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Wine as a Biological Fluid: History, Production, and Role in Disease Prevention

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Wine has been part of human culture for 6,000 years, serving dietary and socio-religious functions. Its production takes place on every continent, and its chemical composition is profoundly influenced by enological techniques, the grape cultivar from which it originates, and climatic factors. In addition to ethanol, which in moderate consumption can reduce mortality from coronary heart disease by increasing high-density lipoprotein cholesterol and inhibiting platelet aggregation, wine (especially red wine) contains a range of polyphenols that have desirable biological properties. These include the phenolic acids (p-coumaric, cinnamic, caffeic, gentisic, ferulic, and vanillic acids), trihydroxy stilbenes (resveratrol and polydatin), and flavonoids (catechin, epicatechin, and quercetin). They are synthesized by a common pathway from phenylalanine involving polyketide condensation reactions. Metabolic regulation is provided by competition between resveratrol synthase and chalcone synthase for a common precursor pool of acyl-CoA derivatives. Polymeric aggregation gives rise, in turn, to the viniferins (potent antifungal agents) and procyanidins (strong antioxidants that also inhibit platelet aggregation). The antioxidant effects of red wine and of its major polyphenols have been demonstrated in many experimental systems spanning the range from in vitro studies (human low-density lipoprotein, liposomes, macrophages, cultured cells) to investigations in healthy human subjects. Several of these compounds (notably catechin, quercetin, and resveratrol) promote nitric oxide production by vascular endothelium; inhibit the synthesis of thromboxane in platelets and leukotriene in neutrophils, modulate the synthesis and secretion of lipoproteins in whole animals and human cell lines, and arrest tumour growth as well as inhibit carcinogenesis in different experimental models. Target mechanisms to account for these effects include inhibition of phospholipase A_2 and cyclo-oxygenase, inhibition of phosphodiesterase with increase in cyclic nucleotide concentrations, and inhibition of several protein kinases involved in cell signaling. Although their bioavailability remains to be fully established, red wine provides a more favourable milieu than fruits and vegetables, their other dietary source in humans. J. Clin. Lab. Anal. 11:287–313, 1997.

Key words: anticarcinogenic agents; cyclo-oxygenase inhibitors; antioxidants; polyphenols; phenolic acids; flavonoids; stilbenes; low-density lipoprotein; biological oxidation; cholesterol; platelet aggregation; xanithine oxidase; nitric oxide; eicosanoids; leukotrienes; thromboxane; p-coumaric acid; cinnamic acid; caffeic acid; gentisic acid, ferulic acid; vanillic acid; resveratrol and polydatin; catechin, epicatechin; quercetin

INTRODUCTION: WINE IN REVIEW

Through the 1990s, a remarkably consistent body of epidemiologic data has accumulated pointing to the reduced incidence of mortality and morbidity from coronary heart disease (CHD) among those who consume alcohol in moderation by comparison with abstainers (1–3). This reduction has been demonstrated for every end-point (death, myocardial infarction, or hospitalisation for CHD), in every one of the diverse populations studied, in both sexes, and at all ages. This protection seems to be due in large measure, if not exclusively, to the ethanol present in those beverages classified as "alcoholic," but there is some evidence that wine confers additional benefits, due to its content of polyphenols, which at least in vitro and in cell culture experiments, act as potent inhibitors of platelet aggregation, eicosanoid synthesis, and biological oxidation reactions associated with the generation of free radicals. Firm evidence sufficient to establish this hypothesis beyond reasonable doubt has been hard to obtain from

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population studies, but there is no disputing the reality that the advocacy of fruit and vegetables as essential components of a healthy diet is based upon their intrinsic content of these same polyphenols, which are deemed to protect against cancer and other chronic and inflammatory diseases as well as CHD (4–7). A major advantage of wine as a matrix for dietary polyphenols is that they are soluble and bioavailable in wine, in contrast to vegetable foodstuffs, which contain their phenolic components in polymeric, insoluble or tightly bound, and compartmentalised forms that render them unavailable for absorption. Indeed, very little is known about the extent or amount of uptake of these dietary constituents in the human alimentary system.

This review is the first time an attempt has been made to present a complete picture of wine—its history, means of production, chemistry, and biological activity, as well as that of its principal phenolic constituents—for a readership of medical scientists. Although the emphasis is placed on the latter two topics, the first two are briefly described, without extensive references, to provide a background for those not versed in enology and viticulture and without which an appreciation and understanding of wine as a biological fluid will be lacking.

HISTORY

Biblical Times

Alcohol had its initiation under the most auspicious circumstances. Life on earth had just been eliminated by the Great Flood so graphically described in Genesis, Chapter VII, all life except Noah and his binary companions, safe in the Ark constructed according to divine prescription. Following his reprieve, when the flood waters receded and the Ark came safely to rest on dry land, human agriculture commenced with Noah's establishment of a vineyard, according to Genesis, Chapter IX, Verse 20). This was the first documented planting of any crop by human hand on earth. Unfortunately, Noah did not have the advantage of advice from the NIAAA or the Bureau of Alcohol, Drugs, and Firearms, because his inebriation is graphically described in the very next verse. But neither this episode nor any of his subsequent drinking adversely affected his longevity, because in Verse 28 of the same chapter, we are informed: And Noah lived after the flood three hundred and fifty years. Not a bad lifespan for a drunkard who narrowly missed death by drowning and was all of 600 years old when the rains started falling.

Many subsequent passages refer to wine in contexts of varying moral propriety. It was employed by Lot's daughters to intoxicate their father prior to his incestuous seduction during the destruction of Sodom (Genesis Chapter XIX, Verse 33). Jacob, impersonating his older brother Esau in order to secure the "Blessing of the First-Born" from his blind father, offered Isaac wine along with venison prior to the performance of the act (Genesis Chapter XXVII, Verse 25). As for the blessing itself two verses later, it was loaded with metaphysical symbolism and contained the promise of material advantage in only one brief passage: So God give thee. . . plenty of corn and wine. Apparently, no greater possessions had any man in those venerable times.

A millennium or so later, Solomon, the wisest of kings, commenced that greatest of love poems, the Song of Songs, with the opening refrain: Let him kiss me with the kisses of his lips, for thy love is sweeter than wine: proof enough that wine is mankind's second-greatest pleasure. Finally, the miracle wrought by Christ during the Wedding Feast at Cana in converting water to wine is all that is needed to sanction its consumption by devout Christians. Since the dawn of civilisation, and subsequently, wine seems to have been an important and integral component of the human diet, although brief historical outbursts such as the rise of the Puritans in sixteenth-century England and Prohibition in the twentieth-century United States disturbed this happy equilibrium.

Post-Biblical Times

Wine has an unequivocally recorded history stretching back nearly 6,000 years, with the earliest evidence dating between 5400 and 5000 BC. A pottery jar recovered from a square mudbrick building at the site of Hajji Firutz, Tepe, Iran, provides the earliest chemical evidence so far discovered (8). Wine was identified by the presence of the calcium salt of tartaric acid, which occurs in large amounts only in grapes, and in the resin from the terebinth tree, which was widely used in ancient times as an additive to wine to inhibit the growth of bacteria. Whereas wild grape pips have been found to originate as far back as the eighth millennium, this archeological discovery marks the earliest scientific record of fermented wine as part of human culture.

Some investigators place the discovery of winemaking, or at least its development, in the southern Caucasus. It is also thought that the domestication of the wine grape (*Vitis vinifera*) initially occurred within this area. It is there that the natural distribution of *Vitis vinifera* most closely approaches that of Western viticulture, suggesting spread from the former to the latter (9).

From its postulated origins in the Caucasus, grape growing and winemaking probably traveled southward to Palestine, Syria, Egypt, and Mesopotamia. From this base, wine consumption, and its socioreligious connections, distributed winemaking around the Mediterranean. Wine was used for sacramental purposes in Egypt no later than the start of the third millennium BC, although evidence indicates that it was not produced there for general consumption for another 2,000 years.

Wines began to take on their modern expressions about the seventeenth century. The widespread use of sulfur in barrel treatment seems to have occurred about this time. This would

have greatly increased the likelihood of producing better quality wines and extending their lives.

North Americans are relatively latecomers to viticulture. Franciscan missionaries planted the first large-scale vineyards in California only 200 years ago, and these had to be reestablished after the repeal of Prohibition.

A by-product of the Californian vineyards in the nineteenth century threatened the extinction of European vines. The plant louse *Phylloxera vitifoliae,* carried to Europe on California rootstocks, caused a pandemic devastating some 1 million hectares (2.5 million acres) of vineyards in France alone. Ultimately, the tide was turned when Europe's vineyards were replanted entirely with Phylloxera-resistant rootstocks (*Vitis labrusca*) native to the eastern United States, onto which vines of the European wine grapes, *Vitis vinifera,* were grafted.

GRAPE SPECIES AND VARIETIES

Grape vines are placed in a single genus, *Vitis,* within the family *Vitaceae,* which contains some 12–14 genera, possessing about 1,000 species. The genus *Vitis* has a primarily temperate zone distribution, occurring more extensively in the Northern Hemisphere, but also in Australia, New Zealand, South America, and South Africa.

Species in the section *Muscadinia* are restricted to the southeastern United States and northeastern Mexico. Information on the origin of the vast majority of cultivars (unique grape species, also known as "varietals") is scarce or nonexistent. Occasionally, name derivation has been used to suggest local origin, e.g., *Semillon* from semis (Fr.), meaning "seed," and *Sauvignon* from sauvage (Fr.), meaning "wild." Substantial evidence comes from morphological (ampelographic) comparison of cultivars, although this is useful only when they have diverged from a common ancestor such as the colour mutants of *Pinot Noir, Pinot Meunier, Pinot Gris,* and *Pinot Blanc* (10). More recently, genetically based chemical indicators have been used to investigate varietal origin and classification. These include analyses of isomeric forms of enzymes as well as the distribution of various anthocyanins, flavonoid and nonflavonoid phenolic compounds. Techniques of DNA cloning are also being used to establish degrees of similarities between cultivars (11,12).

Some grape vine hybridizations were implemented in North America between native species of *Vitis* or between these and imported *Vitis vinifera* cultivars. The parentage of the best-known American cultivars is speculative. Whereas *Catawba* and *Isabella* may be selections from wild *Vitis labrusca* vines, *Concord* is thought to be the result of crossings between *Vitis labrusca* and *Vitis vinifera*. Another product of a possible chance crossing is *Delaware* among *Vitis vinifera, Vitis labrusca,* and *Vitis aestivalis.*

Cultivars derived from crosses originally made in France between *Vitis vinifera* and *Vitis rupestris, Vitis riparia,* or *Vitis lincecumii* are termed French hybrids or French-American hybrids. This designation comes from their ability to grow ungrafted in phylloxera-infested soils. They are of complex parentage, based on subsequent back-crossings to one or more *Vitis vinifera* cultivars and tend to possess *vinifera*-like winemaking qualities.

Although largely rejected in France and the rest of Europe, French hybrids are broadly accepted in much of North America, Brazil, and Japan. In addition to growing well in many areas of the United States and Canada, French hybrids have helped in the production of regionally and varietally distinctive wines. There are approximately 15,000 grapevine cultivars, but no cultivar classification system exists. Attempts have been made in France to rationalize local cultivars into related groups based on ampelographic and physiological properties, as well as assessing relatedness by utilising comparisons of aroma profiles (13).

CHEMICAL COMPOSITION OF GRAPES AND WINE

Our knowledge of the chemical composition of grape and wine has advanced greatly in the last 30–40 years. Although significant progress has been made, much still remains a mystery.

The number of compounds indentified in wine increased dramatically since the development of gas chromatography (GC), high pressure liquid chromatography (HPLC), a thin-layer chromatography (TLC), droplet counter-current chromatography (DCCC), infrared spectroscopy (IRS), and nuclear magnetic resonance (NMR) (14). The interface of mass spectrometry (MS) to GC and to HPLC has been especially valuable in identifying unknown compounds.

More than 500 compounds have been recognized in wine thus far, of which 160 are esters. The concentrations of the majority range between 10^{-1} and 10^{-6} mg/L. At these levels the individual compounds play very little or no role in the human organoleptic (taste) perception, but collectively they may be very significant (15).

The number of aromatic and sapid (taste and mouth-feel) substances derived from grapes are relatively few compared to the vast majority that are the metabolic by-products of yeast activity during fermentation. Wines generally contain 0.8–1.2 g of aromatic compounds per litre, of which the most common are fusel alcohols, volatile acids, and fatty acid esters. Fusel alcohols often constitute 50% of all volatile substances in individual wines. Carbonyls, phenols, lactones, terpenes, acetals, hydrocarbons, sulfur, and nitrogen compounds, although present in much lower concentrations, are more important qualitatively and contribute specific sensory characteristics relevant to the fragrance of a wine.

