Total Antioxidant Capacity of Fruits

Hong Wang,[†] Guohua Cao,[†,‡] and Ronald L. Prior*[,†]

Jean Mayer Human Nutrition Research Center on Aging at Tufts University, Agricultural Research Service, U.S. Department of Agriculture, Boston, Massachusetts 02111, and Nutritional Science Department, University of Connecticut, Storrs, Connecticut 06296

The total antioxidant activity of 12 fruits and 5 commercial fruit juices was measured in this study using automated oxygen radical absorbance capacity (ORAC) assay. On the basis of the wet weight of the fruits (edible portion), strawberry had the highest ORAC activity (micromoles of Trolox equivalents per gram) followed by plum, orange, red grape, kiwi fruit, pink grapefruit, white grape, banana, apple, tomato, pear, and honeydew melon. On the basis of the dry weight of the fruits, strawberry again had the highest ORAC activity followed by plum, orange, pink grapefruit, tomato, kiwi fruit, red grape, white grape, apple, honeydew melon, pear, and banana. Most of the antioxidant capacity of these fruits was from the juice fractions. The contribution of the fruit pulp fraction (extracted with acetone) to the total ORAC activity of a fruit was usually less than 10%. Among the commercial fruit juices, grape juice had the highest ORAC activity followed by grapefruit juice, tomato juice, orange juice, and apple juice.

Keywords: *Free radicals; antioxidant; peroxyl radical; fruits*

INTRODUCTION

The 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 (Doll, 1990; Dragsted et al., 1993; Ames et al., 1993; Willett, 1994a). In animal experiments, vegetables that are common in human diets have been found to have antitumorigenic effects (Belman, 1983; Bingham, 1990; Bresnick et al., 1990; Maltzman et al., 1989; Stoewsand et al., 1988; Stoewsand et al., 1989; Wattenberg and Coccia, 1991). A highly significant negative association between intake of total fresh fruits and vegetables and ischemic heart disease mortality was reported by Armstrong et al. (1975) in Britian and by Verlangieri et al. (1985) in the United States. Similar findings were found among vegetarian groups (Phillips et al., 1978; Burr and Sweetnam, 1982). Vegetarians and nonvegetarians with a high intake of fruits and vegetables also have reduced blood pressure (Ascherio et al., 1992; Sacks and Kass, 1988). A significant negative association was also reported between fruit and vegetable consumption and cerebrovascular disease mortality (Acheson and Williams, 1983).

The protection that fruits and vegetables provide against diseases, including cancer and cardio- and cerebrovascular diseases, has been attributed to the various antioxidants contained in them (Ames, 1983; Gey, 1990; Steinberg et al., 1989, 1991). At present, there is overwhelming evidence to indicate that free radicals cause oxidative damage to lipids, proteins, and nucleic acids. Free radicals may lie at the heart of the etiology or natural history of a number of diseases including cancer and atherosclerosis. Therefore, antioxidants, which can neutralize free radicals, may be of central importance in the prevention of these disease states. Low plasma levels of antioxidant vitamins have been associated with an increased risk of subsequent cancer mortality (Stähelin et al., 1991a,b; Willett, 1994b). Low plasma levels of vitamins E and C increased the risk of angina in Scottish men (Riemersma, 1989). An inverse correlation between plasma vitamin E levels and mortality from ischemic heart disease was reported in cross-cultural epidemiology (Gey et al., 1991).

However, fruits and vegetables contain many different antioxidant components. The majority of the antioxidant capacity of a fruit or vegetable may be from compounds other than vitamin C, vitamin E, or β-carotene. For example, some flavonoids (including flavones, isoflavones, flavonones, anthocyanins, catechin, and isocatechin) that are frequently components of the human diet demonstrated strong antioxidant activities (Bors and Saran, 1987; Bors et al., 1990; Hanasaki et al., 1994). Therefore, it was of interest to measure the total antioxidant capacity of a fruit or vegetable. The objective of this study was to measure the total antioxidant capacity of some common fruits and commercial fruit juices by using the oxygen radical absorbance capacity (ORAC) assay (Cao et al., 1993), as modified and automated on the COBAS Fara II analyzer (Cao et al., 1995).

