FOOD COMPOSITION AND ADDITIVES

Analysis of Botanicals and Dietary Supplements for Antioxidant Capacity: A Review

RONALD L. PRIOR[1] and GUOHUA CAO
U.S. Department of Agriculture, Agricultural Research Service, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111

Free radicals and other reactive species are considered to be important causative factors in the development of diseases of aging such as cancer and cardiovascular diseases. This relationship has led to considerable interest in assessing the antioxidant capacity of foods and botanicals and other nutritional antioxidant supplements. The use of the oxygen radical absorbance capacity (ORAC) assay as a tool for antioxidant assessment is described and proposed as a method for comparing botanical sources and for standardizing nutritional supplements. The free radical or oxidant source is important and direct comparisons cannot be made between procedures that use different sources. The ORAC procedure uses 2,2′-azobis(2-amidinopropane) dihydrochloride as a peroxyl radical source, which is relevant to biological systems because the peroxyl radical is the most abundant free radical. Other oxidant sources (hydroxyl radical and Cu^{++}) can also be used to characterize antioxidants in botanicals. Phenolics or polyphenolics are responsible for most of the antioxidant capacity in fruits, vegetables, and most botanical antioxidant supplements. Although little is known about the absorption and metabolism of these components, improvement in the in vivo antioxidant status has been observed in human subjects following consumption of antioxidant botanicals. The ORAC method provides a basis from which to establish appropriate dietary intakes that might impact health outcomes.

Free radicals and other reactive species (RS) are constantly generated in vivo both by "accidents of chemistry" and for specific metabolic purposes. The most important reactions of free radicals in aerobic cells involve molecular oxygen and its radical derivatives (superoxide anion and hydroxyl radicals), peroxides, and transition metals. RS are thought to play an important role in aging and in the pathogenesis of numerous degenerative or chronic diseases, such as cancer and atherosclerosis (1–3). Although there are many determinants in the development of these diseases, considerable experimental evidence links RS production to biological damage that can potentially provide a mechanistic basis for their initiation and/or progression (4–6). RS are capable of chemically altering virtually all major classes of biomolecules (e.g., lipids, proteins, and nucleic acids) with concomitant changes in structure and function. Humans, along with other aerobic organisms, have developed an antioxidant network to protect themselves from the potentially detrimental effects of RS. The antioxidant network includes antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, and nonenzymatic antioxidants, which include antioxidants of high molecular weight, such as albumin, ceruloplasmin, and ferritin, and an array of antioxidants of low molecular weight, such as ascorbic acid, α-tocopherol, β-carotene, reduced glutathione (GSH), uric acid, bilirubin, and flavonoids. Thus, it is generally thought that oxidative pathology results when the generation of RS exceeds the capacity of the antioxidant network in the body.

Total Antioxidant Capacity Assays

The effectiveness of the antioxidant network in the body depends on the normal function of each antioxidant component in the network. The nonenzymatic antioxidants constitute an important aspect of the body's antioxidant mechanism. Because of the difficulty in measuring each antioxidant component separately and the interactions among these different antioxidant components in the network, several methods (7–12) have been developed to assess the total antioxidant capacity from all of these nonenzymatic antioxidants contained in a biological sample. The total peroxyl radical trapping parameter (TRAP) assay of Wayner et al. (7) was the most widely used assay of antioxidant capacity during the 1980s. The major problem with the TRAP assay lies in the oxygen electrode endpoint; an oxygen electrode will not maintain its stability over the period of time required. Therefore, a high degree of imprecision is inherent in this method (9). More recently, the Trolox equivalent antioxidant capacity (TEAC) assay (8, 9), the ferric-reducing ability of plasma (FRAP) assay (10), and

Received September 17, 1999. Accepted by GL January 21, 2000.

Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.

[1]Address for correspondence: R.L. Prior, USDA, ARS, HNRCA, 711 Washington St, Boston, MA 02111.

our oxygen radical absorbance capacity (ORAC) assay were developed (11, 12). The FRAP assay is simple and inexpensive, but it does not measure the SH group-containing antioxidants (13). The TEAC assay is based on the inhibition by antioxidants of the absorbance of the radical cation of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) and has been commercialized by Randox Laboratories (San Diego, CA). The effect of dilution on the serum TEAC value and the use of inhibition percentage at a fixed time, without considering the length of inhibition time in the quantitation of results, adversely affected the Randox-TEAC assay (13–15). However, there have been some recent modifications to this method that may overcome some of these deficiencies (16).

