Antioxidant properties of phenolic compounds

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There is currently much interest in phytochemicals as bioactive components of food. The roles of fruit, vegetables and red wine in disease prevention have been attributed, in part, to the antioxidant properties of their constituent polyphenols (vitamins E and C, and the carotenoids). Recent studies have shown that many dietary polyphenolic constituents derived from plants are more effective antioxidants *in vitro* than vitamins E or C, and thus might contribute significantly to the protective effects *in vivo*. It is now possible to establish the antioxidant activities of plant-derived flavonoids in the aqueous and lipophilic phases, and to assess the extent to which the total antioxidant potentials of wine and tea can be accounted for by the activities of individual polyphenols.

Studies on the free radical-scavenging properties of flavonoids have permitted characterization of the major phenolic components of naturally occurring phytochemicals as antioxidants. Furthermore, the commercial development of plants as sources of antioxidants that can be used to enhance the properties of foods, for both nutritional purposes and for preservation, is currently of major interest. Numerous epidemiological surveys have shown an inverse relationship between the intake of fruit, vegetables and cereals and the incidence of coronary heart disease and certain cancers. Many constituents of these dietary components may contribute to their protective properties, including: vitamins C and E; selenium and other mineral micronutrients; carotenoids; phytoestrogens; allium compounds; glucosinolates and indoles; dithiolthiones; isothiocyanates; protease inhibitors; fibre; and folic acid. These compounds may act independently or in combination as anti-cancer or cardioprotective agents by a variety of mechanisms. One such protective mechanism, attributed to vitamins C and E and the carotenoids, is antioxidant (radical-scavenging) activity.

Recent work is also beginning to highlight the potential role of other phytochemical components, including the flavonoids, phenylpropanoids and phenolic acids, as important contributing factors to the antioxidant activity of the diet (Ref. 1; see Table 1). The flavonoids are a large class of compounds, ubiquitous in plants, and usually occurring as glycosides. They contain several phenolic hydroxyl functions attached to ring structures, designated A, B and C (Fig. 1). Structural variations within the rings subdivide the flavonoids into several families:

- Flavonols (e.g. quercetin and kaempferol), with the 3-hydroxy pyran-4-one C ring.
- Flavones (e.g. luteolin, apigenin and chrysin), lacking the 3-hydroxyl group.
- Flavanols (e.g. catechin), lacking the 2,3-double bond and the 4-one structure.
- Isoflavones (e.g. genistein), in which the B ring is located in the 3 position on the C ring.

These flavonoids often occur as glycosides, glycosylation rendering the molecule less reactive towards free radicals and more water-soluble, so permitting storage in the vacuole. Common glycosylation positions are: the 7-hydroxyl in flavones, isoflavones and dihydroflavones; the 3- and 7-hydroxyl in flavonols and dihydroflavonols; and the 3- and 5-hydroxyl in

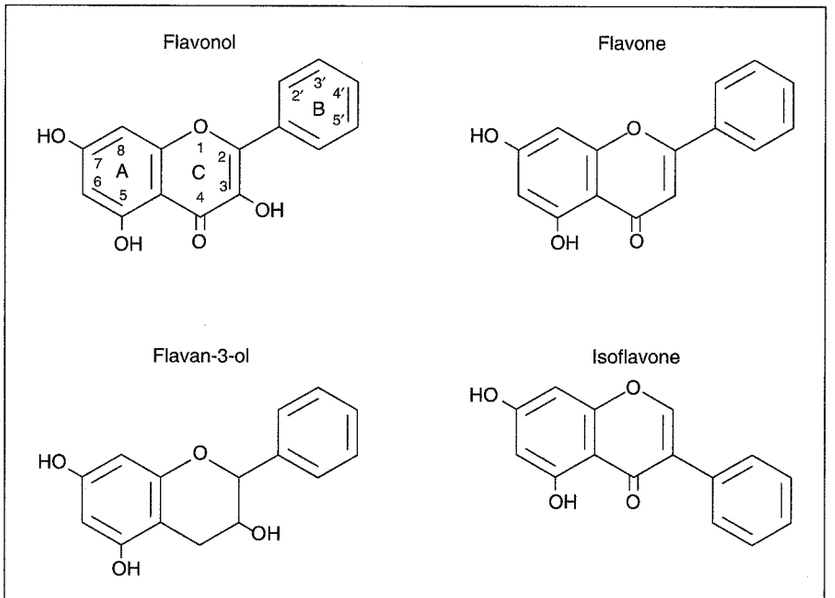

Fig. 1. Structures of the flavonoids. The basic structure consists of the fused A and C rings, with the phenyl B ring attached through its 1′ position to the 2-position of the C ring (numbered from the pyran oxygen). Types shown include: flavonols (3-hydroxyflavones) [e.g. quercetin (3,5,7,3′,4′-hydroxyl) and kaempferol (3,5,7,4′-hydroxyl)]; flavones [e.g. luteolin (5,7,3′,4′-hydroxyl), apigenin (5,7,4′-hydroxyl) and chrysin (5,7,-hydroxyl)]; flavan-3-ols [e.g. catechin (3,5,7,3′,4′-hydroxyl)]; and isoflavones [e.g. genistein (5,7,4′-hydroxyl)].

