Phenolic Compounds: A Review of Their Possible Role as *In Vivo* Antioxidants of Wine*

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Submitted for publication: February 2002
Accepted for publication: June 2002
Key words: Antioxidants, free radical scavenging, lipid peroxidation, flavonoids, phenolic compounds

Phenolic compounds are a large and complex group of chemical constituents found in red and white wines which not only affect their quality, but also contribute to their beneficial health effects. The antioxidant properties of phenolic compounds are important in determining their role as protective agents against free radical-mediated disease processes. This review discusses the principles of oxidative stress and the resultant cellular damage caused by lipid peroxidation *in vivo*. Different groups of wine phenolic compounds are detailed, with specific reference to their *in vitro* antioxidant activity and their relative potency as free radical scavengers. The absorption and bioavailability of phenolic compounds from dietary sources is discussed.

INTRODUCTION

Chronic diseases such as arteriosclerosis and cancer, which are the leading causes of death in the Western world, are likely to be mediated by free radical and lipid peroxidation mechanisms (Halliwell & Gutteridge, 1990). Plant phenolic compounds, such as those occurring in wine, could protect against degenerative diseases involving oxidative damage due to their antioxidant action (Kinsella *et al.*, 1993). A possible illustration of such a scenario is the relatively low incidence of coronary heart disease in the southern regions of France (Renaud & De Lorgeril, 1992). This phenomenon, normally referred to as the French Paradox, is believed to be related to, amongst other factors, the consumption of red wine. Recently the role of phenolic compounds from foods and beverages in the prevention of free radical-mediated diseases has become more important due to the discovery of the link between peroxidation of low-density lipoproteins (LDL) and arteriosclerosis (Esterbauer *et al.*, 1991; Luc & Fruchart, 1991; Steinberg, 2000; Chisolm & Steinberg, 2001). The emphasis placed by the European Commission on enhancing the nutrient content of food crops through traditional plant breeding as well as food-processing technologies confirms the importance of phenolic compounds in terms of health benefits to the international community (Lindsay, 2000).

This review will focus on aspects of oxidative stress and the resultant cellular damage caused by lipid peroxidation, a determining event in the genesis of chronic disease conditions. The *in vitro* antioxidant activity and structural aspects of different groups of wine phenolic compounds in several model systems will be discussed. Key aspects related to the absorption and bioavailability of phenolic compounds from dietary sources will be discussed.

REACTIVE OXYGEN SPECIES AND FREE RADICALS

Oxidative reactions within the cell are tightly controlled and inherent protective mechanisms exist to destroy oxidant by-products of normal cell metabolism. Oxidative stress refers to an imbalance between oxidant by-products and the inherent antioxidant defence system, which is related to metabolism and the antioxidant defence system (Davies, 1995). The inherent antioxidant defence system consists mainly of enzymes such as catalase, superoxide dismutase and glutathione peroxidase (Sies, 1985; Halliwell & Gutteridge, 1990). However, during certain pathophysiological conditions, or when antioxidant deficiencies occur, these control mechanisms are not sufficient and oxidant by-products may cause damage to DNA, proteins and lipids (Cutler, 1991; Hertog *et al.*, 1995; Keli *et al.*, 1996).

Four endogenous sources account for the production of oxidant by-products in cells, namely mitochondrial energy production, activities of phagocytic cells, peroxisomal fatty acid metabolism and the activities of certain metabolic enzymes (Davies, 1995). Exogenous sources, such as excess dietary iron or copper and exposure to environmental toxins and carcinogens such as cigarette smoke, also contribute to oxidative stress (Davies, 1995). The most important oxidant by-products of cells are reactive oxygen species, namely superoxide anion radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\bullet}OH$) and nitric oxide radicals ($^{\bullet}NO$). Of these radical species, $^{\bullet}OH$ is the most reactive and would therefore react at or close to its site of formation if mechanisms for its removal are not available (Davies, 1995). Mitochondria, peroxisomes and a number of cytosolic enzymes generate $O_2^{\bullet -}$ and H_2O_2 during normal metabolic processes, while $^{\bullet}NO$ is produced by endothelial cells in the walls of arteries. Normally, $^{\bullet}NO$ plays a positive role in the regulation of vascular

* *Part of MSc (Food Science) Thesis, Stellenbosch University, March 2002.*

Acknowledgements: We are grateful to the South African Wine Industry (Winetech), the National Research Foundation (NRF) and the Technology and Human Resources for Industry Programme (THRIP) or financial support.

function, but after reaction with $O_2^{\bullet-}$ a very reactive species, namely peroxynitrite ($ONOO^-$), is formed. Production of $^{\bullet}OH$ can also occur when other reactive oxygen species such as $O_2^{\bullet-}$ and H_2O_2, react with iron during Fenton reactions (Sies, 1985; Halliwell & Gutteridge, 1990):

$$Fe^{3+} + O_2^{\bullet-} \rightarrow Fe^{2+} + O_2$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^- + {}^{\bullet}OH$$

Endogenous iron is usually present in chelated or bound forms as part of haemoglobin, myoglobin, several enzymes and the transport protein, transferrin, and therefore not readily available for reaction. During disease conditions, however, iron can be mobilised from endogenous sources (Halliwell & Gutteridge, 1990).

LIPID PEROXIDATION

Lipid peroxidation is an autoxidation process with detrimental effects occurring in foods and metabolically active cells of the body. In foods it can lead to rancidity and loss of nutritional value (Chan, 1987). In the cell, however, lipid peroxidation and products of lipid peroxidation are associated with many conditions of cellular damage and cytotoxicity. This is due to changes in membrane structure and fluidity, increased permeability of membranes, and damage to biologically important molecules such as DNA and proteins, resulting in chronic diseases such as arteriosclerosis and cancer (Halliwell & Gutteridge, 1990; Cutler, 1991; Hertog *et al.*, 1995; Keli *et al.*, 1996). Peroxidation of lipids in foods mostly occurs enzymatically, whereas in the cell it is initiated by reactive oxygen species (Kanner *et al.*, 1987).

Autoxidation is the spontaneous reaction between atmospheric oxygen (triplet state) and organic compounds (Chan, 1987). This process generally follows an autocatalytic free radical chain-reaction mechanism. The overall reaction is the addition of triplet oxygen to an organic compound. Three distinct steps can be distinguished in the free radical chain reaction, namely initiation, propagation and termination (Chan, 1987; Shahidi & Wanasundara, 1992):

Initiation:	$X^{\bullet} + LH$	$\rightarrow$	$L^{\bullet} + XH$
Propagation:	$L^{\bullet} + O_2^3$	$\rightarrow$	$LOO^{\bullet}$
	$LOO^{\bullet} + LH$	$\rightarrow$	$LOOH + R^{\bullet}$
Termination:	$LOO^{\bullet} + LOO^{\bullet}$	$\rightarrow$	non-radical products
	$LOO^{\bullet} + L^{\bullet}$	$\rightarrow$	non-radical products
	$L^{\bullet} + L^{\bullet}$	$\rightarrow$	non-radical products

where $X^{\bullet}$ = initiating radical species; LH = polyunsaturated fatty acid; LOOH = fatty acid hydroperoxide; $L^{\bullet}$ = alkyl radical; $LO^{\bullet}$ = alkoxyl radical; $LOO^{\bullet}$ = peroxyl radical.

