Polyphenols in foods are more complex than often thought[1–3]

Véronique Cheynier

ABSTRACT

Dietary polyphenols show a great diversity of structures, ranging from rather simple molecules (monomers and oligomers) to polymers. Higher-molecular-weight structures (with molecular weights of > 500) are usually designated as tannins, which refers to their ability to interact with proteins. Among them, condensed tannins (proanthocyanidins) are particularly important because of their wide distribution in plants and their contributions to major food qualities. All phenolic compounds are highly unstable and rapidly transformed into various reaction products when the plant cells are damaged (for instance, during food processing), thus adding to the complexity of dietary polyphenol composition. The polyphenol composition of plant-derived foods and beverages depends on that of the raw material used but also on the extraction process and subsequent biochemical and chemical reactions of plant polyphenols. The occurrence of specific tannin-like compounds (ie, thearubigins and theaflavins) arising from enzymatic oxidation is well documented in black tea. Various chemical reactions involving anthocyanins and/or flavanols have been demonstrated to occur during red wine aging. Current knowledge regarding the reaction mechanisms involved in some of these processes and the structures of the resulting products is reviewed. Their effects on organoleptic and nutritional quality are also discussed. *Am J Clin Nutr* 2005;81(suppl):223S–9S.

KEY WORDS Polyphenols, anthocyanins, tannins, reaction products, oxidation, food, wine, tea, organoleptic properties, color, astringency

INTRODUCTION

Phenolic compounds are responsible for major organoleptic characteristics of plant-derived foods and beverages, particularly color and taste properties. They are also reported to contribute to the health benefits associated with consumption of diets high in fruits and vegetables or plant-derived beverages (such as tea and wine).

Innumerable studies have been devoted to polyphenols, their occurrence in plants and plant-derived foods, and their effects on food quality. However, plant polyphenol composition is still poorly understood, because most studies have focused on specific classes of molecules that can be separated and assayed with HPLC and neglected polymers that are not as easily determined. Furthermore, polyphenols are highly reactive compounds and good substrates for various enzymes, including polyphenoloxidases, peroxidases, glycosidases, and esterases. They undergo numerous enzymatic and chemical reactions during postharvest food storage and processing. Although the occurrence of such reactions and their roles in the development or degradation of food quality are well documented, the structures of the resulting products are still poorly understood and their concentrations in food are usually unknown. This article reviews current knowledge regarding the composition of plant-derived foods and beverages, with special emphasis on wine, which was taken as an example of a complex polyphenol–rich food product. The relationships between the structures and properties of genuine plant polyphenols and the reaction products derived from them are also briefly discussed.

POLYPHENOLS IN PLANTS

Plant polyphenols comprise a great diversity of compounds, among which flavonoids and several classes of nonflavonoids are usually distinguished (1). The latter (**Figure 1**) are mostly rather simple molecules, such as phenolic acids (which are subdivided into benzoic acids and hydroxycinnamic acids, based on C1-C6 and C3-C6 skeletons, respectively) and stilbenes, but also include complex molecules derived from them (eg, stilbene oligomers, gallotannins, ellagitannins, and lignins). The former (**Figure 2**) share a common nucleus consisting of 2 phenolic rings and an oxygenated heterocycle. They are divided into several groups differing in the oxidation state of the heterocyclic pyran ring (eg, anthocyanins, flavonols, and flavanols). More than 4000 flavonoids have been identified in plants, and the list is constantly growing (2). This is because of the occurrence of numerous substitution patterns in which primary substituents (eg, hydroxyl, methoxyl, or glycosyl groups) can themselves be substituted (eg, additionally glycosylated or acylated), sometimes yielding highly complex structures. Moreover, flavanols are also encountered as oligomers and polymers, referred to as condensed tannins or proanthocyanidins because they release anthocyanidins (ie, anthocyanin aglycones) when heated under acidic conditions. Proanthocyanidins differ in the nature of their constitutive units (eg, catechin and epicatechin in procyanidins, which release cyanidin after acid-catalyzed cleavage), their sequences, the positions of interflavanic linkages (C4-C6 or C4-C8 in the B-type series, with additional C2-O-C7 or C2-O-C5 bonds in A-type structures), their chain lengths, and the presence of substituents (eg, galloyl or glucosyl groups). Because all constitutive units and linkages can be distributed at random within a

[1] From INRA-Unité Mixte de Recherche Sciences Pour l'Œnologie, Montpellier, France.