The ORAC assay depends on the detection of chemical damage to β- or *R*-phycoerythrin (PE) through the decrease in its fluorescence emission. The fluorescence is highly sensitive to the confirmation and chemical integrity of the protein. Under appropriate conditions, the loss of PE fluorescence in the presence of free radicals is an index of oxidative damage to the protein. The inhibition of the free radical action by an antioxidant, which is reflected in the protection against the loss of PE fluorescence in the ORAC assay, is a measure of its antioxidant capacity against the free radicals. With this fluorescence measurement, as applied in the ORAC assay, there is much less interference by colored compounds than with the absorbance measurements used in other similar methods. This is an important factor to consider particularly when fruits, vegetables, and natural product supplements are analyzed for their antioxidant capacities. In addition to the use of β- or *R*-PE as a sensitive target of free radical attack, and Trolox (a water-soluble α-tocopherol analogue) as a calibrator, the ORAC assay uses 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) as a free radical-generating system and an area-under-curve (AUC) technique for the quantitation of antioxidant capacity. AAPH undergoes spontaneous decomposition and produces peroxyl radicals with a rate primarily determined by temperature. Because of the very high molar ratio (>2000) of AAPH to antioxidant used in this procedure, the ORAC assay has high specificity and thus measures the capacity of an antioxidant to *directly quench free radicals*. The AUC technique combines both inhibition percentage and the length of inhibition time of free radical action by an antioxidant into a single quantity, which makes it superior to other similar methods that use either an inhibition percentage at a fixed time or a length of inhibition time at a fixed inhibition percentage (11, 12, 17). The peroxyl radical is the most common in biological systems, thus, giving the results from the ORAC assay relevance to biological systems. However, the ORAC assay has the additional advantage of being able to test for antioxidant protection against hydroxyl radicals and oxidative damage from transition metals. The ORAC assay has the advantage that it can be adapted to assay lipophilic antioxidants. Because of the high sensitivity of the fluorescence ORAC assay, a dilute emulsion of antioxidant lipid components can be formed in the assay mixture and assayed for antioxidant capacity (G. Cao, unpublished data, 1999). We have measured antioxidant capacities in the range of 0.01–5.0 μmol Trolox equivalents (TE)/mL for various oils used in food preparation and cooking; however, we have not assayed a large number of oil- or lipid-based products at this point to establish a truly representative database. The ORAC assay has been modified to assay the lipophilic components in an organic solvent (18). A disadvantage of this assay is that some automated instruments cannot handle the organic solvents required. The ORAC assay has been used by different laboratories and has provided significant information regarding the antioxidant capacity of various biological samples, from pure compounds such as melatonin and flavonoids, to complex matrixes such as fruits, vegetables, and animal tissues (19–28).

Antioxidant Capacity of Fruits, Vegetables, and Dietary Supplements

Some antioxidants (i.e., ascorbic acid, α-tocopherol, and flavonoids) cannot be synthesized in vivo and must be obtained from diets, mainly fruits, vegetables, or dietary supplements. Consumption of fruits and vegetables has been associated with a lower incidence and lower mortality rates of cancer in several human cohort and case-control studies for all common cancer sites (2, 29–31). The antitumorigenic effects of vegetables were also found in experiments using cells (32) and animals (33–38). There is a highly significant negative association between intake of total fruits and vegetables and cardio- and cerebrovascular disease mortality (39–43). Vegetarians and nonvegetarians with a high intake of fruits and vegetables also have reduced blood pressure (44, 45). The protection that fruits and vegetables provide against diseases has been attributed to antioxidants contained in the fruits and vegetables (46–53).

There are many different antioxidants in fruits and vegetables. Therefore, the determination of total antioxidant capacity is necessary and also critical to evaluate the possible health benefits of a specific fruit or vegetable. Studies from our laboratory represent the first attempt in this area. We measured the total antioxidant capacity of common fruits and vegetables, using the ORAC assay with AAPH as a peroxyl radical generator. Based on the dry weight of the edible portion, kale, blackberries, strawberries, spinach, blueberries, cranberries, and raspberries had an antioxidant capacity of >100 μmol TE/g dry matter (DM). Beets, prunes, plums, red peppers, and Brussels sprouts had an antioxidant capacity between 60 and 100 μmol TE/g DM, whereas garlic, pink grapefruit, onions, cherries, tomato, lettuce, and corn had an antioxidant capacity of 20–60 μmol TE/g DM. Fruits and vegetables such as potato, sweet potato, yellow squash, cucumber, string bean, apple, celery, bananas, and pears had an antioxidant capacity of <20 μmol TE/g DM (54–57). The contribution of vitamin C to the total antioxidant capacity measured as the ORAC of the fruits was <15%, except for kiwifruit and honeydew melon. This suggests that the major source of antioxidant capacity of most fruits may not be vitamin C, but other antioxidant phytochemicals contained in fruits.