The taste and mouth-feel sensations are due primarily to the few compounds that occur individually at concentrations > 100 mg/L. These include water, ethanol, organic acids, sugars, and glycerol. Tannins occur in red wine and rarely in

significant amounts in white wines unless maturation in oak cooperage took place. Unlike the taste and mouth-feel sensations, odour has a much lower olfactory threshold. It is measured as olfactory potential, which represents the number of molecule-grams per litre of air at threshold concentration.

Water

Water is the predominant chemical constituent of grapes and wine and is critical in establishing its fundamental characteristics. It is an essential component in many of the chemical reactions involved in grape growth and juice fermentation and in wine aging. Compounds insoluble or only slightly soluble in water rarely play a significant role in wine.

Sugars

The principal grape sugars are glucose and fructose, and they occur in roughly equal proportions at maturity, whereas overmature grapes often have a higher proportion of fructose. Sucrose is rarely found in *Vitis vinifera* grapes, and other sugars are found in insignificant amounts. *Non-Vitis vinifera* cultivars may contain up to 10% sucrose. Whether added or naturally occurring, sucrose is enzymatically split into glucose and fructose during fermentation. Cultivars of *Vitis vinifera* generally reach a sugar concentration of 22–24 ^{0}Brix (1 ^{0}Brix=1% w/v) or more. This is dependent on cultivar, maturity, and health of berries. At levels above 18 ^{0}Brix sugars become the predominant soluble solids in grapes (16).

The primary wine yeast, *Saccharomyces cerevisiae*, derives most of its metabolic energy from glucose and fructose and has limited ability to ferment other substances. Residual sugars in dry wines, generally below 1.5 g/L, consist mostly of pentoses such as arabinose, rhamnose, and xylose. Their levels may increase slightly during maturation in oak cooperage via the breakdown of glucosides in the wood, as well as from their synthesis and release by yeast cells.

Generally, sweetness is detected at levels higher than 1 ^{0}Brix, and this is influenced by other constituents such as ethanol, acids, and tannins. In addition to being absolutely essential for fermentation and production of ethanol, sugars are metabolized to higher alcohols, fatty acid esters, and aldehydes, which give different wines their individual aromatic character. High sugar concentrations also can increase the volatility of aromatic compounds (17).

Polysaccharides

In finished wines, polysaccharides are generally low. They are partially water soluble and are extracted into the juice during crushing and pressing. Hot pressing of crushed or whole berries enhances skin extraction, but during fermentation polysaccharides form complex colloids in the presence of alcohol and tend to precipitate. The addition of pectolytic enzymes following crushing significantly reduces the pectin content and their precipitation during pressing. In contrast, β-glucans produced in Botrytis-infected grapes can hinder juice and wine clarification by inhibiting the precipitation of other colloidal materials, such as tannins and proteins (18).

Ethanol

The most important and abundant alcohol in wine is ethanol. Under standard fermentation conditions, ethanol can accumulate to ~14–15%, but generally ethanol concentrations in wine range between 10–13%. The primary factors controlling ethanol production are sugars, temperature, and yeast strain.

Ethanol is crucial to the stability, aging, and sensory properties of wine. As its production increases during fermentation, it increasingly limits the growth of most microorganisms, allowing *Saccharyomyces cerevisiae* to dominate the fermentation process. The inhibitory activity of ethanol, combined with the acidity of the wine and the added potassium metabisulfite, allows wine to remain sound for years in the absence of air. During skin fermentation of red grapes, ethanol acts as an important solvent in the extraction of pigments and tannins. It also influences the types and amounts of aromatic compounds produced by affecting the metabolic activity of yeasts.

Furthermore, ethanol acts as an essential reactant in the formation of volatile compounds produced during fermentation and those formed during aging in wood cooperage. The dissolving action of ethanol probably reduces the evaporation of aromatic compounds along with carbon dioxide during fermentation (19). Ethanol plays several roles in the aging of wine. Together with other alcohols, it slowly reacts with organic acids to produce esters and influences their stability. Moreover, it also reacts slowly with aldehydes to produce acetals.

Methanol is a minor constituent of wine (0.1–0.2g/L) and has no direct sensory effect. It is predominantly generated from the enzymatic breakdown of pectins. On degradation, methyl groups associated with pectins are released as methanol. Pectolytic enzymes added to juice or wine to aid clarification inadvertently increase the methanol content of wine. Oxidation of methanol in the body produces formaldehyde and formic acid, which are toxic to the central nervous system. Wine has the lowest concentration of methanol of all fermented beverages (20,21).

Other potentially significant higher alcohols in wine are the straight-chain alcohols: 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, and 3-methyl-1-butanol (22). The formation of higher alcohols occurs as a by-product of yeast fermentation and is markedly influenced by vinification practices such as temperature, presence of oxygen, suspended solids, and yeast strain (23). Higher alcohols may originate from amino acid deamination and grape-derived aldehydes, and by the reductive denitrification of amino acids (24).

Acids

In wine, acids are divided into two categories: volatile and fixed. The first refers to acids that can be readily removed by distillation, whereas the latter refers to the carboxylic acids. The most common volatile acid in wine is ascetic acid. Quantitatively, carboxylic acids such as tartaric, malic, lactic, succinic, oxalic, fumaric, and citric acids control the pH of wine.

Phenols

Phenols are a large and complex group of compounds of particular importance to the characteristics and quality of red wines. Their concentration in white wine is much lower (Table 1). Phenols and related compounds can affect the appearance, taste, mouth-feel, fragrance, and antimicrobial properties of wine. They may come from the fruit (skins and seeds) and vine stems, production by yeast metabolism, or extraction from wood cooperage.

Chemically, phenols are cyclic benzene compounds possessing one or more hydroxyl groups associated directly with the ring structure (Table 2). The two primary phenol groups that occur in grapes and wine are the flavonoids and the nonflavonoids (Table 3). Flavonoids are characterized as molecules possessing two phenols joined by a pyran (oxygen-containing) carbon ring structure. The most common flavonoids in wine are flavonols, catechins (flavan-3-ols), and, in red wines, anthocyanins. Small amounts of free leucoanthocyanins (flavan-3,4-diols) also occur. Flavonoids exist free and polymerized to other flavonoids, sugars, nonflavonoids, or a combination of these compounds. Those esterified to sugars or nonflavonoids are called, respectively, glycosides and acyl derivatives.

TABLE 1. Estimates of Typical Gross Composition (% weight) of Wines[a]

	Table wines		Dessert wines	
Component	White	Red	White	Red
Water (by difference)	87	87	76	74
Ethanol	10	10	14	14
Other volatiiles	0.04	0.04	0.05	0.05
Extract	2.6	2.7	10.1	12.2
Sugars	0.05	0.05	8	10
Pectins	0.3	0.3	0.25	0.25
Glycerol	1.1	1.1	0.9	0.9
Acids	0.7	0.6	0.5	0.05
Ash	0.2	0.2	0.2	0.2
Phenols[b]	0.01	0.2	0.01	0.1
Amino acids	0.25	0.25	0.2	0.2
Fats, terpenoids	0.01	0.02	0.01	0.02
Vitamins, etc.	0.01	0.01	0.01	0.01
Total:	100	100	100	100

[a]From Singleton (93).

[b]Note that apart from sugar content, this is the only major compositional difference between red and white wines of both major categories (Table and Dessert).

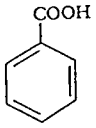
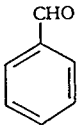
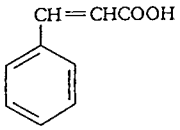
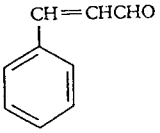
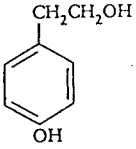
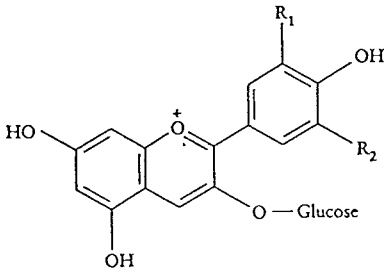
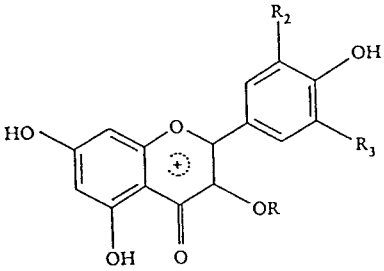
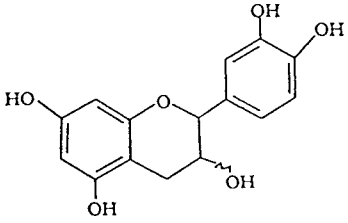
Polymerization of polyhydroxy flavan-3-ol units, (+)-catechin and (-)-epicatechin, and their gallate esters produces oligomers and polymers called proanthocyanidins (often referred to as procyanidins). They are classified based on the nature of the flavonoid monomers, their bonding, esterification to other compounds, or functional properties. The most common structural class in grapes and wine (type B) contains direct carbon-to-carbon linkage between the adjacent procyanidin subunits (Fig. 1). The simplest procyanidins are dimers, and the most common of these in grapes are the 4 → 8 linked compounds (B_1 - B_4) (25). Even though procyanidins occur primarily as dimers in grapes (26), they tend to polymerize and predominate in wine as condensed tannins, mid-size procyanidin polymers containing three to five subunits. In some cultivars, structural differences exist among skin, stem, and seed procyanidins. Also, there are considerable differences in types and concentrations between cultivars (27). The unconjugated hydroxy-phenolic groups on procyanidins give them their distinctive protein-binding property.

Flavonoids are primarily from the skins, seeds, and stems of the fruit. Flavonols and anthocyanins come predominantly from the skins, whereas catechins and leukoanthocyanins originate mainly from the seeds and stems. In red wines, they commonly constitute > 85% of the phenol content (≥ 1,000 mg/L). In white wines, flavonoids typically comprise < 20% of the total phenolic content (≤ 50 mg/L).

The amount of flavonoids extracted during vinification is influenced by many factors, including temperature, length of skin contact, mixing, type of fermentation vessel, ethanol concentrations, SO_2, yeast strain, pH, and pectolytic enzymes. Extraction is ultimately limited by the amount present in the fruit, and this varies with cultivar, vintage, climatic conditions of the region, and vinification process. Carbonic maceration and thermovinification tend to extract less phenolic compounds as compared to traditional skin fermentation.

The primary nonflavonoids, in wines not aged in oak, are derivatives of hydroxycinnamic and hydroxybenzoic acids. They are structurally simpler and are stored primarily in cell vacuoles of grape cells and are easily extracted on crushing. Hydroxycinnamic acid derivatives commonly occur esterified to sugars, organic acids, or various alcohols. Wines aged in oak have higher levels of hydroxybenzoic acid derivatives, especially ellagic acid, which comes from the breakdown of hydrolyzable tannins. These are polymers of ellagic acid, or gallic and ellagic acids, with glucose. Degradation of lignins in wood also liberates various cinnamaldehyde and benzaldehyde derivatives. Wines aged other than in oak wood will differ in their hydrolyzable tannins and nonflavonoids. Under acidic conditions, the nonflavonoid-based, hydrolyzable tannins separate readily into their components. The low pH of wine is responsible for weakening the bonding between the hydrogen and oxygen atoms of associated phenols. In contrast, flavonoid-based condensed tannins are relatively stable under these conditions and are held together by strong covalent bonds.

TABLE 2. Polyphenols in Grapes and Wine[1]

General type	General structure	Examples	Major source[b]
Nonflavonoids			
Benzoic acid		Benzoic acid	G, O
		Vanillic acid	O
		Gallic acid	G, O
		Protocatechuic acid	G, O
		Hydrolyzable tannins	G
Benzaldehyde		Benzaldehyde	G, O, Y
		Vanillin	O
		Syringaldehyde	O
Cinnamic acid		*P*-Coumaric acid	G, O
		Ferulic acid	G, O
		Chlorogenic acid	G
		Caffeic acid	G
Cinnamaldehyde		Coniferaldehyde	O
		Sinapaldehyde	O
Tyrosol		Tyrosol	Y
Flavonoids			
Flavonols		Quercetin	G
		Kaempferol	G
		Myricetin	G
Anthocyanins		Cyanin	G
		Delphinin	G
		Petunin	G
		Peonin	G
		Malvin	G
Flavan-3-ols		Catechin	G
		Epicatechin	G
		Gallocatechin	G
		Procyanidins	G
		Condensed Tannins	G

[a]From Jackson (24a).
[b]G, grape; O, oak; Y, yeast.