[2] Presented at the 1st International Conference on Poplyphenols and Health, held in Vichy, France, November 18–21, 2004.

[3] Address reprint requests to V Cheynier, INRA-Unité Mixte de Recherche Sciences Pour l'Œnologie, 2, place Viala, 34060 Montpellier Cedex, France. E-mail: cheynier@ensam.inra.fr.

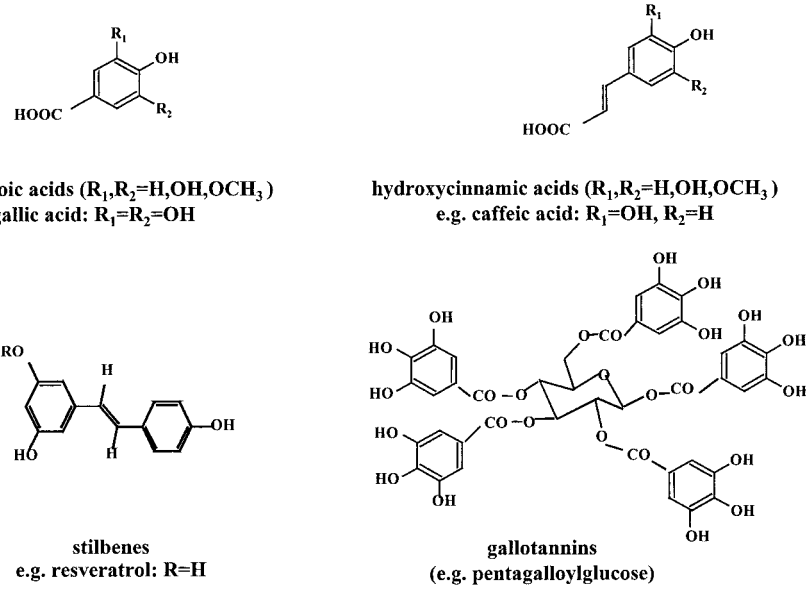

FIGURE 1. Chemical structures of the main classes of nonflavonoid polyphenols.

polymer, the number of possible isomers increases exponentially with the chain length [eg, 8 dimers, 32 trimers, and 128 tetramers in the B-type procyanidin series; more generally, $n^x (n-1)^y$ for a n-mer with x types of constitutive units and y types of linkages].

Plant polyphenol composition is highly variable both qualitatively and quantitatively; some of the compounds are ubiquitous, whereas others are restricted to specific families or species (eg, isoflavones in legumes). Polyphenol diversity in fruits (3) and in plant foods (4) has been described in excellent reviews. Within a single species, large variations may also occur, particularly because of genetic factors, environmental conditions, and growth or maturation stages. For instance, in grapes and apples, anthocyanins are found only in the red varieties and accumulate toward the end of ripening. Pinot noir grapes contain only anthocyanin 3-glucosides, whereas most other grape cultivars also contain acylated anthocyanins (5, 6). Major polyphenols in apples are hydroxycinnamic acids (mostly found as quinic esters, such as chlorogenic acid), flavanols (catechins and procyanidins), and dihydrochalcones (7–10); they are present in substantial amounts in flesh, but their concentrations in peel are higher. Flavonols and anthocyanins are present in smaller amounts and are almost completely restricted to peel. All of these compounds are constituents of most commonly consumed fruits except dihydrochalcones (eg, phloridzin), which are specific to apples.