Many dietary supplements have been developed as a result of the finding of the potential health-promoting aspects of fruits and vegetables. These dietary supplements, based on fruits, vegetables, or other plants rich in antioxidant phytonutrients, are usually marketed as dietary antioxidant supplements. However, good markers have not been applied to indicate the antioxidant potency of these natural products, until recently, when we adapted and automated the ORAC assay and used it to determine the antioxidant capacity in fruits, vegetables, and dietary supplements. We evaluated 46 commercial preparations of antioxidant-related dietary supplements for total antioxidant capacity, using the ORAC assay. Products based on bilberry, cranberry, chokeberry and elderberry were found to have ORAC values ranging from 16 to 3985 μmol TE/g. Proanthocyanin sources such as pine bark and grape seed extracts had antioxidant capacities ranging from 16 to 8392 μmol TE/g (58). The finding of these wide ranges in antioxidant potency of the antioxidant-related supplements underscores the need for quality control of these herbal supplements. This need is especially important in the United States because herbal supplements are not regulated as drugs, but are instead sold as "food supplements."

The presence of antioxidant components in botanicals is quite common. In addition to our work, others have reported antioxidant activity for common fruits and vegetables (59–62) and tea (63–65). Other researchers, using quite varied techniques for assessing antioxidant capacity, have reported antioxidant activity in berry and fruit wines (66, 67), citrus peel and seed extracts (68), evening primrose meal (69), buckwheat groats (70), *Ginkgo biloba* (71), aromatic herbs (72), leaves of the small *Vernonia amygdalina* tree (73), mulberry leaves (74), leaves of *Perilla frutescens*, a popular garnish in Japan (75), and Hawthorn organs (76). Thus, the presence of antioxidant components in a wide variety of botanicals is well documented; however, consistent methods for comparing different sources have not been used, which may not be essential to the demonstration that antioxidant activity exists. If more of the sources are developed as antioxidant dietary supplements in the United States, consistent analytical techniques for evaluating these supplements will be essential.

Importance of Free Radical or Oxidant Source in Assessing Antioxidant Capacity of Botanicals

Considerable confusion is evident in the literature when one attempts to compare antioxidant capacity or relative ranking of different botanicals that have been evaluated for antioxidant capacity by different laboratories. Part of the confusion results from the fact that completely different procedures have been used. Inherent in some of these methodological differences is the difference in free radical or oxidant source used. We have compared the antioxidant capacity of vegetables, using a peroxyl radical source (AAPH), a hydroxyl radical generator (Cu^{++} plus H_2O_2), and Cu^{++} as an oxidant in the ORAC assay (54). Results for a few selected vegetables are presented in Figure 1 to illustrate that components in different vegetables do not respond to the different oxidant sources in a similar manner. Although components in kale are very strong antioxidants against the peroxyl radical, they are not nearly as effective against Cu^{++}. However, on a relative basis, antioxidant components in leaf lettuce, iceberg lettuce, and string beans are more effective against the hydroxyl radical than are some of the other vegetables (Figure 1).

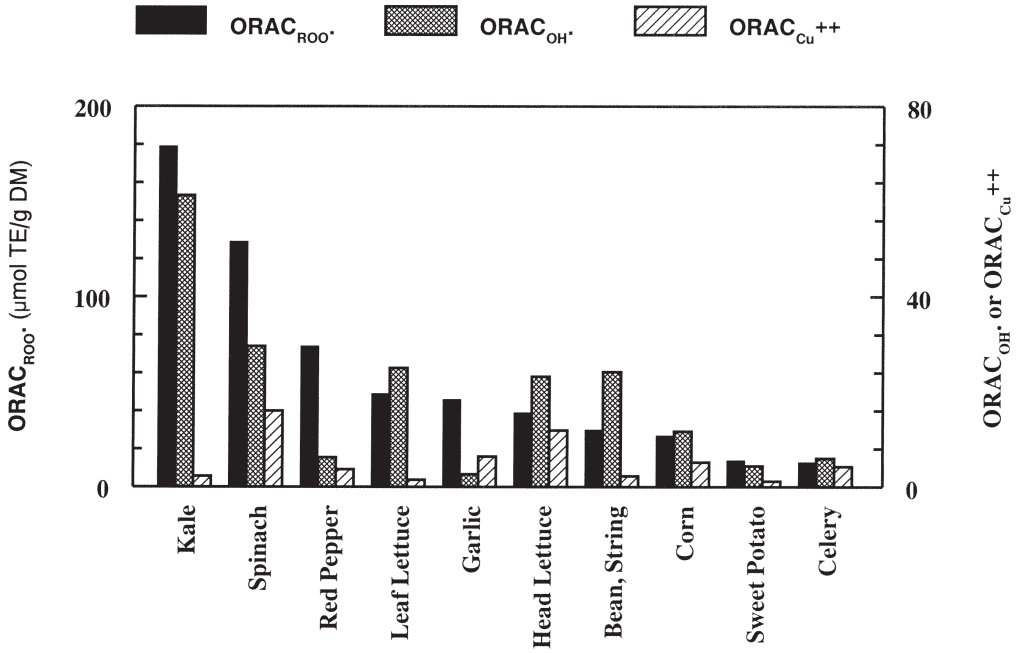

Figure 1. Antioxidant capacity obtained for selected vegetables by using a peroxyl radical generator (AAPH) (ORAC$_{ROO\cdot}$), hydroxyl radical (ORAC$_{OH\cdot}$), or Cu^{++} as the oxidant in the ORAC assay. Data are expressed as μmol Trolox equivalents/g dry matter. Data are from Cao et al. (54).