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anthocyanidins[2]. The sugar most usually involved in the glycoside formation is glucose, although galactose, rhamnose, xylose and arabinose also occur[2], as well as disaccharides such as rutose. The flavonoid variants are all related by a common biosynthetic pathway (Fig. 2), incorporating precursors from both the shikimate and the acetate–malonate pathways[2]. Further modification occurs at various stages, resulting in alteration in the extent of hydroxylation, methylation, isoprenylation, dimerization and glycosylation (producing *O*- or *C*-glycosides)[3–6].

Relationships between the structure and antioxidant activity of phenols

The chemical activities of polyphenols in terms of their reducing properties as hydrogen- or electron-donating agents predicts their potential for action as free-radical scavengers (antioxidants). The activity of an antioxidant is determined by:

- Its reactivity as a hydrogen- or electron-donating agent (which relates to its reduction potential).
- The fate of the resulting antioxidant-derived radical, which is governed by its ability to stabilize and delocalize the unpaired electron[7].
- Its reactivity with other antioxidants.
- The transition metal-chelating potential.

Polyphenols possess ideal structural chemistry for free radical-scavenging activities, and have been shown to be more effective antioxidants *in vitro* than vitamins E and C on a molar basis[8,9]. This is exemplified by studies using pulse radiolysis to investigate the interactions of the hydroxyl radical ($^{\bullet}OH$), azide radical ($N_3^{\bullet}$), superoxide anion ($O_2^{\bullet-}$), lipid peroxyl radical ($LOO^{\bullet}$) and model t-butyl alkoxyl radicals ($tBuO^{\bullet}$) with polyphenols, the rate constants of the reactions and the stability of the antioxidant radical[10]. In addition, the propensity for metal chelation, particularly iron and copper, supports the role of polyphenols as preventative antioxidants in terms of inhibiting transition metal-catalysed free radical formation[11].

There is a hierarchy of flavonoid and isoflavonoid antioxidant activities that is dependent on structure and defines the relative abilities of the compounds to scavenge free radicals. This can be assessed[12,13] by applying the chromogenic redox indicator $ABTS^{\bullet+}$, the radical cation of 2,2′-azino*bis*(3-ethylbenzothiazoline-6-sulphonic acid). The antioxidant activities (Table 1) relative to Trolox, the water-soluble vitamin E analogue, are entirely consistent

Table 1. Relative total antioxidant activities and dietary sources of flavonoids and antioxidant vitamins

Antioxidant	Sources	Antioxidant activity[a] (mM)
Vitamins		
Vitamin C	Fruit and vegetables.	1.0 ± 0.02
Vitamin E	Grains, nuts and oils.	1.0 ± 0.03
Flavonoids		
Anthocyanidins		
Oenin	Black grapes/red wine.	1.8 ± 0.02
Cyanidin	Grapes, raspberries and strawberries.	4.4 ± 0.12
Delphinidin	Aubergine skin.	4.4 ± 0.11
Flavon-3-ols		
Quercetin	Onion, apple skin, berries, black grapes, tea and broccoli.	4.7 ± 0.10
Kaempferol	Endive, leek, broccoli, grapefruit and tea.	1.3 ± 0.08
Flavones		
Rutin	Onion, apple skin, berries, black grapes, tea and broccoli.	2.4 ± 0.12
Luteolin	Lemon, olive, celery and red pepper.	2.1 ± 0.05
Chrysin	Fruit skin.	1.4 ± 0.07
Apigenin	Celery and parsley.	1.5 ± 0.08
Flavan-3-ols		
(Epi)catechin	Black grapes/red wine.	2.4 ± 0.02
Epigallocatechin	Teas.	3.8 ± 0.06
Epigallocatechin gallate	Teas.	4.8 ± 0.06
Epicatechin gallate	Teas.	4.9 ± 0.02
Flavanones		
Taxifolin	Citrus fruit.	1.9 ± 0.03
Narirutin (naringenin-7-rutinoside)	Citrus fruit.	0.8 ± 0.5
Naringenin	Citrus fruit.	1.5 ± 0.05
Hesperidin (hesperetin-7-rutinoside)	Orange juice.	1.0 ± 0.03
Hesperetin	Orange juice.	1.4 ± 0.08
Theaflavins		
Theaflavin	Black tea.	2.9 ± 0.08
Theaflavin-3-gallate	Black tea.	4.7 ± 0.16
Theaflavin-3′-gallate	Black tea.	4.8 ± 0.19
Theaflavin digallate	Black tea.	6.2 ± 0.43
Hydroxycinnamates		
Caffeic acid	White grapes, olive, cabbage and asparagus.	1.3 ± 0.01
Chlorogenic acid	Apple, pear, cherry, tomato and peach.	1.3 ± 0.02
Ferulic acid	Grains, tomato, cabbage and asparagus.	1.9 ± 0.02
p-Coumaric acid	White grapes, tomato, cabbage and asparagus.	2.2 ± 0.06

[a]Measured as the TEAC (Trolox equivalent antioxidant activity) – the concentration of Trolox with the equivalent antioxidant activity of a 1 mM concentration of the experimental substance.

