Antioxidant Phytochemicals in Fruits and Vegetables: Diet and Health Implications

Ronald L. Prior[1] and Guohua Cao

U.S. Department of Agriculture, Agriculture Research Service, Jean Mayer Human Nutrition Research Center on Aging at Tufts University, 711 Washington Street, Boston, MA 02111

Increased consumption of fruits and vegetables has been associated with protection against various age-related diseases (Ames et al., 1993; Steinberg, 1991). What dietary constituents are responsible for this association is not known, but well-characterized antioxidants, including vitamins C and E, or β-carotene, are often assumed to contribute to the observed protection (Ames et al., 1993; Buring and Hennekens, 1997; Gey et al., 1991; Stahelin et al., 1991; Steinberg, 1991; Willett, 1994). However, the results from intervention trials have not been conclusive regarding the protection following supplementation with such antioxidants (Hennekens et al., 1996; Omenn et al., 1996; Priemé et al., 1997; Van Poppel et al., 1995). Recent epidemiological evidence indicates that the putative beneficial effects of a high intake of fruits and vegetables on the risk of diseases of aging may not be exclusively due to these antioxidants (Hertog et al., 1992; Knekt et al., 1997), but other antioxidant phytochemicals contained in fruits and vegetables may be equally important. To critically evaluate the potential roles of these phytochemicals in prevention of age-related diseases, we will discuss the free radical or oxidative stress theory of aging and present data on the antioxidant capacities of fruits, vegetables, and their phytochemical components, mainly flavonoids. Reports on the absorption of these flavonoids and the effects of fruit and vegetable intake on the antioxidant status in humans will be reviewed.

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[1]To whom reprint requests should be addressed (phone: (617) 556-3311; fax: (617) 556-3299; e-mail: prior@hnrc.tufts.edu).

FREE RADICAL OR OXIDATIVE STRESS THEORY OF AGING

Oxygen may be reduced by less than four electrons during normal metabolism to yield partially reduced reactive oxygen metabolites. Many of these reactive species (RS) are free radicals, i.e., they contain an odd number of electrons. Examples of oxygen-derived free radicals include superoxide ($O_2^{-\bullet}$), hydroxyl ($OH^\bullet$), hydroperoxyl ($HOO^\bullet$), peroxyl ($ROO^\bullet$), and alkoxyl ($RO^\bullet$) radicals. Reactive oxygen species that contain an even number of electrons, and thus are not free radicals, include hydrogen peroxide (H_2O_2) and lipid hydroperoxide (ROOH). Other common RS produced in the body include nitric oxide ($NO^\bullet$) and peroxynitrite anion ($ONOO^\bullet$). Reactive species are able to initiate lipid peroxidation, a chain reaction, and oxidize other cellular components, such as DNA and proteins (Halliwell, 1997). To deal with RS, the body is equipped with an effective defense system, which includes: enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP), and glutathione reductase (GR); high-molecular-weight antioxidants such as albumin, ceruloplasmin, and ferritin; and an array of low-molecular-weight antioxidants such as ascorbic acid, α-tocopherol, β-carotene, glutathione (GSH), and uric acid. Oxidative stress is a state of imbalance between RS and antioxidants in favor of the former. The free radical or oxidative stress theory of aging states that oxygen-derived free radicals or oxidative stress is the underlying cause of aging and age-related diseases such as cancer, cardiovascular disease, etc. (Harman, 1956, 1981; Yu, 1996). This theory has now been accepted by many gerontologists.

MEASUREMENT OF ANTIOXIDANT CAPACITY IN FRUITS AND VEGETABLES: OXYGEN RADICAL ABSORBANCE CAPACITY (ORAC) ASSAY

The ability to determine total antioxidant capacity in fruits and vegetables provides an opportunity to evaluate the theory that protection against free radical damage is a component of the protection observed following their consumption. The ORAC assay developed recently by Cao and coworkers (1993, 1995) provides an effective way to evaluate the total antioxidant capacity in fruits and vegetables, in that it combines both inhibition time and inhibition degree of the free radical or oxidant action by an antioxidant into a single quantity using an area under the curve technique for quantitation of the data.