Recent studies established that flavanols are the major polyphenols in apples, accounting for 65–85% of total polyphenol

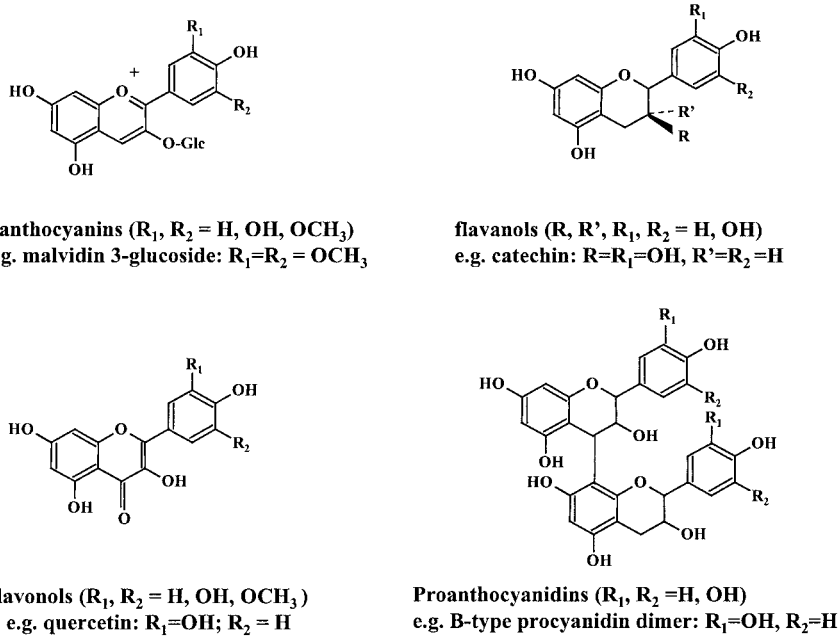

FIGURE 2. Chemical structures of the main classes of flavonoids.

contents in the dessert (8) and cider (9) varieties analyzed. Their concentrations show large variations among apple cultivars, ranging from ~1 g/kg fresh weight in dessert varieties to 5 g/kg in cider varieties. Their chain lengths are also highly variable, averaging ~6 in all studied dessert varieties (8) but ranging up to 45 in some cider apple cultivars (9). Similarly, proanthocyanidins are the major polyphenols in grapes, where they are localized mostly in skins and seeds. Seed proanthocyanidins are partly galloylated procyanidins, with degrees of polymerization in the range of 1 (monomers) to ~20 (11). Skin proanthocyanidins contain both procyanidin and prodelphinidin units and are much larger than skin tannins (~30 units, on average) (12).

It is worth pointing out that difficulties in assaying flavanol polymers, because of their heterogeneity (the large range of molecular weights and the increasing number of isomers as the chain length increases) and the lack of efficient separation techniques, have been overcome only recently. As a consequence, most compositional studies focused only on rather simple molecules, including monomers and flavanol oligomers, which can be individually quantified. In contrast, the concentrations of polymers have been determined in only a few species and the polymers are otherwise generally disregarded, although they are the major polyphenols in most plants (13).

Therefore, available food polyphenol composition data are limited, on the one hand, to the amounts of a limited number of molecules (eg, catechins) and, on the other hand, to approximate estimates of total polyphenol contents. The latter are based on absorbance measurements at 280 nm or the use of more or less specific reagents such as Folin Ciocalteu (assay based on an oxidation reaction), Bate-Smith reagent (proanthocyanidin assay based on the release of anthocyanidins after acid-catalyzed cleavage), or Porter's reagent (vanillin-HCl, enabling detection of flavanols), which is often replaced with dimethylaminocinnamaldehyde. Although each of these methods can be used to compare samples with similar compositions, they are rather imprecise in assays of extracts with different polyphenol profiles, because of different responses of the various polyphenol structures and, for some, interference by nonphenolic compounds.