TABLE 3. Gross Phenol Composition Estimated in mg GAE[a]/L for Typical Table Wines From *Vitis vinifera* Grapes

Phenols class[b]	Source[c]	White wine		Red wine	
		Young	Aged	Young	Aged
Nonflavonoids, total		175	160–260	235	240–500
Cinnamates, deriv	G,D	154	130	165	150
Low volatility benzene deriv	D,M,G,E	10	15	50	60
Tyrosol	M	19	10	15	15
Volatile phenols	M,D,E	1	5	5	15
Hydrolyzable tannins, etc.	E	0	0–100	0	0–260
Macromolecular complexes					
Protein-tannin	G,D,E	10	5	5	10
Flavonoids, total		30	25	1060	705
Catechins	G	25	15	200	150
Flavonols	G,D	tr	tr	50	10
Anthocyanins	G	0	0	200	20
Soluble tannins, deriv	G,D	5	10	550	450
Other flavonoids, deriv	G,D,E,M	?	?	60?	75?
Total phenols		215	190–290	1300	955–1215

[a]GAE = Gallic acid equivalent. From Singleton (42).
[b]Deriv = derivative.
[c]G = grapes; D = degradable product; M = microbes, yeast; E = environment, cooperage.

Nonflavonoids of grape origin are initially synthesized from phenylalanine (28), whereas those of yeast origin are derived from acetic acid (29). Flavonoids are derived from the combination of derivatives synthesized from both phenylalanine (via the shikimic acid pathway) and acetic acid.

The concentration of phenolics in wine increases during skin fermentation and subsequently begins to fall as phenols bond and precipitate with proteins and yeast hulls (cell remnants). During fining and maturation, the phenols continues to decrease, and aging has a further dramatic effect on their reduction.

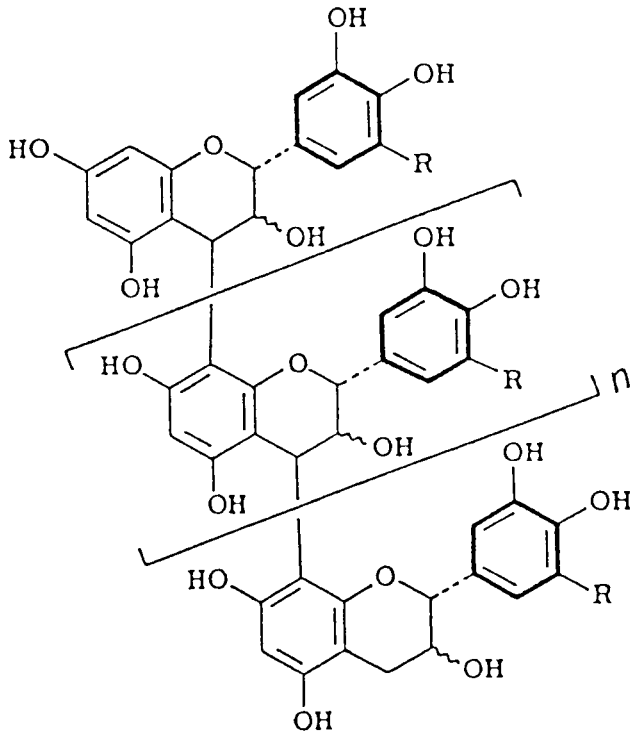


Fig. 1. Structure of B-type procyanidin polymers (R=H,OH), with n=0,1,2,3,4,5.

CLIMATIC INFLUENCES ON BERRY MATURATION

Yield

Overproduction is associated with reduced wine quality. Although specific cause/effect relationships are poorly understood, overproduction delays fruit maturity, retains excessive acidity, retards anthocyanin synthesis and sugar accumulation, and limits flavor development. In France, yield is considered so directly related to wine quality that maximum crop yields have been set for appellation control regions.

Sunlight

Sunlight is the most important climatic factor affecting berry development. Most of the effects of light on berry maturation are either thermal- or phytochrome-induced. Because chlorophyll absorbs light selectively, there is a marked increase in the relative amount of far-red light in vine canopies. Evidence suggests that the proportion of physiologically active phytochrome (P_{fr}) shifts from 60% in sunlight to below 20% in shade (30). This may play a role in delaying the initiation of anthocyanin synthesis and may also decrease sugar accumulation and increase ammonia and nitrate content in the fruit (31). The red/far-red balance (P_r/P_{fr}) of sunlight and occasionally ultraviolet radiation are involved in the control of flavonoid biosynthesis in several plants (32).

Changes in composition of anthocyanins during ripening, due to their different susceptibilities to oxidation, may be sig-

nificant to wine colour stability and total anthocyanin accumulation. Berry pigmentation may influence the P_r/P_{fr} ratio in maturing fruit (33), and this can significantly influence the activity of light-activated enzymes such as phosphoenolpyruvate carboxylase, a critical enzyme in the metabolism of organic acids (34).

Temperature

Termperature influences enzyme function and cell membrane permeability (35). It has a pronounced effect on anthocyanin synthesis and stability in grapes, and this is influenced by warm daytime temperatures and cool nights (20–25°C and 10–15°C, respectively). Temperature also may influence the compositional change in grape anthocyanins during maturation (36). Warm conditions increase sugar accumulation and generally increase the aminoacid content of developing berries as well as potassium accumulation (37).

Nutrients

Soil nitrogen content is important in anthocyanin and amino acid synthesis. High soil nitrogen content suppresses production of anthocyanin but increases amino acids and especially arginine. High potassium levels can reduce berry pH and thereby lower fruit colour and colour stability in red wines (38).

Water

"Excess" irrigation can double fruit yield, increase fruit size, and delay maturity, all potential negatives to quality. A mild water stress following "veraison" (onset of ripening) is beneficial to grape quality. Overirrigation will result in lower sugar concentrations and increased grape acidity. However, this will rarely decrease pH substantially, probably due to the dilution associated with fruit enlargement (39).

GRAPE PROCESSING AND VINIFICATION

Processing and vinification are the series of unit operations leading from the crushing of grapes to the bottling of wines. The common sequence used in the production of white and red wines is shown in Figures 2 and 3.

De-stemming

Immediately after harvest, extraneous material such as stems, leaves, and grape stalks are removed. This minimizes the excessive uptake of phenols and lipids from vine components. Extraction of stem phenols is valuable only when dealing with red grape varieties low in phenol content. Stem phenols generally produce more astringent and bitter tastes than phenols released by the seeds and skins. Leaf removal before crushing limits the production of aldehydes and alcohols generated during the enzymatic oxidation of linoleic and linolenic acids.

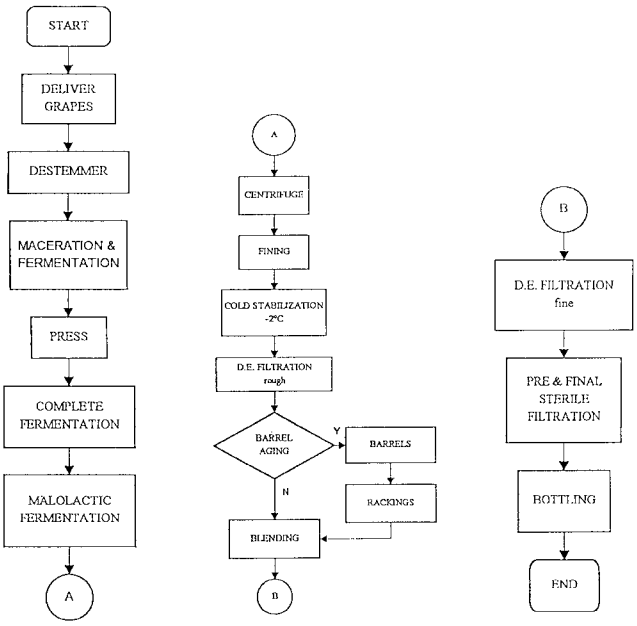


Fig. 2. General structure of unit operations for the production of white wine.

Crushing

The grapes are crushed immediately after de-stemming to avoid microbial contamination and better control of oxidation. The fruit is then pressed against a perforated wall or passed through a set of rollers. The berries are broken, and the juice, pulp seeds, and skins pass through openings and are collected in vats.

Maceration

Following crushing of the grapes, the rupture and release of enzymes from grape cells facilitate the liberation and solubilization of compounds bound in cells of the skin, flesh, and

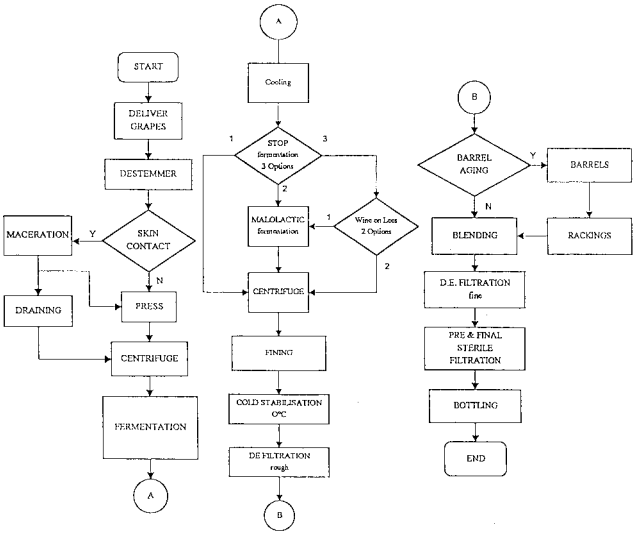


Fig. 3. General structure of unit operations for the production of red wine.

seeds. Maceration is almost always employed in the initial phase of red wine fermentation. Limited maceration is used for white wines, along with slight juice oxidation before fermentation.

During maceration of white grapes, extraction of phenols (flavonoids and nonflavonoids) may be a linear function of the temperature and the length of skin contact (40). Short exposure to high temperature (15 min at 70°C) greatly increases the release of volatile compounds such as monoterpenes (41). Longer warmer maceration generally produces a wine deeper in colour and of fuller flavour that may age more quickly and develop a more complex character (42).

In red wine production, the style of the wine can be altered dramatically by the duration and conditions of maceration. Macerations less than 24 hours commonly produce rose wines. Macerations of 3–5 days generally produce wines for early consumption with good colouration and low tannins. Wines for long aging are usually macerated on the seeds and skins from 7 to as long as 21 days. This may cause a decrease in free anthocyanin content (43), but may enhance colour stability and aging potential.

Pressing

There are three major types of press: horizontal, pneumatic (membrane), and continuous screw press. Winemakers can influence the character of wine by the choice of press. The degree of fining and blending of the various press fractions allows winemakers to adjust the final character of the wine.

Thermovinification

Thermovinification is one technique of improving the colour of red wine. It involves the heating of intact or crushed grapes between 50°C and 80°C. The temperature varies depending on the varietal. For example, with delicate varietals such as *Pinot Noir*, heating may be as low as 32°C for 12 hours (44). Thermovinification is used primarily to produce wines designed for early consumption.

Filtration and Centrifugal Clarification

The clarification of white grape juices prior to fermentation and the removal of microscopic suspended matter of various kinds from wines generally can be achieved by one or more of the following methods: (1) natural settling and racking, (2) filtration (earth or pad), and (3) centrifugal separation (desludging or decanting).

Fining

This is defined as the addition of reactive and/or adsorptive substances to remove or reduce the concentration of undesirable constituents. Fining agents can be classified into the following groups: (1) proteins, e.g., gelatine, casein, isinglass and albumin, (2) clays, e.g., bentonite, kaolin, (3) synthetic polymers, e.g., polyvinylpolypyrolidone (PVPP), nylon, (4) polysaccharides, e.g., agar, gum arabic, (5) carbons, e.g., activated charcoal, (6) silica gels, and (7) other agents, e.g., cufex, metafine.

Membrane Filtration and Bottling

Membrane filtration involves the final filtration and adjustment of dissolved oxygen and carbon dioxide levels. Prefinal filtration is made by using synthetic polymer membrane filters such as cellulose acetate, cellular nitrate, and polysulfone. They have closely controlled pore sizes and can be set up so that the flow is either perpendicular to and through the membrane or parallel to the surface, with a much smaller transmembrane flow as in the "cross-flow" microfilters, ultrafilters, and reverse osmosis membranes. These membranes rely on their pore size to exclude larger particles (microorgansims) or solutes (proteins, gums, or tannins). Generally, wine is prefiltered by filter pads and/or filter cakes before it comes into contact with a membrane.

WINE AND DISEASE PREVENTION

Epidemiological evidence from many studies, involving hundreds of thousands of human subjects of both sexes, overwhelmingly supports the notion that light-to-moderate alcohol consumption is associated with a reduction in overall mortality, due primarily to a reduced risk of CHD (1–3, 45–49).