Another common method for evaluating antioxidant activity of fruits and vegetables has been the oxidation of LDL using either AAPH or Cu^{++} (59, 64, 65). This model is thought to have particular relevance to cardiovascular disease. However, oxidant source is important in this evaluation as well. When we compared the results obtained from ORAC using AAPH as a peroxyl radical source with those obtained from the inhibition of LDL oxidation with AAPH, a significant linear relationship ($Y = 1.74 + 1.00X$; $r_{xy} = 0.91$, where $X = IC_{50}$ for LDL oxidation and Y = ORAC, μmol TE/g) was found between the 2 independent measures of antioxidant capacity of some different extracts from oats (Figure 2; 77). Thus, in this one comparison, there seems to be some agreement between methods if a common free radical source is used. In order to obtain a complete assessment of the antioxidants present in a botanical, it may be necessary to evaluate the material by using the 3 radical sources (peroxyl, hydroxyl, and Cu^{++}).

We have observed a pro-oxidant action of a green tea preparation when Cu^{++} was used as the oxidant in the ORAC assay, but this was not observed for any of the other vegetables tested (54).

Sources of Variability in Antioxidant Capacity of Botanicals and Dietary Supplements

Potential sources of variability in antioxidant capacity in dietary supplements include (*1*) botanical source, (*2*) genetic background, (*3*) environmental effects during growth, and (*4*) processing techniques used in production of the supplement. Clearly, botanical source influences the antioxidant capacity; we have observed a >20-fold difference between those fruits and vegetables with a high antioxidant capacity and those with low activity. Furthermore, we have observed as much as a 3.3-fold difference in the antioxidant capacity of different species and cultivars of *Vaccinium* (57). Similar genetic variation has been observed in strawberries and peaches (unpublished data, Prior et al., 1999). Increasing maturity at harvest of 2 varieties of Rabbiteye blueberries also increased antioxidant capacity by 164 and 224%, respectively (57). Less is known about environmental influences. We did not observe any differences in antioxidant capacity in 2 varieties of blueberries, each grown in different parts of the United States. However, there are indications that conditions which might stress plants (i.e., cold, drought, insects, diseases, etc.) may likely increase the components in plants which account for the measured antioxidant capacity. Extraction and processing techniques are the other factors which may affect the antioxidant capacity measured in supplements. In many cases, the botanical material is merely dried and ground, in which case the activity may be lower than that present in the starting material. If extraction or concentration techniques are used, it is possible to produce supplements that are relatively high in antioxidant capacity. We have observed a range in ORAC values from 16 to 8392 μmol TE/g DM in different natural products and different sources of the same botancial that are being marketed as dietary supplements (58). In many cases, it appears that manufacturers may start with a relatively concentrated or "standardized" source, but in formulation of the final product, various fillers, etc. are added so that the concentration in the final product is diluted, often to levels lower than that of the starting botanical material. Developing a dietary supplement with maximal antioxidant capacity should not be the primary objective; however, the potency of any particular supplement must be known in order to determine the optimal amount and form of delivery.

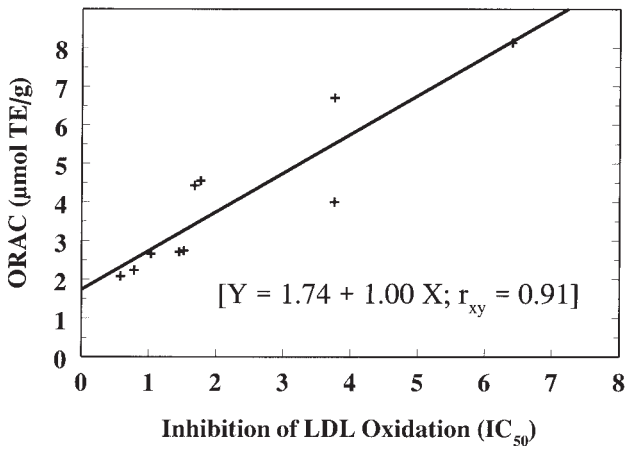

Figure 2. Relationship between antioxidant capacity in different oat fractions as assessed by ORAC and inhibition of LDL oxidation (IC_{50}), using AAPH as the free radical source in both assays. A significant ($P < 0.01$) linear relationship was found, $Y = 1.74 + 1.00X$; $r_{xy} = 0.91$ (77).

