Reviews

Flavonoids as Antioxidants

Pier-Giorgio Pietta*

Institute of Advanced Biomedical Technologies, National Council of Research Via F.lli Cervi 93, 20090 Segrate (MI), Italy

Received September 13, 1999

Flavonoids are phenolic substances isolated from a wide range of vascular plants, with over 8000 individual compounds known. They act in plants as antioxidants, antimicrobials, photoreceptors, visual attractors, feeding repellants, and for light screening. Many studies have suggested that flavonoids exhibit biological activities, including antiallergenic, antiviral, antiinflammatory, and vasodilating actions. However, most interest has been devoted to the antioxidant activity of flavonoids, which is due to their ability to reduce free radical formation and to scavenge free radicals. The capacity of flavonoids to act as antioxidants in vitro has been the subject of several studies in the past years, and important structure−activity relationships of the antioxidant activity have been established. The antioxidant efficacy of flavonoids in vivo is less documented, presumably because of the limited knowledge on their uptake in humans. Most ingested flavonoids are extensively degraded to various phenolic acids, some of which still possess a radical-scavenging ability. Both the absorbed flavonoids and their metabolites may display an in vivo antioxidant activity, which is evidenced experimentally by the increase of the plasma antioxidant status, the sparing effect on vitamin E of erythrocyte membranes and low-density lipoproteins, and the preservation of erythrocyte membrane polyunsaturated fatty acids. This review presents the current knowledge on structural aspects and in vitro antioxidant capacity of most common flavonoids as well as in vivo antioxidant activity and effects on endogenous antioxidants.

Introduction

Oxidation is the transfer of electrons from one atom to another and represents an essential part of aerobic life and our metabolism, since oxygen is the ultimate electron acceptor in the electron flow system that produces energy in the form of ATP.[1] However, problems may arise when the electron flow becomes uncoupled (transfer of unpaired single electrons), generating free radicals. Examples of oxygen-centered free radicals, known as reactive oxygen species (ROS), include superoxide ($O_2^{\bullet -}$), peroxyl ($ROO^{\bullet}$), alkoxyl ($RO^{\bullet}$), hydroxyl ($HO^{\bullet}$), and nitric oxide ($NO^{\bullet}$). The hydroxyl (half-life of 10^{-9} s) and the alkoxyl (half-life of seconds) free radicals are very reactive and rapidly attack the molecules in nearby cells, and probably the damage caused by them is unavoidable and is dealt with by repair processes. On the other hand, the superoxide anion, lipid hydroperoxides, and nitric oxide are less reactive.[2] In addition to these ROS radicals, in living organisms there are other ROS nonradicals, such as the singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and hypochlorous acid (HOCl).

It is accepted that ROS play different roles in vivo. Some are positive and are related to their involvement in energy production, phagocytosis, regulation of cell growth and intercellular signaling, and synthesis of biologically important compounds.[3] However, ROS may be very damaging, since they can attack lipids in cell membranes, proteins in tissues or enzymes, carbohydrates, and DNA, to induce oxidations, which cause membrane damage, protein modification (including enzymes), and DNA damage. This oxidative damage is considered to play a causative role in aging and several degenerative diseases associated with it, such as heart disease, cataracts, cognitive dysfunction, and cancer.[4−6] Humans have evolved with antioxidant systems to protect against free radicals. These systems include some antioxidants produced in the body (endogenous) and others obtained from the diet (exogenous). The first include (a) enzymatic defenses, such as *Se*-glutathione peroxidase, catalase, and superoxide dismutase, which metabolize superoxide, hydrogen peroxide, and lipid peroxides, thus preventing most of the formation of the toxic $HO^{\bullet}$, and (b) nonenzymatic defenses, such as glutathione, histidine-peptides, the iron-binding proteins transferrin and ferritin, dihydrolipoic acid, reduced CoQ_{10}, melatonin, urate, and plasma protein thiols, with the last two accounting for the major contribution to the radical-trapping capacity of plasma. The various defenses are complementary to each other, since they act against different species at different cellular compartments. However, despite these *defense antioxidants* (able either to suppress free radical formation and chain initiation or to scavenge free radical and chain propagation), some ROS still escape to cause damage. Thus, the body antioxidant system is provided also by *repair antioxidants* (able to repair damage, and based on proteases, lipases, transferases, and DNA repair enzymes).[7]

Owing to the incomplete efficiency of our endogenous defense systems and the existence of some physiopathological situations (cigarette smoke, air pollutants, UV radiation, high polyunsaturated fatty acid diet, inflammation, ischemia/reperfusion, etc.) in which ROS are produced in excess and at the wrong time and place, dietary antioxidants are needed for diminishing the cumulative effects of oxidative damage over the life span.[8,9] Well-established antioxidants derived from the diet are vitamins C, E, A, and carotenoids, which have been studied inten-

* To whom correspondence should be addressed. Tel.: 0039 02 26422725. Fax: 0039 02 26422770. E-mail: pietta@itba.mi.cnr.it.

10.1021/np9904509 CCC: $19.00
Published on Web 05/27/2000

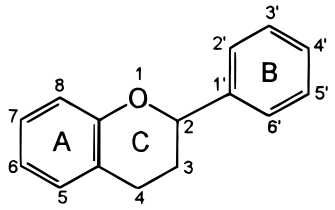


Figure 1. Basic flavonoid structure.

sively.[10] Besides these antioxidant vitamins, other substances in plants might account for at least part of the health benefits associated with vegetable and fruit consumption. Over the past decade evidence has been accumulated that plant polyphenols are an important class of defense antioxidants. These compounds are widespread virtually in all plant foods, often at high levels, and include phenols, phenolic acids, flavonoids, tannins, and lignans.

Flavonoids. Flavonoids are formed in plants from the aromatic amino acids phenylalanine and tyrosine, and malonate.[11] The basic flavonoid structure is the flavan nucleus, which consists of 15 carbon atoms arranged in three rings ($C_6-C_3-C_6$), which are labeled A, B, and C (Figure 1). The various classes of flavonoids differ in the level of oxidation and pattern of substitution of the C ring, while individual compounds within a class differ in the pattern of substitution of the A and B rings. Among the many classes of flavonoids, those of particular interest to this review are flavones, flavanones, isoflavones, flavonols, flavanonols, flavan-3-ols, and anthocyanidins (Table 1). Other flavonoid classes include biflavones, chalcones, aurones, and coumarins. Hydrolyzable tannins, proanthocyanidins (flavan-3-ol oligomers), caffeates, and lignans are all plant phenols, and they are usually classified separately.