Several reasons have been proposed for this cardioprotective effect of alcohol. Approximately half of the reduction in CHD mortality may be attributed to its ability to increase the concentration of high-density lipoprotein cholesterol (HDLC), a well-known negative risk factor for CHD (50,51). Another mechanism that may play a major beneficial role is the ability of alcohol to inhibit or reduce platelet aggregation and blood clotting (52–54). Investigators have demonstrated, both in vitro and in vivo, a reduced coagulability of platelets; reduced concentration of clotting factors; and altered eicosanoid metabolism leading to a reduction in thromboxane synthesis coupled with increased synthesis of vasodilatory prostacyclin (55–61). Other mechanisms may reduce the likelihood of atherosclerosis, such as inhibition of low density lipoprotein (LDL) oxidation, free radical scavenging, and modulation of eicosanoid metabolism. Ethanol itself, the second largest constituent in wine, does not function through these postulated mechanisms. However, wines include polyphenols, especially flavonoids, that can exercise all of these effects (62–67).

In 1979, St. Leger et al. (68) described a strong negative association between CHD mortality and wine consumption in 18 Western countries. Other reports based on analysis of global data have confirmed this inverse correlation between the two (69–71). Supporting evidence came from the observation that the incidence of CHD mortality in France is the

lowest among industrial countries, despite the high incidence of risk factors such as smoking, high fat diet, and lack of exercise generally attributed to its citizens. This phenomenon was believed to be due to the consumption of wine together with the Mediterranean-type diet rich in vegetables and was light-heartedly christened the "French Paradox" (72). Some authors who studied this relationship in a specific population indicated that wine may be more potent than other alcoholic beverages in reducing the incidence of CHD (73–75), although other studies were unable to show a differential effect (76–78).

The intrinsic antibacterial properties of wine have recently been demonstrated by Weisse et al. (79). The ancient practice of including antibacterial elements such as terebinth resin in wine serves as a precursor to current research showing wine to be a useful antibacterial agent (79,80). Until early in this century, wine was universally used as a base for medicinal preparations compounded with various herbs tailored to specific ailments.

Animal Studies

One of the most dramatic demonstrations of the superiority of red wine over other alcoholic beverages in preventing atherosclerosis was provided by Klurfeld and Kritchevsky (81). Rabbits were fed a high cholesterol diet along with water (control), or one of five different beverages containing equal amounts of ethanol. After 3 months, all of the control and beer-drinking rabbits had developed atherosclerotic lesions in the coronary arteries. All alcoholic beverages, except beer, reduced the incidence of atherosclerotic lesions, but red wine showed the most dramatic effect, lowering the incidence to 40% that of the controls.

Yugurani et al. (82) and Tebib et al. (83) demonstrated significant reductions of total and LDL-cholesterol in rats fed a high-cholesterol diet, by simultaneously administering polyphenols present in red wine and polymeric tannins extracted from grape seeds. Ruf et al. (84,85) showed that grape seed tannins also protected rats against rebound platelet aggregation subsequent to acute major ("binge) alcohol ingestion.

Red wine and grape juice, but not white wine, injected in anesthetized dogs, abolished the cyclic flow reductions caused by periodic acute-platelet-mediated thrombus formation. However, it took three times as much grape juice by volume to achieve the same preventative effects (86).

Human Studies

When 16 healthy subjects were given alcohol, white wine, and red wine for a period of 15 days, spirit alcohol-enhanced ADP and adrenaline-induced platelet aggregation increased serum apo-AI and decreased LDL-cholesterol. White wine increased total LDL and HDL-cholesterol, and red wine led to a fall in ADP-induced aggregation and increased HDL-cholesterol (87).

Lavy et al. (88) in a study following 20 healthy males for 2 weeks, 10 of whom consumed 400 mL of white and the rest 400 mL of red wine per day, concluded that red wine intake significantly increased plasma HDLC and plasma apo A-I up to 26% and 12%, respectively. These effects were not observed after the intake of white wine. Struck et al. (89) compared the effects of red versus white wine upon serum lipids, platelet aggregation, and oxidation products in 20 adult hypercholesterolemic subjects over a 3 month period. Both wines significantly reduced thrombin-initiated platelet aggregation, thiobarbituric acid reactive substances (TBARS), and LDL-cholesterol.

In a recent clinical investigation, Furhman et al. (90) studied 17 healthy men who were divided into two groups: 8 received 400 mL red wine/day for 2 weeks, and 9 received the same amount of white wine. Red wine consumption for 2 weeks resulted in a 20% reduction in the propensity of plasma to undergo lipid peroxidation in the presence of a free-radical-generating system as determined by TBARS assay. In parallel, red wine consumption reduced LDL lipid peroxidation in response to copper ions by 46%, 72%, and 54% as determined by decreases in the content of TBARS, lipid peroxides, and conjugated dienes, respectively, as well as by substantial prolongation of the lag phase required for the initiation of LDL oxidation. The concentration of phenolic compounds in the LDL of the red wine drinkers increased by 4.3 fold from basal levels. In contrast, dietary consumption of white wine for 2 weeks resulted in an increase in the propensity of plasma and LDL to undergo lipid peroxidation.

Goldberg and colleagues (91,92) studied 24 healthy males, aged 26–45 years, who consumed 375 mL of red wine or white wine, each for a period of 4 weeks. After a series of tests for lipids, lipoproteins, platelet aggregation, and eicosanoid production, they could find no difference between the two, although both were associated with significant lowering of several major risk factors for CHD. The only consistent difference between red and white wine is that red wine contains much higher concentrations of phenolic compounds, typically 20-fold the concentration found in the average white wine. Among these phenols, the major difference is in the flavonoids, stilbenes, hydroxycinammic, and benzoic acids (93).

POLYPHENOLIC CONSTITUENTS OF WINE

Chemistry and Biosynthesis

Flavonoids are phenolic compounds and act as potent antioxidants and metal chelators. This latter activity is reinforced in the flavonoids, which have both a 4-carbonyl and 1,5- (or 3-) hydroxyl group. They have the ability to sequester and thus reduce the activity of oxidant-inducing metals such as iron and copper. Due to their conjugated aromatic structure, they can act both as potent screens against destructive UV

light and as attenuaters of physiologically active visible light (especially in the 350–450 nm region). Some flavonoids, besides their general properties, act as visible colour signals for important interactions (pollen and fruit dispersal) between higher plants and animals (94). Flavonoids interfere with viral, bacterial, fungal, and animal feeding, reproduction, growth, and development (95).

Both physical and chemical properties of individual compounds strongly affect their antioxidant potency (96). Polyphenols bind with proteins by hydrophobic interactions, hydrogen bonds, and covalent bonds (97). Hydrogen bonding has been proposed to arise between the amide protein linkages and the aromatic phenolic hydroxyls on the polyphenolic compounds. Hydrophobic bonding can also occur between the aromatic rings on the polyphenyls and aromatic amino acids. Changes in pH will affect the hydrogen bonding interactions, but hydrophobic interactions will be largely affected by solvent polarity (98).

The first step in the overall biosynthesis of the flavonoids and related compounds involves the formation of phenylalanine from phenyl pyruvate (via the shikimate pathway) mediated by L-phenylalanine-2-ketoglutarate amino transferase (PKA) in the presence of L-glutanate. The latter acts as an amino donor and is converted to 2-ketoglutarate. Phenylalanine is then transformed by phenylalanine ammonia lyase (PAL) to *trans*-cinnamic acid, and this is accompanied by the release of ammonium ions (Fig. 4) (99).

Cinnamic acid is in turn hydrolyzed by cinnamate-4-hydroxylase (C-4-H) to *p*-coumaric acid. In the final step, p-coumaryl CoA is generated from the free co-enzyme by a specific CoA ligase (Fig. 5) (100). Similarly, the path of cinnamic acid to caffeic acid, ferulic acid, and sinapic acid involves the corresponding CoA esters.

The second essential step in the formation of flavonoids is a modification of the pathway leading to the polyketides via a nonreductive fatty acid-type biosynthesis. As soon as the trimer is formed, there is a tendency for the latter to cyclize to form an aromatic ring. This gives naringenin chalcone as a primary product from *p*-coumaroyl CoA (Fig. 5).

The key to flavonoid formation is the ability of this chalcone to undergo a second cyclization to give the flavanone, naringenin. Bis-noryangonin is an intermediate generated by

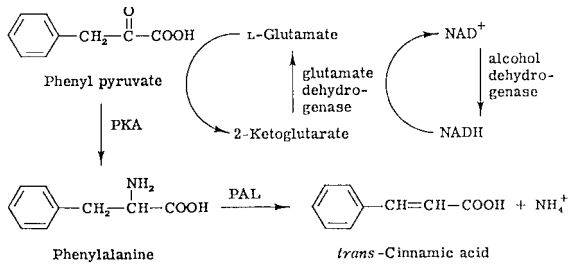


Fig. 4. Biosynthesis of *trans*-cinnamic acid. From Hasegawa et al. (99).

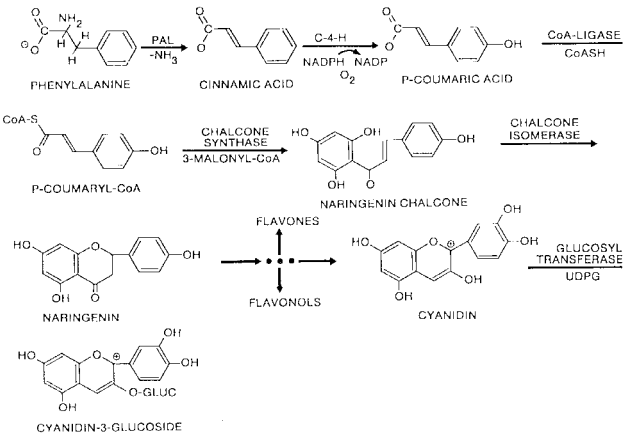


Fig. 5. Biosynthesis of flavonoid compounds. From Hrazdina et al. (100).

the transfer of acetate units from the first two participating malonyl CoA residues, whereas the third acetate transfer reaction completes the formation of naringenin. The tetrahydroxy-chalcone, which serves as the parent compound for flavonoid synthesis, is generated from naringenin through the activity of an independent enzyme, chalcone isomerase (Fig. 6) (101).

Naringenin is the precursor of flavonols, anthocyanidins (commonly referred to as anthocyanins), and the corresponding flavan-3-ols (catechins) and flavan-3, 4-diols, the latter two groups both being precursors of the procyanidins. The

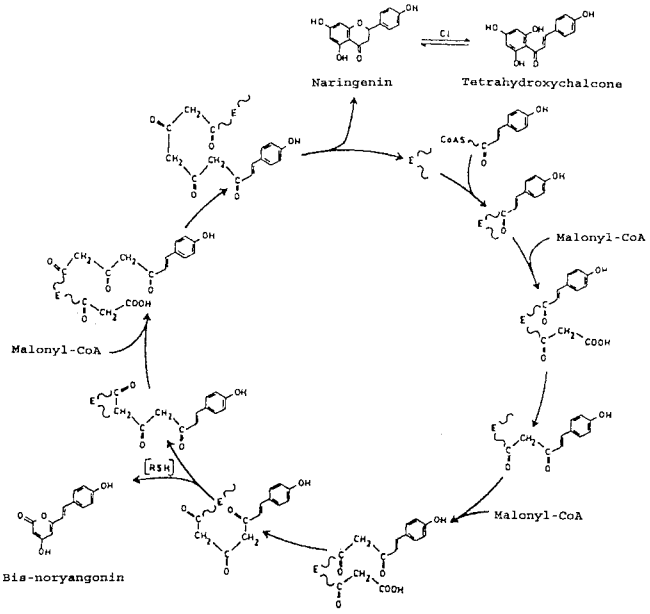


Fig. 6. Scheme for the formation of naringenin and bis-noryangonin from *p*-coumaroyl-CoA and malonyl-CoA. E=Enzyme (Flavone synthase), RSH=mercaptoethanol or dithio-erythritol. The scheme includes the isomerization of naringenin to the corresponding chalcone catalyzed by chalcone somerase (CI). The waved lines represent energy-rich bonds, probably thiol esters. From Kreuzaler et al. (101).

flavonols are much more effective as metal chelators than the corresponding flavones.