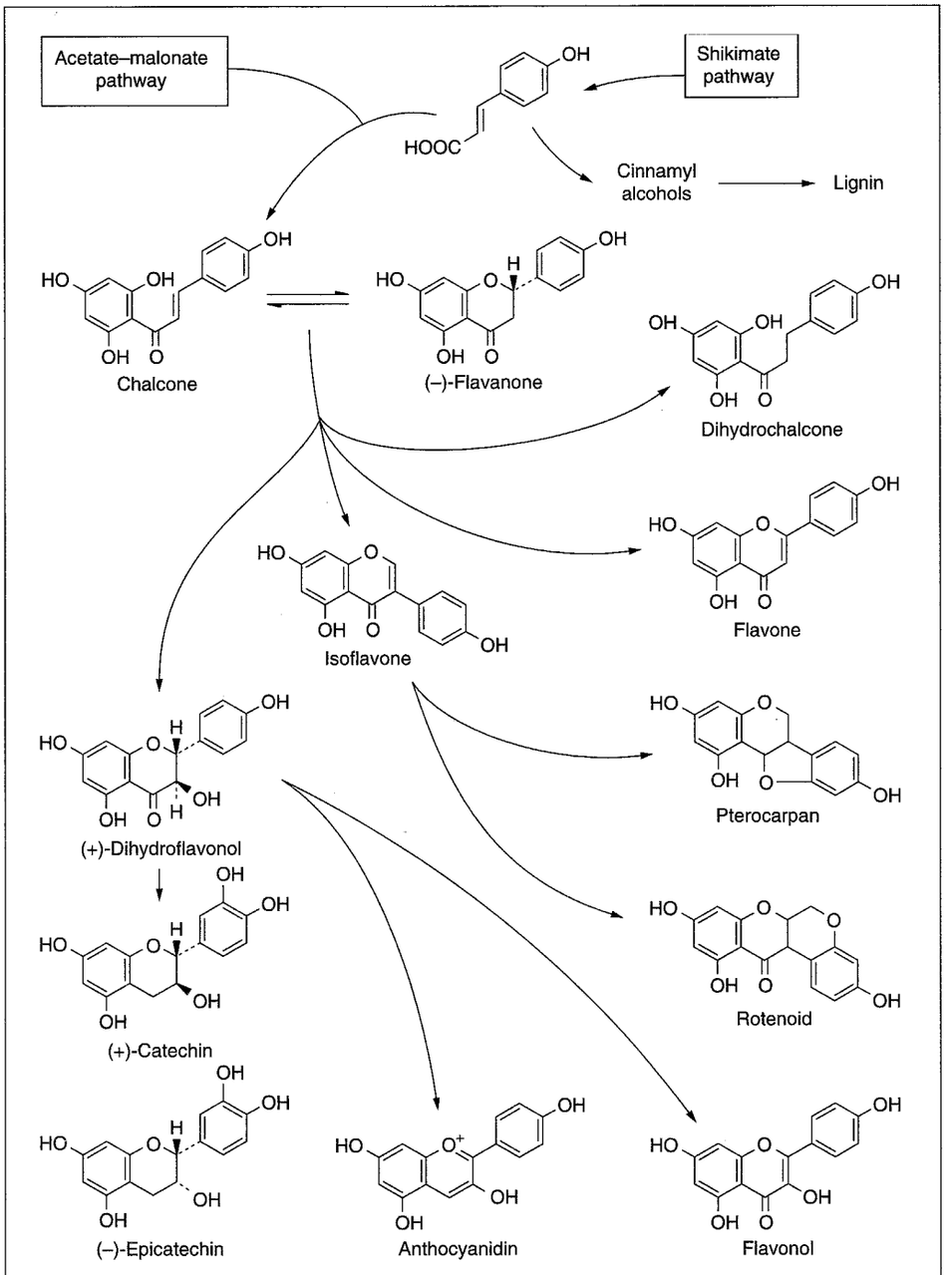

Fig. 2. Inter-relationships between flavonoid classes[2]. Arrows indicate the principal biosynthetic routes.

with their structures. The structural arrangements imparting greatest antioxidant activity as determined from these studies are:

- The *ortho* 3′,4′-dihydroxy moiety in the B ring (e.g. in catechin, luteolin and quercetin).
- The *meta* 5,7-dihydroxy arrangements in the A ring (e.g. in kaempferol, apigenin and chrysin).
- The 2,3-double bond in combination with both the 4-keto group and the 3-hydroxyl group in the C ring, for electron delocalization (e.g. in quercetin), as long as the *o*-dihydroxy structure in the B ring is also present. However, alterations in the arrangement of the hydroxyl groups and substitution of contributing hydroxyl groups by glycosylation decreases the antioxidant activity.
- For metal chelation, the two points of attachment of transition metal ions to the flavonoid molecule are the *o*-diphenolic groups in the 3′,4′-dihydroxy positions in the B ring, and the ketol structures 4-*keto*, 3-hydroxy or 4-*keto* and 5-hydroxy in the C ring of the flavonols. However, it is likely that metals differ with regard to chelation by polyphenols. Glycosylation at all of these crucial hydroxyl positions influences the antioxidant activity of the flavonoids. This is important when considering the relationship between the antioxidant activity of the naturally abundant glycosides and that of the aglycones.

The half peak reduction potential (Ep/2) has also been ascribed as a suitable parameter for representing the scavenging activity of the flavonoids[14]. This was rationalized on the basis that both electrochemical oxidation and hydrogen-donating free radical-scavenging involve the breaking of the same phenolic bond between oxygen and hydrogen, producing the phenoxyl radical and $H^{\bullet}$, which consists of an electron and an H^{+} ion. Thus a flavonoid with a low value for half peak reduction potential (i.e. <0.2 mV) is a good scavenger. The half peak reduction potentials can be compared with the total antioxidant activity, measured as the Trolox Equivalent Antioxidant Capacity (TEAC), by applying the $ABTS^{\bullet+}$ radical cation scavenging assay. Table 2 reveals a broad agreement between the TEAC value (at pH 7.4) and the Ep/2 value (at the same pH), in that flavonoids with efficient scavenging properties have a TEAC value of ≥1.9 mM and Ep/2≤0.2 mV, in comparison with less efficient antioxidants with a TEAC of ≤1.5 mM and Ep/2≥0.3 mV (with the exception of kaempferol). It is interesting to note that the polyphenols with the highest scavenging activities include those with the *o*-dihydroxy structure in the B ring. It has been reported that flavonoids with Ep/2 values of <0.06 mV

undergo redox cycles under physiological conditions[15,16] and are thus able to reduce transition metals[17–19].

Flavonoids in *Citrus* and grapes

The flavonols and their glycosides, the flavanols and the anthocyanins, are the predominant flavonoid classes found in fruit. The genus *Citrus* is characterized by a substantial accumulation of flavanone glycosides, which are not found in many other fruit, at the expense of the accumulation of flavanols and anthocyanins. Some of these flavanone and flavanolol compounds are intermediates in the biosynthetic pathways that lead from hydroxycinnamic acids and malonyl coenzyme A to anthocyanins, flavanones and flavonols (Fig. 2).

Each plant species is characterized by a particular flavanone glycoside pattern[20]. The flavanone aglycones are the 5,7-dihydroxy structures naringenin and hesperetin, and their 4′-methoxylated derivatives, isosakuranetin and hesperetin; the major glycosides are narirutin and hesperidin, the 7-rutinosides of naringenin and hesperetin.

Lemon peel contains two flavanone glycosides, hesperedin and eriocitrin, the 7-rutinosides of hesperetin and eriodictyol, respectively. In grapefruit, naringin (the 7-neohesperidoside of naringenin) is predominant and is accompanied by narirutin (naringenin 7-rutinoside). Naringenin constitutes 88% (v/v) of flavanones in the juice. Naringin also predominates in the juice of sour orange (56–71%, v/v), along with other neohesperidosides – particularly the 7-neohesperidoside of eriodictyol (neoeriocitrin). Only the 7-β-rutinosides are present in sweet orange, hesperidin being the dominant flavanone glycoside. In juice of sweet orange, hesperidin is accompanied by narirutin. *Citrus* fruits also contain several flavones, some of which are polymethoxylated flavones, such as nobiletin and sinensetin in orange peel.

Another family of phenolics found in *Citrus* are the phenylpropanoids, and in particular the hydroxycinnamates. The most widely distributed phenolic components in plant tissues are the hydroxycinnamic acids, *p*-coumaric, caffeic and ferulic acids, which are synthesized via the shikimate pathway (Fig. 3). These are present in many diets at higher concentrations than the flavonoids and anthocyanins. Their occurrence is usually in various conjugated forms resulting from enzymatic hydroxylation, O-glycosylation, O-methylation or esterification of *p*-coumaric acid.

Caffeic acid is frequently the most abundant phenylpropanoid in fruit and is the major representative of hydroxycinnamates in *Citrus* fruit. Although *p*-coumaric acid is less abundant, in general, than caffeic acid in most fruit, it predominates in certain *Citrus* and in the skins of certain red cultivars of *Vitis vinifera*. Ferulic acid usually forms only a small percentage of the total hydroxycinnamic acid in fruit, but, again, can reach 50% (v/v) of the total hydroxycinnamates in certain *Citrus*.