Flavonoids generally occur in plants as glycosylated derivatives, and they contribute to the brilliant shades of blue, scarlet, and orange, in leaves, flowers, and fruits.[12] Apart from various vegetables and fruits, flavonoids are found in seeds, nuts, grains, spices, and different medicinal plants as well in beverages, such as wine (particularly red wine), tea, and (at lower levels) beer.[13] More specifically, the flavones apigenin and luteolin are common in cereal grains and aromatic herbs (parsley, rosemary, thyme), while their hydrogenated analogues hesperetin and naringin are almost exclusively present in citrus fruits.[14] The flavonols quercetin and kaempferol are predominant in vegetables and fruits, where they are found mainly in the skin, with the exception of onions. Isoflavones are found most often in legumes, including soybeans, black beans, green beans, and chick peas. Alfalfa and clover sprouts and sunflower seeds also contain isoflavones.[15] The flavan-3-ols (+)-catechin, (−)-epicatechin, (−)-epigallocatechin, and their gallate esters are widely distributed in plants, although they are very rich in tea leaves. Flavan oligomers (proanthocyanidins) are present in apples, grapes, berries, persimmon, black currant, and sorghum and barley grains.[16] Anthocyanidins and their glycosides (anthocyanins) are natural pigments and are abundant in berries and red grape.[17]

Flavonoids play different roles in the ecology of plants. Due to their attractive colors, flavones, flavonols, and anthocyanidins may act as visual signals for pollinating insects. Because of their astringency, catechins and other flavanols can represent a defense system against insects harmful to the plant.[18] Flavonoids act as catalysts in the light phase of photosynthesis and/or as regulators of iron channels involved in phosphorylation.[19] They can also function as stress protectants in plant cells by scavenging ROS produced by the photosynthetic electron transport system.[20] Furthermore, because of their favorable UV-absorbing properties, flavonoids protect plants from UV radiation of sun and scavenge UV-generated ROS.[21]

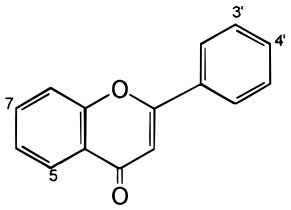
Apart from their physiological roles in the plants, flavonoids are important components in the human diet, although they are generally considered as nonnutrients. Indeed, the level of intake of flavonoids from diet is considerably high as compared to those of vitamin C (70 mg/day), vitamin E (7−10 mg/day), and carotenoids (β-carotene, 2−3 mg/day).[22] Flavonoid intake can range between 50 and 800 mg/day, depending on the consumption of vegetables and fruit, and of specific beverages, such as red wine, tea, and unfiltered beer.[23] In particular, red wine and tea contain high levels (approximately 200 mg per glass of red wine or cup of tea) of total phenols. Thus, variations in consumption of these beverages are mainly responsible for the overall flavonoid intake in different national diets. Another significant source of flavonoids are different medicinal plants and related phytomedicines.[24]

Epidemiological Evidence. Several epidemiological studies provide support for a protective effect of the consumption of fresh fruits and vegetables against cancer,[25,26] heart disease,[27−29] and stroke.[30,31] Normally, high consumers of fruits and vegetables have a healthy lifestyle, which may be an important factor for their resistance against chronic diseases. All in all, fruits and vegetables do play a preventive role, which is due to a variety of constituents, including vitamins, minerals, fiber, and numerous phytochemicals, including flavonoids. Thus, it is possible that also flavonoids contribute to the protective effect of fruits and vegetables. This possibility has been evidenced by several in vitro, ex vivo, and animal studies.[32] Unfortunately, the evidence in humans is still limited and somewhat controversial.[33] Data on biological markers, such as blood levels of flavonoids and their metabolites, are not widely available, thus making it difficult to determine the individual or the combined role of the flavonoids and other antioxidants.

The association between flavonoid intake and cancer protection is (at present) weak. According to some epidemiological studies, there is no evidence that flavonoid intake is protective against some types of cancer.[34] Only one study has shown that the consumption of flavonoids is inversely correlated with lung cancer.[35] In contrast, a possible protective role against coronary heart disease of flavonoid intake (either from fruits and vegetables or red wine and tea) has been reported in four out of six epidemiological studies.[36] The dietary sources of flavonoids were fruits, vegetables, red wine, and tea, and they were found to be inversely correlated with the risk of coronary heart disease and stroke. On the other hand, a weak inverse relationship was observed by Knekt and co-workers,[37] and in the largest prospective cohort study conducted in the United States[38] only a weak but nonsignificant inverse correlation was observed for flavonoid consumption and coronary mortality. Thus, it appears that the effects of flavonoids are strongest for coronary heart disease mortality and not morbidity. Accordingly, the present epidemiological data (although far from conclusive) evidence a possible protective role of dietary flavonoids, thus making desirable a regular consumption of foods and beverages rich in flavonoids.

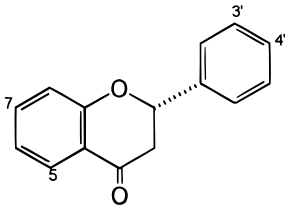
In Vitro Antioxidant Action. According to Halliwell and Gutteridge,[6] mechanisms of antioxidant action can include (1) suppressing reactive oxygen species formation either by inhibition of enzymes or chelating trace elements

Table 1. Structures of Flavonoids



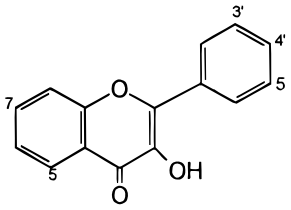
Flavones

	5	7	3′	4′
luteolin	OH	OH	OH	OH
apigenin	OH	OH		OH
chrysin	OH	OH		



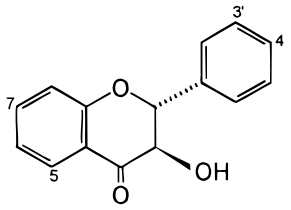
Flavanones

	5	7	3′	4′
hesperetin	OH	OH	OH	OCH_3
naringenin	OH	OH		OH



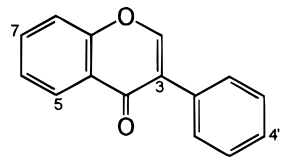
Flavonols

	5	7	3′	4′	5′
quercetin	OH	OH	OH	OH	
kaempferol	OH	OH		OH	
galangin	OH	OH			
fisetin		OH	OH	OH	
myricetin	OH	OH	OH	OH	OH



Flavanonol

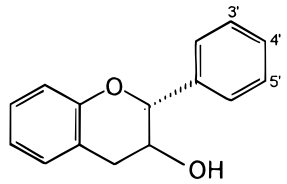
	5	7	3′	4′
taxifolin	OH	OH	OH	OH



Isoflavones

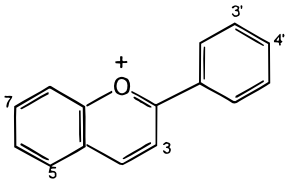
	5	7	4′
genistein	OH	OH	OH
genistin	OH	Oglc	OH
daidzein		OH	OH
daidzin		Oglc	OH
biochanin A	OH	OH	OCH_3
formononetin		OH	OCH_3

Table 1 (Continued)



Flavan-3-ols

	3	5	7	3′	4′	5′
(+)-catechin	βOH	OH	OH	OH	OH	
(−)-epicatechin	αOH	OH	OH	OH	OH	
(−)-epigallocatechin	αOH	OH	OH	OH	OH	OH



Flavylium Salts

	3	5	7	3′	4′
cyanidin	OH	OH	OH	OH	OH
cyanin	O-glc	OH	OH	OH	OH
pelargonidin	OH	OH	OH	−	OH

involved in free radical production; (2) scavenging reactive oxygen species; and (3) upregulating or protecting antioxidant defenses.