Lipid peroxidation is initiated by many mechanisms. The initiating radical, $X^{\bullet}$, abstracting a hydrogen from a polyunsaturated fatty acid, can be a transition metal ion, such as Fe^{2+}, Fe^{3+} or Cu^+ or a reactive oxygen species (Chan, 1987). The most common initiation mechanism *in vivo* is by peroxyl radicals, formed during the decomposition of lipid hydroperoxides involving transition metal ions (Halliwell & Gutteridge, 1990; Marnett & Wilcox, 1995). Unbound iron or copper is normally available in small quantities, although large amounts can be mobilised from bound forms during disease conditions (Halliwell & Gutteridge, 1990). Oxygenation of the alkyl radical formed during initiation, yielding a peroxyl radical, is the first step of the propagation phase. The peroxyl radicals will then abstract another hydrogen from a polyunsaturated fatty acid. The propagation reactions can be repeated indefinitely until the reaction is terminated when radicals combine in radical coupling reactions to form stable non-radical products. The most common products of lipid peroxidation include hydroperoxides, aldehydes, hydroxy acids, hydroperoxy acids and epoxides (Chan, 1987).

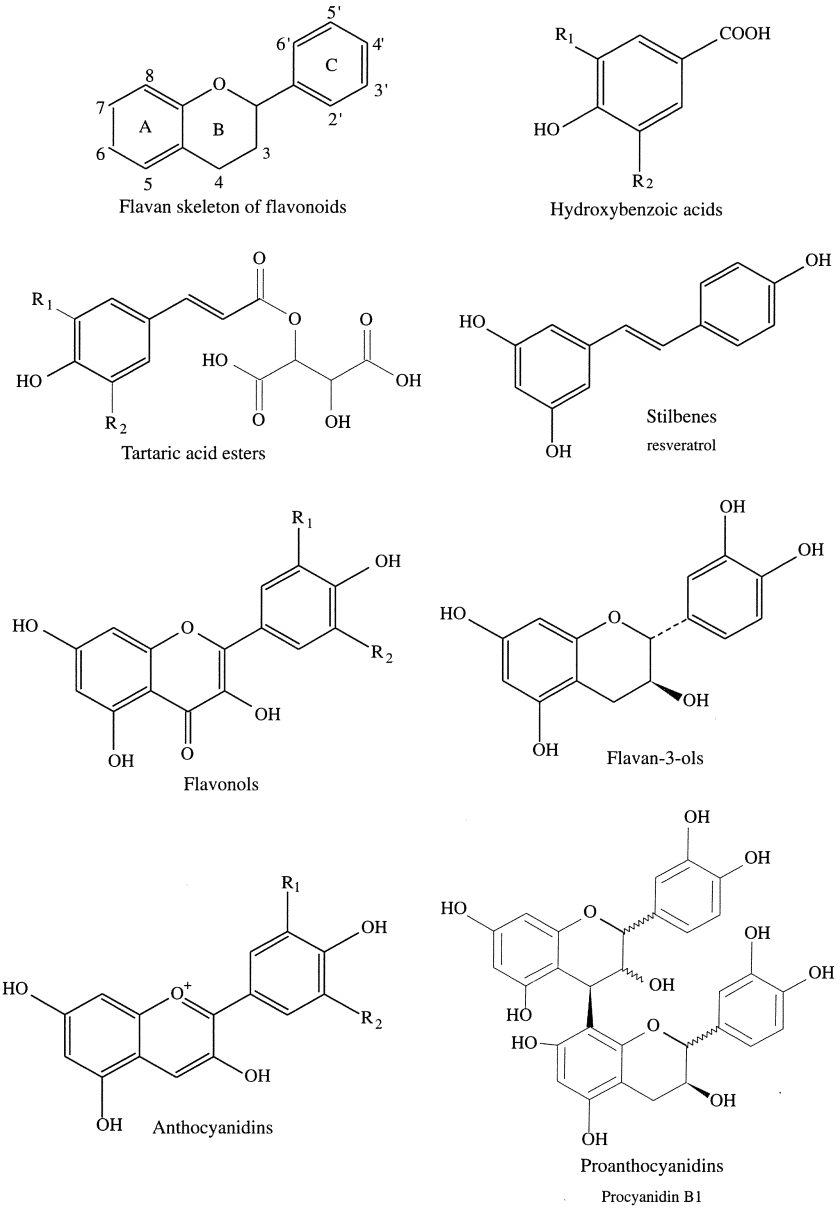
PHENOLIC ANTIOXIDANTS

Halliwell (1995) defines an antioxidant as any substance that, when present at low concentrations relative to that of an oxidisable substrate, significantly delays or prevents the oxidation of that substrate. This definition is especially relevant in biological systems.

Antioxidants can be classified into two groups, namely chain-breaking (primary) antioxidants and preventative (secondary) antioxidants (Namiki, 1990). Chain-breaking antioxidants act by scavenging free radicals and donating hydrogen atoms. Preventative antioxidants are generally metal chelators and reductants capable of sparing other antioxidants *in vivo*. Other functions of antioxidants include peroxide decomposition, singlet oxygen quenching and inhibition of enzymes such as NADH-oxidase, succinoxidase, ATPase and nitric oxide synthase (eNOS) (Namiki, 1990; Hodnick *et al.*, 1994; Chiesi & Schwaller, 1995).

The most common water-soluble antioxidant compounds in plants and foods are the phenolic compounds (Macheix *et al.*, 1990). These secondary metabolites of plants are characterised by an aromatic ring possessing one or more hydroxyl substituents. The flavonoids contain a C_6-C_3-C_6 flavan skeleton (Fig. 1) in which the three-carbon bridge is cyclised with oxygen (Harborne, 1967). The major types of antioxidants found in grapes and wine include phenolic acids and their derivatives, namely hydroxybenzoic acids, hydroxycinnamic acids and hydroxycinnamates, and flavonoids, namely flavan-3-ols (catechins), flavonols, proanthocyanidins and anthocyanidins (Fig. 1). Non-flavonoid compounds such as the stilbene, resveratrol, also occurs in small quantities. Phenolic compounds exhibit structural and functional diversity and can be hydroxylated and methoxylated in various positions. Glycosylation with glucose, galactose, rhamnose, xylose or arabinose on the 3-, 5- and 7-hydroxyl moiety is common among the flavonoids (Macheix *et al.*, 1990).

The phenolic composition of grapes depends on the species (Singleton & Esau, 1969) and cultivar (Etiévant *et al.*, 1988) of grape, climatic conditions related to the mean day temperatures and exposure to sunlight, as well as soil conditions (Jackson & Lombard, 1993). Various parameters influence the phenolic composition of wines, including the phenolic composition of the grapes, the extent to which phenolic compounds are extracted during vinification, the chemical modification of phenolic compounds during maturation and the contribution of phenolic compounds due to contact with wood (Soleas *et al.*, 1997a). The average phenolic composition of red and white wines differs substantially (Table 1). The phenolic composition of wine contributes to its sensory qualities such as colour, flavour, astringency and bitterness, as well as its antioxidant potential (Soleas *et al.*, 1997a). Specific combinations of compounds are important in terms of antioxidant activity as synergistic effects may occur (Saucier & Waterhouse, 1999).



R1 = R2 = H, OH, O-Me or O-sugar

FIGURE 1
Structures of some of the major groups of phenolic compounds.

Phenolic acids

The simplest phenolic compounds commonly found in plants are the derivatives of benzoic and cinnamic acids. Hydroxybenzoic acids (Fig. 1) occurring in grapes and wine include gallic acid, ellagic acid, vanillic acid, protocatechuic acid and syringic acid (Macheix *et al.*, 1990). The presence of ellagic acid and its derivatives is mostly due to their extraction from wood during maturation in wooden barrels. The hydroxycinnamic acids, namely *p*-coumaric, caffeic, ferulic and sinapic acids, rarely occur in the free form in fruits, but can be found in wine due to the vinification process (Macheix & Fleuriet, 1998). The soluble derivatives of these compounds have one of the alcoholic groups esterified with tartaric acid (Fig. 1) and can also be acylated and glycosylated in different positions (Macheix *et al.*, 1990).