There is great interest as to whether the polyphenolic constituents of red wine are an important factor contributing to protection from coronary heart disease. Grapes and wine contain large amounts of polyphenols at concentrations in the range 1.0–1.8 μg ml^{-1}. The major polyphenols of *V. vinifera* are: caftaric acid, the tartaric acid ester of caffeic acid; the blue-red pigment malvidin-3-glucoside, as the

Table 2. Hierarchy of flavonoid antioxidant activities and the relationship with reduction potentials

Flavonoid	Antioxidant activity[a] (mM)	Half peak reduction potential[b] (mV)
Quercetin	4.7	0.03
Rutin	2.4	0.18
Catechin	2.4	0.16
Luteolin	2.1	0.18
Taxifolin	1.9	0.15
Apigenin	1.5	>1
Naringenin	1.5	0.6
Hesperetin	1.4	0.4
Kaempferol	1.3	0.12

[a]Measured as the TEAC (Trolox equivalent antioxidant activity) – the concentration of Trolox with the equivalent antioxidant activity of a 1 mM concentration of the experimental substance[8].
[b]Designated Ep/2 (Ref. 14). An Ep/2 of <0.2 indicates a chemical that is readily oxidized and therefore an efficient free radical scavenger[14].

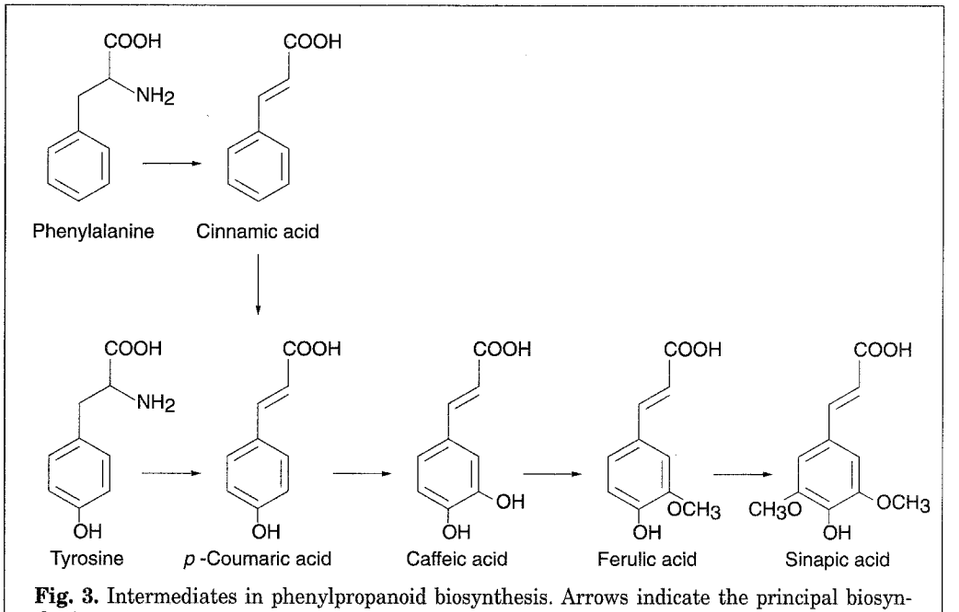

Fig. 3. Intermediates in phenylpropanoid biosynthesis. Arrows indicate the principal biosynthetic routes.

Table 3. Major phenolic constituents of wine[a]

Phenol	Concentration in red wine (mg l^{-1})	Concentration in white wine (mg l^{-1})
Catechin	191	35
Epicatechin	82	21
Gallic acid	95	7
Cyanidin	3	0
Malvidin-3-glucoside	24	1
Rutin	9	0
Quercetin	8	0
Myricetin	9	0
Resveratrol	1.5	0

[a]Data from Ref. 21.

major anthocyanin; and the flavan-3-ol catechin[20]. The mean amounts of phenolic constituents found in a range of wines are shown in Table 3 (Ref. 21). Unlike other classes of flavonoids, monomeric flavan-3-ols are generally found in free rather than glycosylated or esterified form in fruit. Grape is a fruit of high flavan-3-ol content and the major monomers are (+)-catechin and (−)-epicatechin. Epicatechin is reported as being the most abundant flavonoid in skin extracts of several white cultivars.

Black grape skin and the antioxidant activity of wines

The total antioxidant activities of a range of red wines[8] vary from 12–14 mM for Californian Pinot Noir, Rioja and Bouzy Rouge, through to about 16 mM for Australian Shiraz, and 23 mM for red Bordeaux and Chianti. Even though the antioxidant activities of the wines vary over a factor of 2, the ratios of activities to the total phenol content are approximately the same (about a factor of 10), indicating the direct relationship between the two. This is further underlined by the reduced antioxidant activity (total antioxidant activity = 1.5 mM) of a white wine from the same grape as Bouzy Rouge, but without the black grape skin.

