. The green and black teas had much higher antioxidant activity against peroxyl radicals than all of the vegetables tested in this study. However, the tea exhibited a prooxidant activity in the presence of Cu²⁺, which has also been reported for the antioxidants, ascorbic acid and α -tocopherol; this prooxidant activity was not found in the vegetables analyzed in this study. Therefore, the supplementation of natural antioxidants through a balanced diet containing enough fruits and vegetables could be the most effective in protecting the body against various oxidative stressors.

ABBREVIATIONS USED

AAPH, 2,2'-azobis(2-amidinopropane) dihydrochloride; ORAC, oxygen radical absorbance capacity; ORAC-_{ROO}, peroxyl radical absorbance capacity; ORAC_{OH}, hydroxyl radical absorbance capacity; ORAC_{Cu}, antioxidant capacity against Cu^{2+} ; β -PE, β -phycoerythrin; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

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LITERATURE CITED

- Acheson, R. M.; Williams, D. R. R. Does consumption of fruit and vegetables protect against stroke? *Lancet* **1983**, *1* (8335), 1191–1193.
- Ames, B. M. Dietary carcinogens and anticarcinogens: oxygen radicals and degenerative diseases. *Science* **1983**, *221*, 1256–1263.
- Ames, B. M.; Shigena, M. K.; Hagen, T. M. Oxidants, antioxidants and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, 90, 7915–7922.
- Armstrong, B. K.; Mann, J. I.; Adelstein, A. M. Eskin, F. Commodity consumption and ischemic heart disease mortality, with special reference to dietary practices. *J. Chron. Dis.* **1975**, *28*, 455–469.
- Ascherio, A.; Rimm, E. B.; Giovannucci, E. L.; Colditz, G. A.; Rosner, B.; Willett, W. C.; Sacks, F.; Stampfer, M. J. A prospective study of nutritional factors and hypertension among US men. *Circulation* **1992**, *86*, 1475–1484.
- Beach, D. C.; Giroux, E. Inhibition of lipid peroxidation promoted by iron (III) and ascobate. *Arch. Biochem. Biophys.* **1992**, *297*, 258–264.
- Belman, S. Onion and garlic oils inhibit tumor promotion. *Carcinogenesis* **1983**, *4*, 1063–1065.
- Bingham, S. A. Mechanisms and experimental and epidemiological evidence relating dietary fibre (non-starch polysaccharides) and starch to protection against large bowel cancer. *Proc. Nutr. Soc.* **1990**, *49*, 153–171.
- Bors, W.; Saran, M. Radical scavenging by flavonoid antioxidants. Free Radical Res. Commun. 1987, 2, 289–294.
- Bors, W.; Werner, H.; Michel, C.; Saran, M. Flavonoids as antioxiants: determination of radical scavenging efficiencies. *Methods Enzymol.* **1990**, 186, 343–355.
- Bresnick, E.; Birt, D. F.; Wolterman, K.; Wheeler, M.; Markin, R. S. Reduction in mammary tumorigenesis in the rat by cabbage and cabbage residue. *Carcinogenesis* **1990**, *11*, 1159–1163.
- Burr, M. C.; Sweetnam, P. M. Vegetarianism, dietary fibre, and mortality. Am. J. Clin. Nutr. 1982, 36, 673-677.
- Cao, G.; Culter, R. G. High concentrations of antioxidants may not improve defense against oxidative stress. *Arch. Gerontol. Geriatr.* 1993, 17, 189–201.
- Cao, G.; Alessio, H. M.; Culter, R. G. Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biol. Med.* **1993**, *14*, 303–311.
- Cao, G.; Verdon, C. P.; Wu, A. H. B.; Wang, H.; Prior, R. L. Automated oxygen radical absorbance capacity assay using the COBAS FARA II. *Clin. Chem.* **1995**, 41, 1738–1744.
- Cao, G.; Giovanoni, M.; Prior, R. L. Antioxidant capacity decreases during growth but not aging in rat serum and brain. Arch. Gerontol. Geriatr. 1996a, 22, 27–37.
- Cao, G.; Giovanoni, M.; Prior, R. L. Antioxidant capacity in different tissues of young and old rats. *Proc. Soc. Exp. Biol. Med.* **1996b**, *211*, 359–365.
- Cao, G.; Sofic, E.; Prior, R. L. Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. *Free Radical Biol. Med.*, in press.
- Daniel, E. M.; Krupnick, A. S.; Heur, Y. H.; Blinzler, J. A.; Nims, R. W.; Stoner, G. D. Extraction, stability, and quantitation of ellagic acid in various fruits and nuts. *J. Food Comp. Anal.* **1989**, *2*, 338–349.
- Doll, R. An overview of the epidemiological evidence linking diet and cancer. *Proc. Nutr. Soc.* **1990**, *49*, 119–131.