Flavonoids

Flavan-3-ols

Flavan-3-ols (Fig. 1) occur in wine, tea, fruit and chocolate (Arts *et al.*, 2000a; Arts *et al.*, 2000b). This class of compounds differs from other flavonoids, as they do not generally occur as glycosides (Macheix *et al.*, 1990). (+)-Catechin and (-)-epicatechin are the most common members, although gallate esters are also found in teas. Much greater quantities of flavan-3-ols are found in red wines than white wines due to extraction from grape seeds and skins during vinification (Table 1) (Oszmianski *et al.*, 1986).

TABLE 1

Relative concentrations of phenolic acids and flavonoids in wine[a].

Phenolic group/compound	Concentration (mg/L)	
	Red wine	White wine
Non-flavonoids	**240 – 500**	**160 – 260**
Hydroxybenzoic acids	*0 – 260*	*0 – 100*
p-Hydroxybenzoic acid	20.0[b]	–[c]
Gallic acid	63.8 (3.1 – 320)	6.4 (2.8 – 11)
Total gallates	49.0 (38.6 – 58.7)	6.9 (6.8, 7)
Syringic acid	11.5 (4.9, 18)	–[c]
Protocatechuic acid	88.0[b]	–[c]
Hydroxycinnamic acids	*143.1 (74.1 – 226)*	*130 – 154*
p-Coumaroyl tartaric acid	52.2 (21 – 137)	1.8[b]
Caffeoyl tartaric acid	80.9 (13.4 – 178)	5 (3, 7)
Caffeic acid	8.7 (4.7 – 18)	3.171 (1.5 – 5.2)
p-Coumaric acid	4.7 (0.9 – 22)	2.2 (1 – 3.2)
Ferulic acid	10.9 (2.9 – 19)	–[c]
Stilbenes	*11.1 (4 – 19)*	*1.8 (0.04 – 3.5)*
Resveratrol	1.2 (0.09 – 3.2)	0.04 (0 – 0.1)
Flavonoids	**750 – 1060**	**25 – 30**
Flavonols	*127.8 (65.3 – 238.3)*	*traces*
Quercetin	11.5 (0.5 – 28.5)	0.55 (0 – 1.2)
Myricetin	12.3 (0 – 64.5)	0.1 (0 – 0.3)
Kaempferol	1.0 (0.1 – 6)	0.1[b]
Rutin	7.4 (0 – 31.7)	0.3 (0 – 0.9)
Flavan-3-ols	*208.8 (27.3 – 557)*	*11.5 (2 – 29)*
Catechin	94.0 (15.3 – 390)	15.4 (1.5 – 46)
Epicatechin	44.3 (9.2 – 62)	8.7 (0.5 – 60)
Procyanidins	215.0 (30.9 – 367.1)	0
Anthocyanins	*270.9 (39.4 – 469)*	*0*
Delphinidin-3-glucoside	10.9 (2.3 – 22)	0
Cyanidin-3-glucoside	38.0[b]	0
Petunidin-3-glucoside	21 (18, 24)	0
Peonidin-3-glucoside	19 (6, 32)	0
Malvidin-3-glucoside	46.7 (0 – 206)	0
Malvidin-3-glucoside-acetate	38.2 (13.2 – 129)	0
Malvidin-3-glucoside-*p*-coumarate	15.1 (8.3 – 44)	0
Total polyphenols	**1686.4 (700 – 4059)**	**177.6 (96 – 331)**

[a]Values are averages from all values reported in Arts *et al.* (2000b), Carando *et al.* (1999), Ricardo da Silva *et al.* (1990), Fogliano *et al.* (1999), Frankel *et al.* (1995), German & Walzem (2000), Ghiselli *et al.* (1998), Goldberg *et al.* (1998a), Goldberg *et al.* (1998b), Goldberg *et al.* (1999), Lamuela-Raventós & Waterhouse (1993), Mazza (1995), Mazza *et al.* (1999), Pellegrini *et al.* (2000), Ritchey & Waterhouse (1999), Simonetti *et al.* (1997), Soleas *et al.* (1997b). Values in parentheses indicate the range of values reported; [b]Only one value was found in the literature; [c]No values reported in literature.

Flavonols

Flavonols (Fig. 1) occur in fruit and vegetables, as well as in beverages such as wine and tea (Hollman & Arts, 2000). They generally occur as glycosides with the sugar attached preferably to the 3-position. Although glucose is usually the main sugar, glycosides comprising galactose, rhamnose, arabinose and xylose are also formed. The most common flavonols in wine and grapes include quercetin, kaempferol, myricetin and their glycosides (Ribéreau-Gayon, 1972). White wines contain only small quantities of flavonols (Table 1).

Anthocyanidins

Anthocyanidins (Fig. 1) and anthocyanins (the glycoside derivatives of anthocyanidins) are common in red, blue and purple fruit and flowers (Mazza, 1995). These compounds are responsible for the intense colour of red wine. Copigmentation of anthocyanins with other flavonoids and phenolic acids occurs and contributes to the colour of red wine (Osawa, 1982; Brouillard & Dangles, 1994; Markovíc *et al.*, 2000; Darias-Martín *et al.*, 2001). The site of glycosylation in anthocyanins, as in the case of flavonols, is usually C-3. Acylated anthocyanins, where an organic acid (namely *p*-coumaric acid, caffeic acid or ferulic acid) is attached to the sugar molecule, are also found in grapes and wine (Ribéreau-Gayon, 1972; Wulf & Nagel, 1978). Only the monoglucosides of anthocyanins occur in red cultivars of *Vitis vinifera* grapes. Grapes from other species can therefore be distinguished by the presence of anthocyanin diglucosides (Singleton & Esau, 1969).

Proanthocyanidins

The name of this group of compounds is derived from the fact that these compounds yield anthocyanidins by cleavage of a carbon-carbon bond when heated in the presence of a mineral acid (Porter *et al.*, 1986). Proanthocyanidins (Fig. 1) are complex flavonoids naturally present in cereals, legumes, some fruits, cocoa and bev-

erages such as wine and tea (Santos-Buelga & Scalbert, 2000). The structure of this group of compounds is based on flavan-3-ol subunits [(+)-catechin and (-)-epicatechin] linked through the 4- and 8-positions or through the 4- and 6-positions (Haslam, 1980). Procyanidin dimers such as procyanidin B1, B2, B3 and B4 occur in wine along with small amounts of trimers such as C1 and T2, and tetramers (De Pascual-Teresa *et al.*, 2000).