Based on the data[21,22] for the phenolic composition of wine, and data for the TEAC values of polyphenols[8,9], a figure can be calculated for the contribution that these constituents make to the antioxidant activity of wine. Calculating the mean total antioxidant activity from the measured antioxidant activities of the individual constituents, only 25% of the measured value can be accounted for. Although Frankel's compositional figures are low compared with the data of others[20,21], and in view of the constancy of the total antioxidant activity:polyphenol ratios, the remainder of the activity is presumably derived from unidentified antioxidants such as vitamin C, other polyphenols and phenolic acids, but especially polymers formed from the polyphenols.

Flavonoids as contributors to the antioxidant activity of tea

The importance of catechins, gallocatechins, catechin-gallate esters and related phytochemicals as dietary antioxidants has been investigated. Both *in vivo* and *in vitro* studies on the effects of green and black teas, and their polyphenolic constituents, have been undertaken in models of cardiovascular diseases and cancers, and as markers of lipid metabolism. Green tea consumption has been associated with lowered cardiovascular risk through decreased serum cholesterol and triacylglyceride, increased high density lipoprotein and a decrease in indicators of atherogenesis[23]. Animal studies *in vivo* demonstrated that green tea stimulates hepatic UDP-glucuronyl transferase activity[24] and provides protection against kidney lipid peroxidation, whereas black tea showed enhanced protection against peroxidation of liver[25]. Theaflavins are formed during the manufacture of black and oolong teas from the enzymatic oxidation of the flavanols, catechin and gallocatechin, by polyphenol oxidase. The reaction involves the oxidation of the B rings to quinones, followed by a 'Michael' addition of the gallocatechin quinone to the catechin quinone, prior to carbonyl addition across the ring and subsequent decarboxylation[26]. Theaflavins also possess *in vitro* antioxidative properties against lipid peroxidation, in erythrocyte membranes and microsomes[27], and suppress mutagenic effects induced by hydrogen peroxide (the gallic acid moiety of the theaflavins is essential for this activity[27]). Epigallocatechin gallate, one of the major polyphenolic constituents of green tea, suppresses the production of superoxide radicals and hydrogen peroxide by tumour promoter-activated human neutrophils[28].

The flavanols catechin and epicatechin, their gallo forms with three hydroxyl groups adjacently placed on the B ring and their gallate esters are major constituents of green tea. Polyphenols constitute about 42% of the dry weight composition of green tea extract, of which 26.7% are catechin–gallate components: epigallocatechin gallate (11.16%); epicatechin gallate (2.25%); epigallocatechin (10.32%); epicatechin (2.45%); and catechin (0.53%) (Ref. 29). As seen in the parent catechin structure (Fig. 1), there is no delocalization of electrons across the structures between the A and B rings because of saturation of the heterocyclic pyran ring.

Studies on the antioxidant activities of the individual constituent catechin and catechin–gallate esters of green tea have shown that *in vitro* they are more effective antioxidants on a molar basis than vitamin C (Ref. 30). This ranking of reactivity follows the order: epicatechin gallate ≈epigallocatechin gallate>epigallocatechin>epicatechin≈ catechin. This is consistent with the number and arrangement of phenolic hydroxyl groups[8]. These findings are in agreement with the hierarchies of antioxidant activities against the 1,1-diphenyl-2-picrylhydrazyl radical[31] and superoxide radical reduction[32].

The enhanced values for the catechin gallate esters in relation to the catechins reflect the additional contribution from the trihydroxybenzoate, gallic acid. Epigallocatechin is structurally similar to catechin and epicatechin, with an additional hydroxyl group adjacent to the *o*-diphenolic structure in the B ring enhancing the antioxidant activity. The linkage of gallic acid to the epicatechin or epigallocatechin structure via esterification at the 3 position again increases the antioxidant potential, as in epicatechin gallate or epigallocatechin gallate. The total antioxidant activity of catechin gallate-rich green tea extract at 1000 ppm (mg l^{-1}) gives a value of 3.78 ± 0.03 mM (n = 9). The antioxidant capacities of the polyphenolic constituents in green tea in relation to their concentrations are used to calculate their predicted contributions to the antioxidant potential, and the result of 2.95 mM is reasonably consistent with the measured value of the proportionately combined catechin

gallate constituents of 2.78 ± 0.03 mM. Thus 78% of the antioxidant activity of green tea extracts can be accounted for by the catechins and catechin gallate esters. Taking into account the antioxidant activity of the polyphenolic constituents of green tea and their relative abundances, the order of contribution to the antioxidant effectiveness within green tea is: epigallocatechin≈epigallocatechin gallate>epicatechin gallate=epicatechin>catechin.