Flavonoids have been identified as fulfilling most of the criteria described above. Thus, their effects are twofold.

1. Flavonoids inhibit the enzymes responsible for superoxide anion production, such as xanthine oxidase[39] and protein kinase C.[40] Flavonoids have been also shown to inhibit cyclooxygenase, lipoxygenase, microsomal monooxygenase, glutathione *S*-transferase, mitochondrial succinoxidase, and NADH oxidase, all involved in reactive oxygen species generation.[41,42]

A number of flavonoids efficiently chelate trace metals, which play an important role in oxygen metabolism. Free iron and copper are potential enhancers of reactive oxygen species formation, as exemplified by the reduction of hydrogen peroxide with generation of the highly aggressive hydroxyl radical,

$$H_2O_2 + Fe^{2+}(Cu^{+}) \rightarrow {}^{\bullet}OH + OH^{-} + Fe^{3+}(Cu^{2+})$$

or by the copper-mediated LDL (low-density lipoprotein) oxidation,

$$LH \rightarrow L^{\bullet} \rightarrow LOO^{\bullet}$$

where LH represents LDL.

Nevertheless, it has to be remembered that these metal ions are essential for many physiological functions, as constituents of hemoproteins and cofactors of different enzymes, including those involved (iron for catalase, copper for ceruloplasmin and *Cu,Zn*-superoxide dismutase) in the antioxidant defense.[43]

The proposed binding sites for trace metals to flavonoids are the catechol moiety in ring B, the 3-hydroxyl, 4-oxo groups in the heterocyclic ring, and the 4-oxo, 5-hydroxyl groups between the heterocyclic and the A rings (Figure 2). However, the major contribution to metal chelation is due to the catechol moiety, as exemplified by the more pronounced bathochromic shift produced by chelation of

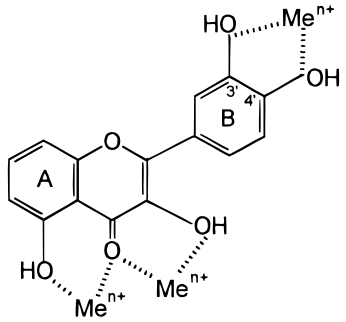


Figure 2. Binding sites for trace metals.

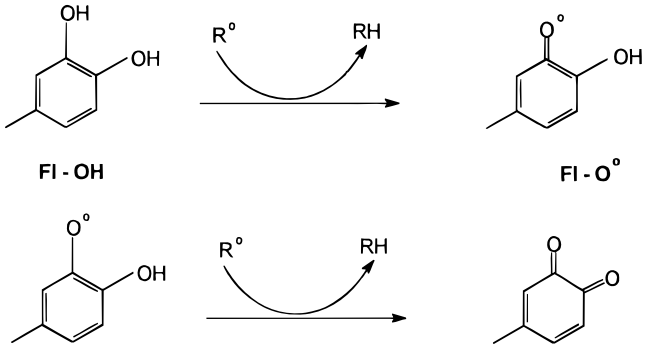


Figure 3. Scavenging of ROS (R•) by flavonoids.

copper to quercetin compared to that of kaempferol (similar in structure to quercetin except that it lacks the catechol group in the B ring).[44]

2. Due to their lower redox potentials ($0.23 < E_7 < 0.75$ V),[45] flavonoids (Fl-OH) are thermodynamically able to reduce highly oxidizing free radicals with redox potentials in the range 2.13−1.0 V,[46] such as superoxide, peroxyl, alkoxyl, and hydroxyl radicals by hydrogen atom donation:

$$\text{Fl-OH} + \text{R}^\bullet \rightarrow \text{Fl-O}^\bullet + \text{RH}$$

where R• represents superoxide anion, peroxyl, alkoxyl, and hydroxyl radicals.[47−49]

The aroxyl radical (Fl-O•) may react with a second radical, acquiring a stable quinone structure (Figure 3).

The aroxyl radicals could interact with oxygen, generating quinones and superoxide anion, rather than terminating chain reactions. The last reaction may take place in the presence of high levels of transient metal ions and is responsible for the undesired prooxidant effect of flavonoids.[50] Thus, the overall capacity of flavonoids to act as antioxidants depends not only on the redox potential of the couple Fl-O•/Fl-OH but also on possible side reactions of the aroxyl radical. Scavenging of superoxide is particularly important, because this radical is ubiquitous in aerobic cells and, despite its mild reactivity, is a potential precursor of the aggressive hydroxyl radical in the Fenton and Haber−Weiss reactions.[51] Besides scavenging, flavonoids may stabilize free radicals involved in oxidative processes by complexing with them.[52]

Many studies have been performed to establish the relationship between flavonoid structure and their radical-scavenging activity, and the most relevant are briefly described. Rice-Evans et al.[53] developed a valuable assay that allows for the determination of the hierarchy of radical-scavenging ability of flavonoids (and related phenolic acids). This assay is based on the ability of an antioxidant to scavenge (at pH 7.4) a preformed radical cation chromophore of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS•+) in relation to that of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), an aqueous soluble vitamin E analogue. The Trolox equivalent antioxidant capacity (TEAC) is defined as the concentration of Trolox with the same antioxidant capacity as a 1 mM concentration of the antioxidant under investigation. Jovanovic et al.[45] adopted the same approach described for determining the priority[46] (hierarchy of one-electron reduction potentials at pH 7, Table 2) of radical couples and evaluated the in vitro antioxidant potential of flavonoids on the basis of the one-electron reduction potential at pH 7 (E_7) of the Fl-O•/Fl-OH pair. In contrast, the half-peak oxidation potentials ($E_p/2$) of flavonoids have been proposed as suitable parameters to evaluate the scavenging activity. This assumes that both the electrochemical oxidation Fl-OH → Fl-O• + e^- + H^+ and the hydrogen atom donating reaction Fl-OH → Fl-O• + H• involve the breaking of the same O−H bond.[54] According to this approach, flavonoids with $E_p/2 < 0.2$ are defined as readily oxidable and therefore good scavengers. TEAC, E_7, and $E_p/2$ values of selected flavonoids are compared in Table 3.