MATERIALS AND METHODS

Fruits and Commercial Fruit Juices. Twelve fruits and five commercial fruit juices were purchased on three separate occasions from local supermarkets during the winter. The 12 fruits were strawberry, orange, apple, pink grapefruit, plum, red grape, white grape, kiwi fruit, banana, tomato, pear, and honeydew melon. The 5 commercial fruit juices were orange juice (Tropicana Pure Premium original 100% orange juice), grapefruit juice (Florida's Natural ruby red premium 100% grapefruit juice), tomato juice (Welch's homogenized 100% tomato juice), grape juice (Welch's 100% Concord grape juice), and apple juice (Stewart's fresh pressed 100% apple juice).

Chemicals. β-Phycoerythrin (β-PE) from *Porphydium cruentum* was purchased from Sigma (St. Louis, MO). 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was pur-

* Author to whom correspondence should be addressed [telephone (617) 556-3310; fax (617) 556-3299; e-mail RPrior@infonet.tufts.edu].

[†] Jean Mayer USDA Human Nutrition Research Center on Aging.

[‡] University of Connecticut.

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Table 1. Effect of Acetone Extraction Time on ORAC (Nanomoles of Trolox Equivalents per Gram) Measured in Fruit Pulp

	strawberry[a]	white grape[a]	orange[a]
2 min	847 ± 29 (3)	248 ± 22 (3)	3556 ± 154 (3)
30 min	1264 ± 32 (3)*	306 ± 32 (3)	6041 ± 63 (3)*
1 h	1116 ± 21 (3)*	340 ± 11 (3)	5782 ± 147 (3)*
4 h	1129 ± 113 (3)	398 ± 80 (3)	5572 ± 182 (3)*

[a] The data are presented as mean ± SE (*n*). * denotes $P < 0.05$ using ANOVA in comparison with 2 min extraction.

chased from Wako Chemicals USA Inc. (Richmond, VA). 6-Hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox) was obtained from Aldrich (Milwaukee, WI). Acetone was from Sigma or Aldrich.

Sample Preparation. All of the fruits except white and red grapes, strawberries, plums, and tomatoes were peeled. The edible portion of the fruit was weighed and then homogenized by using a blender after deionized water was added (1:2 w/v). The homogenate was centrifuged at 4500*g* for 15 min (4 °C). The supernatant (juice fraction) was recovered and used directly for the ORAC assay after suitable dilution with phosphate buffer (75 mM, pH 7.0). The pulp (insoluble fraction) was further extracted by using pure acetone (1:7 w/v) with shaking at room temperature for 30 min. Acetone has been used by other investigators to extract antioxidants from fruit pulp (Daniel et al., 1989; Mass et al., 1991). Acetone itself has only a very small effect on the ORAC assay (Cao et al., 1995). The acetone extract was recovered after centrifugation (4500*g*, 15 min, 4 °C), and the sample was used for the ORAC assay after suitable dilution with phosphate buffer. The dry matter of fruit was determined by weighing the water lost after drying at 40 °C for 1 week.

Automated ORAC Assay. The automated ORAC assay was carried out on the COBAS FARA II centrifugal analyzer (Roche Diagnostic System Inc., Branchburg, NJ) with a fluorescence attachment; fluorescent filters were set to pass the light with an excitation wavelength of 540 nm and an emission wavelength of 565 nm. The procedure was based on a previous paper of 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 (1.67×10^{-8} M) was used as a target of free radical damage, AAPH (4 mM) as a peroxyl radical generator, and Trolox as a control standard. The analyzer was programmed to record the fluorescence every 2 min after AAPH addition. Final results were calculated using the differences of areas under the quenching curves of β-PE between a blank and a sample and are expressed as micromoles of Trolox equivalents per gram or milliliter. The ORAC activity of a fruit was calculated by adding the ORAC activity from the juice fraction and the pulp fraction extracted with acetone.

