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Phenolic Bioactives in Grapes and Grape-Based Products

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Introduction

Polyphenols are a large and complex group of compounds responsible for the characteristics, quality, and color of grape and wine, especially for red ones. The polyphenolic composition has been extensively studied and a large family of structures have been identified in grapes and wine (Ribereau-Gayon 1965; Somers 1971; Nagel and Wulf 1979; Slinkard and Singleton 1984; Bourzeix et al. 1986; Cheynier and Rigaud 1986; Da Silva et al. 1991b; Prieur et al. 1994; Souquet et al. 1996) These compounds can affect the appearance, taste, mouth-feel, fragrance, and antimicrobial properties of wine. In addition, polyphenols were confirmed to be the key compounds responsible for the antioxidant potential of wine (Burns et al. 2000).

Wines and grapes contain a number of polyphenolic constituents classified as flavonoids and nonflavonoids that contribute to wine sensory characteristics, especially to color, flavor, and astringency and therefore, to the differences between red and white wines. The family of wine flavonoids includes anthocyanins, flavan-3-ols, flavonols, and dihydroflavonols, whereas the nonflavonoids include phenolic acids (hydroxybenzoic and hydroxycinnamic acids (HCA) and their derivatives) and stilbenes. Red wines contain all the above phenolics, while white wines contain mainly phenolic acids and flavanols. In particular, flavan-3-ols (monomeric flavan-3-ols and proanthocyanidins) confer the astringency and structure to the wine (Sarni-Manchado et al. 1999) and anthocyanins, as red pigments, are responsible for the color of the wines (Wulf and Nagel 1978).

The grape phenolic composition and content is affected by several factors such as grape variety, ripening stage, climate, soil, place of growing, and vine cultivation. In addition, wine-making practices (maceration time, temperature, intensity of pressing, yeast, SO_2-doses) affect the extraction of phenolic compounds from grapes and also enological treatments and aging when subsequent reactions in wine occur to influence the wine phenolic composition.

Flavonoids

Flavonoids exist as free or polymerized with other flavonoids, sugars, and nonflavonoids, or in combination with nonflavonoids, such as HCA derivatives and occur esterified to sugars, organic acids, or various alcohols. The main structure of flavonoids that consists of two benzene rings (A and B) linked by an oxygen-containing pyrane ring condensed with the A ring, is presented in Figure 8.1. Flavonoids may be divided into the following groups.

Anthocyanins

Anthocyanins are red compounds, responsible for the color of red grapes and wines. They are mainly located in the vacuoles of grape skins, with the exception of the teinturier varieties that contain anthocyanins in the pulp. Anthocyanins identified in grape skins and wines from *Vitis vinifera* L. are based on five main anthocyanidins—delphinidin, cyanidin, petunidin, peonidin, and malvidin. The most abundant anthocyanins are the 3-*O*-glucosides and among them, malvidin-3-glucoside is the major compound. Acylated monoglucoside anthocyanins: 3-*O*-acetylglucosides, 3-*O*-*p*-coumroylglucosides, and 3-*O*-caffeoylglucosides have also been identified in grapes and wines. Acylation occurs in the C-6 position of the glucose molecule with acetic, *p*-coumaric, and caffeic acid (Ribereau-Gayon 1965; Somers 1971; Wulf and Nagel 1978; Baldi et al. 1995; Núñez et al. 2004;, Alcalde-Eon et al. 2006). The amount of acylated anthocyanins is largely influenced by the grape variety. The non-*V. Vinifera* grapes, such as *V. labrusca*, *V. rotundifolia,* and *V. upestris* and hybrid grape varieties contain anthocyanin 3,5-diglucosides, as well as 3-(*p*-coumaroylglucoside)-5-glucosides (Giusti et al. 1999; Favretto and Flamini 2000). The structure of antocyanins is presented in Figure 8.2.

Enological practices affect the extraction of phenolic compounds from grapes and their subsequent reactions in wine. For red wine production, the grapes are crushed after the separation of the leaves and stalks and then maceration is applied on the pomace. Maceration means contact between the grape juice and pomace (hard parts of the grape skins and seeds) and its duration varies depending on the grape that is processed and type of wine that is produced.