Another class of compounds are the stilbenes and stilbene glycosides known to occur naturally in a rather restricted distribution, predominantly among spermatophytes (102). These compounds make up the bulk of the phenolic antifungal phytoalexins, compounds that are usually synthesized only in response to infection or injury. The essential structural skeleton comprises two aromatic rings joined by a methylene bridge. There is an array of compounds varying in the number and position of hydroxyl groups; the extent to which these groups become substituted with sugars, methyl, methoxy, and other residues; the steric configuration of chemically identical molecules; and their ability to enter into reactions forming dimers, trimers, or larger polymers. The most extensively studied of the stilbenes is *trans*-resveratrol (3,5,4´-trihydroxystilbene). The main significance of resveratrol in plant biology lies in its role as the parent molecule of a family of polymers given the name viniferin (Fig. 7). These compounds are able to inhibit the progress of fungal infection. The production of trans-resveratrol in grapes is accomplished by the condensation of *p*-coumaroyl CoA with three molecules of malonyl CoA through the activity of the stilbene synthase, resveratrol synthase (Fig. 8).

Antioxidant Activity of Polyphenols: Structure-Function Relationships

There is a considerable body of literature relating the structure of flavonoids to antioxidant activity. Pratt et al. (103) showed that the dihydroxylation in both rings and in the 3-position of catechin, myricetin, and quercetin are required for antioxidant activity reported in various lipid systems. Hydroxylation of the 3,5,7-positions of the A-ring and 3´,4´-dihydroxylation of the B-ring of flavone were also considered important to antioxidant activity. Hexahydroxy myricetin had greater antioxidant activity in a lard system oxidized at 60°C than the pentahydroxy quercetin, followed by the pentahydroxy catechin (104). In another experiment, 3-glycosylation of the 3-hydroxy flavones did not alter the antioxidant activity of myricetin and quercetin (105). Darmon et al. (106) confirmed a relationship between increased hydroxylation and increased antioxidant activity of phenolic compounds. The presence of hydroxyl groups on quercetin, namely, at the 5- and 7-positions of the A ring, at the 3´- and 4´-positions of the B ring, and at the 3-positions of the C-ring, enhanced its antioxidant activity (107).

In an LDL oxidation system, Teissedre et al. (Fig. 9) showed that catechin, which lacks a keto group in the 4-position, was more inhibitory than the flavonol myricetin (with 3´,4´,5´-trihydroxylation of the B-ring), which was more active than quercetin (with dihydroxylation in the

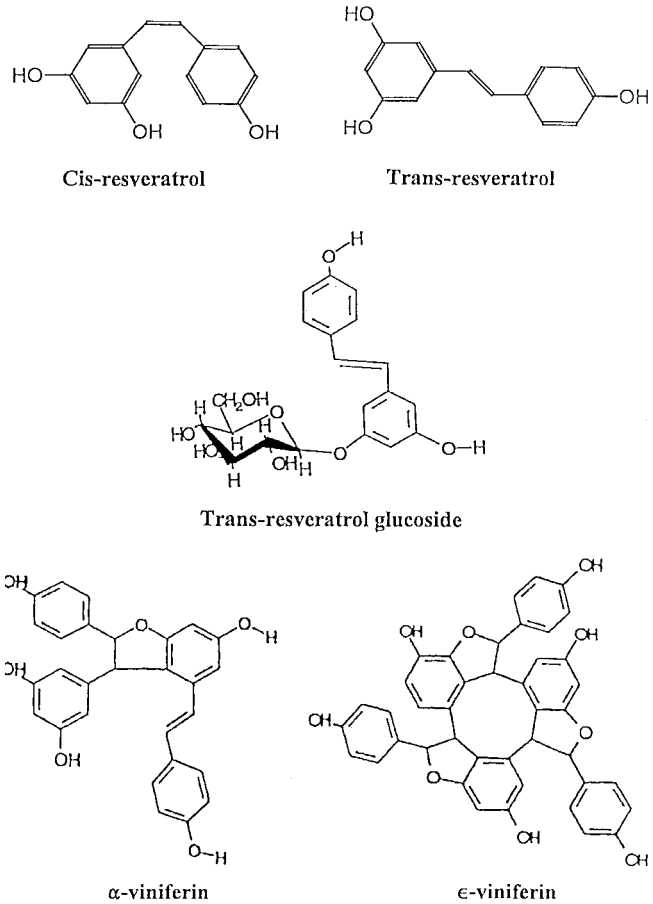


Fig. 7. Chemical structures of resveratrol isomers, *trans*-resveratrol glucoside, α-viniferin and ε-viniferin.

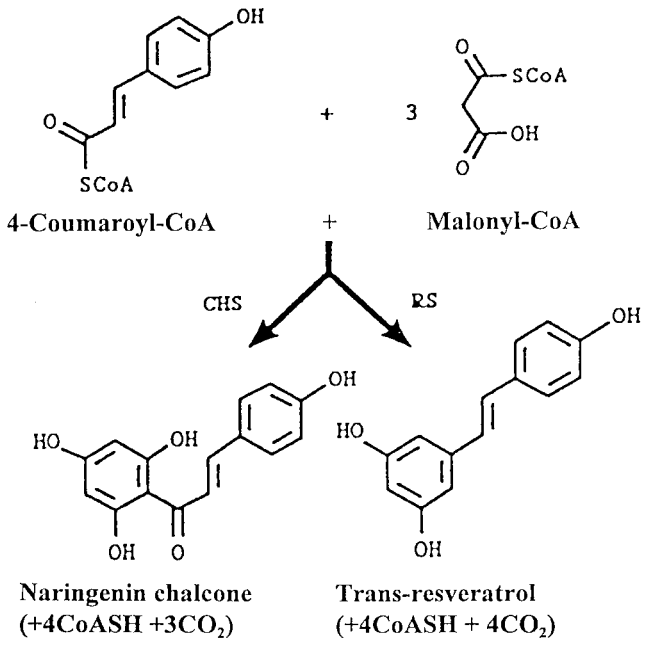


Fig. 8. Biosynthesis of resveratrol; reactions performed by chalcone (CHS) and resveratrol (RS) synthases.

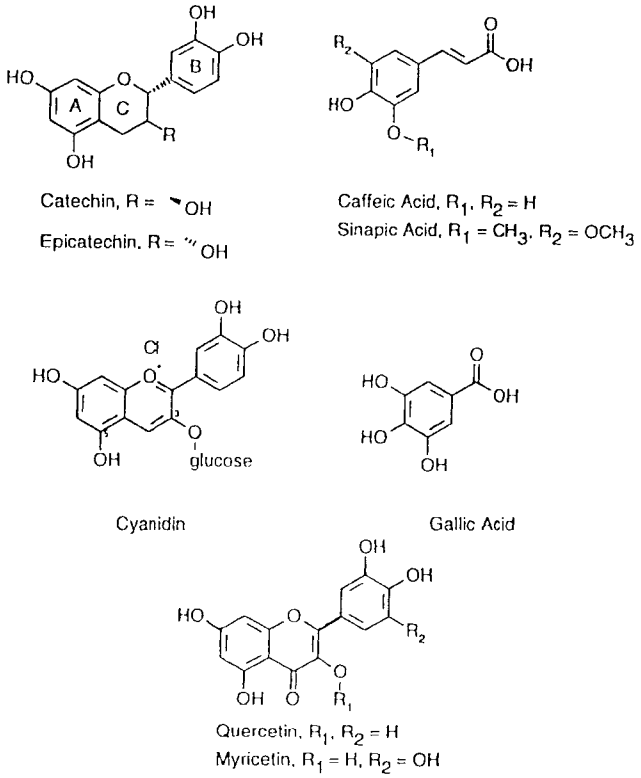


Fig. 9. Monomeric wine phenolics. From Teissedre et al. (108).

B-ring), both having a keto group in the 4-position (108). In the same study, caffeic acid had higher antioxidant activity than synapic acid, confirming that methoxylation of ortho-dihydroxy groups decreases antioxidant activity (Table 4).

BIOLOGICAL ACTIVITY OF POLYPHENOLS

Biological Oxidation

Damage to tissues by free radicals derived from molecular oxygen (including superoxide, hydrogen peroxide, hydroxyl radicals, and lipid peroxides) is a concept that has progressed beyond hypothesis and established fact to become a dogma that dominates much current thinking about what causes diseases and how best to prevent them (109,110). Dietary antioxidants have taken on a sanctity previously reserved for vitamins such as α-tocopherol, which owes its present status as a recommended or even essential food constituent virtually exclusively to its antioxidant potential (111,112). Its possible role in reducing the risk of atherosclerosis and CHD has recently received strong emphasis (113).

In the debate surrounding the relative merits of different alcoholic beverages, the antioxidant capacity of red wine has been promulgated as an important attribute contributing to its perceived superiority and supported by human in vivo studies demonstrating increased antioxidant activity in blood following oral ingestion of red wines, but not other beverages (90,114,115). In contrast, the increment in antioxidant activity in these experiments was less marked and of shorter duration that the following 1 g of ascorbic acid according to the techniques used by these investigators (115).

Low Density Lipoprotein (LDL)

In atherosclerosis, a major target of oxidation is LDL, which is generated in the circulation by remodeling of VLDL through the mechanisms of lipolysis and the exchange of lipids and proteins with HDL. This natural (or native) form of LDL is subject to receptor-mediated endocytosis through a tightly regulated system, the LDL-receptor. During its transit in the

TABLE 4. Inhibition of LDL Oxidation by Phenolic Compounds in Wine[a]

Phenolic compounds	Average content (mg litre^{-1})[b]		Inhibition (%)[c]		
	Red wines	White wines	5 μM (GAE)[d]	10 μM (GAE)[d]	20 μM (GAE)[d]
Catechin	191.3	34.9	74.9 ± 0.3	98.2 ± 1.1	98.9 ± 0.2
Myricetin	8.5	0.0	68.1 ± 8.7	97.4 ± 1.3	97.6 ± 0.9
Epicatechin	82.0	21.2	67.6 ± 7.6	96.4 ± 1.5	ND[d]
Rutin	9.1	0.0	67.6 ± 15	98.2 ± 0.2	99.1 ± 0.1
Gallic acid	95.0	6.8	63.3 ± 3.4	71.8 ± 3.0	97.8 ± 3.0
Quercetin	7.7	0.0	61.4 ± 1.4	97.7 ± 0.4	98.7 ± 0.1
Caffeic acid	7.1	2.8	58.5 ± 12	98.1 ± 0.1	98.5 ± 0.5
Ellagic acid	ND	ND	36.6 ± 0.9	ND	ND
Sinapic acid	1.8	0.1	35.1 ± 0.9	ND	ND
Cyanidin	2.8	0.0	27.0 ± 4.6	54.6 ± 7.1	89.4 ± 4.2
α-tocopherol	ND	ND	32.6 ± 1.8	54.7 ± 7.1	73.8 ± 3.7

[a]From Teissedre et al. (108).
[b]Average contents in 14 California red wines and 6 white wines.
[c]Inhibition of hexanal formation in human LDL oxidised in the presence of Cu^{2+}, mean ± SD.
[d]Molar concentrations in gallic acid molecular weight equivalents.
ND = not determined.

circulation, oxidative changes may occur affecting the lipids and also the free lysine groups of apolipoprotein B. This "oxidised" LDL is taken up by endocytosis mediated by a "scavenger" receptor system, which is unregulated, and results in overaccumulation of LDL in subendothelial cells, which derive from the monocyte lineage, but because of their engorgement with lipid have come to be known as "foam cells" (116). They play a major role in the accumulation of cholesterol in atheromatous lesions. Oxidized LDL has certain other attributes conducive to atheromatous changes, including blocking of reverse cholesterol transport (uptake) by HDL, and chemotactic properties promoting adhesion of platelets to the superficial endothelium (117, 118).

Oxidation of LDL represents "bad news" from the perspective of CHD risk, and at least one therapeutic drug, probucol, has been developed to treat or prevent the disease based, predominately, upon its antioxidant properties. Alpha-tocopherol is also believed to exercise its beneficial effects by protecting LDL against oxidative changes.

The research undertaken by the group at the University of California, Davis, has made some important and original contributions to our knowledge of the relationship between wine phenolics and LDL oxidation. By means of an ex vivo system whereby conjugated dienes were generated from native LDL during Cu^{2+}-mediated oxidation, they showed that the phenolic constituents of red wine, but not white wine, prevented these oxidative changes and were several-fold more potent than α-tocopherol in this respect (119). They extended these observations using pure quercetin, epicatechin, and *trans*-resveratol. The latter was not as potent as the first two compounds in blocking Cu^{2+}-induced oxidation of human LDL, but was nevertheless superior to α-tocopherol on a molar basis (120).

These results were elaborated by demonstrating that the antioxidant effects of wine phenolics also inhibited lipid peroxidation by biological catalysts such as myoglobin, cytochrome, and iron ascorbate (121). The ability of certain red wines to inhibit LDL oxidation strongly correlated with their content of gallic acid (r=0.92), catechin (r=0.76), myricetin (r=0.70), quercetin, (r=0.68), caffeic acid (r=0.63), and several other phenolics, but not with resveratrol (122).