Statistical Analysis. The effect of acetone extraction time on the ORAC activity of a fruit pulp was analyzed by analysis of variance (ANOVA) using SYSTAT (SYSTAT, Inc., Evanston, IL). Pairwise multiple comparisons were evaluated by Tukey's significant difference (HSD) test used in SYSTAT. Differences at $P < 0.05$ were considered significant.

RESULTS

The ORAC activity of a fruit (edible portion) was calculated by adding the ORAC activity from its juice fraction and its pulp fraction extracted with acetone. Thirty minutes of extraction with acetone was found to be sufficient to extract the antioxidants contained in the pulp of a fruit (strawberry, orange, and white grape were tested in this study; Table 1). The ORAC activities measured in the pulp of strawberries and oranges following 2 min of acetone extraction at room temperature were significantly ($P < 0.05$) lower than those measured after 30 min of acetone extraction. However, the ORAC activities measured in the pulp of strawberries and oranges following 30 min of acetone extraction were not significantly different from those measured by using a 1 or 4 h acetone extraction. The ORAC activity measured in the pulp of white grapes following 2 min of acetone extraction was not significantly different from that measured following 30 min or 1 or 4 h of acetone extraction (Table 1).

Table 2. ORAC of Selected Fruits Using AAPH as a Peroxyl Radical Generator[a]

item	dry matter (%)	total ORAC		juice extract ORAC[b]
		as is basis[b]	DM basis[c]	
strawberry	10.0	15.36 ± 2.38	153.6 ± 7.5	12.44
plum	12.0	9.49 ± 0.67	79.1 ± 1.9	8.35
orange	14.5	7.50 ± 1.01	51.7 ± 2.7	6.82
grape, red	20.5	7.39 ± 0.48	36.0 ± 1.1	3.99
kiwi fruit	16.5	6.02 ± 0.52	36.5 ± 1.3	5.54
grapefruit, pink	10.0	4.83 ± 0.18	48.3 ± 0.6	4.54
grape, white	17.0	4.46 ± 1.06	26.2 ± 2.6	2.89
banana	24.5	2.21 ± 0.19	9.0 ± 0.4	2.10
apple	16.5	2.18 ± 0.35	13.2 ± 0.9	1.92
tomato	5.0	1.89 ± 0.12	37.8 ± 0.5	1.57
pear	14.0	1.34 ± 0.06	9.6 ± 0.2	1.23
melon	7.5	0.97 ± 0.15	12.9 ± 0.5	0.88

[a] Data expressed as means ± SEM of three samples purchased and analyzed independently. [b] Data expressed as micromoles of Trolox equivalents per gram of fruit (as is basis). [c] Data expressed as micromoles of Trolox equivalents per gram of dry matter (DM) (DM basis).

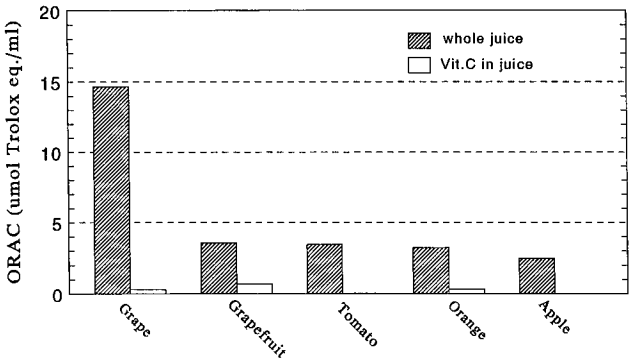

Figure 1. ORAC (micromoles of Trolox equivalent per milliliter) of common commercial fruit juices. The ORAC contributed by vitamin C in a commercial juice was based on the ORAC activity of vitamin C (Cao et al., 1993) and the content of vitamin C obtained from the commercial juice label. No data were available for apple juice. Vitamin C in tomato juice accounted for 0.02 μmol of Trolox equivalent/mL.