Antioxidants Identified in Fruits, Vegetables, Dietary Supplements, and Other Botanicals

As suggested earlier, our studies on the antioxidant capacities of fruits suggest that the major source of antioxidant capacity of these fruits may not be vitamin C, but other antioxidant phytochemicals. The measurement of total phenolics and/or anthocyanins (a group of phenolics belonging to the broad class of flavonoids) along with the ORAC assay demonstrated further that phenolics are the main antioxidant constituents contained in fruits, vegetables, and dietary natural product supplements. The total antioxidant capacity, measured as ORAC, was significantly correlated with total phenolic content in teas, fruits, and antioxidant-related natural product supplements, with a linear correlation coefficient of >0.85 (57, 58, 63). In berries and berry-based dietary supplements, ORAC was also significantly correlated with total anthocyanin content. The total phenolic content ranged from 1.9 to 804 mg/g in the 46 commercial dietary supplements (58). For the berry-based (bilberry, cranberry, chokeberry, and elderberry) products, total anthocyanin concentrations ranged from 0.2 to 204 mg/g, and total phenolic concentrations ranged from 1.8 to 464 mg/g. The total phenolics as anthocyanins in these berry-based products also ranged from 1.4 to 72.7%. The supplements of proanthocyanin sources (pine bark extracts and grape seed extracts) did not contain any anthocyanins (58).

Phenolics or polyphenols, the primary components responsible for antioxidant capacity in fruits, vegetables, and dietary natural product supplements, constitute one of the most numerous and widely distributed groups of substances in the plant kingdom, with more than 8000 phenolic structures currently known (78). Natural phenolics can range from simple molecules, such as phenolic acids, to highly polymerized compounds, such as tannins. Flavonoids represent the most common and widely distributed group of plant phenolics. Their basic structure is that of diphenylpropanes (C_6-C_3-C_6) and consists of 2 aromatic rings linked through 3 carbons that usually form an oxygenated heterocycle. The common family members of flavonoids include flavones, isoflavones, flavanones, flavanols, anthocyanins, and proanthocyanidins (procyanidins or condensed tannins). Phenolic antioxidants function as terminators of free radicals by rapid donation of a hydrogen atom to radicals. They can also act as chelators of transition metals that are involved in free radical production and oxidative reactions (79, 80). We have demonstrated, along with others, that many phenolics, including phenolic acids and flavonoids, have antioxidant capacities that are much stronger than those of vitamins C and E (25, 81).

Absorption of Dietary Phenolic Antioxidants and Their Effects on in vivo Antioxidant Status in Humans

Absorption of phenolics from the diet was long assumed to be negligible, because most flavonoids, except some simple phenolics and 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 cumulative evidence indicates that flavonoid glycosides are well absorbed in humans and rats without prior hydrolysis by microorganisms (82–86). Anthocyanins, the main flavonoids found in red grapes, red wine, berries, and berry-based dietary supplements, have also been shown to be absorbed (84, 86–90). Absorption of ferulic acid, a phenolic acid and a strong antioxidant, has also been demonstrated (90).

The absorbed dietary phenolic antioxidants can improve antioxidant status 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 resulted in a significant increase in plasma antioxidant capacity (91), as did increasing the consumption of strawberries (92), spinach (92), grape juice (93), and red wine (92, 94). A reduced sensitivity to oxidation of plasma and/or low-density lipoprotein was observed by several research groups in human subjects consuming red wine (95–97).

Intake of Antioxidants from Fruits, Vegetables, and Dietary Supplements

Based on the food intake survey conducted by the U.S. Department of Agriculture on consumption of fruits and vegetables, the average intake of total antioxidant capacity (expressed in ORAC units) from fruits and vegetables in the United States is 1200–1640 μmol TE/day (R.L. Prior [unpublished data, 1999], 56). Based on our studies, an increase in antioxidant intake from fruits and vegetables equivalent to 1000–2000 μmol TE/day may be needed to bring about some of the beneficial effects associated with fruit and vegetable consumption. When a dietary supplement is used to increase the intake of natural antioxidants, a measure of the antioxidant capacity of the supplement is needed to arrive at a reasonable intake of the supplement to provide an efficacious dose. This requires that a standard procedure be adopted by the nutritional supplement industry for determining antioxidant capacity. The measurement of ORAC and total phenolics provides a good measure of total potency. However, liquid chromatographic procedures will be needed if the botanical source is to be verified.

Conclusions

The ORAC method has been used to assess antioxidant capacity in botanicals and dietary supplements. Results demonstrate that the total antioxidant capacity of fruits, vegetables, and antioxidant-related dietary supplements varies considerably from one product to another. When a dietary supplement is used to increase the intake of natural antioxidants, the antioxidant capacity of the supplement is needed in order to arrive at a reasonable intake of the supplement to provide an efficacious dose; thus, a standard procedure should be adopted by the nutritional supplement industry for determining antioxidant capacity.