During the maceration period when the grape juice and pomace are in contact, anthocyanins and the other phenolic compounds are transferred from the solid parts of the grape to the must. After a few days of fermentation, anthocyanins reach a maximum level followed by a decrease of their concentration as a result of precipitation with tartaric salts in a form of colloidal material, adsorption on yeast cell walls, or elimination during filtration and fining. Anthocyanin pigments extracted from the grapes give the color of the young wines, but during the winemaking and aging of wine, anthocyanins may be modified to create stable, C4-substituted pigments through reactions involving pyruvic acid (Bakker et al. 1997; Fulcrand et al. 1998) and vinyl phenol derivatives (Fulcrand et al. 1996). A number of oligomeric pigments resulting from a condensation reaction involving acetaldehyde and from direct reactions of anthocyanins with flavanols have also been described (Somers 1971; Fulcrand et al. 1996; Berg and Akiyoshi 1975; Bakker and Timberlake 1986). Recently, the existence of anthocyanins acylated with lactic acid, formed in wine, has been reported (Alcalde-Eon et al. 2006). Despite all these changes, the wine anthocyanin profile in some studies has also been used as criteria for characterizing and classifying grape varieties in order to establish differences between them (González-San José et al. 1990; Arozarena et al. 2000).

The color of anthocyanins is directly linked to the pH of the medium, as several studies have shown the presence of colored and uncolored forms of anthocyanins depending on the pH (Somers 1971; Brouillard and Dubois 1977; Brouillard et al. 1978). Flavylium cations are mainly present in highly acidic media, which lose their color as pH increases by formation of colorless carbinol bases as a result of addition of water and loss of proton. Carbinol bases are in equilibrium with the open ring chalcone yellow form. The equilibrium between Carbinol and chalcone forms is very slowly attained at room temperature in a slightly acidic media, while increased temperature displaces the equilibrium toward the chalcone forms. In neutral and alkaline medium, as a result of deprotonation (acid-base reaction), the flavylium is transformed into a purple quinoidal anhydrobase (Figure 8.3).