Table 2. One-Electron Reduction Potentials at pH 7 (E_7, V) for Selected Radical Couples (Adapted from Ref 46)

HO•, H^+/HO	2.310
RO•, H^+/ROH (alkoxyl)	1.600
ROO•, H^+/ROOH (peroxyl)	1.000
PUFA•, H^+/PUFA-H	0.600
HU•, H^+/UH_2 (urate)	0.590
TO•, H^+/TOH	0.480
ascorbate•−, H^+/ascorbate−	0.282

The data obtained by the three different approaches mentioned above provide clear evidence that the radical-scavenging activity depends on the structure and the substituents of the heterocyclic and B rings, as suggested by Bors et al.[55] More specifically, the major determinants for radical-scavenging capability are (i) the presence of a catechol group in ring B, which has the better electron-donating properties and is a radical target, and (ii) a 2,3-double bond conjugated with the 4-oxo group, which is responsible for electron delocalization.

The presence of a 3-hydroxyl group in the heterocyclic ring also increases the radical-scavenging activity, while additional hydroxyl or methoxyl groups at positions 3,5 and 7 of rings A and C seem to be less important. These structural features contribute to increase the stability of the aroxyl radical, i.e., the antioxidant capacity of the parent flavonoid. Thus, flavonols and flavones containing a catechol group in ring B are highly active, with flavonols more potent than the corresponding flavones because of the presence of the 3-hydroxyl group. Glycosylation of this group, as in rutin, reduces greatly the radical-scavenging capacity. An additional hydroxyl group in ring B (pyrogallol group) enhances further the antioxidant capacity, as exemplified by myricetin. On the contrary, the presence of only one hydroxyl in ring B diminishes the activity. Flavanonols and flavanones, due to the lack of conjugation provided by the 2,3-double bond with the 4-oxo group, are weak antioxidants. Antioxidant activities of flavan monomers are comparable to those of flavanonols (catechin vs taxifolin). However, the presence of a pyrogallol group in ring B (like in epigallocatechin) or galloylation of the 3-hydroxyl group (as for epigallocatechin gallate and epicatechin gallate) enhances the antioxidant capacity.

Anthocyanidins and their glycosides (anthocyanins) are equipotent to quercetin and catechin gallates, provided that a catechol structure is present in ring B (like in cyanidin). Removal of the 3-hydroxyl group from ring B, as in pelargonidin, reduces the antioxidant capacity at the same

Table 3. Trolox Equivalent Antioxidant Capacity (mM), E_7 (V), and $E_p/2$ (V) of Flavonoids (the Hydroxylation Pattern Is Shown in Parentheses for Each Component)

	TEAC (mM)	E_7 (V)	$E_p/2$ (V)
Flavonols			
quercetin (3, 5, 7, 3′, 4′)	4.7	0.33	0.06
quercetin 3-rutinoside	2.42	0.6	0.18
kaempferol (3, 5, 7, 4′)	1.34	0.75	0.12
myricetin (3, 5, 7, 3′, 4′, 5′)	3.10	0.36	
galangin (3, 5, 7)	1.49	0.62	0.32
Flavanonols			
taxifolin (3, 5, 7, 3′, 4′)	1.9	0.5	0.15
dihydrokaempferol (3, 5, 7, 3′)	1.39		
Flavones			
luteolin (5, 7, 3′, 4′)	2.09	0.6	0.18
luteolin 4′-glucoside	1.74		
apigenin (5, 7, 4′)	1.45		>1
chrysin (5, 7)	1.43		>1
Flavanones			
eriodictyol (5, 7, 3′, 4′)	1.8		
hesperetin [5, 7, 3′, 4′(och$_3$)]	1.4		0.4
naringenin (5, 7, 4′)	1.5		0.6
naringenin 7-rutinoside	0.8		
Catechins and Catechin Gallates			
catechin (3, 5, 7, 3′, 4′)	2.4	0.57	0.16
epicatechin (3, 5, 7, 3′, 4′)	2.5		
epigallocatechin (3, 5, 7, 3′, 4′, 5′)	3.8	0.42	
epicatechin gallate	4.93		
epigallocatechin gallate	4.75	0.43	
Anthocyanidins			
cyanidin (3, 5, 7, 3′, 4′)	4.4		−0.23
cyanidin 3-rutinoside	3.2		
pelargonidin (3, 5, 7, 4′)	1.3		

Table 4. Trolox Equivalent Antioxidant Capacity (mM) of Isoflavones and Selected Flavonoid Metabolites (the Hydroxylation Pattern Is Shown in Parentheses for Each Compound)

Isoflavones	
genistein (5, 7, 4′)	2.90
biochanin A (5, 7, 4′OCH$_3$)	1.16
daidzein (7, 4′)	1.25
formononetin (7, 4′OCH$_3$)	0.11
genistein 7-glucoside	1.24
Selected Flavonoid Metabolites	
3,4-dihydroxyphenylacetic acid (I)	2.16
3-methoxy-4-hydroxyphenylacetic acid (II)	1.63
3-methoxy-4-hydroxybenzoic acid (III)	1.19
3,4-dihydroxybenzoic acid (IV)	1.01
3-methoxy-4-hydroxyhippuric acid (V)	1.29

level of kaempferol (which differs from quercetin because it has a lone hydroxyl group in ring B). These data confirm further that the catechol structure in the B ring is the major determinant for radical-scavenging capacity of the flavonoids.

In the case of isoflavones, the location of ring B at the 3-position of the heterocyclic ring greatly affects the radical-scavenging capacity, as determined by the TEAC assay (Table 4). Thus, genistein is 2 times more potent than its flavone relative apigenin. The single 4′-hydroxy group is required for scavenging, and on methoxylation, as in biochanin A, diminishes the potency. The 5,7-dihydroxy structure in ring A is also important, as evidenced by comparing the pairs genistein/daidzein and biochanin A/formononetin. As for the other flavonoid classes, glycosylation negatively influences the radical-scavenging capacity.

A different approach to evaluating the antioxidant potential of flavonoids is based on their ability to increase the resistance of isolated LDL to copper oxidation in vitro. This approach stems from the oxidative theory of atherogenesis, which states that it is not LDL or VLDL that is atherogenic but the oxidized form of these lipoproteins.[56] Indeed, several studies[57−60] have shown that most of dietary flavonoids are effective against the oxidative modification of LDL in vitro, provided that they are added before the initiation of the oxidation.[61] What is interesting is that the most active flavonoids possess the same structural features that guarantee TEAC efficacy or low redox potentials.