Most recently, the U.C. Davis team has once more confirmed the antioxidant properties of phenolic compounds from grapes and wines (108). Oxidation of LDL was measured by analysing hexanal, a specific volatile oxidation product of n-6 polyunsaturated lipids (123). In this LDL oxidation system, most phenols tested exhibited much more potent antioxidant properties than α-tocopherol at 10 μM concentration (Table 4).

Another group of phenols found in wine are the hydroxycinnamic acids (caffeic, ferulic, and p-coumaric acids). Their antioxidant properties were investigated in a lipid peroxidising system mediated by metmyoglobin. The results showed that the order of effectiveness in increasing the resistance of LDL to peroxidation, in protecting LDL cholesterol from oxidation and preventing the oxidative modification of apolipoprotein B100 is caffeic>ferulic>p-coumaric acid. Assessment of the rates of reaction of the hydroxycinnamates with ferrylmyoglobin, a product of the reductive decomposition of lipid hydroperoxides, revealed that the compounds are more effective as peroxyl radical scavengers than reductants of ferrylmyoglobin in LDL peroxidising systems mediated by haem proteins (124). A study by Vinson et al. (125) using hamster and human LDL and measuring TBARS and hexanal production demonstrated that all wines, red and white, were better antioxidants than ascorbic acid or α-tocopherol at 3 μM.

In a system where endogenous α-tocopherol of LDL particles exposed to ferrylmyoglobin (iron in the form of FeIV=0) vanishes as a function of myoglobin concentration, subsequent heavy lipid peroxidation was prevented by caffeic acid and *p*-coumaric acid (126). This was accomplished by a mechanism involving the one-electron transfer reaction between phenols and ferrylmyoglobin, with formation of metmyoglobin and the corresponding phenoxyl radicals from caffeic and *p*-coumaric acids. It was shown that caffeic acid delays α-tocopherol consumption when present before oxidation challenge and restores α-tocopherol when added halfway during the reaction. In LDL enriched with α-tocopherol, caffeic acid inhibited the initiation of oxidation longer than that expected from the sum of discrete periods characteristic of the phenolic acid and α-tocopherol. Similar effects of these phenolics were observed in a Triton X-100 micellar system. These results suggest that caffeic acid acts synergistically with α-tocopherol, extending the antioxidant capacity of LDL by recycling of α-tocopherol from the α-tocopheroxyl radical. In spite of the fact that *p*-coumaric acid accelerated the rate of α-tocopherol consumption when added either before or during the oxidation reaction, it decreased the peroxidation chain rate. In a study of the kinetics of the depletion of α-tocopherol in human LDL during macrophage-mediated and cell-free oxidation, the addition of the flavonoid morin prevented both α-tocopherol consumption and oxidative modification of LDL (127).

In a comparison of the protective effects of catechin, probucol, and α-tocopherol against cell injury due to LDL oxidation, the ultraviolet-induced lipid peroxidation of LDL was strongly inhibited by 10 μmol/L catechin and 25 μmol/L probucol, but only poorly by 100 μmol/L α-tocopherol. The ultraviolet-treated LDL protected by catechin or probucol were much less "cytotoxic" than unprotected ultraviolet-treated LDL. In contrast, LDL treated by ultraviolet irradiation in the presence of 1000 μmol/L α-tocopherol were just as "cytotoxic" as unprotected LDL. Catechin (10 μmol/L) together with α-tocopherol (100 μmol/L) were very effective cytoprotective agents in combination, whereas probucol (up to 50 μmol/L) was completely ineffective. Porbucol was very protective in preventing lipid peroxidation of LDL and their

subsequent cytotoxicity, but could not protect cells against the cytotoxicity of previously oxidized LDL. The cytoprotective effect of α-tocopherol was associated with a complete inhibition of cellular TBARS formation induced by UV-treatment of LDL, whereas the cytoprotective effect of catechin was relatively independent of TBARS inhibition. Thus catechin (10 μmol/L) exhibited two types of protective effects: inhibition of the lipid peroxidation of LDL (and their subsequent "cytotoxicity") and effective protection of the cells against the "toxicity" of previously oxidized LDL (128).

Cholesterol

In an in vitro study (129) on micellar solubility of cholesterol, a mixture of (-)-epicatechin (EC) and (-)-epigallocatechin (EGC) and their gallates [(epicatechingallate) (ECG) and (epigallocatechingallate)EGCG)] precipitated cholesterol solubilized in mixed bile salt micelles in a dose-dependent manner. The effects of epicatechin and epigallocatechin were weaker than that of their gallate esters. The amount of coprecipitated EGCG and cholesterol were positively correlated. In the same study, Ikeda et al. (129) demonstrated the hypocholesterolemic effects of tea catechins, epicatechin, and EGC mixture, and a mixture of ECG and EGCG. Both mixtures markedly lowered lymphatic cholesterol absorption in rats with a cannulated thoracic duct.

Igarashi et al. (13) investigated the effects of isorhamnetin, rhamnetin, and quercetin on TBARS content and antioxidative enzyme activities or rats fed on cholesterol-enriched and cholesterol-free diets. The concentration of total serum cholesterol in the rats fed with all three flavonoids was significantly lower than that of control rats, although there was no significant difference between the individual flavonoid-fed groups. The total liver cholesterol concentration and TBARS content in the rats fed the cholesterol-free diet were decreased by feeding all three flavonoids. These compounds did not affect the activities of liver superoxide dismutase and catalase, suggesting the possibility that the lower liver TBARS content in those rats fed the cholesterol-free diet with added flavonoids is ascribable in part to the direct antioxidative and superoxide anion generation-suppressing activities of flavonoids and/or their metabolites absorbed from the gastrointestinal tract.

Other Lipids, Sugars, and Proteins

In an attempt to correlate the inhibitory activities of several structurally-related flavonoids against cyclo-oxygenase and 5-lipoxygenase, Laughton et al. (131) tested them as inhibitors of iron-dependent lipid peroxidation in a rat liver microsomal system. They concluded that the flavonol class (quercetin, fisetin, myricetin, morin, naringenin) inhibit iron-ascorbate lipid peroxidation by a mechanism that probably involves the scavenging of intermediate peroxyl and alkoxyl radicals. Their activity is structure dependent: polyhydroxylated flavonoids are the most active, with high activity among compounds possessing two or more hydroxyl sustituents in the B ring.

Yutin et al. (132) measured the superoxide scavenging activity and antioxidation properties of seven flavonoids, including quercetin, rutin, and morin. The superoxide anions were generated in a phenazine methosulphate-NADH system and assayed by reduction of nitroblue tetrazolium. The concentration values yielding 50% inhibition of lipid peroxidation in mouse liver homogenate were in the order of 10^{-6} M for all three, and no correlation was found between the antioxidative and scavenging activities of these flavonoids.

The antioxidant abilities of ferulic acid and catechins to scavenge the reactive oxygen species hydroxyl radical, hypochlorous acid, and peroxyl radicals was evaluated by Scott et al. (133). Ferulic acid tested at concentrations up to 5 μM inhibited the peroxidation of phospholipid liposomes. Both (+/-) and (+) catechin and (-) epicatechin were much more effective. All three compounds reacted with trichloromethyl peroxyl radical. In the same experiment the three compounds were shown to react with hydroxyl radicals, preventing deoxyribose damage, in a system whereby hydroxyl radicals generated are detected by their ability to cause damage to the sugar deoxyribose. Furthermore, (+) catechin and (-) epicatechin inhibited the reduction of cytochrome c in a concentration-dependent manner, in a mixture of hypoxanthine and xanthine oxidase, which generates superoxide, thus reducing cytochrome c to ferrocytochrome c. All three compounds tested were able to scavenge hypochlorous acid at a rate sufficient to protect α-1-antiproteinase against inactivation.

Salah et al. (134) measured the relative antioxidant potentials of several flavonoids against radicals generated in the aquous phase and against propagating lipid peroxyl radicals. The results showed that in the aqueous phase their order of effectiveness as radical scavengers is epicatechin gallate> epigallocatechin gallate>epigallocatechin>gallic acid> epicatechin=catechin. Against propagating lipid peroxyl radical species, epicatechin and catechin were as effective as the others and least efficacious were epigallocatechin and gallic acid.

Catechins and quercetin served as powerful antioxidants against lipid peroxidation when phospholipid bilayers were exposed to aqueous oxygen radicals (135). Measuring the inhibition of lipid peroxidation in large unilamellar liposomes composed of egg yolk phosphatidylchoine (PC), catechol-type flavonoids retarded the accumulation of PC-hydroperoxides, depending on their concentrations, when the suspension was exposed to a water-soluble radical initiator. Their inhibitory effects lasted longer than that of α-tocopherol.

In an in vitro study measuring the inhibition of hydroperoxidation of methyl linoleate initiated by a radical initiator, quercetin exhibited the highest peroxyl radical-scavenging activity with its glucosides slightly less active (136). In the same investigation, quercetin and its glucosides retarded the

accumulation of PC-hydroperoxides to a lesser extent, and the induction period was increased in phospholipid bilayers. The authors concluded that quercetin acts as an antioxidant more efficiently than its monoglucosides when phopholipid bilayers are exposed to aqueous oxygen radicals.

Cells

Ex-vivo studies

The antioxidant fraction of green tea extract (GTA), which is high in catechins, was shown to scavenge H_2O_2 and O_2 in a concentration-dependent fashion in a xanthine/xanthine oxidase superoxide (O_2^-) production system (137). GTA prevented the cytotoxicity of paraquat and glucose oxidase and/or the inhibition of intercellular communications by noncytotoxic concentrations of paraquat, glucose oxidase, phenobarbital and 12-0-tetradecanoylphorbol-13-acetate in mouse hepatocytes and human keratinocytes.

Ten newly synthesized flavones possessing varied hydroxyl substitution patterns (Table 5) were studied by Wallet et al. (138) in order to establish the effect of the hydroxyl groups on biological activities. Using chemiluminescence in two biological processes (the oxidative burst of alveolar macrophages, and the xanthine/xanthine oxidase system), they studied radical formation by electron spin resonance spectroscopy. They demonstrated that flavones devoid of a C-3 hydroxyl group also can be efficient radical scavengers and antioxidants and speculated that they should be useful in the treatment of human disease processes, including ischemia, in which both reactive oxygen species and xanthine oxidase are implicated.

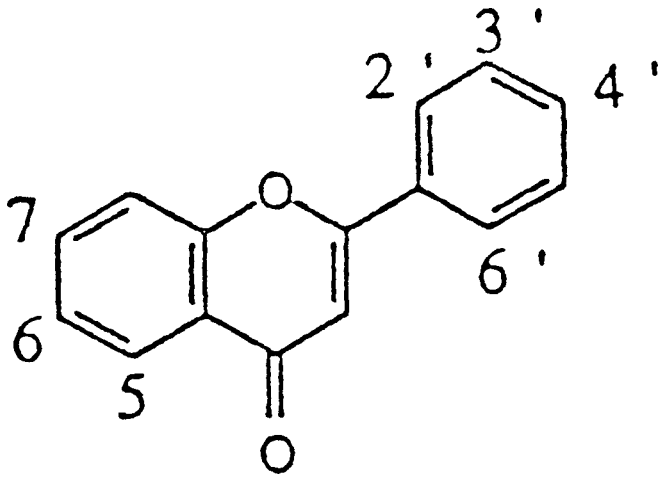
Gallate-green tannin from green tea, (-) epigallocatechin, (-)-epicatechin, and (+)-catechin showed significant dose-dependent inhibition of smooth muscle cell proliferation (139).

Primary cultures

The hydroperoxide-induced cytotoxicity on Chinese hamster V79 cells, Salmonella TA 104, and *Echerichia coli* PQ37 was suppressed by caffeic acid ester, gallic acid ester, quercetin, and catechin. In contrast, neither ferulic acid ester bearing o-methoxyphenol structure nor α-tocopherol were effective, indicating that o-dihydroxy or its equivalent structure in flavonoids is essential for the protection (140).

Using 3T3 Swiss fibroblasts, the effects of catechin were studied under different conditions of oxidative stress leading to cell proliferation or cytotoxicity. Various levels of reactive oxygen species (ROS) (O^{2-}, H_2O_2), generated extracellularly

TABLE 5. Flavones Investigated to Establish the Effect of the Hydroxyl Groups on Biological Activities



n°	5	6	7	2′	3′	4′	6′
1			OMe	OMe	OMe	OMe	
2			**O**H	OMe	OMe	OMe	
3		OMe		OMe	OMe	OMe	
4		OH		OMe	OMe	OMe	
5	OH			OMe	OMe	OMe	
6	OH		OH	OMe	OMe	OMe	
7	OH		OH	OH	OH	OH	
8				OH	OH	OH	
9	OH			OMe	OMe		
10				OMe			OMe

[a]From Wallet et al. (138).

by a xanthine-xanthine oxidase system, inhibited DNA synthesis at high levels of ROS, the cytotoxicity being proportional to the level of H_2O_2 generated by the enzyme system. Catechin did not significantly alleviate the inhibition of DNA synthesis induced by low levels of ROS, but protected in a dose-dependent manner against the cytotoxicity of high levels of ROS (141).