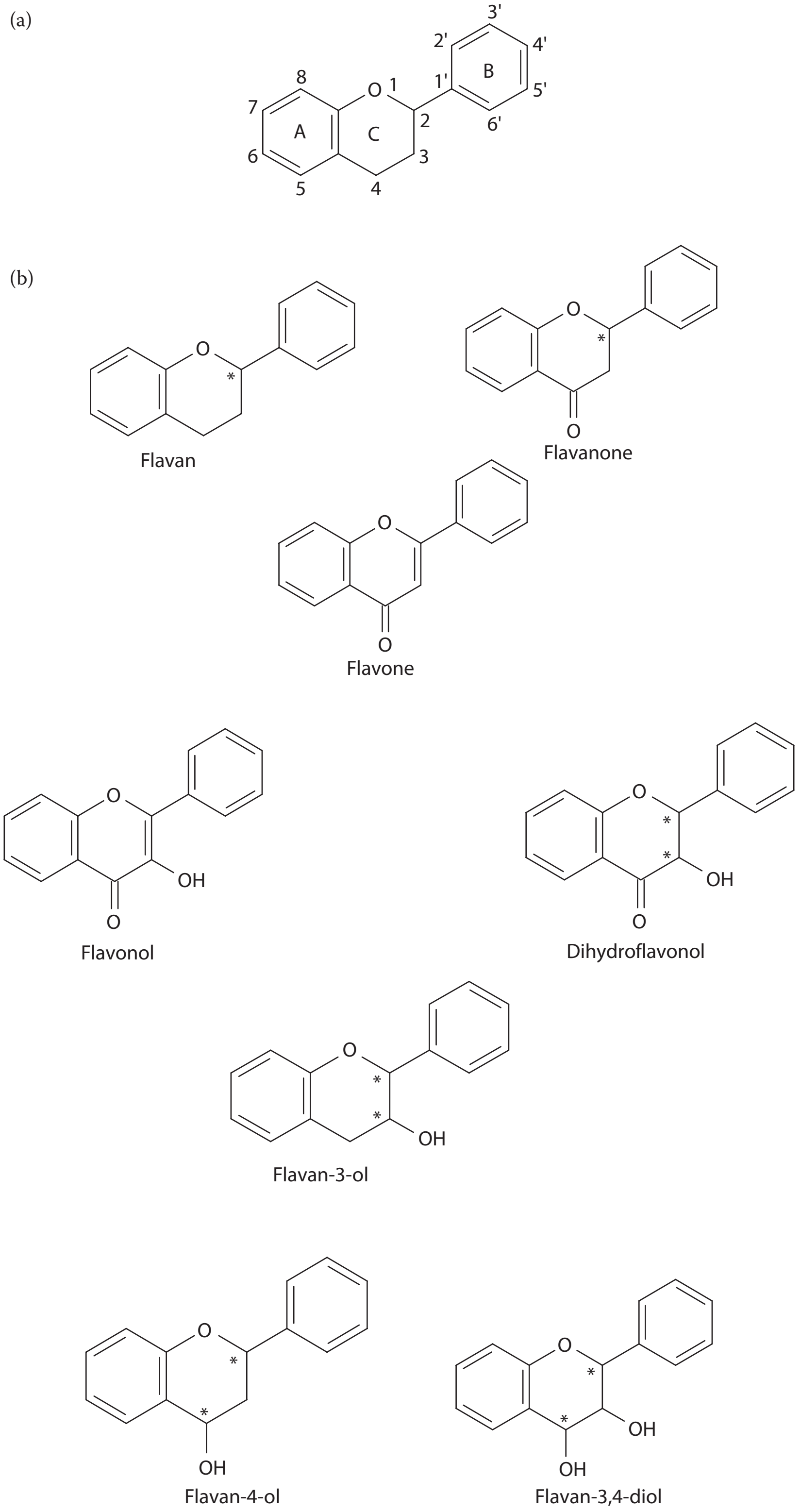
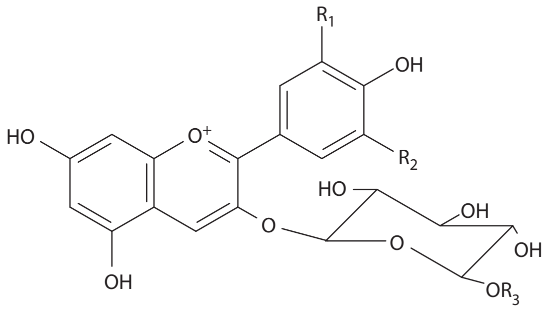


FIGURE 8.1 (a) Flavonoid structure, (b) flavonoid groups.



Anthocyanins	R_1	R_2	M_r
Delphinidin-3-glucoside	OH	OH	465
Cyanidin-3-glucoside	OH	H	449
Petunidin-3-glucoside	OCH_3	OH	479
Peonidin-3-glucoside	OCH_3	H	463
Malvidin-3-glucoside	OCH_3	OCH_3	493

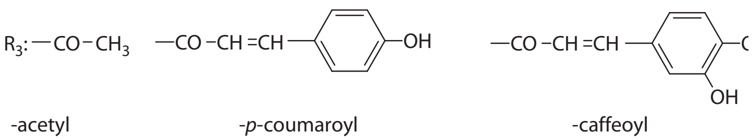


FIGURE 8.2 Structures of anthocyanins. (Adapted from Giusti, M. M., Rodriguez-Saona, L. E., Griffin, D., and Wrolstad, R. E., *J. Agric. Food Chem.*, 47, 4657–64, 1999; Adapted from Favretto, D., and Flamini, R., *Am. J. Enol. Vitic.*, 51, 55–64, 2000.)

Anthocyanins can act as electrophiles in their flavylium form through the C-2 and C-4 positions (C-ring) accepting an electron pair in a chemical reaction. In addition, they can act as nucleophiles in hemiketal form through their C-6 and C-8 positions (A-ring) by donating electrons to the reaction partner, forming a chemical bond.