In Vivo Flavonoid Antioxidant Potential. Despite the increasing evidence for the in vitro antioxidant potential of flavonoids, little is known about their efficacy in vivo, and this may be ascribed to the only sketchy knowledge on their bioavailability in humans. Only recently has it been proved that flavonoids from dietary sources are absorbed at an extent that may promote an antioxidant effect. According to most authors, flavonol, flavone, and isoflavone glycosides are initially hydrolyzed to their respective aglycons.[62,63] However, glycosides are absorbable, as recently proved by the LC-MS detection of quercetin 3-rutinoside in blood of volunteers after consumption of tomato purèe[64] and of naringin (4′,5,7-trihydroxyflavanone-7-rhamnoglucoside) in urine of subjects who received orally naringin.[65] In the case of catechins, epigallocatechin gallate and epicatechin gallate have been detected in human blood after intake of green tea, decaffeinated green tea extracts, and dark chocolate.[66−69] Glucuronide and sulfate conjugates of (+)-catechin and 3′-*O*-methyl-(+)-catechin have been determined in human plasma after consumption of red wine.[70]

The percentage of absorption normally does not exceed a few percent of the ingested dose, as determined by measuring the blood levels of intact flavonoids and their conjugates. Food composition may represent an important factor that affects bioavailability. Proteins may bind to polyphenols,[71] reducing their availability; by contrast, alcohol may improve it,[72] as evidenced by the increased uptake of red wine phenolics as compared with levels resulting after the consumption of alcohol-free red wine.[73] In addition, recent data support an improved absorption of specific flavonoids in the presence of fats. Thus, catechins from green tea, oligomeric proanthocyanidins from grape seeds, and silibinin from milk thistle are absorbed at higher extent when administered as phospholipid complexes rather than when free.[74]

During absorption across the intestinal membrane, flavonols, flavones, isoflavones, and catechins are partly transformed in their glucuronides and sulfates.[75] Subsequently, this small fraction of the absorbed flavonoids is metabolized by the liver enzymes, resulting in more polar conjugates being excreted in the urine or returned to the duodenum via the gallbladder. However, the major part of ingested flavonoids is not absorbed and is largely degraded by the intestinal microflora. The bacterial enzymes catalyze several reactions, including hydrolysis, cleavage of the heterocyclic oxygen-containing ring, dehydroxylation, and

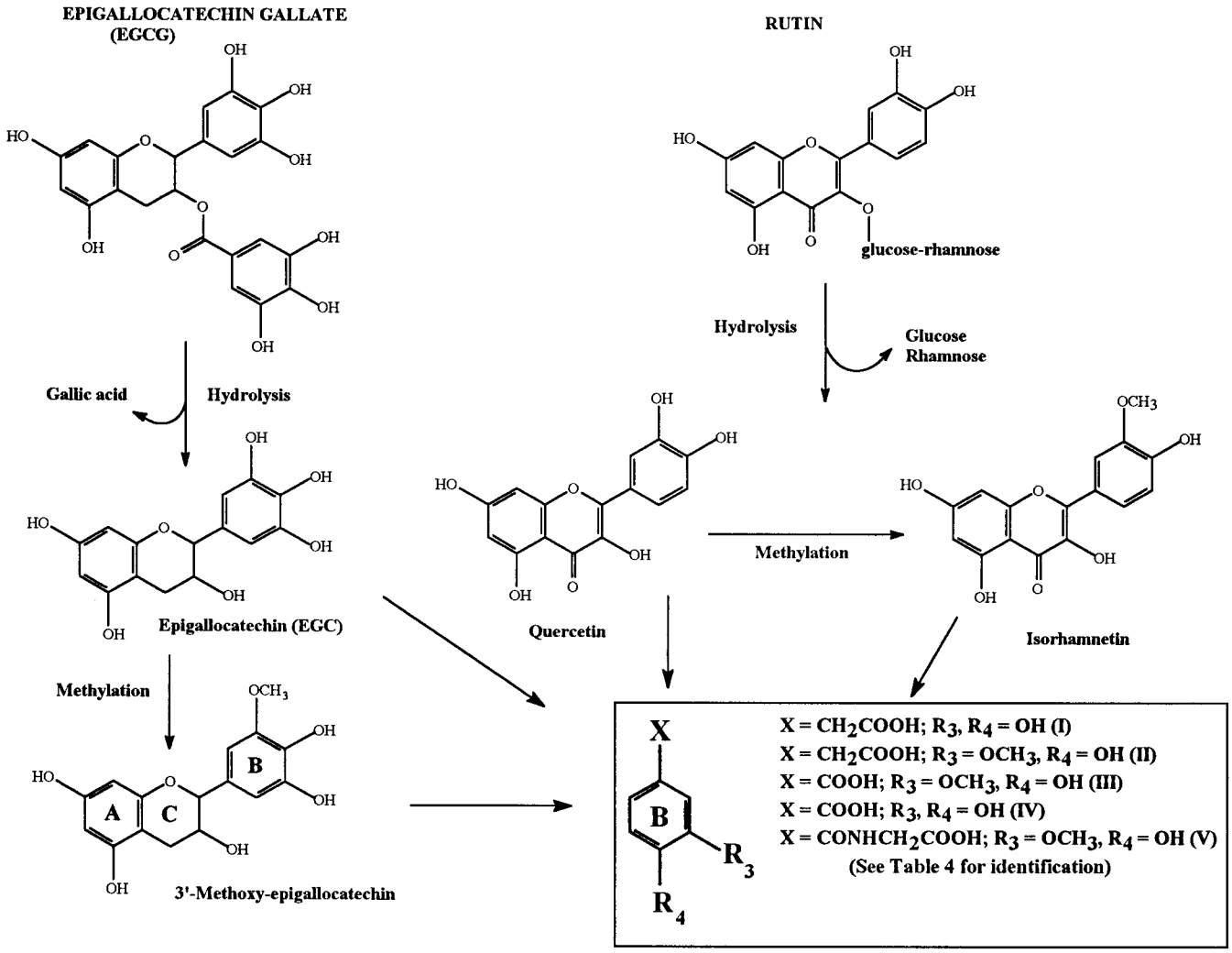


Figure 4. Metabolic conversion of epigallocatechin gallate and rutin.

decarboxylation. Several phenolic acids are produced, depending on the structure of the flavonoid involved (Figure 4).[76] These phenolic acids can be reabsorbed and subjected to conjugation and *O*-methylation in the liver and then may enter into the circulation. This aspect is relevant for antioxidant protection, mainly for two reasons. The first one is that phenolic acids may account for a large fraction of the ingested flavonoids (30−60%), and the second is that some of these acids, because of their catechol structure, possess a radical-scavenging ability comparable to that of their intact precursors.[77] This suggests that these metabolites may take part in the antioxidant protection, as suggested by their TEAC values (Table 4).[78]