The ORAC activities of 12 fruits are shown in Table 2. The ORAC assays of these fruits were carried out on three independent occasions using fruit purchased on three separate occasions. The results are presented as mean ± SE. On the basis of the wet weight of fruit (edible portion), strawberry had the highest ORAC activity, followed by plum, orange, red grape, kiwi fruit, pink grapefruit, white grape, banana, apple, tomato, pear, and melon. On the basis of the dry weight of fruit, strawberry also had the highest ORAC activity followed by plum, orange, pink grapefruit, tomato, kiwi fruit, red grape, white grape, apple, honeydew melon, pear, and banana. In most fruits, the contribution of the fruit pulp fraction (extracted with acetone) to the total ORAC activity of a fruit was usually less than 10%.

The ORAC activities of five commercial fruit juices are shown in Figure 1. Among the commercial fruit juices tested, grape juice had the highest ORAC activity, followed by grapefruit juice, tomato juice, orange juice, and apple juice. The calculated contribution of vitamin C to the total ORAC activity of those commercial fruit

juices was less than 30%. Data on vitamin C content of the commercial juices were from their labels except for apple juice, which had no such datum. The ORAC activity of 1.0 μmol of vitamin C is 0.52 μmol of Trolox equiv (Cao et al., 1993).

DISCUSSION

Reactive oxygen species (ROS) are constantly generated in vivo, both by "accidents of chemistry" and for specific purposes. To counteract ROS and to prevent their possible damage to biological molecules, especially to DNA, lipids and proteins, all oxygen-consuming organisms are endowed with well-integrated antioxidant systems, which include enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, and macromolecules, such as albumin, ceruloplasmin, and ferritin, and an array of small molecules, such as ascorbic acid, α-tocopherol, β-carotene, and reduced glutathione.

There are many different antioxidant components in animal and plant tissues, and it is relatively difficult to measure each antioxidant component separately. Therefore, several methods (Cao et al., 1993; Glazer, 1990; Miller et al., 1993; Wayner et al., 1985; Whitehead et al., 1992) have been developed in recent years to evaluate the total antioxidant capacity of biological samples. In these methods, the inhibition of free radical action by an antioxidant contains two components: the inhibition time and the inhibition degree. We used the ORAC assay system because it combines both inhibition time and inhibition degree into a single quantity (Cao et al., 1993, 1995). All other methods 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.

Fruits are good sources of antioxidants. Considerable attention has been focused on the vitamin C, vitamin E, and β-carotene content of fruits. However, fruits also contain many other substances that have antioxidant activities. By using the ORAC assay, we have measured, for the first time, the total antioxidant capacity of some common fruits. On the basis of the data from USDA handbooks (USDA, 1986) the vitamin C contents of kiwi fruit, strawberry, orange, grapefruit, honeydew melon, and tomato are 5.0, 2.9, 2.7, 1.8, 1.3, and 0.9 μmol/g (wet wt), respectively. The other fruits including plum, red grape, white grape, banana, apple, and pear contain less than 0.49 μmol of vitamin C/g (wet wt). The ORAC activity of 1.0 μmol of vitamin C is 0.52 μmol of Trolox equiv (Cao et al., 1993). Therefore, it was calculated that the contribution of vitamin C to the total ORAC activity of a fruit was usually less than 15% except for kiwi fruit and honeydew melon. This suggests that the major source of antioxidant capacity of most fruits and commercial fruit juices may be not from vitamin C.

We did not measure the vitamin C content of the fruits directly but used vitamin C data from the USDA handbooks (USDA, 1986), realizing that this may introduce some added variability in the estimate. However, since the estimated contribution of vitamin C to the total ORAC activity is relatively low, we feel confident in concluding that this contribution is low and that other antioxidants in fruits should be considered as significant contributors to the total ORAC activity. The vitamin C values contained in the USDA handbook generally will represent a diverse sampling, and thus one would not expect severalfold fluctuations in the amount of vitamin C concentration in the food. The objective of this study was to determine the total antioxidant capacity (ORAC activity) of fruits and not the contribution of any individual antioxidant to the total antioxidant capacity. Our data in fact indicate that some unknown antioxidants may need to be identified in these fruits.

