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## **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 ( $\mu$ 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  $Cu^{2+}$ , 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  $\alpha$ -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  $\beta$ -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 (ROO¹) generator, (ii) Cu²+-H<sub>2</sub>O<sub>2</sub>, mainly a hydroxyl radical (OH¹) generator, and (iii) Cu²+, a transition metal.

#### MATERIALS AND METHODS

**Chemicals.**  $\beta$ -Phycoerythrin ( $\beta$ -PE) from *Porphydium cruentum* was purchased from Sigma (St. Louis, MO). The  $\beta$ -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<sup>a</sup>

	dry matter	ORAC <sub>ROO</sub> .b		ORAC <sub>OH</sub> .b		$\mathrm{ORAC_{Cu}}^c$		antioxidant
item	(%)	WM basis	DM basis	WM basis	DM basis	WM basis	DM basis	$\mathbf{score}^d$
green tea			$814\pm30$		$35.8 \pm 6.0$		$\textbf{-41.9} \pm 7.1$	
black tea			927		$NM^e$		NM	
garlic	$42.9 \pm 2.7$	$19.4 \pm 3.1$	$46\pm 9$	$1.1\pm0.4$	$2.7\pm0.9$	$2.7\pm0.41$	$6.4\pm1.1$	23.2
kale	$10.4\pm1.7$	$17.7\pm0.6$	$179\pm32$	$6.2\pm0.3$	$61.3 \pm 7.5$	$0.2\pm0.03$	$2.3\pm0.5$	24.1
spinach	$9.8 \pm 0.6$	$12.6\pm0.3$	$129 \pm 6$	$2.8 \pm 0.4$	$29.6 \pm 6.1$	$1.6\pm0.19$	$16.0\pm1.9$	17.0
Brussels sprouts	$14.0\pm0.5$	$9.8\pm1.8$	$70\pm10$	$5.4\pm0.8$	$38.5 \pm 4.7$	$0.6\pm0.09$	$4.3\pm0.9$	15.8
alfalfa sprouts	$8.0\pm0.2$	$9.3\pm0.7$	$117\pm12$	$4.6\pm0.5$	$58.1 \pm 6.9$	$0.6\pm0.05$	$7.0\pm0.7$	14.5
broccoli flowers	$15.1\pm0.3$	$8.9\pm1.0$	$59\pm 5$	$2.4\pm0.3$	$15.6\pm1.8$	$1.6\pm0.09$	$10.5\pm0.4$	12.9
beets	$12.0\pm2.7$	$8.4\pm0.2$	$81\pm28$	$3.1\pm0.1$	$36.0 \pm 7.7$	$0.2\pm0.03$	$2.2\pm0.7$	11.7
red bell pepper	$9.8\pm0.5$	$7.1\pm0.5$	$74\pm 9$	$0.6\pm0.1$	$6.2\pm0.9$	$0.4\pm0.08$	$3.7\pm0.7$	8.1
onion	$11.2\pm0.7$	$4.5\pm0.5$	$40\pm2$	$0.5\pm0.1$	$4.1\pm0.9$	$0.6\pm0.17$	$5.4\pm1.4$	5.6
corn	$18.6 \pm 2.4$	$4.0\pm0.5$	$22\pm4$	$2.2\pm0.2$	$11.7\pm0.5$	$1.0\pm0.15$	$5.2\pm0.7$	7.2
eggplant	$5.3\pm1.1$	$3.9 \pm 0.3$	$80\pm22$	$1.1 \pm 0.1$	$22.4 \pm 3.5$	$0.1 \pm 0.03$	$1.3\pm0.2$	5.1
cauliflower	$8.3\pm0.9$	$3.8\pm1.0$	$46\pm11$	$1.1\pm0.1$	$13.6 \pm 2.3$	$0.2\pm0.07$	$2.7\pm0.6$	5.1
potato	$22.7 \pm 2.1$	$3.1\pm1.0$	$15\pm 5$	$1.0\pm0.2$	$4.4\pm1.2$	$0.5\pm0.11$	$2.3\pm0.5$	4.6
sweet potato	$21.8\pm1.7$	$3.0 \pm 0.3$	$14 \pm 2$	$1.0 \pm 0.1$	$4.4 \pm 0.3$	$0.3 \pm 0.03$	$1.2\pm0.2$	4.3
cabbage	$9.5\pm0.7$	$3.0 \pm 0.3$	$32\pm2$	$1.5\pm0.1$	$15.8 \pm 0.5$	$0.3\pm0.02$	$3.4\pm0.4$	4.8
leaf lettuce	$5.4 \pm 0.5$	$2.6\pm0.2$	$49\pm7$	$1.4\pm0.2$	$25.0\pm1.4$	$0.1 \pm 0.03$	$1.5\pm0.4$	4.1
string bean	$7.4\pm1.5$	$2.0\pm0.5$	$30\pm 8$	$1.7\pm0.2$	$24.2 \pm 3.3$	$0.2 \pm 0.04$	$2.3\pm0.6$	3.9
carrot	$7.7 \pm 0.6$	$2.1\pm0.7$	$26\pm 8$	$0.8 \pm 0.1$	$10.3\pm0.4$	$0.5\pm0.06$	$7.2\pm1.0$	3.4
yellow squash	$12.0\pm3.1$	$1.5\pm0.3$	$17\pm3$	$1.1\pm0.2$	$12.5\pm1.5$	$0.2\pm0.02$	$1.7\pm0.2$	2.8
iceberg lettuce	$3.7\pm1.2$	$1.2\pm0.2$	$39\pm12$	$0.7\pm0.1$	$23.2 \pm 6.9$	$0.4 \pm 0.08$	$11.9 \pm 3.2$	2.3
celery	$5.0 \pm 0.4$	$0.6\pm0.1$	$13\pm2$	$0.3\pm0.1$	$6.0\pm1.0$	$0.2\pm0.09$	$4.3\pm2.0$	1.1
cucumber	$3.5\pm0.2$	$0.5\pm0.1$	$15\pm2$	$0.3\pm0.1$	$7.1\pm1.4$	$0.3\pm 0.02$	$9.2 \pm 0.8$	1.1