It is assumed that dietary flavonoids may display their first antioxidant defense in the digestive tract, by limiting ROS formation[79] and scavenging them. Once absorbed, either as aglycons and glycosides or, to a larger extent, as phenolic acids, they continue to exert an antioxidant effect, although other systemic activities are possible. The in vivo antioxidant effect may be evidenced by measuring the increase of the total antioxidant potential of plasma after a single (and, often, large) intake of flavonoid-containing food, beverages, or herbs and correlating this value to the time course of plasma flavonoids. Alternatively, plasma flavonoids (and/or their metabolites) and specific markers of the body antioxidant status (ascorbate, glutathione, α-tocopherol, β-carotene, polyunsaturated fatty acids, malondialdehyde, 8-hydroxydeoxyguanosine) can be followed during a long-term consumption of normal dosages. According to this approach and in agreement with other authors,[80−83] we have proved that long-term consumption of green tea improves the levels of α-tocopherol in RBC (red blood cell) membranes and LDL. Plasma α-tocopherol and hydrophilic antioxidant levels remained constant, while β-carotene sligthly increased, possibly due to the protection from oxidation exerted by α-tocopherol.[84] The content of polyunsaturated fatty acids in RBC membranes was improved, confirming that α-tocopherol, β-carotene, and catechins (as co-antioxidants) act as effective lipid peroxidation inhibitors. These results suggest that long-term intake of green tea guarantees a baseline plasma concentration of catechins and their metabolites, which is able to induce an improvement of lipophilic vitamin levels. This modification may be explained assuming that the antioxidant protection can be exerted through a cascade involving endogenous antioxidants, which react differently according to their polarity and redox potential.[85] More specifically, flavonoids and their metabolites are capable of reducing the highly oxidizing ROS, becoming less aggressive aroxyl radicals. Some of these aroxyl radicals (those with $E_7 >$ 0.282 V) from their hydrophilic character may oxidize ascorbate, which in turn is regenerated by glutathione. This could be the reason why, after a single dose of green tea catechins either free or as phospholipid complexes, plasma ascorbate and total glutathione decrease transiently.[74] This decrease is concomitant with the time course of plasma catechin (Cat-OH) concentration and the rise of plasma antioxidant capacity. On the basis of its redox potential,

α-tocopherol (α-TOH, E_7 = 0.5V) could be oxidized by radicals with E_7 > 0.5 V (reaction 1), becoming a potential pro-oxidant (reaction 2):

$$R^{\bullet} + \alpha\text{-TOH} \rightarrow RH + \alpha\text{-TO}^{\bullet} \quad (1)$$

$$\alpha\text{-TO}^{\bullet} + LH(LDL) \rightarrow \alpha\text{-TOH} + L^{\bullet} \rightarrow LOO^{\bullet} \quad (2)$$

Prevention of this pro-oxidant activity depends on the rapid elimination of the α-tocopheroxyl radical (α-TO•).[86] This requires the presence of reductants (YH) capable of interacting with the lipophilic α-TO•:

$$\alpha\text{-TO}^{\bullet} + HY \rightarrow \alpha\text{-TOH} + Y^{\bullet}$$

HY should have a strong reducing capacity (i.e., a redox potential lower than 0.5 V) and generate a harmless aqueous radical Y•, thereby preventing LOO• formation. A variety of natural reductants can play this role, including ascorbate, ubiquinol-10, some flavonoids, and phenolic acids (e.g., caffeic acid).

Among flavonoids, quercetin and tea catechins (E_7 0.22 and ~0.4 V, respectively) should be able to regenerate α-tocopherol from the α-tocopheroxyl radical:

$$\alpha\text{-TO}^{\bullet} + \text{Cat-OH} \rightarrow \alpha\text{-TO}^{\bullet} + \text{Cat-OH}$$

This may explain the improvement/maintainance of α-tocopherol levels after intake of green tea catechins. It is quite conceivable that this sparing effect requires consumption of green tea for a while, and during this period homeostatic recovery of the hydrophilic antioxidants ascorbate and glutathione takes place, as found experimentally.[85]

Concerning the protection of LDL in vivo, the situation is far from conclusive. According to Vinson et al.,[87] red wine and black tea polyphenols are absorbed and protect both LDL and VLDL against oxidation by enrichment of lipoproteins. However, some other in vivo studies have shown that ingestion of tea[88,89] or red wine does not protect LDL against oxidation. The findings reinforce the hypothesis[91] that polyphenols are bound to plasma proteins other than LDL and, thus, are unable to preserve LDL.

Conclusions. Dietary flavonoids represent an important source of antioxidants, since their intake may reach 800 mg/day. In the last years, many papers have been published on the in vitro antioxidant activity of flavonoids, and a correlation between the antioxidant capacity and chemical structure has been assessed. However, the antioxidant efficacy in vivo of flavonoids has been less thoroughly documented, possibly due to the limited knowledge on their pharmacokinetics. Only recently has it been proved that a small fraction of the ingested dietary flavonoids is absorbed in either the aglycon or glycoside form, while the major part is extensively degraded to different phenolic acids. Both the absorbed flavonoids and their metabolites may display an in vivo antioxidant activity, which seems to involve differently the physiological antioxidants, resulting in a sparing effect on α-tocopherol and β-carotene.

Acknowledgment. The author wishes to acknowledge Specchiasol s.r.l. (Verona, Italy) and Indena S.p.A. (Milan, Italy) for financial support.