Flavan-3-ols

Flavan-3-ols are a large family of polyphenolic compounds mainly responsible for the astringency, bitterness, and structure of the wine (Arnold et al. 1980). These compounds can be found as monomers, but also as oligomers and polymers. The major flavan-3-ol monomers in grapes are (+)-catechin and (–)-epicatechin, and, to a lesser extent, the gallic ester of (–)epicatechin and (–)-epicatechin-3-*O*-gallate (Su and Singleton 1969). In *Vitis vinifera* grapes, gallocatechin has been detected and also catechin-3-*O*-gallate and gallocatechin-3-*O*-gallate have been identified (Piretti et al. 1976; Lee and Jaworski 1987). The structure of flavan-3-ol monomers is presented in Figure 8.4.

Flavanol oligomers and polymers are better known as condensed tannins or proanthocyanidins. The term tannin also refers to their capacity to interact or react with proteins and precipitate them out. When heated under acidic and oxidative conditions, these molecules release red anthocyanidin pigments by an acid-catalyzed cleavage (Bate-Smith 1954), hence the term proanthocyanidins is used.

Grape and wine proanthocyanidins belong to two groups, procyanidins and prodelphinidins that release cyanidin and delphinidin, respectively. Procyanidins consist of catechin- and epicatechin-based polymers, and prodelphinidins contain gallocatechin and epigallocatechin units in addition to catechin and epicatechin (Czochanska et al. 1979; Porter et al. 1986). Proanthocyanidins composed of flavan-3 -ol constitutive units containing a single interflavan linkage and linked by C4-C8 and/or C4-C6 bonds are named B-type (Da Silva et al. 1991a) whereas, A-type proanthocyanidins contain double interflavan linkages, with the C2-O-C7 or C2-O-C5 bond in addition to the C4-C6 or C4-C8. Figure 8.5 shows the structure of procyanidin dimers identified in grapes and wines.