- Dragsted, L. O.; Strube, M.; Larsen, J. C. Cancer-protective factors in fruits and vegetables: biochemical and biological background. *Pharmacol. Toxicol.* **1993**, *72* (Suppl. 1), 116–135.
- Gey, K. F. The antioxidant hypothesis of cardiovascular disease: epidemiology and mechanisms. *Biochem. Soc. Trans.* **1990**, *18*, 1041–1045.
- Gey, K. F.; Puska, P.; Jordan, P.; Moser, U. K. Inverse correlation between plasma vitamin E and mortality from ischemic heart disease in cross-cultural epidemiology. *Am. J. Clin. Nutr.* **1991**, *53*, 326S–334S.
- Ghiselli, A.; Serafini, M.; Maiani, G, Assini, E.; Ferro-Luzzi, A. A fluorescence-based method for measuring total plasma antioxidant capability. *Free Radical Biol. Med.* **1994**, *18*, 29–36.
- Glazer, A. N. Phycoerythrin flourescence-based assay for reactive oxygen species. *Methods Enzymol.* **1990**, *186*, 161– 168.
- Grisham, M. B. Reactive metabolites of oxygen and nitrogen in biology and medicine; R. G. Landes Co.: Austin, TX, 1992; p 10.
- Hanasaki, Y.; Ogawa, S.; Fukui, S. The correlation between active oxygen scavenging and antioxidative effects of flavonoids. *Free Radical Biol. Med.* **1994**, *16*, 845–850.
- International Agency for Research on Cancer. Coffee, tea, methylxanthines, and methylglyoxal. *IARC Monogr.* **1991**, *51*, 1–513.
- Iwatsuki, M.; Niki, E.; Stone, D.; Darley-Usmar, V. M. Alphatocopherol mediated peroxidation in the copper (II) and metmyoglobin induced oxidation of human low density lipoprotein: the influence of lipid hydroperoxides. *FEBS Lett.* **1995**, *360*, 271–276.
- Maeda, H.; Katsuki, T.; Akaike, T.; Yasutake, R. High correlation between lipid peroxide radical and tumor-promoter effect: suppression of tumor promotion in the Epstein-Barr virus/B-lymphocyte system and scavenging of alkyl peroxide radicals by various vegetable extracts. *Jpn. J. Cancer Res.* **1992**, *83*, 923–928.
- Maiorino; Zamburlini, A.; Roveri, A.; Ursini, F. Prooxidant role of vitamin E in copper induced lipid peroxidation. *FEBS Lett.* **1993**, *330*, 174–176.
- Maltzman, T. H.; Hurt, L. M.; Elson, C. E.; Tanner, M. A.; Gould, M. N. The prevention of nitrosomethylurea-induced mammary tumors by d-limonene and orange oil. *Carcino*genesis **1989**, 10, 781–783.
- Mass, J. L.; Wang, S. Y.; Galletta, G. J. Evaluation of strawberry cultivars for ellagic acid content. *Hort. Sci.* **1991**, *26*, 66–68.
- Miller, N. J.; Rice-Evans, C.; Davies, M. J.; Gopinathan, V.; Milner, A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonatoes. *Clin. Sci.* **1993**, *84*, 407– 412.
- Parthasarathy, S.; Wieland, E.; Steinberg, D. A role for endothelial cell lipoxygenase in the oxidative modification of low density lipoprotein. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 1046–1050.
- Phillips, R. L.; Lemon, F. R.; Beeson, W. L.; Kuzma, J. W. Coronary heart disease mortality among Seventh-Day Adventists with differing dietary habits: a preliminary report. *Am. J. Clin. Nutr.* **1978**, *31*, 191–198.
- Pieri, C.; Marra, M.; Moroni, F.; Recchioni, R.; Marcheselli, F. Melatonin: a peroxyl radical scaveger more effective than vitamin E. *Life Sci.* **1994**, *55*, PL 271–276.
- Riemersma, R. A.; Wood, D. A.; Macintyre, C. C. A.; Elton, R.; Gey, K. F.; Oliver, M. F. Low plasma vitamin E and C increased risk of angina in Scottish men. *Ann. N.Y. Acad. Sci.* **1989**, *570*, 291–295