IN VITRO ANTIOXIDANT ACTIVITY OF WINES AND WINE PHENOLICS

Measurement of antioxidant activity

Different assays are available to evaluate antioxidant properties of compounds and foods. The antioxidant assays can be divided into free radical scavenging, reducing capacity (Benzie & Strain, 1996), metal chelating (Aruoma *et al.*, 1987; Decker & Welsh, 1990), and lipid peroxidation assays. The free radical scavenging assays can be categorised as those using synthetic radicals, such as 2,2-diphenyl-1-picrylhydrazyl radicals ($DPPH^{\bullet}$) (Brand-Williams *et al.*, 1995), 2,2'-azinodi-(3-ethylbenzthialozine sulphonate) radical cations ($ABTS^{\bullet+}$) (Miller *et al.*, 1993) and *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride radicals ($DMPD^{\bullet}$) (Fogliano *et al.*, 1999) or biological radicals, such as superoxide radical anions (Robak & Gryglewski, 1988), hydroxyl radicals (Halliwell *et al.*, 1987) or peroxyl radicals (Wayner *et al.*, 1985; Cao *et al.*, 1993). The lipid peroxidation assays include assays using pure oils (Kosugi *et al.*, 1989), fatty acids (Kosugi *et al.*, 1989), model membranes (Pryor *et al.*, 1993), biological membranes (Beuge & Aust, 1978) or other biologically oxidisable substrates such as LDL (Frankel *et al.*, 1992). Free radical scavenging assays using synthetic radicals offer an easy and rapid way to screen foods and beverages for *in vitro* antioxidant activity. However, the use of biologically relevant assays involving biological substrates and free radicals commonly occurring in the body is important (Halliwell, 1995). Knowledge about the absorption and metabolism of active compounds is also needed for a complete understanding of possible *in vivo* antioxidant activity (Halliwell, 1995). Some antioxidants can also have prooxidant activity by recycling transition metal ions, thereby promoting lipid peroxidation (Sevanian & Ursini, 2000). This is one of the potentially toxic effects cautioning against excessive flavonoid intake (Halliwell *et al.*, 1987; Skibola & Smith, 2000). The antioxidant activity of some phenolic compounds in four selected antioxidant assays is listed in Table 2. It is important to note that the assay used will affect the perceived antioxidant

TABLE 2
Relative antioxidant activity of selected phenolic compounds.

Compounds	TEAC[a]	EC_{50} (DPPH)[b]	IC_{50} (LDL)[c]	Prooxidant activity[d]
Phenolic acids				
Gallic acid	3.01[e]		1.25[i]	+[j]
p-Coumaric acid	2.22[f]	ineffective[h]	>16[i]	–[j]
Ferulic acid	1.90[f]	407[h]		–[j]
Vanillic acid	1.43[e]			–[j]
Syringic acid	1.36[e]	218[h]		
Caffeic acid	1.26[f]	110[h]	0.24[i]	+[j]
Protocatechuic acid	1.19[e]	172[h]		
p-Hydroxybenzoic acid	0.08[e]	ineffective[h]		–[j]
Flavan-3-ols				
Epicatechin gallate	4.90[g]		0.14[i]	
Epigallocatechin gallate	4.80[g]		0.08[i]	
Epigallocatechin	3.80[g]		0.10[i]	
Epicatechin	2.50[g]	135[h]		
Catechin	2.40[g]	149[h]	0.19[i]	+[k]
Flavonols				
Quercetin	4.72[f]	91[h]	0.23[i]	+[k]
Myricetin	3.10[g]		0.48[i]	+[k]
Rutin	2.40[g]	136[h]	0.51[i]	
Kaempferol	1.34[f]		1.82[i]	+[k]
Anthocyanidins				
Delphinidin	4.44[f]			
Cyanidin	4.40[f]		0.21[i]	
Peonidin	2.22[g]			
Malvidin	2.06[f]			
Malvidin-3-glucoside	1.78[g]			
Pelargonidin	1.30[g]			
Plasma antioxidants				
Ascorbic acid	0.99[f]		1.45[i]	+[j]
α-Tocopherol	0.97[f]	304[h]	2.40[i]	
Synthetic antioxidants				
Trolox	1.00[f]	284[h]	1.26[i]	–[l]

[a]Trolox equivalent antioxidant activity in mM as measured by the ABTS (2,2'-azinodi-(3-ethylbenzthialozine sulphonate)) radical scavenging assay; [b]Concentration (μM) needed to scavenge 50% of DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals; [c]Concentration needed for 50% inhibition of low-density lipoprotein oxidation; [d]Ability to promote hydroxyl radical generation in the deoxyribose assay; [e]Miller & Rice-Evans, 1997; [f]Re *et al.*, 1999; [g]Rice-Evans *et al.*, 1996; [h]Winterton, 1999; [i]Vinson *et al.*, 1995; [j]Moran *et al.*, 1997; [k]Puppo, 1992; [l]Aruoma *et al.*, 1990.

activity of phenolic compounds or mixtures of them as different aspects of antioxidant activity can be measured individually or in combinations (Halliwell, 1995; Baderschneider *et al.*, 1999; Prior & Cao, 1999; Frankel & Meyer, 2000).

Structure-activity relationships

The chemical structures of phenolic compounds are predictive of their antioxidant potential in terms of radical scavenging, hydrogen- or electron-donating and metal-chelating capacities (Rice-Evans *et al.*, 1997). The antioxidant potency of a compound is also affected by the stability of the resulting phenoxyl radical. The unique structure of phenolic compounds facilitates their role as free radical scavengers due to resonance stabilisation of the captured electron (Shahidi & Wanasundara, 1992). Free radical scavenging occurs by hydrogen donation to lipid radicals competing with the chain propagation reaction (Shahidi & Wanasundara, 1992):

$$LOO^{\bullet} + AH \rightarrow LOOH + A^{\bullet}$$
$$LO^{\bullet} + AH \rightarrow LOH + A^{\bullet}$$
$$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet}$$
$$A^{\bullet} + LH \rightarrow AH + L^{\bullet}$$

where $LOO^{\bullet}$ = peroxyl radical; AH = phenolic antioxidant; LOOH = fatty acid hydroperoxide; $A^{\bullet}$ = phenoxyl radical; $LO^{\bullet}$ = alkoxyl radical; LOH = alcohol; LH = polyunsaturated fatty acid; $L^{\bullet}$ = alkyl radical.

Many researchers (Bors *et al.*, 1990; Foti *et al.*, 1996; Rice-Evans *et al.*, 1996; Liao & Yin, 2000) have studied the structure-activity relationships (SAR) of antioxidants in various test systems. All the structurally related effects could not be explained due to differences in mechanisms, end-points used, substrates and concentrations of antioxidants in the methods used. Most lipid peroxidation assays use metal ions as initiators, therefore the SAR derived from such test systems includes both free radical scavenging and metal chelation (Van Acker *et al.*, 1996). A study by Van Acker *et al.* (1998) making use of a microsomal lipid peroxidation assay, however, reported that metal chelation did not play a role in the antioxidant activity of a number of phenolic compounds. On the other hand, a study by Sugihara *et al.* (1999) showed that the activity of phenolic compounds to inhibit lipid hydroperoxide dependent peroxidation in cultured hepatocytes differed depending on the metal ion used. In systems containing both lipid and aqueous phases, partitioning of compounds between these phases also plays a contributing role in the antioxidant activity (Foti *et al.*, 1996; Liao & Yin, 2000). Porter (1980; 1993) introduced the concept of the polar paradox, stating that hydrophilic antioxidants are more effective in pure oil systems, while lipophilic constituents are more effective in systems containing both lipid and aqueous phases.

More effective comparisons of the structure-activity relationships for phenolic compounds can be made if only one aspect of antioxidant activity, such as free radical scavenging activity or metal-chelating ability, is investigated at a time. The importance of the chemical structure in the antioxidant potency of phenolic compounds can be illustrated by considering the Trolox equivalent antioxidant capacity (TEAC) values of compounds differing in only one structural aspect. The TEAC values of compounds are their free radical scavenging activity in relation to a reference compound, Trolox, measured using the ABTS radical cation scavenging assay (Miller *et al.*, 1993).

Phenolic acids

The antioxidant activity of phenolic acids is related to the acid moiety and the number and relative positions of hydroxyl groups on the aromatic ring structure (Rice-Evans *et al.*, 1996; Hall III & Cuppett, 1997). Hydroxycinnamic acids are more effective antioxidants than hydroxybenzoic acids due to increased possibilities for delocalisation of the phenoxyl radical (Chen & Ho, 1997; Moon & Terao, 1998; Silva *et al.*, 2000). Substitution patterns of some hydroxybenzoic and hydroxycinnamic acids are shown in Table 3.