ANTIOXIDANT CAPACITY OF FRUITS AND VEGETABLES

Studies from our laboratory represent the first attempt to measure total antioxidant capacity of fruits and vegetables (Cao et al., 1996; Guo et al., 1997; Prior et al., 1998; Wang et al., 1996). The antioxidant capacity of common fruits and vegetables, and drinks including green and black teas [*Camellia sinensis* (L.) Kuntze], commercial fruit juices and wines, were measured with the automated ORAC assay using a peroxyl radical generator ($ORAC_{ROO}$). Based upon the weight of an edible portion, prunes (*Prunus ×domestica* L.), raisins (*Vitis* sp.), blueberries (*Vaccinium* sp.), cranberries (*Vaccinium oxycoccos* L.), and blackberries (*Rubus* sp.) had an antioxidant capacity of >20 $\mu mol \cdot g^{-1}$ Trolox equivalents (TE) (Trolox: 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). Strawberries (*Fragaria ×ananassa* Duch.), red raspberries (*Rubus idaeus* L.), garlic (*Allium sativum* L.), kale (*Brassica oleracea* L. Acephala Group), and spinach (*Spinacia oleracea* L.) had an antioxidant capacity between 10 and 20 $\mu mol \cdot g^{-1}$ of TE while brussels sprouts (*Brassica oleracea* L. Gemmifera Group), alfalfa sprouts (*Medicago sativa* L.), plums, broccoli (*Brassica oleracea* L. Botrytis Group) florets, beets (*Beta vulgaris* L. ssp. *vulgaris*), oranges [*Citrus sinensis* (L.) Osbeck], red grapes (*Vitis* sp.), red bell pepper (*Capsicum annuum* L.), red cherries (*Prunus* sp.), and kiwifruit (*Actinidia chinensis* Planch.) had an antioxidant capacity between 5 and 9.9 $\mu mol \cdot g^{-1}$ of TE. Fruits and vegetables, such as pink grapefruit (*Citrus ×paradisi* Macfad.), white grape (*Vitus* sp.), onion (*Allium cepa* L.), corn (*Zea mays* L.), eggplant (*Solanum melongena* L.), cauliflower (*Brassica oleracea* L. Botrytis Group), potato (*Solanum tuberosum* L.), sweetpotato (*Ipomoea batatas* (L.) Lam.), cabbage (*Brassica oleracea L.* Capitata Group), leaf lettuce (*Lactuca sativa* L.), bananas (*Musa acuminata* Colla), apples (*Malus ×domestica* Borkh.), carrot (*Daucus carota* L.), string bean (*Phaseolus vulgaris* L.), tomatoes (*Lycopersicon esculentum* Mill.), yellow squash (*Cucurbita* sp.), pears (*Pyrus* sp.), iceberg lettuce (*Lactuca sativa* L.), honeydew melon (*Cucumis melo* L. Inodorus Group), celery [*Apium graveolens* L. var. *dulce* (Mill) Pers.], and cucumber (*Cucumus sativus* L.) had an antioxidant capacity <5 $\mu mol \cdot g^{-1}$ of TE (Cao et al., 1996; Prior et al., 1998; Wang et al., 1996).

Antioxidant capacity of fruits and vegetables may be influenced by genetics as well as environmental factors. We have observed as much as a 3.3-fold difference in antioxidant capacity as well as total phenolics and anthocyanins among species and cultivars of *Vaccinium* (Prior et al., 1998) and among cultivars of strawberries (Prior et al., unpublished data). Antioxidant capacity, total phenolics, and anthocyanins of two cultivars of rabbiteye blueberry (*V. ashei* Reade) also increased with maturity at harvest (Prior et al., 1998). Total antioxidant capacity may be an effective tool for use in fruit and vegetable breeding programs designed to increase antioxidant components available for human consumption.