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

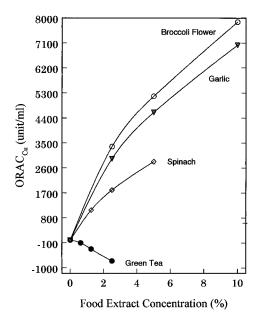
at 34000g for 30 min (4 °C). The supernatant (water soluble fraction) was recovered and used directly for the ORAC assay after suitable dilution with the phosphate buffer. The pulp (water insoluble fraction) was washed twice with deionized water and further extracted by using pure acetone (1:4, w/v) 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),  $\beta$ -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<sub>ROO</sub> assay), (ii) H<sub>2</sub>O<sub>2</sub>-Cu<sup>2+</sup>  $[H_2O_2, 0.3\%; Cu^{2+} (as CuSO_4), 9 \mu M]$  as mainly a hydroxy radical generator (ORAC<sub>OH</sub> assay), or (iii) Cu<sup>2+</sup> (as CuSO<sub>4</sub>) (18  $\mu$ M) as a transition metal oxidant (ORAC<sub>Cu</sub> 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  $\beta$ -PE every 2 min after AAPH, H<sub>2</sub>O<sub>2</sub>-Cu<sup>2+</sup>, or Cu<sup>2+</sup> was added. All fluorescent measurements were expressed relative to the initial reading. Final results were calculated using the differences of areas under the  $\beta$ -PE decay curves between the blank and a sample and are expressed as  $\mu$ 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 Cu<sup>2-</sup> 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<sub>Cu</sub> 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  $\beta$ -PE decay curve by 100% in the ORAC<sub>Cu</sub> assay. A negative ORAC<sub>Cu</sub> value indicated a Cu<sup>2+</sup>-initiated prooxidant activity.