Benzoic and cinnamic acid, neither of which possesses free hydroxyl groups, have no free radical scavenging activity (Miller & Rice-Evans, 1997). Di- and trihydroxylation increase the activity over a single hydroxyl group with the position of the hydroxyl groups being the most important factor. Hydroxylation in the 2- and 4-positions or in the 3-, 4- and 5-positions confers the greatest antioxidant activity. Adjacent hydroxyl groups, as found in protocatechuic acid (TEAC = 1.2), are less favourable for antioxidant activity than those *meta*-orientated with respect to each other, as is the case for α-resorcylic acid (TEAC = 2.15) (Miller & Rice-Evans, 1997).

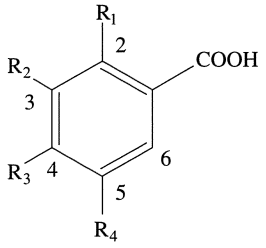
Substituents increasing the electron density on the hydroxyl groups cause a decrease in the dissociation energy of the O-H bond. Therefore electron-donating substituents will increase the antioxidant activity, as in the case of vanillic acid (TEAC = 1.4)

TABLE 3
Substitution patterns for phenolic acids.

Compounds	2	3	4	5	6	TEAC[a]
Hydroxybenzoic acids[1]						
Salicylic acid	OH	H	H	H	H	
m-Hydroxybenzoic acid	H	OH	H	H	H	
p-Hydroxybenzoic acid	H	H	OH	H	H	0.08
Protocatechuic acid	H	OH	OH	H	H	1.19
Gallic acid	H	OH	OH	OH	H	3.01
Vanillic acid	H	O-Me	OH	H	H	1.43
Syringic acid	H	O-Me	OH	O-Me	H	1.36
Hydroxycinnamic acids[2]						
p-Coumaric acid	H	H	OH	H	H	2.22
Caffeic acid	H	OH	OH	H	H	1.26
Ferulic acid	H	O-Me	OH	H	H	1.90
Sinapic acid	H	O-Me	OH	O-Me	H	

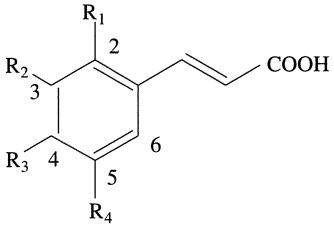
[a]mM Trolox equivalent antioxidant activity as measured by the ABTS (2,2'-azinodi-(3-ethylbenzthialozine sulphonate)) radical scavenging assay.

$R_1 = R_2 = R_3 = R_4$ = H, OH, OMe



[1] Hydroxybenzoic acids

$R_1 = R_2 = R_3 = R_4$ = H, OH, OMe



[2] Hydroxycinnamic acids

relative to *p*-hydroxybenzoic acid (TEAC = 0.1) (Miller & Rice-Evans, 1997).

Hydroxycinnamic acid esters, such as caffeoyltartaric acid, *p*-coumaroyltartaric acid and chlorogenic acid, exhibit greater antioxidant activity than the parent hydroxycinnamic acids, possibly due to increased possibilities for electron delocalisation (Meyer *et al.*, 1998; Silva *et al.*, 2000).

Flavonoids

The structural characteristics imparting the highest antioxidant activity in flavonoids have been found to be the following (Fig. 2) (Bors *et al.*, 1990):

1) the *ortho* 3',4'-dihydroxy moiety in the B-ring for electron delocalisation and stability of the phenoxyl radical;
2) the 2,3-double bond in combination with the 4-keto group for electron delocalisation in the C-ring; and
3) the 3- and 5-hydroxyl groups in the C- and A-ring, respectively, in combination with the 4-keto group in the C-ring for maximum scavenging potential.

Substitution patterns of some flavan-3-ols, flavonols and anthocyanins are shown in Table 4. Quercetin (TEAC = 4.7), one of the most effective flavonoid antioxidants, satisfies all of the above-mentioned criteria. Catechin (TEAC = 2.4), which lacks the 2,3-double bond and the 4-keto group in the C-ring, is therefore a less effective free radical scavenger than quercetin (Jørgensen *et al.*, 1998; Rice-Evans & Miller, 1998). The 3',4'-dihydroxy moiety in the B-ring of most flavonoids is an important structural criterion for effective free radical scavenging activity (Hall III & Cuppett, 1997). This function provides increased stability due to participation in electron delocalisation of the phenoxyl radical increasing the acidity of the 4'-hydroxyl moiety. As an example, kaempferol (TEAC = 1.3), lacking the 3'-hydroxyl group, has a much lower antioxidant activity than quercetin (TEAC = 4.7) due to decreased acidity of its 4'-hydroxyl moiety (Rice-Evans *et al.*, 1996).

The significant reduction in antioxidant activity due to glycosylation at the 3-position of the C-ring as found in rutin (TEAC = 2.4) confirms the importance of the 3-hydroxyl group in quercetin (TEAC = 4.7) (Rice-Evans *et al.*, 1996).

Retaining the *o*-dihydroxy structure of the B-ring with saturation of the 2,3-double bond as seen in flavanonols, eliminates delocalisation of the B-ring phenoxyl radical. An example of this effect can be observed when comparing the antioxidant activity of quercetin (TEAC = 4.7) and dihydroquercetin (TEAC = 1.9) (Rice-Evans *et al.*, 1996). In the absence of the 3',4'-dihydroxy moiety in the B-ring, as is the case with kaempferol (TEAC = 1.3), the reduction of the 2,3-double bond as found in dihydrokaempferol (TEAC = 1.4) has little effect on the antioxidant activity (Rice-Evans & Miller, 1998). Therefore, these combined structural features are critical for maximum antioxidant activity.