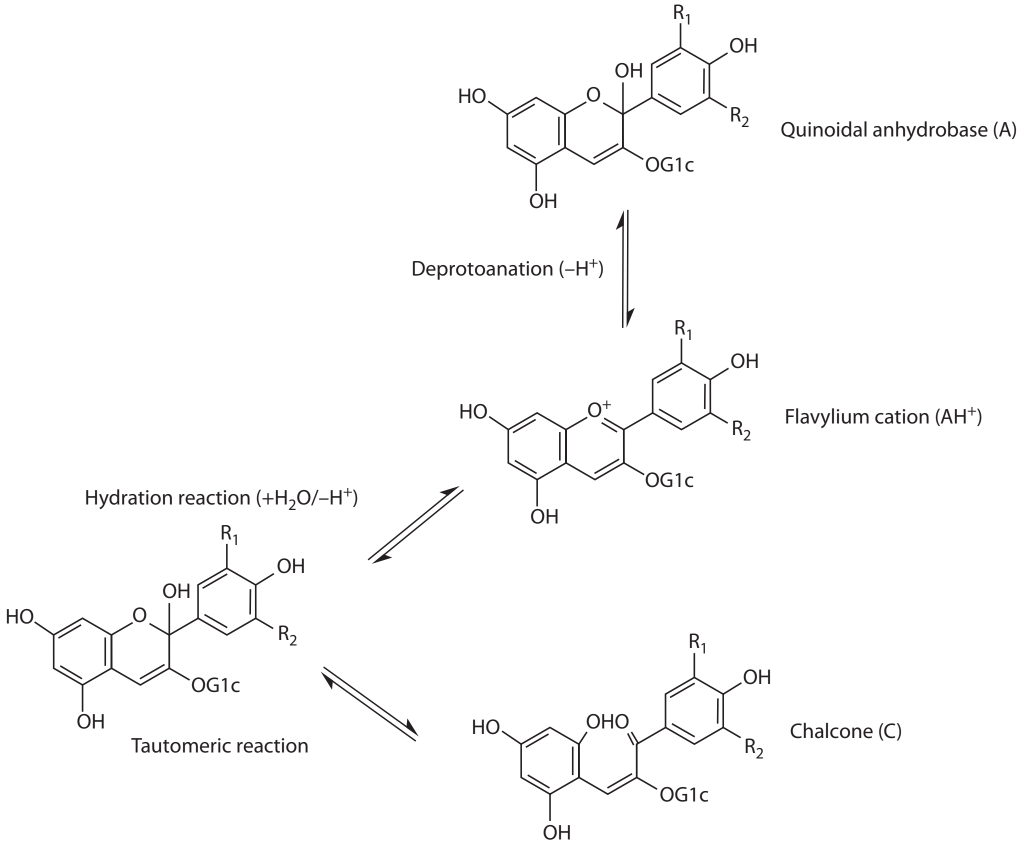
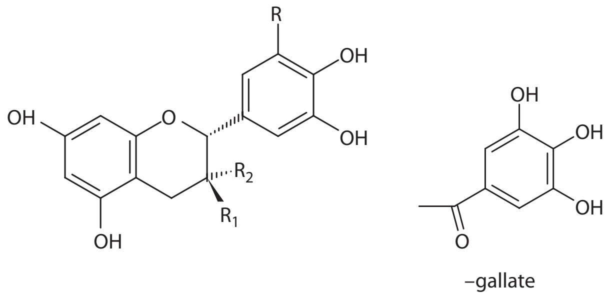
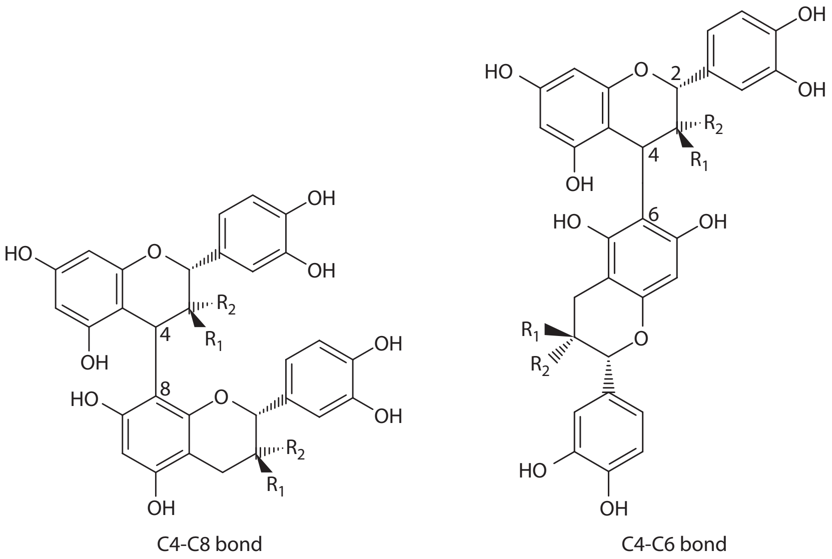


FIGURE 8.3 Anthocyanin transformations depending on pH of the medium. (Adapted from Somers, T. C., *Phytochemistry*, 10, 2175–86, 1971; Adapted from Brouillard, R., and Dubois, J. E., *J. Am. Chem. Soc.*, 99, 1359–64, 1977; Adapted from Brouillard, R., Delaporte, B., and Dubois, J. E., *J. Am. Chem. Soc.*, 100, 6202–5, 1978.)

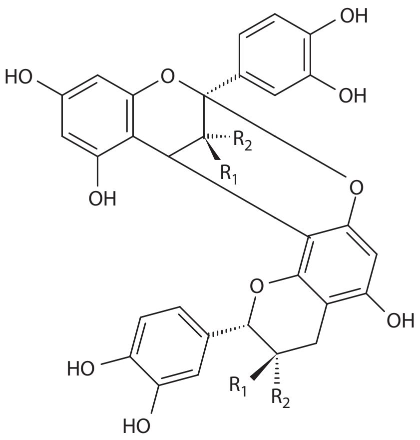


Flavan-3-ols	R	R_1	R_2	M_r
(+)-Catechin	H	OH	H	290
(–)-Epicatechin	H	H	OH	290
(+)-Gallocatechin	OH	OH	H	306
(–)-Epigallocatechin	OH	H	OH	306
(–)-Epicatechin-3-gallate	H	H	OGallate	442

FIGURE 8.4 Structures of flavan-3-ol monomers.