References

(1) Halliwell, B., Gutteridge, J.M.C., & Cross, C.E. (1992) *J. Clin. Lab. Med*. **119**, 598–620
(2) Ames, B.N., Shigensaga, M.K., & Hagen, T.M. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 7915–7922
(3) Maxwell, S.R. (1995) *Drugs* **49**, 345–361
(4) Halliwell, B., & Chirico, S. (1993) *Am. J. Clin. Nutr*. **57**, 715S–725S
(5) Loft, S., & Pousen, H.E. (1996) *J. Mol. Med.* **74**, 297–312
(6) Stahl, W., & Sies, H. (1997) *Diabetes* **46**, S14–S18
(7) Wayner, D.D.M., Burton, G.W., Ingold, K.U., & Locke, S. (1985) *FEBS Lett.* **187**, 33–37
(8) Miller, N.J., Rice-Evans, C., Davies, M.J., Gopinathan, V., & Milner, A. (1993) *Clin. Sci.* **84**, 407–412
(9) Rice-Evans, C., & Miller, N.J. (1994) *Methods Enzymol.* **234**, 279–293
(10) Benzie, I.F.F., & Strain, J.J. (1996) *Anal. Biochem*. **239**, 70–76
(11) Cao, G., Alessio, H.M., & Cutler, R.G. (1993) *Free Rad. Biol. Med.* **14**, 303–311