Other Oxidation Reactions

Xanthine oxidase

Xanthine oxidase (XO) is a highly versatile enzyme that catalyzes the hydroxylation of many purine substances and converts hypoxanthine to xanthine and then uric acid in the presence of molecular oxygen to yield superoxide anion (O_2^-) (142). XO-derived superoxide anion has been linked to reperfusion injury and edema (143), as well as changes in vascular permeability (144), and limitation of superoxide anion generation by this enzymatic pathway would be beneficial in the case of ischemia. Furthermore, serum XO levels are increased in hepatitis and mild hepatotoxicity (145); they are also significantly increased in brain tumor tissues as compared with normal brain tissue (146), and the degree of brain edema and injury is dependent on the level of XO. Gout is also caused by the deposition of urate crystals, a by-product of the XO-catalyzed reaction, in the joints.

In a study by Chang et al. (147), nine flavonoids were investigated, among them quercetin, catechin, and epicatechin, for inhibition of XO and compared with allopurinol, an XO inhibitor. The inhibitory effect of quercetin at low concentration was by far the best among all the flavonoids and exceeded that of allopurinol.

Nitric oxide

A related issue is the ability of wine phenolics to modulate the production of nitric oxide (EDRF:endothelial-derived relaxing factor) by vascular endothelium. This agent, generated from arginine by a complex enzymatic system in which guanosine 3′,5′-cyclic monophosphate plays a stimulatory role, is considered to be a major promoter of vascular relaxation and also inhibits platelet adherence to the endothelium (148). Fitzpatrick and colleagues (149) have shown that the contraction of rat aortic rings (but not of aorta from which the endothelium had been removed) could be inhibited by wines, grape juices and grape skin extracts. Quercetin and tannic acid alone, but not resveratrol, could reproduce these effects.

Polyphenols and Platelet Aggregation

The role of platelets in initiating chemical signals that set in motion the complex cellular events resulting in atherosclerosis, following their adherence to the endothelial surface of the arteries, as well as in triggering lumenal occlusion leading to acute CHD, is well established (52,150). The potential of platelets to adhere to vascular endothelium as well as to participate in blood coagulation and thrombus formation can be measured by their ability to aggregate in vitro in response to a number of agonists. Resistance to aggregation is evidenced by an increase in the concentration of agonist required to aggregate 50% of the cells under standard condition (IC_{50}).

Early investigations on the biological effects of flavonoids indicated that they were quite potent inhibitors of cAMP phosphodiesterase (151) and of cGMP phosphodiesterase (152). Kinetic and structure-activity studies suggested that these effects were attributable, at least in part, to competitive inhibition of substrate binding so that the cyclic nucleotides tended to accumulate. Quercetin (153) and catechin (152) were among the most active of the phosphodiesterase inhibitors tested by these authors. Beretz et al. (153) showed that quercetin blocks the ADP-induced aggregation of washed human platelets in vitro and also potentiates the antiaggregatory effects of prostacyclin. Both phenomena could be explained by increased intracellular cAMP concentrations in the treated platelets. A different conclusion was reached by Mower et al. (154), who could not find a good correlation between inhibition by flavonoids of platelet aggregation induced by arachidonic acid, epinephrine, and ADP and changes in intracellular cAMP concentrations. Instead, they concluded that inhibition of cyclo-oxygenase and therefore of thromboxane A_2 production was the most likely mechanism involved in this inhibition. In a later publication, these authors modified this view (155), obtaining evidence that different flavonoid classes showed striking differences in their interaction with platelets. The glycones of flavonoids and the flavonone derivatives tested did not affect platelet function. Most flavones inhibited platelet aggregation by depressing cyclooxygenase activity and also reduced cAMP response to prostacyclin, probably by inhibition of adeylate cyclase, but the flavones myricetin and quercetin increased the rise of platelet cAMP stimulated by prostacyclin and also inhibited the 12-lipoxygenase pathway leading to production of 12-HETE (12-hydroxyeicosatetraenoate)

Subsequently, Corvazier and Maclouf (156) demonstrated that of 10 flavonoids examined for their effect on arachidonic acid metabolism of human platelets and neutrophils, some had no effect at concentrations as high as 1 mM, but others inhibited both thromboxane B_2 and 12-HETE formation. All compounds tested (including quercetin and catechin) inhibited platelet aggregation in parallel with inhibition of thromboxane B_2, but not morin and rutin. Some (including quercetin and morin, but not catechin) inhibited the synthesis of 5-HETE and leukotriene B_4 by human platelets. The authors concluded that eicosanoid metabolism was altered by flavonoids in general and at several different pathways, but each compound had its own spectrum of effects based upon chemical structure as pointed out previously (155). This concept was further strengthened by Grylewski et al. (157) who found that

flavonols but not flavanes were bound to platelet membranes and prevented platelet adherence to rabbit vascular endothelium as well as dispersing platelet thrombi already formed.

More recently, the dose-dependent inhibition by both *trans*-resveratrol and quercetin of the aggregation of platelets prepared from healthy human subjects was demonstrated using ADP and thrombin as agonists (158). The IC_{50} concentrations were rather high but were three orders of magnitude less than that of ethanol, although the antiplatelet activity of the latter has been advanced as one of the mechanisms involved in its protection against CHD (54). None of a wide range of other wine phenolics or established dietary antioxidants (including α-tocopherol) that were similarly tested had any antiaggregatory activity. Indeed, by applying information obtained from dose-response curves, the antiaggregatory effect of de-alcoholized red wines could be computed as approximately that expected from its concentrations of resveratrol and quercetin.

Gentisic acid (2,5-dihydroxybenzoic acid) is another likely candidate for the prevention of platelet aggregation and anti-inflammatory response. This compound has been described repeatedly in wine (159–161), at levels of 2.25 mg/L in white wine and at slightly higher concentrations in red wine (162). According to one report (163), the body produces gentisic acid upon absorption of aspirin. Gentisic acid, due to its striking structural similarity with the active principle of salicylic acid (2-hydroxy benzoic acid), would be expected to exert a pharmacological action similar to aspirin: inhibition of the cyclo-oxygenase pathway and vasodilatory effects. Indeed, gentisic acid has been prescribed as a substitute for aspirin in clinical medicine (164). The antioxidant activity of this compound has been well established (163,165).

Eicosanoid Metabolism

Neutrophil leukotriene production

The major pathways for arachidonate metabolism in human neutrophils involve the 5-lipoxygenase and 15-lipoxygenase enzyme systems, the activity of which can be measured by quantitating the rate of formation of their stable products 5-HETE and 15-HETE, respectively (166). The former pathway is particularly important, because it accounts for the synthesis of leukotrienes. These are powerful mediators of inflammatory reactions and are thought to play a role in certain of the cellular processes contributing to atherosclerosis (167,168).

Corvazier and Maclouf (156) demonstrated that some flavonoids, including quercetin, rutin, and morin, blocked the 5-lipoxygenase pathway (and therefore leukotriene synthesis) by human neutrophils. The inhibitory action of certain glucoside and aglycone flavonoids for eicosanoid generation via the 5-lipoxygenase and cyclo-oxygenase pathways was subsequently studied by Moroney et al. (169) in elicited rat peritoneal leukocytes stimulated with calcium ionophore. The results (Table 6) showed that addition of sugar residues greatly reduces inhibitory potency while retaining selectivity against 5-lipoxygenase. In aglycone flavonoids, the inhibitory potency and selectivity against 5-lipoxygenase are conferred by the presence of 3′ 4′-vicinal diol (catechol) in ring B as part of a 3,4-dihydroxycinnamoyl structure and by the incorporation of additional hydroxyl substituents. In contrast, crossover of inhibitory selectivity is observed in compounds containing few hydroxyl substituents (with one in ring B) that are selective against cyclo-oxygenase.

In a recent investigation of a large range of polyphenols and antioxidants tested, using freshly isolated washed neutrophils from human subjects, only resveratrol and quercetin were effective inhibitors of these pathway (170). Dose-response studies yielded IC_{50} values of 22.4 μM (resveratrol) and 0.75 μM (quercetin) for inhibition of the 15-lipoxygenase pathway.

Platelets

Two major pathways for the synthesis of eicosanoids from arachidonic acid are present in human platelets. The cyclooxygenase pathway leads to the production of thromboxane A_2, which plays an important role in propagating aggregation once this process has been initiated. The half-life of this component is extremely short, so that meaningful results are very difficult to obtain. The overall activity of the pathway can, however, be assessed by measuring the production from labeled arachidonate of thromboxane B_2 (TxB_2) and hydroxyheptadecatrienoate (HHT), which are stable products formed from thromboxane A_2. Quercetin was reported to potentiate the inhibitory effect of prostacyclin on ADP-induced platelet aggregation (153), whereas flavone and a number of other flavonoids directly inhibited the synthesis of thromboxanes from labeled arachidonate due to suppression of the cyclooxygenase pathway (154–156). *Trans*-resveratrol strongly inhibited this pathway, ~60% at a concentrations of 10 μM (158). Neither quercetin alone or any of the other wine phenolics or antioxidants tested in the same study had any major effect at this concentration.

The other pathway from arachidonate expressed in platelets involves the 12-lipoxygenase enzyme system, which synthesises a family of eicosanoids defined as hepoxillins and which are mediators of calcium mobilization, vascular permeability, and neturophil activation (171). Its overall activity is most frequently assessed by measuring the production of 12-HETE, a stable compound thought to be pro-atherogenic by impairing endothelial function and prostacyclin production (172). At a concentration of 10 μM, quercetin abolished the production of 12-HETE by 70%, but none of the other compounds tested were effective at this concentration, although at higher concentrations *trans*-resveratrol exerted modest inhibition (158).

TABLE 6. Inhibition of 5-lipoxygenase and Cyclo-oxygenase by Flavonoids[a]

Compound	Class	OH group positions	5-lipoxygenase (~IC_{50} value mM)[b]	cyclo-oxygenase (~IC_{50} value mM)[b]	Comment
Aglycones					
Morin	flavonol	3,5,7,2´,4´	160	180	nonselective
Myricetin	flavonol	3,5,7,3´,4´,5´	13	56	LO-selective
Fisetin	flavonol	3,7,3´,4´	11	80	LO-selective
Kaempferol[c]	falvonol	3,5,7,4´	20	20	nonselective
Quercetin[c]	flavonol	3,5,7,3´,4´	3.5	16	LO-selective
Gossypetin	flavonol	3,5,6,7,3´,4´	10	not active	LO-selective
Naringenin[c]	flavanone	5,7,4´	16	100	LO-selective
Hypolaetin[c]	flavone	5,7,8,3´,4´	4.5	70	LO-selective
Galangin	flavonol	3,5,7	20	7	CO-selective
Chrysin	flavone	5,7	18	5	CO-selective
3-Hydroxy-flavone	flavonol	3	16	1	CO-selective
Flavone	flavone	(none)	32	8	CO-selective
Glycosides					
Rutin*	flavonol	3-sugar, 5,7,3´,4´	45	ca 450	LO-selective
Gossypin	flavonol	8-sugar, 3,5,7,3´,4´	250	630	LO-selective
Naringin[c]	flavanone	7-sugar, 5,4´	>500	320	nonselective
Hypolaetin-8-glycoside	flavone	8-sugar, 5,7,3´,4´	56	>1000	LO-selective

[a]From Moroney et al. (169).
[b]IC_{50} values based on tests at 6 concentrations; each in triplicate.
[c]Indicates compounds tested also using radio-TLC methodology. Inhibition of 5- lipoxygenase in terms of production of leukotriene B_4; inhibition of cyclo-oxygenase in terms of thromboxane B_2.

The flavonols quercetin and rutin were studied with respect to their antithrombotic action in vivo and their efficacy at influencing the platelet-endothelium interaction in vitro (157). Both flavonols inhibited the ascorbate-stimulated formation of malondialdehyde by boiled rat liver microsomes, inhibited platelet lipoxygenase activity, stimulated cyclo-oxygenase, and were bound to platelet membranes. They both dispersed platelet thrombi, which were adhering to rabbit aortic endothelium ex vivo and prevented platelets from aggregating over blood-superfused collagen strips (ED_{50} for quercetin was 5 nmol/kg and for rutin 33 nmol/kg i.v.). It was concluded that activated platelets adhering to vascular endothelium generate lipid peroxides and oxygen free radicals that inhibit endothelial biosynthesis of prostacyclin and destroy EDRF. These flavonols were antithrombotic, because they are selectively bound to mural platelet thrombi. By scavenging free radicals, they stimulate biosynthesis and action of endothelial prostacyclin and EDRF. Thus flavonols release the thrombolytic and vasoprotective endothelial mediators only in these vascular segments that are covered by a carpet of aggregating platelets.