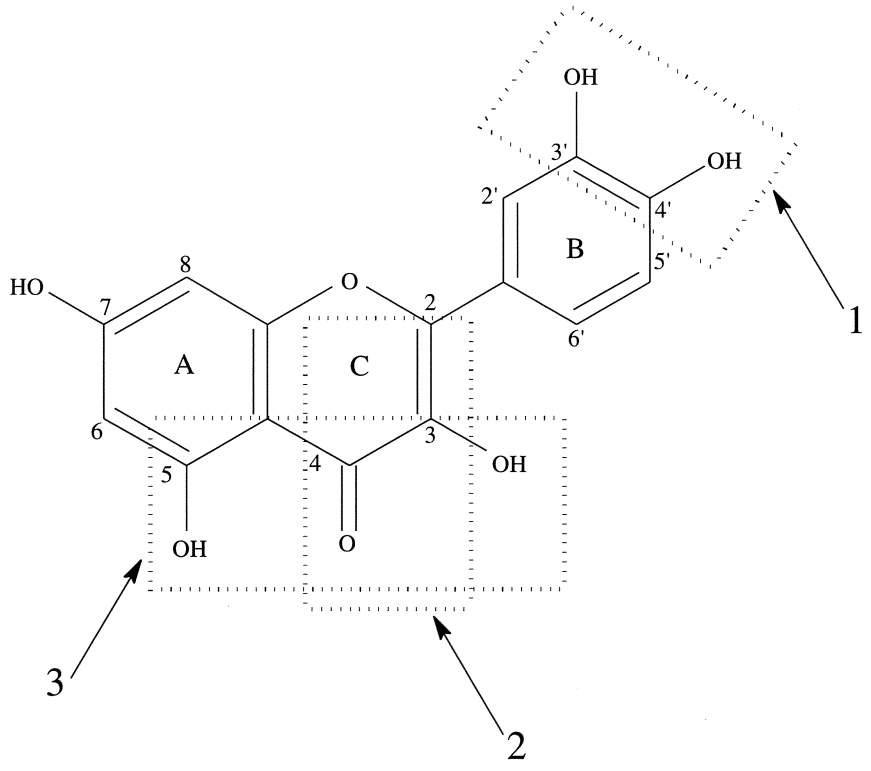


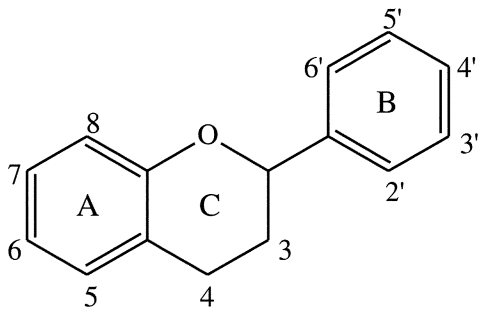
FIGURE 2

Structural characteristics of flavonoids conferring maximum antioxidant activity:
1 The ortho-3', 4'-dihydroxy moiety in the B-ring
2 The 2,3-double bond in combination with the 4-keto group
3 The 3- and 5-hydroxyl groups in the C- and A-ring respectively in combination with the 4-keto group in the C-ring

TABLE 4
Substitution patterns of flavonoids.

Compounds	5	7	3	4	2'	3'	4'	5'	TEAC[a]
Flavan-3-ols									
Catechin	OH	OH	H,OH	H,H	H	OH	OH	H	2.40[b]
Epicatechin	OH	OH	H,OH	H,H	H	OH	OH	H	2.50[b]
Flavonols									
Quercetin	OH	OH	OH	O	H	OH	OH	H	4.72[b]
Dihydroquercetin	OH	OH	H,OH	O	H	OH	OH	H	1.90[b]
Myricetin	OH	OH	OH	O	H	OH	OH	OH	3.10[b]
Kaempferol	OH	OH	OH	O	H	H	OH	H	1.34[b]
Dihydrokaempferol	OH	OH	H, OH	O	H	H	OH	H	1.39[b]
Rutin	OH	OH	O-Rut[c]	O	H	OH	OH	H	2.40[b]
Anthocyanins									
Malvidin	OH	OH	OH	H	H	O-Me	OH	O-Me	2.06[b]
Malvidin-3,5-diGlc[d]	OH	OH	O-Glc	H	H	O-Glc	OH	O-Me	1.78[b]
Cyanidin	OH	OH	OH	H	H	OH	OH	H	4.40[b]
Cyanidin-3-Glc	OH	OH	O-Glc	H	H	OH	OH	H	
Peonidin	OH	OH	OH	H	H	O-Me	OH	H	2.22[b]
Peonidin-3-Glc	OH	OH	O-Glc	H	H	O-Me	OH	H	
Delphinidin	OH	OH	OH	H	H	OH	OH	OH	4.44[b]
Delphinidin-3-Glc	OH	OH	O-Glc	H	H	OH	OH	OH	
Pelargonidin	OH	OH	OH	H	H	H	OH	H	1.30[b]
Pelargonidin-3-Glc	OH	OH	O-Glc	H	H	H	OH	H	

[a]mM Trolox equivalent antioxidant activity as measured by the ABTS (2,2'-azinodi-(3-ethylbenzthialozine sulphonate)) radical scavenging assay; [b]Rice-Evans & Miller, 1998; [c]Rutinose; [d]Glucose



Flavan skeleton of flavonoids

ACTIVITY OF WINES IN SELECTED ANTIOXIDANT ASSAYS

The antioxidant activity of a wine is largely dependent on its phenolic content and composition, as different compounds and combinations of them exhibit varying degrees of activity. The phenolic content, on the other hand, is determined by the phenolic composition of the grapes used (Singleton & Esau, 1969; Etiévant *et al.*, 1988), the vinification process (Oszmianski *et al.*, 1986; Macheix *et al.*, 1990; Ricardo da Silva *et al.*, 1993; Bakker *et al.*, 1998; Sun *et al.*, 1999) and the maturation processes (Somers & Pocock, 1990). Any variation in the vinification process that would introduce a difference in phenolic composition of the wine should influence its antioxidant activity.

The antioxidant activity of wines has been studied using free radical scavenging assays such as the ABTS radical cation (Campos & Lissi, 1996; Simonetti *et al.*, 1997; Soleas *et al.*, 1997b; Fogliano *et al.*, 1999; Pellegrini *et al.*, 2000), the DPPH radical (Manzocco *et al.*, 1998; Larrauri *et al.*, 1999; Sánchez-Moreno *et al.*, 1999) and the superoxide anion radical (Sato *et al.*, 1996) scavenging assays, as well as lipid peroxidation assays such as the LDL assay (Kanner *et al.*, 1994; Frankel *et al.*, 1995; Vinson & Hontz, 1995; Teissedre *et al.*, 1996).

Total antioxidant activity of wines as measured using the ABTS radical cation scavenging assay was 7 – 33 mM Trolox equivalents for red wines and 0 – 5 mM Trolox equivalents for white wines (Table 5). When utilising the DPPH radical (Sánchez-Moreno *et al.*, 1999) and the superoxide radical anion (Sato *et al.*, 1996) scavenging assays, red wines also exhibited more effective free radical scavenging activity than white wines, with rosé wines exhibiting intermediate activity. The free radical scavenging activity has been correlated to total phenol (Simonetti *et al.*, 1997; Fogliano *et al.*, 1999) and flavan-3-ol (Simonetti *et al.*, 1997) contents, as well as to the content of specific compounds (Soleas *et al.*, 1997b) such as vanillic acid, gallic acid and catechin. Despite these findings, Saint-Cricq de Gaulejac *et al.* (1999) reported that the fraction of wine which contains anthocyanins exhibits the highest superoxide radical anion scavenging activity.

TABLE 5
Total antioxidant activity of wines from different countries.

Type of wine	Vintage	TAA[a] (number of wines)	Reaction time	Reference
Red, Chile	1991 – 1992	25.1 – 33.3[b] (10)	6 min.	Campos & Lissi, 1996
Red, France	1991 – 1999	9.6 – 29.9[c] (34)	4 min.	Landrault *et al.*, 2001
Red, Canada	1991 – 1994	7.5 – 28.6[c] (14)	3 min.	Soleas *et al.*, 1997b
Vini Novelli, red, Italy	1997	10.9 – 22.9[c] (8)	3 min.	Pellegrini *et al.*, 2000
Red, Italy	1991 – 1994	7.8 – 19.8[c] (10)	3 min.	Simonetti *et al.*, 1997
Red, South Africa	1998	9.2 – 19.5[d] (46)	4 min.	De Beer, 2002
Red, Spain	1992	14.1[d] (1)	6 min.	Verhagen *et al.*, 1996
Red, Italy	1989 – 1996	6.1 – 11.6[c] (3)	1 min.	Fogliano *et al.*, 1999
Rose, Chile	1994	5.0[b] (1)	6 min.	Campos & Lissi, 1996
Rose, Spain	1993	2.4[d] (1)	6 min.	Verhagen *et al.*, 1996
White, Chile	1991 – 1994	2.9 – 5.2[b] (3)	6 min.	Campos & Lissi, 1996
White, Italy	1994 – 1995	0.0 – 3.6[c] (3)	3 min.	Simonetti *et al.*, 1997
White, Italy	1996	1.4 – 1.9[c] (4)	1 min.	Fogliano *et al.*, 1999
White, South Africa	1999	0.5 – 1.4[d] (40)	4 min.	De Beer, 2002
White, Spain	1993	0.8[d] (1)	6 min.	Verhagen *et al.*, 1996

[a]Total antioxidant activity as mM Trolox equivalents; [b]Generation of $ABTS^{\bullet+}$ with 2,2'-azobis(2-amidinopropane) before assay; [c]Generation of $ABTS^{\bullet+}$ with ferrylmyoglobin during assay; [d]Generation of $ABTS^{\bullet+}$ with manganese dioxide before assay.