(12) Cao, G., & Prior, R.L. (1999) *Methods Enzymol.* **299**, 50–62
(13) Cao, G., & Prior, R.L. (1998) *Clin. Chem.* **44**, 1309–1315
(14) Schofield, D., & Braganza, J.M. (1996) *Clin. Chem.* **42**, 1712–1714
(15) Strube, M., Haenen, G.R.M.M., Van Den Berg, H., & Bast, A. (1997) *Free Rad. Res.* **26**, 515–521
(16) Nicoletta, R.R., Protsogente, P.A., Pannala, A., Yang, M., & Rice-Evans, C. (1999) *Free Rad. Biol. Med.* **26**, 1231–1237
(17) Cao, G., Verdon, C.P., Wu, A.H.B., Wang, H., & Prior, R.L. (1995) *Clin. Chem.* **41**, 1738–1744
(18) Naguib, Y.M.A. (1998) *Anal. Biochem.* **265**, 290–298
(19) Pieri, C., Maurizio, M., Fausto, M., Recchioni, R., & Marcheselli, F. (1994) *Life Sci.* **55**, PL271–276
(20) Testa, R., Testa, I., Manfrini, S., Bonfigli, A.R., Piantanelli, L., & Marra, M. (1996) *Life Sci.* **59**, 43–49
(21) Miller, J.W., Selhub, J., & Joseph, J.A. (1996) *Free Rad. Biol. Med.* **21**, 241–249
(22) Lin, Y.L., Juan, I.M., Chen, Y.L., Liang, Y.C., & Lin, J.K. (1996) *J. Agric. Food Chem.* **44**, 1387–1394
(23) Sharma, H.M., Hanna, A.N., Kauffman, E.M., & Newman, H.A.I. (1995) *Free Rad. Biol. Med.* **18**, 687–697
(24) Yu, J., Fox, J.G., Blanco, M.C., Yan, L., Correa, P., & Russell, R.M. (1995) *J. Nutr.* **125**, 2493–2500
(25) Cao, G., Sofic, E., & Prior, R.L. (1997) *Free Rad. Biol. Med.* **22**, 749–760
(26) Atanasiu, R.L., Stea, D., Mateescu, M.A., Vergely, C., Dalloz, F., Briot, F., Maupoil, V., Nadeau, R., & Rochette, L. (1998) *Mol. Cell Biochem.* **189**, 127–135
(27) Ninfali, P., & Aluigi, G. (1998) *Free Rad. Res.* **29**, 399–408
(28) Vergely, C., Walker, M.K., Zeller, M., Rademakers, J.R., Maupoil, V., Schiavi, P., Guez, D., & Rochette, L. (1998) *Mol. Cell Biochem.* **178**, 151–155
(29) Doll, R. (1990) *Proc. Nutr. Soc.* **49**, 119–131
(30) Dragsted, L.O., Strube, M., & Larsen, J.C. **(YEAR?)** *Pharmacol. Toxicol.* **72** (suppl. 1), 116–135
(31) Willett, C.W. (1994) *Science* **264**, 532–537
(32) Maeda, H., Katsuki, T., Akaike, T., & Yasutake, R. (1993) *Jpn. J. Cancer Res.* **83**, 923–928
(33) Belman, S. (1983) *Carcinogenesis* **4**, 1063–1065
(34) Bresnick, E., Birt, D.F., Wolterman, K., Wheeler, M., & Markin, R.S. (1990) *Carcinogenesis* **11**, 1159–1163
(35) Maltzman, T.H., Hurt, L.M., Elson, C.E., Tanner, M.A., & Gould, M.N. (1989) *Carcinogenesis* **10**, 781–783
(36) Stoewsand, G.S., Anderson, J.L., & Munson, L. (1988) *Cancer Lett.* **39**, 199–207
(37) Stoewsand, G.S., Anderson, J.L., Munson, L., & Lisk, D.J. (1989) *Cancer Lett.* **45**, 43–48
(38) Wattenberg, L.W., & Coccia, J.B. (1991) *Carcinogenesis* **12**, 115–117
(39) Acheson, R.M., & Williams, D.R.R. (1983) *Lancet* **1**, 1191–1193
(40) Armstrong, B.K., Mann, J.I., Adelstein, A.M., & Eskin, F. (1975) *J. Chron. Dis.* **28**, 455–469
(41) Burr, M.C., & Sweetnam, P.M. (1982) *Am. J. Clin. Nutr.* **36**, 673–677
(42) Phillips, R.L., Lemon, F.R., Beeson, W.L., & Kuzma, J.W. (1978) *Am. J. Clin. Nutr.* **31**, 191–198
(43) Verlangieri, A.J., Kapeghian, J.C., el-Dean, S., & Bush, M. (1985) *Med. Hypotheses* **16**, 7–15
(44) Ascherio, A., Rimm, E.B., Giovannucci, E.L., Colditz, G.A., Rosner, B., Willett, W.C., Sacks, F., & Stampfer, M.J. (1992) *Circulation* **86**, 1475–1484
(45) Sacks, F.M., & Kass, E.H. (1988) *Am. J. Clin. Nutr.* **48**, 795–800
(46) Ames, B.M. (1983) *Science* **221**, 1256–1263
(47) Gey, K.F. (1990) *Biochem. Soc. Trans.* **18**, 1041–1045
(48) Gey, K.F., Puska, P., Jordan, P., & Moser, U.K. (1991) *Am. J. Clin. Nutr.* **53**, 326S–334S
(49) Stähelin, H.B., Gey, K.F., Eichholzer, M., Lüdin, E., Bernasconi, F., Thurneysen, J., & Brubacher, G. (1991) *Am. J. Epidemiol.* **133**, 766–775
(50) Stähelin, H.B., Gey, K.F., Eichholzer, M., & Lüdin, E. (1991) *Am. J. Clin. Nutr.* **53**, 265S–269S
(51) Steinberg, D., Parthasarathy, S., Carew, T.E., Khoo, J.C., & Witztum, J.L. (1989) *N. Engl. J. Med.* **320**, 915–924
(52) Steinberg, D. (1991) *Circulation* **84**, 1420–1425
(53) Willett, C.W. (1994) *Am. J. Clin. Nutr.* **59**, 162S–165S
(54) Cao, G., Sofic, E., & Prior, R.L. (1996) *J. Agric. Food Chem.* **44**, 3426–3431
(55) Wang, H., Cao, G., & Prior, R.L. (1996) *J. Agric. Food Chem.* **44**, 701–705
(56) Cao, G., Booth, S.L., Sadowski, J.A., & Prior, R.L. (1998) *Am. J. Clin. Nutr.* **68**, 1081–1087
(57) Prior, R.L., Cao, G., Martin, A., Sofic, E., McEwen, J., O'Brien, C., Lischner, N., Ehlenfeldt, M., Kalt, W., Krewer, G., & Mainland, C.M. (1998) *J. Agric. Food Chem.* **46**, 2686–2693
(58) Prior, R.L., & Cao, G. (1999) *J. Am. Nutraceut. Assoc.* **2**, 36–46
(59) Vinson, J.A., Hao, Y., Su, X., & Zubik, L. (1998) *J. Agric. Food Chem.* **46**, 3630–3634
(60) Vinson, J.A. (1998) in *Flavonoids in the Living System*, J. Manthey & B. Buslig (Eds), Proceedings of American Chemical Symposium, Orlando, FL, pp 151–164
(61) Wang, H., Nair, M.G., Strasburg, G.M., Booren, A.M., & Gray, J.I. (1999) *J. Agric. Food Chem.* **47**, 840–844
(62) Furauta, S., Nishiba, Y., & Suda, I. (1997) *J. Food Sci.* **62**, 526–528
(63) Prior, R.L., & Cao, G. (1999) *Proc. Soc. Exp. Biol. Med.* **220**, 255–261
(64) Vinson, J.A., & Dabbash, Y.A. (1998) *Nutr. Res.* **18**, 1067–1075
(65) Vinson, J.A., Dabbagh, Y.A., Serry, M.M., & Jang, J. (1995) *J. Agric. Food Chem.* **43**, 2800–2802
(66) Heinonen, I.M., Lehtonen, P.J., & Hopia, A.I. (1998) *J. Agric. Food Chem.* **46**, 25–31
(67) Kondo, Y., Ohnnishi, M., & Kawaguchi, M. (1999) *J. Agric. Food Chem.* **47**, 1781–1785
(68) Bocco, A., Cuvelier, M.E., Richard, H., & Berset, C. (1998) *J. Agric. Food Chem.* **46**, 2123–2129
(69) Wettasinghe, M., & Shahidi, F. (1999) *J. Agric. Food Chem.* **47**, 1801–1812
(70) Watanabe, M. (1998) *J. Agric. Food Chem.* **46**, 839–845
(71) Akiba, S., Kawauchi, T., Oka, T., Hashizume, T., & Sato, T. (1998) *Biochem. Mol. Biol. Int.* **46**, 1243–1248
(72) Dapkevicius, A., Venskutonis, R., van Beek, T.A., & Linssen, J.P.H. (1998) *J. Sci. Food Agric.* **77**, 140–146