POLYPHENOLS IN PROCESSED FOODS

Overview

Polyphenols are highly unstable species that undergo numerous reactions in the course of food processing and storage. These changes are well known to food chemistry and technology specialists and have large effects on food quality. The resulting products account for large proportions of the polyphenolic contents in some foods and beverages, but they are overlooked in most studies addressing food composition. However, some of the new compounds formed in these processes may show particular properties different from those of their precursors.

An illustration of such changes can be found in a recent study comparing catechin contents of fresh and processed fruit and vegetables (14). Total catechin contents decreased by 28, 58, and 26% in rhubarb, broad beans, and cooking pears, respectively, after home preparation according to standard recipes. The concentrations measured in industrially canned products were also much lower than those in the equivalent fresh products.

Distribution in plant tissues and selective extraction

Changes associated with food processing involve selective extraction of some particular compounds with juice or wine technology but also removal of some parts (eg, peel and seeds) before consumption. For instance, hydroxycinnamic acids are the only phenolic compounds present in the flesh of most grape varieties (except teinturiers), whereas seeds contain galloylated procyanidins and skins contain flavonols and, in red varieties, anthocyanins, in addition to hydroxycinnamic acids and proanthocyanidins. Consequently, white grape juice made through direct pressing of the grapes contains only hydroxycinnamic acids and preparation of a red juice or wine requires a maceration step to extract red pigments from the skins. Monitoring of the phenolic composition of the fermenting red must enabled demonstration that anthocyanins diffuse faster than proanthocyanidins and that, among the latter, proanthocyanidins from skins that can be traced through their specific epigallocatechin units are extracted earlier than those from seeds, because of either their localization or their higher water solubility (15). Tannins with higher molecular weights also diffuse more slowly than oligomers. Extending the maceration time after the end of fermentation thus leads to increased extraction of proanthocyanidins and especially of higher-molecular-weight and highly galloylated structures. Higher-molecular-weight proanthocyanidins (beyond the decamer) have been reported to be insoluble in aqueous media. However, this has not been confirmed experimentally. In fact, polymers with average degrees of polymerization of $>$ 20 were shown to be present in wine (16), and apple procyanidin fractions containing 70 units, on average, also proved to be soluble in wine-like hydroalcoholic medium (17).

Polyphenol reactions in food processing

Reactions involving polyphenols in food processing and storage include biochemical and chemical processes. The most important biochemical process is enzymatic oxidation, which starts as soon as the integrity of the cell is broken, but other types of enzymes, such as esterases, glycosidases, and decarboxylases, may also catalyze transformations and degradations of polyphenolic compounds. Enzymatic oxidation is ubiquitous in plant-derived foods. The resulting browning is usually detrimental to quality, particularly in postharvest storage of fresh fruits or juice and puree technology, but may be desirable for some products (eg, tea, coffee, cocoa, and raisins). Chemical reactions take place simultaneously and gradually become prevalent as the enzymatic activities decrease.

These mechanisms and the structures of the resulting products have been particularly studied in black tea and red wine. Enzymatic oxidation and subsequent reactions in black tea have been extensively reviewed (18, 19) and are not developed herein. Briefly, tea (*Camelia sinensis* L.) is particularly rich in polyphenols; they represent 30% of the leaf dry matter (18). Major polyphenols in the fresh tea leaves are flavanol monomers, among which (−)-epicatechin, (−)-epigallocatechin, and their gallic esters are particularly abundant. The so-called "fermentation" of black teas actually consists of enzymatic oxidation of native green tea polyphenols, catalyzed by polyphenoloxidase, followed by chemical reactions of the resulting quinones (18). A major part of tea leaf flavanols are thus converted into various types of products, including thearubigins and theaflavins, which are responsible for the characteristic dark brown color of black