Reports on the relative efficacy of red and white wines to inhibit the *in vitro* peroxidation of LDL have been conflicting. Frankel *et al.* (1995) reported that red wine inhibits LDL peroxidation to a greater extent than white wine when measured at the same total phenol content, while Vinson & Hontz (1995) reported exactly the opposite. Consumption of wine is thought to reduce the susceptibility of LDL to peroxidation. An *ex vivo* study, where LDL had been isolated from volunteers consuming red wine, showed that red wine protects LDL from peroxidation (Fuhrman *et al.*, 1995), while another observed no protective effect (De Rijke *et al.*, 1996). This illustrates the need to characterise the individual phenolic compounds and specific combinations of them, as the total phenol content alone could not clarify these differences. Teissedre *et al.* (1996) reported the fractions of wine containing the flavan-3-ols and procyanidins to be more effective antioxidants than the fractions containing phenolic acids, flavonols or anthocyanins, when compared at the same total phenol concentration. Correlation of total phenols, as well as the content of specific phenolic compounds such as gallic acid, catechin and myricetin, with inhibitory activity in the LDL peroxidation assay has also been reported (Frankel *et al.*, 1995).

The effect of factors such as cultivar and vinification on the free radical scavenging activity of wines has also been investigated. Pellegrini *et al.* (2000) investigated the effect of carbonic maceration on the total phenol and flavan-3-ol contents of wines and their free radical scavenging activity as measured using the ABTS radical cation scavenging assay. Higher free radical scavenging activity was reported for wines prepared using carbonic maceration as opposed to those prepared in the traditional manner. Skin contact during the making of white wines was found to increase the protective ability against LDL peroxidation (Hurtado *et al.*, 1997). Conflicting reports have been given in terms of the effect of in-bottle ageing on the free radical scavenging activity of wines. Manzocco *et al.* (1998) reported a decrease in free radical scavenging activity detected using the DPPH radical scavenging assay with increasing time, while Larrauri *et al.* (1999) reported an increase in free radical scavenging activity using the same assay.

A study evaluating the effect of in-bottle ageing of red and white cultivar wines under accelerated storage conditions on the free radical scavenging activity was completed recently. This study concluded that storage of bottled wines at 0°C, 15°C and 30°C results in complex changes in phenolic composition with a concomitant decrease in total antioxidant activity over a period of one year (De Beer, 2002). South African red (Cabernet Sauvignon, Ruby Cabernet, Pinotage, Shiraz and Merlot) and white (Sauvignon blanc, Chenin blanc, Chardonnay and Colombard) cultivar wines were also compared in terms of antioxidant activity, which was related to their phenolic composition. Several phenolic groups in wines correlated with their antioxidant activity in different test systems (De Beer, 2002). The Ruby Cabernet wines exhibited the lowest antioxidant activity of all the red cultivar wines despite its high anthocyanin content, while Chardonnay and Chenin blanc represented the highest and lowest antioxidant activity of the white cultivar wines, respectively (De Beer, 2002). Very little attention has been directed to the influence of cultivar and vintage on the phenolic composition and antioxidant activity of wines in the past. Most studies investigated the effect of a limited number of parameters using insufficient repetitions on which to base comparative statistical analysis (Hurtado *et al.*, 1997; Manzocco *et al.*, 1998; Pellegrini *et al.*, 2000).

ABSORPTION AND BIOAVAILABILITY

The metabolic fate and pharmacokinetics of most phenolic compounds have not yet been extensively studied in humans. A number of studies on the detection of plasma and urinary metabolites of certain phenolic compounds, such as quercetin, quercetin glycosides, kaempferol, catechin, epicatechin, ferulic acid, caffeic acid and cyanidin glucosides, are summarised in Table 6. Phenolic compounds in wine, present as soluble forms, should be more bioavailable than those in fruits and vegetables, where they are present as polymeric, insoluble or tightly bound and compartmentalised forms (Soleas *et al.*, 1997a).

Intakes of phenolic acids and flavonoids in humans have been estimated to range from as much as 170 mg/day (Kühnau, 1976) to 23 mg/day (Hertog *et al.*, 1993). Average intakes are, however,

TABLE 6

Bioavailability of phenolic compounds in human and rat *in vivo* studies.

Source	Amount administered	Human / Rat	Absorption detection parameters	Excretion	Metabolites	Maximum plasma concentrations	Kinetics	References
Phenols from red wine, whisky or new make spirit	100 mL	Human	Increase in plasma total phenol content and antioxidant capacity	Urine conc. = 32 µg GAE/L (red wine), 22 µg GAE/L (whisky) and 14 µg GAE/L (new make spirit)	–	–	–	Duthie *et al.*, 1998
Red wine (catechin)	120 mL red wine (RW) or dealcoholised red wine (DRW)	Human	Presence of metabolites in plasma	–	Catechin glucuronides and sulfates and 3-MC glucuronides (20% of total catechin at 1h) in plasma	91 nM total catechin after 1h (red wine); 81 nM total catechin after 1h (dealcoholised red wine)	Max. absorption after 1h; elimination half-life (t1/2) less for RW than for DRW	Donovan *et al.*, 1999
Red wine (catechin)	120 mL dealcoholised red wine reconstituted in water (DRW) or alcohol and water (ARW)	Human	Presence of metabolites in plasma	–	Conjugate forms predominant in plasma	40 – 130 nM total catechin (DRW); 30 – 110 nM total catechin (ARW)	Max. absorption after 1h; elimination half-life (t1/2) less for ARW than for DRW	Bell *et al.*, 2000
Quercetin and catechin	0.25% of diet directly into stomach	Rat	Presence of metabolites in plasma	–	Catechin glucuronides and Q sulfo- and glucuronosulfo derivatives; methylation rate higher for Q	50 mM Q metabolites (12h); 38 mM catechin metabolites (12h)	Absorption of catechin faster than Q; plasma conc. of Q stable between 8 and 24h	Manach *et al.*, 1999
Quercetin-4'-Glc and quercetin-3-Glc	331 µmol Q-4'-Glc or 325 µmol Q-3-Glc	Human	Presence of metabolites in plasma	–	–	Peak plasma concentration = 4.5 µM after Q-4'-Glc and 5.0 µM after Q-3-Glc	Peak content reached after 27 min. for Q-4'-Glc and after 37 min. for Q-3-Glc; t1/2(elimination) = 17.7h for Q-4'-Glc and 18.5h for Q-3-Glc	Olthof *et al.*, 2000
Quercetin, rutin or onion	100 mg Q, rutin (100 mg Q equiv.), onions (89 mg Q equiv.)	Human (ileostomy volunteers)	24 ca. 9% from Q, 17 ca. 15% from rutin and 52 ca. 15% from onion	Illeostomy effluent and urine – 0.12% for Q, 0.07% for rutin and 0.31% for onion (13h)	–	–	–	Hollman *et al.*, 1995
Cyanidin-3-Glc and cyanidin-3,5-diGlc or tea catechin (EGCg)	2.7 mg/kg body weight Cy-3-Glc + 0.25 mg/kg body weight Cy-3,5-diGlc; 2.6 mg/kg body weight EGCg	Human	Presence of metabolites in plasma	–	No glucuronides or sulfates or aglycone of anthocyanins, but peonidin-3-Glc (methylated Cy-3-Glc) found; glucuronides and sulfates of EGCg	29 nM Cy-3-Glc after 60 min; 725 nM EGCg after 60 min.	Absorption of anthocyanins faster than tea catechin (EGCg)	Miyazawa *et al.*, 1999
Cyanidin-3-Glc and cyanidin-3,5-diGlc or tea catechin (EGCg)	320 mg/kg body weight Cy-3-Glc + 40 mg/kg body weight Cy-3,5-diGlc; 320 mg/kg body weight EGCg	Rat	Presence of metabolites in plasma	–	No glucuronides or sulfates or aglycone of anthocyanins, but peonidin-3-Glc found (methylated Cy-3-Glc); glucuronides and sulfates of EGCg	1560 mg/L of Cy-3-Glc and 195 µg/L of Cy-3,5-diGlc; 3620 mg/L of EGCg	Absorption of anthocyanins faster than tea catechin (EGCg)	Miyazawa *et al.*, 1999
Anthocyanins from red wine	300 mL water, white wine or red wine (218 mg Mv-3-Glc equiv.)	Human	Presence of urinary metabolites	1.5 – 5.1% exctreted in urine after 12 h	Detection of anthocyanin dimers and unidentified derivatives of Mv-3-Glc in urine	–	Max. anthocyanin level in urine after 6 h	Lapidot *et al.*, 1998
Ferulic acid from tomato	8 g/kg body weight fresh tomato (ca. 21 – 44 mg ferulic acid)	Human	Presence of urinary metabolites	11 – 25% excreted in urine	Ferulic acid and feruloylglucuronide	–	Maximal urinary excretion after 7 h	Bourne & Rice-Evans, 1998
Epicatechin	172 µmol/kg body weight	Rat	Presence of metabolites in plasma	–	EC, methylated EC and glucuronide and sulfate conjugates of EC and MEC found in plasma	Free EC = 1.2 mM; MEC sulfate/glucuronide = 11.5 mM; EC glucuronide = 10.7 mM	Max. plasma concentration of all metabolites reached within 2 h	Piskula & Terao, 1998