Little has been done to determine effects of cooking and/or processing on the measured antioxidant capacity. Cooking of kale produced a small (10% to 15%) decline in antioxidant capacity, whereas steaming of blueberries for 5 min seemed to increase the quantities of antioxidant phytochemicals (unpublished data) that could be extracted and measured using the ORAC assay.

We calculated that the contribution of vitamin C to the total $ORAC_{ROO}$ activity of these fruits was usually <15%, except for kiwifruit and honeydew melon (Prior et al., 1998; Wang et al., 1996). This suggests that the major source of antioxidant capacity of most fruits and commercial fruit juices may not be vitamin C, but other antioxidant phytochemicals. There have been very few other published attempts at quantitating antioxidant capacity in foods. However, there has been some interest, using various analytical techniques, in quantitating antioxidant activity in grape wines, garlic extracts, and herbs. Whitehead et al. (1995), who used a chemiluminescent reaction to measure the antioxidant capacity of red wine, reported a variation of 10.0 to 20.7 $\mu mol \cdot mL^{-1}$ of TE in antioxidant capacity in nine different red wines with a mean of 15.4 $\mu mol \cdot mL^{-1}$ of TE. This value is similar to the value of 12.3 $\mu mol \cdot mL^{-1}$ of TE which Cao and coworkers (1995) measured in a red wine using the $ORAC_{ROO}$ method. Whitehead et al. (1995) also reported that four white wines had antioxidant capacities of <2.0 $\mu mol \cdot mL^{-1}$ of TE, which agrees closely with the value of 2.3 $\mu mol \cdot mL^{-1}$ of TE observed by Cao et al. (1995). Campos and Lissi (1996) used the Trolox Equivalent Antioxidant Capacity (TEAC) method to measure total antioxidant capacity of Chilean wines and found that red wines had values between 25.1 and 33.3 $\mu mol \cdot mL^{-1}$ of TE, and white wines ranged from 2.9 to 5.2 $\mu mol \cdot mL^{-1}$ of TE. Using a phenol antioxidant index based upon phenol concentration and inhibition of low-density lipoprotein (LDL) oxidation, Vinson and Hontz (1995) concluded that red wines had a significantly higher antioxidant index than white wines and thus were a better source of antioxidants. Frankel and coworkers (1995) also found that red wines were more effective in the inhibition of LDL oxidation than white wines. Vinson and coworkers (1998) used similar techniques to evaluate vegetables. A high antioxidant activity has also been found in a garlic extract (Popov et al., 1994), selected oriental herb extracts (Kim et al., 1994), and leaves from *Vernonia amygdalina*, a small tree that grows throughout tropical Africa (Igile et al., 1994).

FLAVONOIDS PRESENT IN FRUITS AND VEGETABLES

One hypothesis that has been advanced is that the protection against diseases, such as cancer and cardiovascular diseases, can be attributed to a large class of antioxidant phytochemicals, termed flavonoids, contained in fruits and vegetables. The phytochemicals considered for the purposes of this review will, thus, refer primarily to the flavonoids found in fruits, vegetables, nuts, seeds, stems, and flowers, as well as in tea and wine. Flavonoids are diphenylpropanes that commonly occur in plants; >4000 flavonoids have been identified. The common family members of flavonoids include flavones, isoflavones, flavanones, anthocyanins, flavans, and proanthocyanidins.