The effectiveness of theaflavin as an antioxidant is increased by esterification with gallate, and is further enhanced as the digallate ester[33]. This can be predicted from the previous studies on the catechins and catechin–gallate esters showing that, in the case of the flavanols, increasing numbers of hydroxyl groups as *ortho* diphenolics or triphenolics, as with incorporation of gallate esters or in the gallocatechins, progressively augments the antioxidant activities of these polyphenols against radicals generated in the aqueous phase. Thus the hierarchy of antioxidant activities of these phytochemicals can be rationalized by consideration of their structural chemistry.

Antioxidant activity of polyphenols in the lipophilic phase

One of the key mechanisms relating the antioxidant status of the blood and decreased risk of coronary heart disease relates to the postulate that oxidation of the low density lipoproteins (LDL) is an early event in atherosclerosis. Biochemical and clinical studies have suggested that oxidized LDL has an array of atherogenic properties through the formation of lipid hydroperoxides and the products derived from these. Oxidatively modified LDL can be taken up readily by macrophages via the scavenger receptor to form foam cells, an early marker of the development of atherosclerotic lesions (see Ref. 34).

The antioxidant status of LDL and plasma are important determinants of the susceptibility of the LDL to peroxidation. The major antioxidant localized in LDL is the dietary phenolic α-tocopherol, the level of which is an important contributor to the resistance of LDL to oxidation. Of the other dietary antioxidants, the extent of incorporation of the flavonoids and polyphenols is unclear, although interesting studies[35–37] have suggested a relationship between cardioprotection and the increased consumption of flavonoids from dietary sources such as onions, apples, tea and red wine. The constituents of red wine are a source of particular interest because of the French paradox[37], that the Southern French show low mortality from coronary heart disease, despite their high fat intake and smoking tendencies.

Because oxidation of LDL is implicated in the pathogenesis of atherosclerosis, the enhancement of the resistance of LDL to oxidation is one of the models used by many researchers for investigating the efficacy of dietary phytochemicals as antioxidants against radicals generated in the lipophilic phase. The LDL particle contains α-tocopherol, the major lipophilic antioxidant in plasma, in its outer monolayer, and carotenoids in the inner core. Free radical-mediated peroxidation of polyunsaturated fatty acids leads to the formation of lipid hydroperoxides through peroxidation. Oxidative and reductive decomposition of hydroperoxides amplifies the peroxidation process. The presence of chain-breaking phenolic antioxidants provides a means of intercepting the peroxidation process by reducing the alkoxyl or peroxyl radicals to alkoxides or hydroperoxides, respectively. In order to study the antioxidant activity of polyphenols as scavengers of propagating peroxyl radicals, no initiating radical species need be added – as long as redox-cycling agents, such as copper ions or heme proteins, are applied to propagate the decomposition of the minimal levels of endogenous pre-existing hydroperoxides[38].

Assessing the relative efficacies of the polyphenolic constituents of green tea to protect LDL from oxidation demonstrates the low concentration (<1 μM) required for 50% inhibition of LDL oxidation[30]. Specifically, whereas 0.75 μM epigallocatechin and 1.2 μM gallic acid were required, for epicatechin, catechin, epicatechin gallate and epigallocatechin gallate concentrations between 0.25 and 0.38 μM were effective. The three- to fivefold differences in concentration required to inhibit LDL oxidation by 50% for epigallocatechin or gallic acid and the rest presumably relate to the relative partitioning abilities of the constituents within the LDL particle and their accessibility to the lipid peroxyl radicals: epigallocatechin and gallic acid are more hydrophilic and therefore have less access to the lipid peroxyl radicals. Miura *et al.*[39] also found that epigallocatechin was the least effective catechin in protecting LDL from copper-mediated oxidation and epigallocatechin gallate the most effective, with a threefold decrease in the concentration required for 50% inhibition of oxidation. The catechin/gallate family of tea components was also studied for its ability to conserve the α-tocopherol located within the LDL particles and protect it from oxidation. Monitoring the consumption of α-tocopherol within the LDL, when challenged with a pro-oxidant in the presence of catechin polyphenols, confirms that epigallocatechin is indeed the least effective in protecting vitamin E. This reflects its lesser contribution to increasing the resistance of LDL to oxidation, whereas the consumption of α-tocopherol was delayed and prolonged by epigallocatechin gallate and epicatechin gallate. Their abilities to enhance the resistance of low density lipoprotein to oxidation and to prolong the lifetime of α-tocopherol within LDL is in the sequence: epigallocatechin gallate=epicatechin gallate≈epicatechin=catechin>epigallocatechin>gallic acid. This is consistent with their partition coefficients, gallic acid and epigallocatechin being preferentially localized in the aqueous phase and thus having less access to the lipid peroxyl radicals.