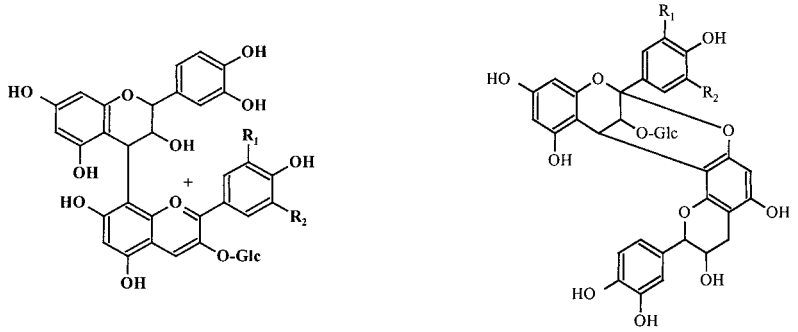

FIGURE 3. Structures of anthocyanin-flavanol dimeric adducts (R = OH or OCH_3; R' = H, OH, or OCH_3).

tea (20), and smaller quantities of theaflavic acids (21) and bisflavanols, which are also called theasinensins (22). The number of compounds is still increasing because new structures are regularly being identified (23–25), but no information is available on their respective concentrations in tea.

Additional complexity: changes during red wine aging

Chemical reactions of polyphenols are particularly important in wine, because they are responsible for the color and taste changes that occur during aging. Grape polyphenols, namely, anthocyanins, flavonols, hydroxycinnamic acids, and flavanols, including catechins and proanthocyanidins, represented only approximately one-half of the polyphenol content of a 2-y-old red wine polyphenol extract (26). The other one-half consisted of unknown phenolic species derived from grape polyphenol reactions during winemaking and aging. Genuine anthocyanins determined with HPLC contributed 50% of the red color intensity measured spectrophotometrically after dilution with 1% HCl; therefore, the other one-half could be attributed to anthocyanin-derived pigments, in agreement with earlier observations (27). Fractionation of the wine polyphenol extract with chromatography on a Fractogel column (TOSOH Biosep, Stuttgart, Germany) allowed separation of lower-molecular-weight polyphenols (fraction 1) from higher-molecular-weight polyphenols (fraction 2) (28, 29). Although fraction 1 was thought to consist mainly of simple polyphenols, its HPLC tracing at 520 nm showed a hump under the elution profile of grape anthocyanins, indicating that it contained other pigments (29). Extraction of this fraction with isoamyl alcohol, as proposed by Somers (27), allowed recovery of monomeric polyphenols in the organic phase, whereas unresolved oligomeric compounds were retained in the aqueous phase. The fraction 1 organic phase, fraction 1 aqueous phase, and fraction 2 each contained approximately one-third of the wine polyphenolic material.

Analysis of the fraction 1 aqueous phase and fraction 2 with HPLC after acid-catalyzed cleavage in the presence of toluene-α-thiol (thiolysis) showed that genuine proanthocyanidin units accounted for ~50% and 70%, respectively, of the material present. Average degrees of polymerization of 2.9 in the fraction 1 aqueous phase and 7.3 in fraction 2 were calculated, confirming that they were oligomeric and polymeric fractions, respectively. Color determinations indicated that anthocyanin derivatives represented ~6% of the polymeric material, whereas they accounted for 40% of polyphenols in the oligomeric fraction. Therefore, it seems that the major part of anthocyanin-tannin adducts are oligomeric rather than polymeric molecules. In addition, glucose determinations after hydrolysis, performed as described previously (30), indicated that a large proportion of anthocyanin derivatives were colorless, because most of the glucose released was assumed to be linked to flavonoid and particularly anthocyanin moieties. This suggests that anthocyanin-derived pigments exist both as red flavylium forms and as colorless hydrated hemiketal forms, as demonstrated for genuine anthocyanins (31).