Flavonoids have long been recognized to possess antiallergic, antiinflammatory, antiviral, antiproliferative, and anticarcinogenic activities (Middleton and Kandaswami, 1993). No attempt will be made in this review to cover the diverse enzymes and metabolic processes that are affected by the numerous flavonoids. These studies have been summarized in several reviews (Cook and Samman, 1996; Dakora, 1995; Formica and Regelson, 1995; Middleton and Kandaswami, 1993; Stavric, 1994). Flavonoids are generally considered nonessential dietary nutrients. However, of historical significance, in light of possible nutritional implications of the consumption of flavonoids, is the early observation that a mixture of two flavonoids called citrin (eriodictyol and hesperidin) was considered to possess vitamin-like activity. The term "vitamin P" was coined to indicate that this material could decrease capillary permeability (and fragility), prolong the life of marginally scorbutic guinea pigs, and reduce the signs of hypovitaminosis C in experimental animals. Although so-called vitamin P ultimately did not fulfill the definition of a vitamin and the term was subsequently abandoned, there was nonetheless a strong indication that these two flavonoids had potent antioxidant-dependent, vitamin-C-sparing activity. We do not know much about possible synergistic or additive effects of flavonoids and the nutrient antioxidants, such as vitamins C or E. The view that flavonoids are irrelevant to human health and/or disease may need to be modified in view of the potentially health-promoting activities of the flavonoids that have recently been reported in experimental (Middleton and Kandaswami, 1993) and epidemiological studies (Hertog et al., 1993a, 1993b).

ANTIOXIDANT ACTIVITIES OF FLAVONOIDS

Because of the prevalence of flavonoids in foods, we and others (Cao et al., 1997; Rice-Evans et al., 1996; Wang et al., 1997) have undertaken studies to determine the antioxidant activity associated with flavonoids of different chemical structures. We found that, generally, the more OH substitutions, the stronger the $ORAC_{ROO}$ activity. Weak $ORAC_{ROO}$ activity (0.2–0.6 μmol·mol^{-1} of TE) was observed for flavones with single OH substitutions on the 3, 6, 2′, or 4′ position and in flavanones with single OH substitutions in the 7, 2′, 3′, 4′, or 7′ position (Cao et al., 1997). A flavone with a single OH substitution on the 5 position, however, had no detectable $ORAC_{ROO}$ activity, while a flavanone with a single OH substitution on the 6 position had an $ORAC_{ROO}$ activity of 1.36 TE, even stronger than Trolox. With compounds having the same basic chemical structure, the $ORAC_{ROO}$ activity was proportional to the number of OH substitutions on the flavone or flavanone structure. Kaempherol, quercetin, and myricetin, which have 4, 5, and 6 OH substitutions, respectively, had a $ORAC_{ROO}$ activity of 2.7, 3.3, and 4.3 TE, respectively (Cao et al., 1997). This is consistent with the inhibitory effects of these flavones (kaempherol < quercetin < myricetin) on platelet aggregation induced by a combination of adenosine diphosphate (ADP), collagen, and platelet activating factor (Tzeng et al., 1991). Measurement of antioxidant activity by the TEAC method did not rank these flavones in a similar order (Rice-Evans et al., 1995, 1996). However, several techniques have identified the importance of 3′, 4′ di-OH substitution in the B ring to the antioxidant activity of flavonoids, including techniques such as: 1) the formation and decay of the flavonoid aroxyl radicals (Bors et al., 1990); 2) the ability of flavonoids to quench the chromogenic radical cation, 2,2′-azinobis(3-ethylbenzothiazoline 6-sulfonate ($ABTS.^{+}$) (Rice-Evans et al., 1995); 3) the protection of lysosomes by flavonoids against oxygen radicals (Decharneux et al., 1992); and 4) the inhibitory effects of flavonoids on the release of reactive oxygen species by stimulated human neutrophils (Limassett et al., 1993).

The flavones and flavanones that contain multiple OH substitutions have very strong antioxidant activities against peroxyl radicals. For example, the $ORAC_{ROO}$ activities of myricetin, quercetin, luteolin, fustin, eriodictyol, and taxifolin were 4.32, 3.29, 3.57, 3.91, 3.41, 3.59 TE, respectively, while α-tocopherol, ascorbic acid, β-carotene, GSH, uric acid, and bilirubin had $ORAC_{ROO}$ values of 1.0, 0.52–1.12, 0.64, 0.68, 0.92, and 0.84 TE, respectively (Cao et al., 1993; Pieri et al., 1994). This observation means that the stoichiometric factor (i.e., the number of peroxyl radicals trapped per molecule antioxidant) of these flavonoids is ≈6–9, since the stoichiometric factor of Trolox is 2 (Burton et al., 1983). Copper-initiated prooxidant actions were observed for some flavonoids, as well as for other antioxidants, including ascorbic acid and α-tocopherol. However, this may not be important in vivo, where copper ions will be largely sequestered, except perhaps in certain metal overload diseases. The prevention of iron-increased lipid peroxidation in hepatocytes by some flavonoids, including quercetin, has been reported (Morel et al., 1993; 1994).