The ability of the hydroxycinnamates to enhance the resistance of LDL to oxidation shows that chlorogenic and caffeic acids have a higher peroxyl radical scavenging ability than monophenolics such as *p*-coumaric acid[40,41]. Methoxylation of one hydroxyl group of caffeic acid to form ferulic acid decreases the efficiency of the scavenging reaction with peroxyl radicals (i.e. caffeic is substantially more effective in inhibiting LDL oxidation). Ferulic acid is much more effective than *p*-coumaric acid. The electron-donating methoxy group allows increased stabilization of the resulting aryloxyl radical through electron delocalization after hydrogen donation by the hydroxyl group. These data are again consistent with the partitioning characteristics of the hydroxycinnamates, and with results from studies undertaken in lipophilic systems to establish the structural criteria for the activity of polyhydroxyflavonoids in enhancing the stability of fatty acid dispersions (especially methyl linoleate), lipids, oils and low density lipoproteins[7,11,30,39,42].

Bioavailability

There is a paucity of information on the absorption, pharmacokinetics, metabolism and excretion of dietary polyphenols in humans and their handling by the

Table 4. Absorption and uptake of flavonoids in humans[a]

Flavonoid	Dose	Observation
Catechin[44]	5.8 g (based on 83 mg kg^{-1} for a 70 kg man).	26% of administered dose excreted within 24 h. Major metabolite *m*-hydroxyphenyl propionic acid, detected in plasma after 6 h.
3-*O*-methyl-catechin[45]	2 g.	Plasma levels, 11–18 μg ml^{-1} within 2 h; urine, 38% excreted within 120 h as glucuronides and sulphates.
Quercetin[46,47]	64 mg from fried onions, 89–100 mg equivalent from supplements.	Plasma concentration about 1 μM, 2 h later.
Quercetin[48]	4 g supplement.	Not detected in urine or plasma after oral dosage.
Decaffeinated green tea[29]	88 mg epigallocatechin gallate, 82 mg epigallocatechin, 33 mg epicatechin gallate, and 32 mg epicatechin.	Plasma levels, 46–268 ng ml^{-1}, 82–206 ng ml^{-1}, undetectable and 40–80 ng ml^{-1}, respectively.

[a]Basal flavonoid plasma levels in nonsupplemented humans[49]: rutin 0.72 μM; combined other glycosides of flavonols 0.6–3.8 μM; and phloridzin 0.6–1.6 μM.

gastrointestinal tract. Much early evidence[43] indicated that absorption of the hydroxyaromatic acids, benzoates, phenylacetates and hydroxycinnamates might occur, but there was a requirement for splitting of the polyphenols in the gastrointestinal tract to lower molecular mass forms prior to absorption and conversion to hydroxyaromatic acids[43]. Hydrolysis of flavonoid glycosides, ring fission and the reductive metabolism of phenyl acyl fragments are carried out by intestinal microorganisms. The catabolism of quercetin, for example, is proposed to form hydroxyaromatic acids with two-carbon side chains on the aromatic ring, caused by the presence of the 3-hydroxyl group on the C ring of quercetin. However, in the absence of a 3-hydroxyl group, as in hesperidin, hydroxyaromatic acids with three-carbon side chains are formed, which then undergo β-oxidation to benzoic acid derivatives. The metabolic transformation of caffeic acid, for example, has been proposed to proceed through the methylation of the phenolic hydroxyl groups, dehydroxylation in the *para* position, hydrogenation of the side chain, β-oxidation and formation of conjugates, as in the metabolism of flavonoids. These studies suggest that the conjugation reaction with glucuronide or sulphate is perhaps the most common final step in the metabolic pathway with intact flavonoids.

Table 4 summarizes the current (limited) available information concerning the absorption and uptake of a range of polyphenols in humans. Evidence exists that catechin is absorbed by the human gut[44], and other investigations involving oral adminstration of 3-*O*-methyl-[U-^{14}C]-catechin in three volunteers showed that the supplement occurred in plasma[45]. Peak levels were observed within 2 h of administration. The time course of plasma quercetin was studied in two subjects after ingestion of fried onions containing quercetin glycosides to the level of quercetin aglycone equivalent to 64 mg, and on supplementation with pure quercetin glycosides[46,47]. Quercetin was detected in plasma after hydrolysis, whereas, in one early study, supplementation with quercetin suggested that there was no absorption of this compound[48]. Green tea consumption led to the detection of all the major constituents, with the exception of epicatechin gallate, in plasma[29]. More recently, studies[49] have shown the presence of several dietary flavonoids in human plasma from nonsupplemented subjects, including phloridzin, quercetin glucosides and quercetin rutinosides.

It should also be emphasized, however, that certain flavonoids in chemical systems autoxidize readily, especially quercetin, myricetin, quercetagetin and delphinidin[16,50]. Furthermore, there are some reports of the prooxidant activity of some polyphenols in the presence of metal ions in which excessive concentrations (25–100 μM) accelerate hydroxyl radical formation and DNA damage *in vitro*[51].

Conclusions

The studies described in this review demonstrate the antioxidant properties of polyphenolic constituents of plants, and will help in the identification of the active constituents in beverages, vegetables and fruit that may help sustain antioxidant status and protect against free radical damage. This should be useful information for identifying foods that are rich in these protective components, for the development of safe food products and additives with appropriate antioxidant properties.

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