Analysis of the oligomeric fraction with HPLC coupled to ultraviolet/visible spectrophotometry and mass spectrometry showed the presence of 2 series of masses corresponding to anthocyanin-(epi)catechin adducts (29). The first group, in which the anthocyanin is in the red flavylium form, presumably corresponds to flavanol-anthocyanin adducts (**Figure 3**, left). Such adducts were also obtained in wine-like model solutions containing anthocyanins and procyanidins. Their formation involves cleavage of the tannin interflavanic linkage, followed by nucleophilic addition of the anthocyanin hemiketal to the carbocation thus released and dehydration of the resulting adduct to the corresponding flavylium (32). The second group (Figure 3, right) consists of colorless adducts in which the anthocyanin and flavanol units are linked by both carbon-carbon (C4-C8) and ether (C2-O-C7) bonds (A-type), formed by nucleophilic addition of the flavanol to the anthocyanin flavylium cation (33). Both types of dimeric adducts were also released with thiolysis of the oligomeric and polymeric fractions, indicating that these moieties were also incorporated in higher-molecular-weight molecules.

Various other anthocyanin and tannin reactions have been shown to occur in wines. These include reactions involving acetaldehyde, arising from yeast metabolism or ethanol oxidation. Acetaldehyde-induced reactions yield ethyl-linked species, including flavanol anthocyanin adducts (34), flavanol oligomers (35), and anthocyanin oligomers (36), as well as another group of pigments based on a pyranoanthocyanin structure (37, 38) (**Figure 4**). Pyranoanthocyanins are formed through reactions of anthocyanins with compounds with a polarizable double bond, such as vinyl or enol derivatives. Addition of vinylphenol (39), pyruvic acid (40), or acetaldehyde (41, 42), which are yeast metabolites formed during fermentation, to anthocyanins takes place spontaneously in wine, yielding phenyl-pyranoanthocyanins (R = phenyl), carboxy-pyranoanthocyanins (R = COOH), and pyranoanthocyanins (R = H), respectively.

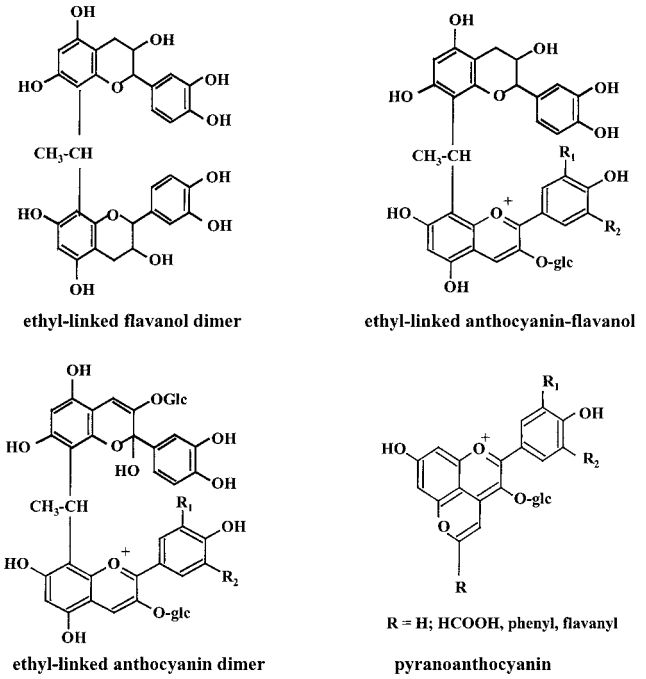

FIGURE 4. Structures of products formed in wine through reactions of tannins and/or anthocyanins with acetaldehyde and other yeast metabolites (eg, vinylphenol: R = phenyl; pyruvic acid: R = COOH) (R_1 = OH or OCH_3; R_2 = H, OH, or OCH_3).

Other aldehydes, such as glyoxylic acid (resulting from oxidation of tartaric acid, which is present in wine in large amounts) or furfural (extracted from oak barrels), can replace acetaldehyde in polymerization reactions (43, 44). The adducts thus formed then proceed to new xanthylium salt pigments (45). Finally, degradation of anthocyanins releases lower-molecular-weight compounds after cleavage of the heterocyclic ring (eg, syringic acid from malvidin derivatives).