- Sacks, F. M.; Kass, E. H. Low blood pressure in vagetarians: effects of specific foods and nutrients. *Am. J. Clin. Nutr.* **1988**, *48*, 795–800.
- Sato, K.; Akaike, T.; Kohno, M.; Ando, M.; Naeda, H. Hydroxyl radical production by H_2O_2 plus Cu,Zn-superoxide dismutase reflects the activity of free copper released from the oxidatively damaged enzyme. *J. Biol. Chem.* **1992**, *67*, 25371–25377.
- Stähelin, H. B.; Gey, K. F.; Eichholzer, M.; Lüdin, E.; Bernasconi, F.; Thurneysen, J.; Brubacher, G. Plasma antioxidant vitamins and subsequent cancer mortality in the 12year follow-up of the prospective basel study. *Am. J. Epidemiol.* **1991a**, *133*, 766–775.
- Stähelin, H. B.; Gey, K. F.; Eichholzer, M.; Lüdin, E. β-Carotene and cancer prevention: the Basel study. *Am. J. Clin. Nutr.* **1991b**, *53*, 265S–269S.
- Steinberg, D. Antioxidants and atherosclerosis: acurrent assessment. *Circulation* **1991**, *84*, 1420–1425.
- Steinberg, D.; Parthasarathy, S.; Carew, T. E.; Khoo, J. C.; Witztum, J. L. Beyond cholestrol: Modification of lowdensity lipoprotein that increase its atherogenicity. *N. Engl. J. Med.* **1989**, *320*, 915–924.
- Stoewsand, G. S.; Anderson, J. L.; Munson, L. Protective effect of dietary Brussels sprouts against mammary carcinogenesis in Sprague-Dawleys rats. *Cancer Lett.* **1988**, *39*, 199–207.
- Stoewsand, G. S.; Anderson, L. J.; Munson, L.; Lisk, D. J. Effect of dietary Brussels sprouts with increased selenium content on mammary carcinogenesis in the rat. *Cancer Lett.* **1989**, *45*, 43–48.
- Verlangieri, A. J.; Kapeghian, J. C.; el-Dean, S.; Bush, M. Fruit and vegetable consumption and cardiovascular mortality. *Med. Hypoth.* **1985**, *16*, 7–15.
- Wang, H.; Cao, G.; Prior, R. L. Total antioxidant capacity of fruits. J. Agric. Food Chem. 1996, 44, 701–705.
- Wattenberg, L. W.; Coccia, J. B. Inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone carcinogenesis in mice by D-limonene and citrus fruit oils. *Carcinogenesis* **1991**, *12*, 115–117.
- Wayner, D. D. M.; Burton, G. W.; Ingold, K. U.; Locke, S. Quantitative meaurement of the total, peroxyl radicaltrapping antioxidant capacity of human plasma by controlled peroxidation. *FEBS Lett.* **1985**, *187*, 33–37.
- Whitehead, T. P.; Thorpe, G. H. G.; Maxwell, S. R. J. Enhanced chemoluminescent assay for antioxidant capacity in biological fluids. *Anal. Chim. Acta* **1992**, *266*, 265–277.
- Willett, C. W. Diet and health: what should we eat? *Science* **1994a**, *264*, 532–537.
- Willett, C. W. Micronutrients and cancer risk. Am. J. Clin. Nutr. **1994b**, 59, 162S–165S.
- Yang, C. S.; Wang, Z.-Y. Tea and cancer: A review. J. Natl. Cancer Inst. 1993, 58, 1038–1049.
- Yoshida, Y.; Tsuchiya, J.; Niki, E. Interaction of alphatocopherol with copper and its effect on lipid peroxidation. *Biochim. Biophys. Acta* **1994**, *1200*, 85–92.

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