#### **RESULTS**

The antioxidant activities against peroxyl radicals (ORAC<sub>ROO</sub> 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<sub>ROO</sub> measured in the 22 vegetables. Spinach, Brussels sprouts, alfalfa sprouts, broccoli flowers, beets, red bell pepper, onion, corn, and eggplant had ORAC<sub>ROO</sub>· 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<sub>ROO</sub> activities of the vegetables measured. However, based on the dry weight of a vegetable, kale had the highest ORAC<sub>ROO</sub> 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<sub>ROO\*</sub> activities expressed on a dry matter basis. Green and black teas had much higher ORAC<sub>ROO</sub> 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  $Cu^{2+}$  (without  $H_2O_2$ ) (Table 1). This  $Cu^{2+}$ -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  $Cu^{2+}$  (ORAC<sub>Cu</sub> 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<sub>ROO</sub> (umol of Trolox equiv), ORAC<sub>OH</sub> (μmol of Trolox equiv), and ORAC<sub>Cu</sub> (10<sup>3</sup> units), based on the wet weight of the vegetable. One nanomole of Trolox equivalent calculated from ORAC<sub>ROO</sub> assay and 1 ORAC<sub>Cu</sub> unit calculated from ORAC<sub>Cu</sub> assay represent a similar area difference under the  $\beta$ -PE decay curve between the blank and a sample, which was used in the ORAC quantification. Because ORAC<sub>ROO</sub> activity of a vegetable weights the score more heavily than ORACOH or ORACCu 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<sub>ROO</sub> 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  $Cu^{2+}$ -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  $Cu^{2+}$  (without  $H_2O_2$ ), 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<sup>2+</sup>, 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 (ROO¹) 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 OH¹ 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<sub>ROO¹</sub> assay (Wang et al., 1996), which measures all *traditional* antioxidants including ascorbic acid,  $\alpha$ -tocopherol,  $\beta$ -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<sup>2+</sup>-H<sub>2</sub>O<sub>2</sub> (a "OH•" generator) and Cu<sup>2+</sup> 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 H<sub>2</sub>O<sub>2</sub>, 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<sup>2+</sup>-H<sub>2</sub>O<sub>2</sub> or Cu<sup>2+</sup> alone is frequently used in inducing oxidative damage to protein and nucleic acids (Parthasarathy et al., 1989; Sato et al., 1992). The ORAC<sub>OH</sub> assay with Cu<sup>2+</sup>-H<sub>2</sub>O<sub>2</sub> 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.

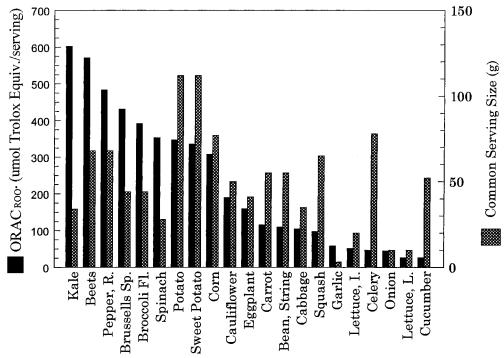


Figure 2. Amount of ORAC<sub>ROO</sub> activity consumed ( $\mu$ mol of Trolox equiv) (left y axis) per common serving or measured quantity (g) (right y axis). Common serving sizes were obtained from USDA Agriculture Handbook No. 8-11 (Composition of Foods: Vegetables and Vegetable Products).

The ORAC<sub>Cu</sub> assay using copper alone measures not only the antioxidant activity (positive ORAC<sub>Cu</sub> value) of a compound which can sequester transition metals but also the transition metal-initiated *prooxidant* activity (negative ORAC<sub>Cu</sub> 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<sub>ROO</sub>, ORAC<sub>OH</sub>, and ORAC<sub>Cu</sub> values, based on wet weight of the vegetable, since 1 nmol of Trolox equiv calculated from ORAC<sub>ROO\*</sub> assay and 1 ORAC<sub>Cu</sub> unit calculated from ORAC<sub>Cu</sub> assay represent a similar area difference under the  $\beta$ -PE decay curve between the blank and a sample. The ORAC<sub>ROO</sub> value of a vegetable weights the score more heavily than the ORAC<sub>OH</sub> or ORAC<sub>Cu</sub> 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<sub>ROO</sub> 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<sup>2+</sup>), although their ORAC<sub>ROO\*</sub>, ORAC<sub>OH</sub>, and ORAC<sub>Cu</sub> 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 Cu<sup>2+</sup>-H<sub>2</sub>O<sub>2</sub> in the ORAC assay, it was also found that ascorbic acid and Trolox (at a relatively high concentration), a water soluble  $\alpha$ -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,  $\alpha$ -tocopherol, and  $\beta$ -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<sub>ROO</sub> 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<sub>ROO</sub> 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<sub>ROO</sub> 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<sub>OH</sub> activity of the green tea, based on the dry weight, was only 58% of that measured in kale. The ORAC<sub>OH</sub> activity of the tea was actually compromised by the Cu<sup>2+</sup> used in the assay, since the ORAC<sub>Cu</sub> 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<sup>2+</sup> and H<sub>2</sub>O<sub>2</sub>. Some tea components apparently can produce reactive species through direct reactions among these tea components, Cu<sup>2+</sup>, and O<sub>2</sub>, when Cu<sup>2+</sup>-H<sub>2</sub>O<sub>2</sub> 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  $\alpha$ -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  $\alpha$ -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<sub>ROO</sub> 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 Cu<sup>2+</sup>, which has also been reported for the antioxidants, ascorbic acid and  $\alpha$ -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  $Cu^{2+}$ ;  $\beta$ -PE,  $\beta$ -phycoerythrin;

Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

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