The total antioxidant capacity varies considerably from one kind of fruit to another. For example, on the basis of the wet weight of a fresh fruit, the total antioxidant capacity of strawberry was 2 times the capacity measured in oranges or red grapes, 7 times the capacity measured in apple and banana, 11 times the capacity measured in pears, and 16 times the capacity measured in honeydew melon. The antioxidant capacities of the five commercial fruit juices were not always similar to those measured in respective fresh fruits used in this study. The commercial grape juice and tomato juice had much higher ORAC than the fresh red grapes and the fresh tomatoes, while the commercial orange juice had much lower ORAC than the fresh oranges. However, the commercial grape juice was made from Concord grapes which have a *dark* color and thick skin and which are different from the *red* grapes tested in this study. Red wine had an ORAC activity of 12.34 ± 0.47 mmol/L (Cao et al., 1995), which is similar to that measured in grape juice. The varieties of tomatoes and oranges used in the commercial juices could also have been very different from the fresh tomatoes and oranges. In addition, we do not know whether vitamin C was added into these commercial fruit juices. However, other commercial processing factors, such as the procedures used for juice preparation (diluting and concentrating) and storage, cannot be excluded in explaining the observed difference.

Part of the antioxidant capacity of these fruits may be from flavonoids. Flavonoids are low molecular weight polyphenolic compounds that are widely distributed in vegetables and fruits (Hertog et al., 1992; Ortuño et al., 1995). Many flavonoids, such as kaempherol, quercetin, luteolin, myricetin, eridictyol, and catechin, have been shown to have antioxidant (Bors and Saran, 1987; Bors et al., 1990; Hanasaki et al., 1994), anti-inflammatory, antiallergic, anticancer, and antihemorrhagic properties (Das, 1994). Hertog et al. (1992) measured the flavonoid content (including kaempherol, quercetin, luteolin, and myricetin) of important fruits, vegetables, and beverages in the Dutch diet and related the baseline intake of dietary flavonoids to subsequent coronary heart disease mortality and the incidence of myocardial infarction in the Zutphen Elderly Study during a 5-year followup period. They found that flavonoid intake was significantly inversely related to mortality from coronary heart disease and of borderline significance ($P < 0.08$ for trend) with the incidence of a first fatal or nonfatal myocardial infarction (Hertog et al., 1993). This further suggests that other antioxidants besides vitamins E and C and β-carotene are also responsible for the protection provided by fruits and vegetables against various diseases.

The antioxidant defense system of the body is composed of different antioxidant components. The supplementation of one or a few antioxidants may not be very effective. Fruits contain a group of natural antioxidants that could have not only a high antioxidant activity but also a good combination or mixture of antioxidants. For example, 1 lb of fresh strawberries (454 g) has an ORAC activity (6973 μmol of Trolox equiv) equal to about 1.7

g of Trolox, 3.0 g of α-tocopherol (the ORAC activity of 1 μmol of α-tocopherol = 1 μmol of Trolox equiv; Cao et al., 1993) or 2.3 g of vitamin C (the ORAC activity of 1 μmol of vitamin C = 0.52 μmol of Trolox equiv; Cao et al., 1993). A high intake of vitamin C may act in some situations as a pro-oxidant in the body when free transition metals are available at the same time. Therefore, the supplementation of these natural antioxidants through a balanced diet containing enough fruits could be much more effective and also economical than the supplementation of an individual antioxidant, such as vitamin C or vitamin E, in protecting the body against oxidative damage under different conditions.

In summary, the total antioxidant activities of 12 fruits and 5 commercial fruit juices were measured by using an automated ORAC assay with a peroxyl radical generator. On the basis of the wet weight of fruits (edible portion), strawberry had the highest ORAC activity followed by plum, orange, red grape, kiwi fruit, pink grapefruit, white grape, banana, apple, tomato, pear, and honeydew melon. On the basis of the dry weight of fruits, strawberry also had the highest ORAC activity followed by plum, orange, pink grapefruit, tomato, kiwi fruit, red grape, white grape, apple, honeydew melon, pear, and banana. Among the commercial fruit and vegetable juices, grape juice had the highest antioxidant activity, followed by tomato juice, orange juice, and apple juice.

ABBREVIATIONS USED

AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; ORAC, oxygen radical absorbance capacity; β-PE, β-phycoerythrin; ROS, reactive oxygen species; Trolox, 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid.

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