Using the automated $ORAC_{ROO}$ assay, we have also determined the antioxidant capacity of 14 anthocyanins, including the aglycones delphinidin, cyanidin, pelargonidin, malvidin, and peonidin, and the glycosides kuromanin (cyanidin-3-glucoside), keracyanin (cyanidin-3-rhamnoglucoside), ideain (cyanidin-3-galactoside), cyanin (cyanidin-3,5-diglucoside), callistephin (pelargonidin-3-glucoside), pelargonin (pelargonidin-3,5-diglucoside), oenin (malvidin-3-glucoside), malvin (malvidin-3,5-diglucoside), and peonidin-3-glucoside. All of these compounds showed strong antioxidant activities (Wang et al., 1997). Among them, kuromanin had the highest ORAC, which was 3.5× stronger than Trolox. Pelargonin had the lowest ORAC but was still as potent as Trolox (Wang et al., 1997).

ABSORPTION OF ANTIOXIDANT FLAVONOIDS AND EFFECTS OF INCREASING FRUIT AND VEGETABLE INTAKE ON ANTIOXIDANT STATUS IN HUMANS

Although strong antioxidant properties of the various flavonoids have been demonstrated in numerous biological systems in vitro, little information is available on their absorption and contribution to the overall antioxidant status in vivo. Absorption of flavonoids from the diet was long assumed to be negligible, as most of the flavonoids, except catechins, are present in plants bound to sugars as glycosides, and these glycosides were considered nonabsorbable. Contrary to the common belief that only flavonoid aglycones can be absorbed, the accumulating evidence indicates that flavonoid glycosides are easily absorbed in humans and rats without prior hydrolysis by microorganisms.

To their surprise, Hollman and coworkers (1995) found in ileostomy subjects that the quercetin glycosides from onions were absorbed far better than the pure aglycones. They also found in healthy subjects that such glycosides were absorbed and were eliminated slowly through the day (Hollman et al., 1996). The bioavailability of both quercetin from apples and pure quercetin-3-rutinoside was both 30% relative to onions (Hollman et al., 1997). Rutin, and other quercetin glycosides, as well as an anthocyanin, were detected simultaneously in plasma from nonsupplemented humans (Paganga and Rice-Evans, 1997). Recently, Prior and Cao (1999a) observed the absorption and appearance in plasma within 60 min of two cyanidin glucosides from elderberry (*Sambucus nigra* L.) fruits in normal human subjects. What appeared to be the same compounds also were observed unmetabolized in the urine (Prior and Cao, 1999a), based upon HPLC retention times and absorption spectra. Absorption of flavonoid glycosides has also been observed in rats (Horwitt, 1933; Morazzoni et al., 1991). A recent study suggests that quercetin glucosides are capable of interacting with the sodium-dependent glucose transport receptors in the mucosal epithelium and may therefore be absorbed by the small intestine in vivo (Gee et al., 1998).

The effects of fruit and vegetable intake on in vivo antioxidant status also support the absorption of flavonoids in humans. We found in 36 healthy nonsmokers that daily intake of the total antioxidants, measured as ORAC, from fruits and vegetables was significantly correlated with the fasting plasma antioxidant capacity. Increasing the consumption of fruits and vegetables from the usual five servings/day to the experimental 10 servings/day resulted in a significant increase of plasma antioxidant capacity (Cao et al., 1998a). Increased plasma antioxidant capacity and/or plasma total polyphenols in humans were also reported after the consumption of red wine (Cao et al., 1998b; Serafini et al., 1998; Whitehead et al., 1995) and grape juice (Day et al., 1997). A reduced sensitivity to oxidation of plasma and/or low density lipoprotein was observed in subjects consuming red wine in one study (Fuhrman et al., 1995) but not in another (de Rijke et al., 1996)