References and Notes

(1) Davies, K. J. *Biochem. Soc. Symp*. **1995**, *61*, 1−31.
(2) Ames, B. N. Shigenaga, M. K.; Hagen, T. M. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 7915−7922.
(3) Halliwell, B. *Nutr. Rev*. **1997**, *55*, S44−S52.
(4) Blake, D.; Winyard, P. G. *Immunopharmacology of Free Radical Species*; Academic Press: San Diego, 1995.
(5) Harman, D. J. *Gerontology* **1956**, *11*, 289−300.
(6) Halliwell, B.; Gutteridge, J. M. C. In *Free Radicals in Biology and Medicine*; Oxford University Press: Oxford, 1998.
(7) Warma, S. D.; Devamanoharan, P. S.; Morris, S. M. *Crit. Rev. Food Sci. Nutr*. **1995**, *35*, 111−129.
(8) Wayner, D. D. M.; Burton, G. W.; Ingold, K. U.; Barclay, L. R. C.; Locke, S. J. *Biochem. Biophys. Acta* **1987**, *924*, 408−419.
(9) Halliwell, B. *Lancet* **1994**, *344*, 721−724.
(10) Sies, H. *Antioxidants in Disease Mechanisms and Therapy, Advances in Pharmacology*, Vol. 38; Academic Press: San Diego, 1997.
(11) Harborne, J. B. In *Plant Flavonoids in Biology and Medicine*; Cody, V., Middleton, E., Harborne, J. B., Eds.; Alan R. Liss: New York, 1986; pp 15−24.
(12) Brouillard, R.; Cheminat, A. *Prog. Clin. Biol. Res*. **1988**, *280*, 93−106.
(13) Kuhnau, J. *World Rev. Nutr. Diet*. **1976**, *24*, 117−191.
(14) Pietta, P. G.; Mauri, P. L.; Simonetti, P.; Testolin, G. Fresenius J. *Anal. Chem*. **1995**, *352*, 788−792.
(15) Herrman, K. J. *Food Technol*. **1976**, *11*, 433−448.
(16) Franke, A.; Custer, L. J.; Cerna, C. M.; Narala, K. K. *J. Agric. Food Chem*. **1994**, *42*, 1905−1913
(17) Haslam, E. *Plant Polyphenols*; Cambridge University Press: Cambridge, U.K., 1989.
(18) Mazza, G.; Miniati, E. *Anthocyanins in Fruits, Vegetables and Grains*; CRC Press: Boca Raton, FL, 1993.
(19) Pietta, P. G.; Simonetti, P. In *Antioxidant Food Supplements in Human Health*; Packer, L., Hiramatsu, M., Yoshikawa, T., Eds.; Academic Press: San Diego, 1999; pp 283−308.
(20) Harborne, J. B. In *Flavonoids: Advances in Research Since 1986*; Harborne, J. B., Ed.; Chapman and Hall: London, 1994; pp 589−618.
(21) Shirley, B. W. *Trends Plant Sci*. **1996**, *31*, 377−382.
(22) Yamasaki, H.; Sakihama, Y.; Ikehara, N. *Plant Physiol*. **1997**, *115*, 1405−1412.
(23) Larson, R. A. *Phytochemistry* **1988**, *27*, 969−978.
(24) Pietta, P. G. In *Flavonoids in Health and Disease*; Rice-Evans, C. A., Packer, L., Eds.; Marcel Dekker: New York, 1998; pp 61−110.
(25) Ingram, D.; Sanders, K.; Kolybaba, M.; Lopez, M. *Lancet* **1997**, *983*, 990−994.
(26) Block, G.; Patterson, B.; Subar, A. *Nutr. Cancer* **1992**, *17*, 1−29.
(27) Frei, B. C. *Crit. Rev. Food Sci*. **1995**, *35*, 83−97.
(28) Gei, K. F. *J. Nutr. Biochem*. **1995**, *6*, 206−236.
(29) Gillman, M. W.; Cupples, L. A.; Gagnon, D.; Posner, B. M.; Ellison, C.; Castelli, W. P.; Wolf, P. *J. Am. Med. Assoc*. **1995**, *273*, 1113−1117.
(30) Ness, A. R.; Powles, J. W. *Int. J. Epidemiol*. **1997**, *6*, 1−13.
(31) Peterson, J.; Dwyer, J. *Nutr. Res*. **1998**, *12*, 1995−2018
(32) Gorinstein, S.; Barnitkowska, E.; Kulasek, G.; Zemser, M.; Trachtenberg, S. *J. Nutr*. **1998**, *128*, 2023−2027.
(33) Wang, W.; Goodman, M. T. *Nutr. Res*. **1999**, *19*, 191−202.
(34) Hertog, M. G. L. *Proc. Nutr. Soc*. **1996**, *55*, 385−396.
(35) Knekt, P.; Jarvinen, R.; Seppanen, R. Heliovaara, M.; Teppo, L.; Pukkala.; Aromaa, A. *Am. J. Epidemiol*. **1997**, *146*, 223−230.
(36) Hertog, M. G. L.; Katan, M. B. In *Flavonoids in Health and Disease*; Rice-Evans, C. A., Packer, L., Eds.; Marcel Dekker: New York, 1998; pp 447−467.
(37) Knekt, P.; Jarvinen, R.; Reunanen, A.; Maatela, J. *Br. Med. J*. **1996**, *312*, 478−481.
(38) Rimm, E. B.; Katan, M. B.; Ascherio, A.; Stampfer, M.; Willet, W. C. *Ann. Int. Med*. **1996**, *125*, 384−389.
(39) Hanasaki, Y.; Ogawa, S.; Fukui, S. *Free Rad. Biol. Med*. **1994**, *16*, 845−850.
(40) Ursini, F.; Maiorino, M.; Morazzoni, P.; Roveri, A.; Pifferi, G. *Free Rad. Biol. Med*. **1994**, *16*, 547−553.
(41) Korkina, L. G.; Afanas'ev, I. B. In *Antioxidants in Disease Mechanisms and Therapy*; Sies, H., Ed.; Academic Press: San Diego, 1997; pp 151−163.
(42) Brown, J. E.; Khodr, H.; Hider, R. C.; Rice-Evans, C. *Biochem. J*. **1998**, *330*, 1173−1178.
(43) Morris, C. J.; Earl, J. R.; Trenam, C. W.; Blake, D. R. *Int. J. Biochem. Cell Biol*. **1995**, *27*, 109−122.
(44) van Acker, S. A. B. E.; van den Berg, D. J.; Tromp, M. N. J. L.; Griffoen, D. H.; van Bennekom, W. P.; van der Vijgh, W. J. F.; Bast, A. *Free Rad. Biol. Med*. **1996**, *20*, 275−283.
(45) Jovanovic, S. V.; Steenken, S.; Tosic, M.; Marjanovic, B.; Simic, M. G. *J. Am. Chem. Soc*. **1994**, *116*, 4846−4851.
(46) Buettner, G. R. *Arch. Biochem. Biophys*. **1993**, *300*, 535−543.
(47) Robak, J.; Gryglewski, R. J. *Biochem. Pharmacol*. **1988**, *37*, 83−88.
(48) Hussain, S. R.; Cillard, J.; Cillard, P. *Phytochemistry* **1987**, *26*, 2489−2491.
(49) Torel, J.; Cillard, J.; Cillard, P. *Phytochemistry* **1986**, *25*, 383−385.
(50) McCord, J. M. *Proc. Exp. Biol. Med*. **1995**, *202*, 112−118.
(51) Cao, G.; Sofic, E.; Prior, R. L. *Free Rad. Biol. Med*. **1997**, *22*, 749−760.
(52) Shadidi, F.; Janitha, P. K.; Wanasundara, P. D. *Crit. Rev. Food Sci*. **1992**, *32*, 67−103.
(53) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. *Free Rad. Biol. Med*. **1996**, *20*, 933−956.
(54) van Acker, S. A. B. E.; van den Berg, D. J.; Tromp, M. N. J. L.; Griffoen, D. H.; van der Vijgh, W. J. F.; Bast, A. *Free Rad. Biol. Med*. **1996**, *20*, 331−342.
(55) Bors, W.; Heller, W.; Michel, C.; Saran, M. *Methods Enzymol*. **1990**, *186*, 343−355.