Despite the variety of mechanisms and the large number of products demonstrated, wine composition remains largely unknown. In fact, each of the elucidated derived structures is present in only small amounts and, taken together, they account for only a small proportion of wine polyphenols. It should be emphasized that all flavanols initially present can be similarly involved in all of these reactions, so that each of the flavanol derivatives detected is in fact the head of a large family. In addition, although most new pigments identified are based on malvidin 3-glucoside, the major anthocyanin in grapes, other anthocyanins are expected to react in the same way, yielding a series of derivatives. Furthermore, sequences of cleavage and addition reactions may yield extremely complex molecules combining the various types of structures described above, leading to increased structural diversity. Proanthocyanidins and ethyl-linked flavanols are particularly labile. It was shown that acid-catalyzed cleavage of proanthocyanidins takes place spontaneously at wine pH values. When large amounts of lower-molecular-weight phenolic compounds (eg, anthocyanins and flavanol monomers) are present, random cleavage and subsequent addition reactions result in reduction of the average size of tannins and derived species (46, 47). Ethyl-linked flavanols also undergo acid-catalyzed cleavage at wine pH values (48). Reaction of the intermediates thus released with anthocyanins leads either to ethyl-linked flavanol anthocyanin adducts (48) or to flavanyl-pyranoanthocyanins (49) (Figure 4, R = flavanyl). All of these processes take place simultaneously, with their respective kinetics depending on the proportions of phenolic precursors (which are themselves determined by the grape composition and extraction process) and also on several other factors, including the concentrations of some nonphenolic compounds (eg, aldehydes), oxygen exposure and the presence of oxidation catalysts, pH, and temperature.

PROPERTIES OF FOOD POLYPHENOLS

Polyphenols exhibit a wide range of properties, depending on their particular structures. They include yellow, orange, red, and blue pigments, as well as various compounds involved in food flavor. Some volatile polyphenols, such as vanillin and eugenol (which is responsible for the characteristic odor of cloves), are extremely potent odorants, but the major flavors associated with polyphenols are bitterness and astringency. Other major polyphenol characteristics include their radical-scavenging capacity, which is involved in antioxidant properties, and their ability to interact with proteins. The latter is responsible for astringency perception (resulting from interactions of tannins with salivary proteins), for formation of haze and precipitates in beverages, and for inhibition of enzymes and reduced digestibility of dietary proteins.

Major polyphenol pigments in plants are anthocyanins, which exhibit red, purple, or blue color, and, to a lesser extent, the yellow flavonols and flavones. Anthocyanins are highly reactive species. When dissolved in water, the anthocyanin red flavylium cations are converted to several other forms through proton transfer and hydration reactions (31, 50). In slightly acidic media such as encountered in plant foods, simple anthocyanins are present mostly in the colorless hemiketal form, in equilibrium with the yellow chalcone isomer (31). Conversion of genuine anthocyanins to other molecules during the course of food processing results in either loss or stabilization of color and increases the range of available hues. Among the anthocyanin derivatives formed in wine, pyranoanthocyanins are orange pigments, whereas ethyl-linked species and tannin-anthocyanin adducts are purple. All 3 types of molecules are more resistant to discoloration through hydration reactions than are genuine grape anthocyanins. It was recently shown that ethyl-linked anthocyanin dimers in wine are in a single form, in which one anthocyanin unit is colorless and the other is red (51). This means that conversion of grape pigments (75–80% colorless in the wine pH range) to ethyl-linked dimers (one colorless unit and one red unit, ie, 50% colorless) not only leads to a slight shift from red to purple but also greatly enhances color intensity and stability. Reactions of anthocyanins also yield colorless products, such as A-type anthocyanin-flavanol adducts (33) and syringic acid, whereas noncolored flavonoids may also proceed to pigment species, in particular through enzymatic browning, as mentioned above in the case of black tea.