DIETARY INTAKE OF FLAVONOIDS

Kuhnau (1976) estimated that the average intake of all flavonoids combined was ≈1 g·d^{-1}. These results have been widely quoted; however, more recent results (Hertog et al., 1993a, 1993b) suggest that these values may be ≈5-fold higher than the typical intake in a Western population. Hertog and coworkers (1993a, 1993b) determined that the intake of flavonoids (quercetin, kaempferol, myricetin, apigenin, and luteolin) among 4112 adults was 23 mg·d^{-1}, with the most important flavonoid being quercetin with a mean intake of 16 mg·d^{-1}. However, a limitation in these data is a lack of quantitative information on all the flavonoid compounds in fruits and vegetables.

Anthocyanins are a group of flavonoids that have not received much attention in terms of possible implications for human nutrition. The anthocyanins (i.e., cyanidin, delphinidin, malvidin, peonidin, and pelargonidin) have a high antioxidant capacity as measured by $ORAC_{ROO}$ (Wang et al., 1997). Mazza and Miniati (1993) have completed a comprehensive review of anthocyanins present in fruits, vegetables, and grains; however, much of the earlier literature cited lacks quantitative data. The content of anthocyanins in fruit clearly seems high compared with that of the other flavonoids evaluated by Hertog et al. (1993a, 1993b) (quercetin, kaempferol, myricetin, apigenin, and luteolin). In fruits such as blackberry, blueberry, cranberry, raspberry, strawberry, and boysenberry (*Rubus* sp.), anthocyanin content may range from 200 to 4950 mg·kg^{-1} of fresh fruit. In blueberries, the anthocyanin content is 10 to 200× the quercetin content. Consumption

of 100 to 150 g (≈1 cup) of these fruits could easily result in an intake of 100 to 200 mg of anthocyanins, which is well above the average intake of flavonoids indicated by Hertog et al. (1993a, 1993b).

Intake of total antioxidant capacity expressed as ORAC equivalents ($\mu mol \cdot d^{-1}$ of TE) is in the range of 1200 to 1640 ORAC equivalents per day for individuals consuming two to five servings of fruits and vegetables per day (Cao et al., 1998a). From ORAC measurements of individual flavonoids, we have observed a ratio of antioxidant capacity (ORAC) to total phenols ($\mu mol \cdot mg^{-1}$ of TE) ranging from 2 to 13 with a mean of 7.2 ± 0.4 (n = 33) (Prior and Cao, 1999b). The same ratio in polyphenolics extracted from food samples is ≈ 11 $\mu mol \cdot mg^{-1}$ TE of total phenolics (mean of 20 samples) (Prior et al., unpublished data). Based upon this ratio, a total antioxidant capacity intake of 1200 to 1640 ORAC equivalents would provide a total phenolic intake of 109 to 149 mg for individuals consuming two to five servings of fruits and vegetables per day and up to 336 mg if 10 servings (3700 ORAC equivalents) of fruits and vegetables are consumed (Cao et al., 1998a). Vinson et al. (1998) estimated that the per capita consumption of total and free phenolics from vegetables is 242 and 116 $mg \cdot d^{-1}$, respectively. Based upon our data from individuals consuming 3700 ORAC equivalents on in vivo antioxidant capacity, an increase of 1000 to 2000 ORAC equivalents per day to the average diet may be needed to bring about some of the beneficial health effects of fruit and vegetable consumption.

CONCLUSION

Due to accumulating evidence demonstrating the absorption of dietary flavonoids in humans and significant contributions of phytochemicals such as flavonoids to the antioxidant capacity measured in fruits and vegetables, we conclude that these phytochemicals can be an important source of dietary antioxidants and may be responsible for the health benefits observed with increased consumption of fruits and vegetables.

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