(56) Steinberg, D.; Parathasarathy, S.; Carew, T. E.; Khoo, J. C.; Witzum, J. L. *N. Engl. J. Med.* **1989**, *320*, 915−924.
(57) deWhalley, C. V.; Rankin, S. M.; Hoult, J. S. P.; Leake, D. S. *Biochem. Pharmacol.* **1990**, *39*, 1743−1750.
(58) Morel, I.; Lescoat, G.; Cogrel, P.; Cillard, J. *Biochem. Pharmacol.* **1993**, *45*, 13−19.
(59) Igarashi, K.; Ohmuma, M. *Biosci. Biotech. Biochem.* **1995**, *59*, 595−601.
(60) Vinson, J.; Dabbagh, Y. A.; Serry, M. M.; Jang, J. *J. Agric. Food Chem.* **1995**, *43*, 2800−2802.
(61) Otero, P.; Viana, M.; Herrera, E.; Bonet, B. *Free Rad. Res.* **1997**, *27*, 619−626.
(62) Manach, C.; Regerat, T.; Texier, O.; Agullo, G.; Demigne, C.; Remesy, C. *Nutr. Res.* **1996**, *16*, 517−544.
(63) Nielsen, S. A.; Kall, M.; Justesen, U.; Schon, A.; Dragsted, L. O. *Cancer Lett.* **1997**, *114*, 173−174.
(64) Mauri, P. L.; Iemoli, L.; Gardana, C.; Riso, P.; Simonetti, P.; Porrini, M.; Pietta. P. G. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 924−931.
(65) Ishii, K.; Furuta, T.; Kasuya, Y. *J. Agric. Food Chem.* **2000**, *48*, 56−59.
(66) Pietta, P. G.; Simonetti, P.; Gardana, C.; Brusamolino, A.; Morazzoni, P.; Bombardelli, E. *Biofactors* **1998**, *8*, 111−118.
(67) Unno, T.; Kondo, K.; Itakura, H.; Takeo, T. *Biosci. Biotech. Biochem.* **1996**, *60*, 2066−2068.
(68) Nakagawa, K.; Miyazawa, T. *Anal. Biochem.* **1997**, *248*, 41−49.
(69) Michelle, M.; Tavazzi, T.; Enslen, M.; Offord, E. A. *Eur. J. Clin. Nutr.* **1999**, *53*, 22−26.
(70) Bell, J. R. C.; Donovan, J. L.; Wong, R.; Waterhouse, A. L.; German, J. B.; Walzem, R. L.; Kasim-Karakas, S. E. *Am. J. Clin. Nutr.* **2000**, *71*, 103−108.
(71) Ichikawa, M.; Tsao, S. C.; Lin, T. H.; Miyahuchi, S.; Sawada, Y.; Iga, T.; Hanano, M.; Sugiyama, Y. J. *Hepatology* **1992**, *16*, 38−49.
(72) Serafini, M.; Maiani, G.; Ferro-Luzzi, A. *J. Agric. Food Chem.* **1997**, *45*, 3148−3151.
(73) Simonetti, P.; Gardana, C.; Mauri, P. L.; Pietta, P. G. *Methods Enzymol.* **2000**, in press.
(74) Pietta, P. G.; Simonetti, P.; Gardana, C.; Brusamolino, A., Morazzoni, P.; Bombardelli, E. *Biochem. Mol. Biol. Int.* **1998**, *46*, 895−903.
(75) King, R. A.; Bursill, D. B. *Am. J. Clin. Nutr.* **1998**, *67*, 867−872.
(76) Pietta, P. G.; Gardana, C.; Mauri, P. L. *J. Chromatogr.* **1997**, *693*, 249−255.
(77) Merfort, I.; Heilmann, J.; Weiss, M.; Pietta, P. G.; Gardana, C. *Planta Med.* **1996**, *62*, 289−292.
(78) Pietta, P. G.; Simonetti, P.; Gardana, C.; Mauri, P. L. *J. Pharm. Biomed. Anal.* **2000**, in press.
(79) Papas, A. M. *Lipids* **1996**, *31*, S77−S82.
(80) Maffei-Facino, R.; Carini, A.; Aldini, G.; Calloni, M. T.; Bombardelli, E.; Morazzoni, P. *Planta Med.* **1998**, *64*, 343−347.
(81) Brown, J. E.; Rice-Evans, C. A. *Free Rad. Res.* **1998**, *29*, 247−255.
(82) Fremont, L.; Gozzelino, M. T.; Franchi, M. P.; Linard, A. *J. Nutr.* **1998**, *128*, 1495−1502.
(83) Pietta, P. G.; Simonetti, P.; Roggi, C.; Brusamolino, A.; Pellegrini, N.; Maccarini, L.; Testolin, G. In *Natural Antioxidants and Food Quality in Atherosclerosis and Cancer Prevention*; Kumpulainen, J. T., Salonen, J. T., Eds.; Royal Society of Chemistry: Cambridge, UK, 1996; pp 249−255.
(84) Palozza, P.; Krinsky, N. I. *Arch. Biochem. Biophys.* **1992**, *297*, 184−187.
(85) Pietta, P. G.; Simonetti, P. *Biochem. Mol. Biol. Int.* **1998**, *44*, 1069−1074.
(86) Thomas, S. R.; Neuzil, J.; Mohr, D.; Stocker, R. *Am. J. Clin. Nutr.* **1995**, *62*, 1357S−1364S.
(87) Vinson, J. A.; Jang, J.; Dabbagh, Y. A.; Serry, M. M.; Cai, S. *J. Agric. Food Chem.* **1995**, *43*, 2798−2799.
(88) van het Hof, K.; de Boer, H. S. M.; Wiseman, S. A.; Westrate, J. A.; Tijburg, L. B. M. *Am. J. Clin. Nutr.* **1997**, *66*, 1125−1132.
(89) McAnlis, G. T.; McEnemy, J.; Pearce, J.; Young, I. Y. *Eur. J. Clin. Nutr.* **1998**, *52*, 202−206.
(90) Caccetta, R. A.; Croft, K. D.; Beilin, L. J.; Puddey, I. B. *Am. J. Clin. Nutr.* **2000**, *71*, 67−74.
(91) McAnlis, G. T.; McEnemy, J.; Pearce, J.; Young, I. Y. *Eur. J. Clin. Nutr.* **1999**, *53*, 92−96.

NP9904509