Other major polyphenol properties, such as the ability to complex with proteins and free radical-scavenging capacity, are primarily related to the number and accessibility of phenol (in particular, *o*-diphenol) moieties. The oxygen radical-scavenging capacity of procyanidin dimers and trimers was shown to increase with galloylation and to a lesser extent with longer chain

length but was also influenced by the position of galloyl substituents (52). Similar results were obtained for scavenging of the radical cation 2,2'-azinobis(3-ethyl-benzothiazoline-6-sulfonate) in the aqueous phase, but antioxidant activity decreased from the trimer to the tetramer. Conversely, in the lipid phase, radical-scavenging activity decreased as the molecular weight increased (53). These conflicting results suggest that antioxidant effects are exerted through different mechanisms in the different assays. One should be careful in assessing the relevance of in vitro tests as predictors of in vivo situations.

The affinity of proanthocyanidins for proteins (54) and their astringency (17) increase with both the degree of polymerization and the extent of galloylation. Complex transformation products of plant polyphenols can be similarly expected to act as radical scavengers and bind to proteins. Very few studies have addressed this issue, because of the difficulty of isolating the compounds in sufficient amounts. Flavanol dimers arising from catechin oxidation showed enzyme inhibition effects similar to those of their procyanidin isomers, whereas yellow products obtained after another oxidation step were more active (55). It is not known whether other tannin-like molecules, such as ethyl- or ethanoic-linked flavanol oligomers, behave like tannins, but conversion of grape tannins to new products, particularly tannin-anthocyanin adducts, is generally reported to reduce wine astringency (27). This was classically ascribed to an increase in molecular weight, because larger tannins were thought to be insoluble and thus non-astringent. However, recent studies showed that higher-molecular-weight proanthocyanidins are both soluble and more astringent than the oligomeric proanthocyanidins (17). Consequently, the decrease in astringency observed during wine aging is likely to involve acid-catalyzed processes leading to lower-molecular-weight species, as described above, rather than polymerization reactions. However, the taste of polyphenol reactions products and the effect on astringency of incorporating anthocyanin units into a tannin structure remain to be investigated.

Properties of polyphenols are also greatly affected by their interactions with other constituents of the food matrix. Color intensification resulting from interactions of anthocyanins with other compounds (ie, copigmentation) is well documented (56, 57). The astringency of tannins may also be altered by the presence of various molecules, including polysaccharides and proteins. A mechanism involving interactions of tannins with soluble pectins released during ripening, impeding their binding to salivary proteins, has been proposed to explain changes that occur during fruit ripening (58). The formation of soluble and colloidal polysaccharide-tannin complexes in wine-like model systems was demonstrated with light-scattering measurements (59). Similarly, analyses of wines before and after protein fining suggested that the reduction of astringency induced by fining was partly attributable to the incorporation of tannins in soluble tannin-protein complexes.

Many products arising from various reaction pathways have been determined in different foods and beverages, but their composition is still largely unknown. The properties of complex genuine polyphenols and derived molecules also remain to be established. Additional important questions are related to their bioavailability. In fact, absorption of complex structures in the small intestine, as described for phenolic acids, flavanol monomers, and flavonols, and their circulation in conjugated forms appears very unlikely. However, they may be metabolized by the gut microflora, yielding the same lower-molecular-weight metabolites as those arising from genuine polyphenols and also possibly others. The new linkages formed include labile ones that should not resist the digestive process but also extremely strong ones. For example, catechin dimers arising from enzymatic oxidation contain extremely resistant interflavanic linkages (60). Their fermentation by the digestive flora should consequently yield both metabolites identical to those obtained from catechin or procyanidins after cleavage of the heterocyclic ring and additional metabolites derived from the biphenyl moiety. Finally, strong interactions with other constituents of the food matrix are likely to interfere with the metabolism of polyphenols and should be taken into account in bioavailability studies. Indeed, interactions of polyphenols with food proteins and digestive enzymes are well known to reduce protein digestibility and can be expected to alter polyphenol bioavailability similarly.

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