Oxygenation of arachidonic acid catalyzed by prostaglandin H synthase (PGH synthase) generates two protaglandin products: prostaglandin G_2 (cyclo-oxygenase function), and its alcohol derivative, prostaglandin H_2 (peroxidase function) (173,174). Bakovic et al. (175) demonstrated that the cinnamic acid derivatives (caffeic acid>ferulic acid>p-coumaric acid) in addition to inhibiting 5- and 12-lipoxygenase pathways by reducing active site iron, as do many other lipoxygenase inhibitors (176,177), also stimulate PGH synthase cyclo-oxygenase, serving as a reducing substrate for PGH synthase peroxidase. Consequently, good inhibitors of leukotriene biosynthesis potentiate prostaglandin biosynthesis, acting as reducing agents in both cases. This suggests that structurally similar catechins may possess therapeutic effects different to conventional nonsteroidal anti-inflammatory drugs.

Phospholipase A_2

This key enzyme (PLA_2) initiates eicosanoid synthesis by liberating arachidonic acid from membrane-bound phospholipids. Quercetin was found to inhibit group I PLA_2 from porcine pancreas in lower concentrations (<100μM) than indomethacin (>100 μM) (178). It was also a potent inhibitor of one of two PLA_2 fractions purified from the plasma of septic shock patients (185). PLA_2 is regarded as an important enzyme in inflammation as it plays a pivotal role in the formation of inflammatory mediators, including prostaglandins, leukotrienes, platelet-activating factor, and lysophospholipids (179). The importance of this extracellular PLA_2 has been emphasized and PLA_2 has been described as an acute-phase protein (180) that may be a key mediator between proximal and distal effectors of inflammation (181).

POLYPHENOLS AND CANCER

Quercetin

In addition to a wide spectrum of pharmacological properties, phenolic compounds, and specifically quercetin, have been shown to inhibit the growth of cells derived from human and animal cancers, such as leukemia and Ehrlich ascites tumors (182), the estrogen receptor-positive breast carcinoma (MCF-7 (183), squamous cell carcinoma of head and neck origin (184), gastric cancer (185), and colon cancer (186), as well as human leukemia HL-60 cells in culture (187).

Quercetin treatment arrested human gastric cancer cells in the G_1-phase (185) and human melanoma as well as leukemic T cells in late G_1-phase prior to the beginning of S-phase (188). More recently, quercetin has been reported to downregulate mutant p53 levels, which can block apoptosis (189), and to induce apoptosis through a pathway involving heat-shock proteins (190).

The fact that quercetin and other phenolic compounds exhibit growth-inhibitory effects may be a consequence of their ability to interfere with enzymatic activities involved in the regulation of cellular proliferation. Part of the growth inhibitory effects of these compounds may be explained by their biological activities reported in various experimental systems, such as the inhibition of Na^+K^+ATPase (191); the increase of cAMP levels (192,193); the inhibition of protein tyrosine kinases (194,195) and protein kinase C (196,197); Ca^{2+}-ATPase of sarcoplasmic reticulum (198); $pp60^{src}$ kinase (199); the modulation of estrogenic response through an interaction with type II binding sites (183); the inhibition of the synthesis of a 17-kDa protein (cell-cycle related) (186) and HIV reverse transcriptase (with a K_i of 0.08 μM) (200). Furthermore, quercetin is also a potent inhibitor of phosphatidyl-3 kinase (201) and 1-phosphatidylinositol-4 kinase (202), important enzymes in proliferation signaling pathways.

Quercetin has antiproliferative activity against breast (203) and stomach (204) cancer cell lines and human ovarian cancer primary cultures (205) and can potentiate the action of cisplatin ex vivo (206). It has been shown to potentiate the cytotoxic action of 1-β-D-arabinofuranosylcytosine in primary cultures of human acute myeloid leukemia cells (207). Furthermore, in vivo synergy of quercetin with cisplatin against Walker lung cancer xenografts in nude mice has been described (208).

The bioavailability and the plasma transport of flavonols in rats fed quercetin or rutin were studied by Manach et al. (209). The concentration of circulating flavonols was significantly increased, and quercetin and rutin administration resulted in similar concentrations of quercetin in cecal contents. Their results demonstrated that dietary flavonols are recovered in rat plasma as conjugated metabolites in non-negligible concentrations.

Only a few pharmacokinetic studies have been conducted with humans. Xu et al. (210) reported that in adult women the average plasma concentration of total isoflavones reached 4.4 μmol/L at 6.5 hours after a dose of 2 mg soybean isoflavones/kg body weight.

In a recent phase I clinical trial using quercetin, administered by short i.v. infusion at increasing doses, in 9 of 11 patients lymphocyte protein tyrosine phosphorylation was inhibited at 1 hour, persisting up to 16 hours. In one patient with ovarian cancer refractory to cisplatin, following two courses of quercetin, the serum CA 125 concentration (a protein marker for ovarian cancer) had decreased by six-fold, and in another patient with hepatoma the serum α-fetoprotein (a marker correlating with tumor burden) was diminished (211).

Other Flavonoids

Hydroxylated flavonoids, including myricetin, quercetin, morin, kaempferol, and rutin, directly interacted with and potently inhibited the mutagenic activity of metabolites (diol-epoxides) of polycyclic aromatic hydrocarbons, well established to be powerful mutagens and carcinogens (212). The genotoxic effects of a number of cooked-food mutagens were inhibited in a bacterial mutation assay, in a dose-dependent manner equally by myricetin and quercetin and much better by morin (213).

The possibility that the effects of flavonoids and other antioxidants on the TPA-mediated induction of ornithine decarboxylase (ODC) and tumour promotion being parallel with their activities of lipoxygenase inhibition was investigated in an animal study using CD-1 mice. Morin, fisetin, and kaempferol, were shown potently to inhibit epidermal lipoxygenase activity as well as 12-0-tetradecanoylphorbol-13-acetate (TPA)-mediated ODC induction. TPA-mediated DNA synthesis was not inhibited by morin, but TPA-induced skin tumor promotion was markedly inhibited by morin and slightly by α-tocopherol (214).

Catechin

In an in vitro study, addition of (+)-catechin to rat hepatocytes cultured with 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a tobacco-specific carcinogen, inhibited the formation of DNA-damaging intermediates by selectively impairing the enzymatic activation of NNK, suggesting that (+)-catechin could be an effective preventive agent against NNK hepatocarcinogenicity (215). In another experimental study, Fan (216) investigated the effect of catechin on cell growth of HeLa, KB, and 3T3 cell lines and reported that the first two were sensitive to catechin. The survival curve of HeLa cells treated with catechin varied with the dose and duration of exposure, and the ^{3}H-TdR incorporation rate into HeLa cells was inhibited by catechin at a rate that was also dose and exposure-dependent. It is, therefore, possible that one of the mechanisms of cell growth inhibition by catechin may be inhibition of DNA synthesis.

Hydroxycinnamic and Benzoic Acids

Caffeic and ferulic acids may play a role in the body's defense against carcinogenesis by inhibiting the formation of N-nitroso compounds. Caffeic and ferulic acids were reported to react with nitrite in vitro and to inhibit nitrosamine formation in vivo. In simulated gastric fluid, caffeic acid and ferulic acid reacted rapidly and completely with an equimolar quantity of sodium nitrite. Caffeic acid was more effective than ferulic acid in both the in vitro (reaction with nitrite) and in vivo (inhibition of hepatotoxicity) systems (217).

Gentisic acid, due to its antioxidant activity and its ability to inactivate hydroxyl free radicals (163,165), might also prevent, as has been suggested for aspirin, the development of some forms of cancer (163).

Gallic acid, caffeic acid, and other phenolic compounds inhibited aflatoxin B_1 (AFB1)-induced mutagenesis in a Salmonella typhimurium strain TA 98 in a suspension assay in the presence of rat liver microsomes (218). The inhibitory effect was observed only when the phenolic compound and the mutagen were administered concurrently and in a dose-dependent manner.

Caffeic and ferulic acids inhibited the induction of ODC by TPA by 42% and 46%, respectively, in mice (219). In a similar study, the ability of m-chloroperoxybenzoic acid to induce the ODC marker of skin tumor promotion was inhibited by gallic acid and other polyphenols in mouse epidermis in vivo (22). The same two compounds, when added to the diet of mice on benzo(a)pyrene-induced neoplasia of the forestomach, suppressed neoplasia (221). In a related study (222) using mice fed on powdered food with or without caffeic acid, some of each group were injected with 7,12-dimethyl benz[α] anthracene (DMBA) after 1 week. Using a bone-marrow micronucleus test, it was observed that caffeic acid inhibited the DMBA-induced chromosomal breakage by 50%, suggesting that caffeic acid provides significant protection against the genotoxicity of DMBA.

CONCLUSIONS

Many phenolic compounds present in food and vegetables demonstrate potent and desirable biological activities. The most universal property relates to their function as antioxidants, manifested by their ability to trap free radicals and inhibit their enzymatic generation, and to block the oxidation of cellular and extracellular components such as membranes and LDL. More selectively, certain of these polyphenols can prevent or diminish aggregation of platelets and their synthesis of pro-aggregatory eicosanoids such as thromboxane A_2, as well as synthesis by leukocytes of pro-inflammatory leukotrienes. However, some polyphenols are able to promote the synthesis of prostacyclin and nitric oxide and in this way may play a role in optimizing blood flow through the arterial system. Several, especially quercetin, have anticancer potential despite their mutagenic capabilities (223), and others may have lipid lowering properties, although such effects appear to be relatively weak.

This remarkable spectrum of biochemical and cellular functions has earned the polyphenols a privileged role in human nutrition. The resistance to certain diseases, such as atherosclerosis and CHD, manifested by a number of population groups, especially those of the Mediterranean basin, is widely attributed to the high proportion of fresh fruit and vegetables together with the moderate consumption of wine, which are considered to be the characteristic features of their diet. Advanced industrial societies whose diets generally lack these components are being urged to modify their lifestyles accordingly.

It must be kept in mind that virtually all of the evidence supporting these notions comes from direct experiments in artificial systems or from epidemiology-driven population studies. Investigations with isolated cells such as leukocytes, primary cell cultures, and cell lines, in vitro preparations such as LDL, and purified enzymes can provide valuable clues to the intrinsic structure-function relationship of the polyphenols and how they might potentially behave in the human body, but such evidence is a long way from definitive proof. Issues such as the absorption and bioavailability of these compounds have barely been addressed. Unlike vitamins and essential minerals, daily dietary requirements for these compounds have not been established; no specific clinical syndromes are observed among individuals or group whose intake seems to be particularly low. With an exception cited earlier (211), their deliberate administration singly or collectively had not led to amelioration or cure of any disease or symptom complex once this has become established (224). Likewise, their putative ability to prevent or protect against disease rests upon vague inferences projected by epidemiologists from observations on populations whose variables are impossible to control adequately.

Nevertheless, the notion that dietary polyphenols enhance health and prevent disease, actively promoted but not fully established by nutritional scientists, has gained growing acceptance among medical practitioners and other health professionals and has infiltrated the consciousness of the informed lay public through its wide dissemination in the media. There is almost a collective desire to see this hypothesis achieve the status of established truth, because it is consistent with the presumed benefits of healthy lifestyle practices. It is not our intention to adopt a cynical posture in this debate, since we align ourselves with those who support the hypothesis, but we would be irresponsible to our readers if we were to terminate this review without raising some of these cautionary matters.

So, what about wine? This much can be confidently asserted: an intake of two glasses of red wine per day will provide ~40% of the total antioxidant polyphenols present in a healthy diet (225,226), as well as a number such as resveratrol that are virtually absent from commonly consumed fruit and

vegetables (1,227). Moreover, the natural way in which wine is produced and the various processes that contribute to its production as described in this review allow plenty of scope for further enrichment of its polyphenol content (228–230). Finally, fermentation and the increasing presence of alcohol leads to their liberation and solubilisation such that they are likely to be much more bioavailable in this milieu than in solid foodstuffs. Only tea can approach the advantages of wine in this respect. The study of wine phenolics is, therefore, a valid scientific enterprise that deserves to proceed in parallel with broader studies to establish and confirm their advantage to human health.

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