GAE = gallic acid equivalents; max. = maximum; conc. = concentration; equiv. = equivalents; Q = quercetin; Q-4'-Glc = quercetin-4'-glucoside; Q-3-Glc = quercetin-3-glucoside; EC = epicatechin; MEC = methyl epicatechin; Mv-3-Glc = malvidin-3-glucoside; Cy-3-Glc = cyanidin-3-Glc; Cy-3,5-diGlc = cyanidin-3,5-diglucoside; peonidin-3-Glc = peonidin-3-glucoside; EGCg = epigallocatechin gallate.

linked to the dietary habits of the populations studied. In countries where red wine or coffee is consumed regularly, intakes may be much higher. Dietary intakes of hydroxybenzoic acid derivatives, hydroxycinnamates, anthocyanins and flavonols have been estimated as approximately 48.9 – 95 mg (Tomás-Barberán & Clifford, 2000), 25 – 1000 mg (Clifford, 2000a), 180 – 215 mg (Clifford, 2000b) and 4 – 26 mg (Hollman & Arts, 2000). Recently Teissedre & Landrault (2000) estimated the average intake of phenolic compounds by the French population per day from red wine. The estimated daily consumption of 180 mL red wine (1995 consumption figures) equates to between 400 mg phenols (as gallic acid equivalents) per person per day, while 180 mL white wine equates to only 44 mg phenols per person per day.

The bioavailability of a compound is not only affected by the extent of absorption, but factors such as distribution, metabolism (bioconversion in the gut and the liver) and excretion also play important roles in determining its *in vivo* protective ability (Wiseman, 1999). Degradation of phenolic compounds in gastric and intestinal fluids could decrease the amount available for absorption. A study by Martínez-Ortega *et al.* (2001) showed that phenolic compounds in wine are more stable with respect to gastric and intestinal fluids than purified phenolic compounds in a 10% ethanol solution. Absorption of phenolic compounds occurs mostly in the small intestines prior to microbial degradation (Hollman & Katan, 1998). After compounds and degradation products are absorbed into the bloodstream from the intestines, biotransformations by enzymes in the liver and the kidneys can occur. Major conjugates formed are methylated derivatives, glucuronides, sulphates and conjugates with both glucuronide and sulphate moieties (Table 6) (Hollman & Katan, 1998).

Bacteria in the colon are able to hydrolyse β-glycosidic bonds to release aglycones, which are more active antioxidants *in vitro* than the glycoside parent molecules (Bokkenheuser *et al.*, 1987). Colonic bacteria release glucuronidases and sulphatases, which hydrolyse the phenolic conjugates. Ring fission of flavonoids in various positions by microbial enzymes produces a variety of phenolic acids (Hollman & Katan, 1998). These also possess antioxidant activity and can contribute to the biological activity after absorption (Manach *et al.*, 1998). The specific hydroxylation pattern of the flavonoids determines susceptibility to ring fission and the products of ring fission (Hollman & Katan, 1998). Some phenolic compounds such as epicatechin (Piskula & Terao, 1998), catechin (Hollman & Katan, 1998), myricetin (Hollman & Katan, 1998) and ferulic acid (Bourne & Rice-Evans, 1998) have also been detected in plasma unmetabolised (Table 6).

A few studies have correlated the presence of flavonoid metabolites with intake of specific phenolic compounds (De Vries *et al.*, 1998; Hodgson *et al.*, 2000; Noroozi *et al.*, 2000). The measurement of metabolites of quercetin and kaempferol in urine and plasma can be used to distinguish between high and low flavonol consumption in epidemiological studies (De Vries *et al.*, 1998). Short-term intake of these flavonols can also be inferred from the plasma concentrations of metabolites as elimination has been shown to be less than 24 h (Hollman *et al.*, 1996; Young *et al.*, 1999; Olthof *et al.*, 2000). Plasma flavonol concentration and 24 h urine excretion were significantly correlated to dietary intake of flavonols (Noroozi *et al.*, 2000). This will enable the determination of dietary intakes of flavonols for use in epidemiological studies, eliminating the need to rely on food intake questionnaires.

Many studies show increases in plasma antioxidant activity, measured by a variety of methods, after the ingestion of foods containing phenolic compounds (Cao *et al.*, 1998a; Cao *et al.*, 1998b; Duthie *et al.*, 1998; Serafini *et al.*, 1998; McAnlis *et al.*, 1999; Sung *et al.*, 2000). This suggests that phenolic compounds are absorbed and circulate in plasma in bioactive forms.

As long as information on the absorption, distribution, metabolism and excretion of phenolic compounds is limited to a few specific compounds, it will remain unclear whether phenolic compounds are retained in the body in bioactive forms at sufficient levels to provide *in vivo* protection.

CONCLUDING REMARKS

Oxidative stress in the cell occurs during disease conditions or when optimal nutrition is lacking. Under these circumstances reactive oxygen species are available to initiate lipid peroxidation and damage other biomolecules. Antioxidants, such as phenolic compounds, can play a protective role to inactivate harmful reactive oxygen species. Antioxidant assays measure different aspects of antioxidant activity and the need exists to use several different test systems to fully characterise the antioxidant properties of compounds or foods. The activity of antioxidants depends on their ability to scavenge free radicals and chelate metal ions, which strongly relates to their chemical structure. The many phenolic compounds present in wine, having different antioxidant activities, preclude the prediction of antioxidant activity from the total phenol content alone. Differences in phenolic composition due to cultivar, vinification processes, maturation in wood and in-bottle ageing will affect the antioxidant potential of wines. Factors such as absorption and distribution of antioxidant molecules in the body, as well as structural changes occurring during metabolism, could also influence potential bioactivity.

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