(73) Igile, G.O., Oleszek, W., Jurzysta, M., Burda, S., Fafunso, M., & Fasanmade, A.A. (1994) *J. Agric. Food Chem.* **42**, 2445–2448

(74) Yen, G.C., Wu, S.C., & Duh, P.D. (1996) *J. Agric. Food Chem.* **44**, 1687–1690

(75) Tada, M., Matsumoto, R., Yamaguchi, H., & Chiba, K. (1996) *Biosci. Biotech. Biochem.* **60**, 1093–1095

(76) Bahorum, T., Gressier, B., Trotin, F., Brunet, C., Dine, T., Luyckx, M., Vasseur, J., Cazin, M., Cazin, J.C., & Pinkas, M. (1996) *Arzneim.-Forsch.* **46**, 1086–1089

(77) Handelman, G., Cao, G., Walter, M., Nightingale, Z., Paul, G., Prior, R., & Blumberg, J. (1999) *J. Agric. Food Chem.* **47**, 4888–4893

(78) Bravo, L. (1998) *Nutr. Rev*. **56**, 317–333

(79) Morel, I., Lescoat, G., Cillard, P., & Cillard, J. (1994) *Methods Enzymol.* **243**, 437–443

(80) Morel, I., Lescoat, G., Cogrel, P., Sergent, O., Pasdeloup, N., Brissot, P., Cillard, P., & Cillard, J. (1993) *Biochem. Pharmacol*. **45**, 13–19

(81) Rice-Evans, C.A., Miller, N.J., Bolwell, P.G., Bramley, P.M., & Pridham, J.B. (1995) *Free Rad. Res.* **22**, 375–383

(82) Hollman, P.C., de Vries, J.H., van Leeuwen, S.D., Mengelers, M.J., & Katan, M.B. (1995) *Am. J. Clin. Nutr.* **62**, 1276–1282

(83) Hollman, P.C., v.d. Gaag, M., Mengelers, M.J., van Trijp, J.M., de Vries, J.H., & Katan, M.B. (1996) *Free Radical Biol. Med.* **21**, 703–707

(84) Paganga, G., & Rice-Evans, C.A. (1997) *FEBS Lett*. **401**, 78–82

(85) Morazzoni, P., Livio, S., Scilingo, A., & Malandrino, S. (1991) *Arzneim.-Forsch.* **41**, 128–131

(86) Horwitt, M.K. (1933) *Proc. Soc. Exptl. Biol. Med.* **30**, 949–951

(87) Cao, G., & Prior, R.L. (1999) *Clin. Chem.* **45**, 574–576

(88) Miyazawa, T., Nakagawa, K., Kudo, M., Muraishi, K., & Someya, K. (1999) *J. Agric. Food Chem*. **47**, 1083–1091

(89) Lapidot, T., Harel, S., Granit, R., & Kanner, J. (1999) *J. Agric. Food Chem.* **46**, 4297–4302

(90) Bourne, L.C., & Rice-Evans, C. (1998) *Biochem. Biophys. Res. Comm.* **253**, 222–227

(91) Cao, G., Booth, S.L., Sadowski, J.A., & Prior, R.L. (1998) *Am. J. Clin. Nutr*. **68**, 1081–1087

(92) Cao, G., Russell, R.M., Lischner, N., & Prior, R.L. (1998) *J. Nutr.* **128**, 2383–2390

(93) Day, A.P., Kemp, H.J., Bolton, C., Hertog, M., & Stansbie, D. (1997) *Ann. Nutr. Metab*. **41**, 353–357

(94) Whitehead, T.P., Robinson, D., Allaway, S., Syms, J., & Hale, A. (1994) *Clin. Chem*. **41**, 32–35

(95) Miyagi, Y., Miwa, K., & Inoue, H. (1997) *Am. J. Cardiol*. **80**, 1627–1631

(96) Nigdikar, S.V., Williams, N.R., Griffin, B.A., & Howard, A.N. (1998) *Am. J. Clin. Nutr*. **68**, 258–265

(97) Fuhrman, B., Lavy, A., & Aviram, M. (1995) *Am. J. Clin. Nutr*. **61**, 549–554