B-type procyanidins



A-type procyanidin

FIGURE 8.5 Structures of flavan-3-ol dimmers.

Determination of proanthocyanidins is generally based on acid-catalyzed depolymerization in the presence of strong nucleophilic reagent (Thompson et al. 1972; Foo et al. 1983) followed with formation of adducts, which are analyzed by reversed phase high-performance liquid chromatography (Shen et al. 1986; Rigaud et al. 1991; Koupai-Abyazani et al. 1992). In acidic media, proanthocyanidins are depolymerized, releasing terminal subunits, as flavan-3-ols and extensional subunits, as electrophilic flavan-3-ol intermediates (Figure 8.6). These electrophilic intermediates can be trapped by a nucleophilic reagent, followed by formation of adducts (Kennedy and Jones 2001).

The most commonly used electrophilic reagents are phloroglucinol (Kennedy and Jones 2001) and benzylhydrosulfide (Remy et al. 2000). After the performed acid-catalyzed depolymerization and HPLC analysis, the proanthocyanidin composition, the mean degree of polymerization (mDP), and the concentration of released units can be determined. According to literature data, the mDP in grape skins is around 30 and in seeds and stems around 10 (Souquet et al. 2000).

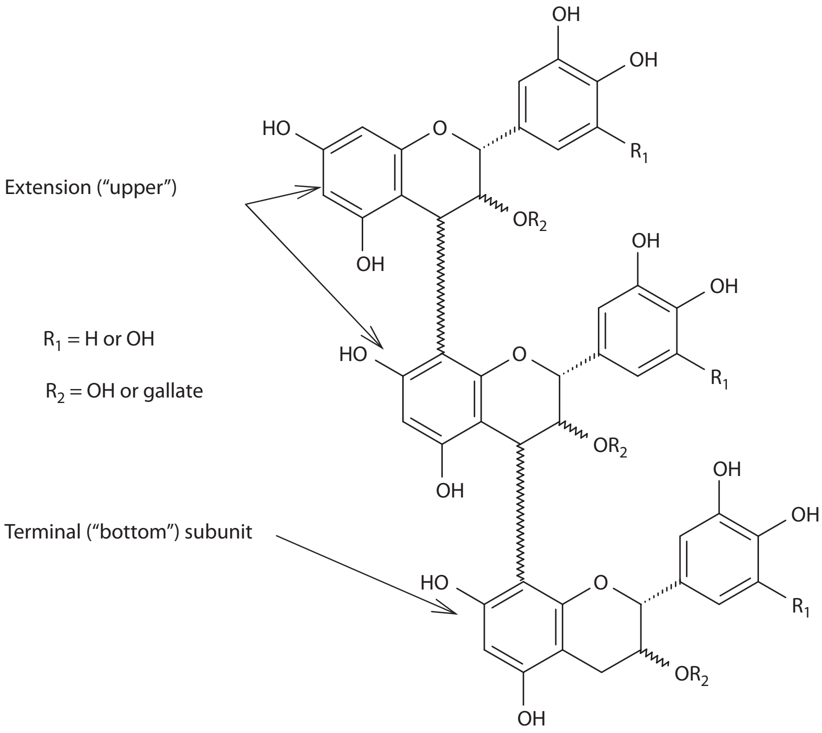


FIGURE 8.6 Basic structure of condensed tannins.

Flavonols and Dihydroflavonols

Flavonols, which are located in epidermal and hypodermal grape skin vacuoles (together with the anthocyanins present in red grapes), absorb the UV radiation and play a protective role. In this regard, the flavonol biosynthesis is increased in the grapes highly exposed to daylight (Spayd et al. 2002). Flavonols present in red grape *Vitis vinifera* L. varieties, are mainly 3-glucosides of myricetin, quercetin, kaempferol, and isorhamnetin (Cheynier and Rigaud 1986; Figure 8.7). Recently, the methoxylated flavonols, laricitrin and syringetin, and their 3-glucoside forms have been identified (Wang et al. 2003; Amico et al. 2004; Castillo-Muñoz et al. 2007).

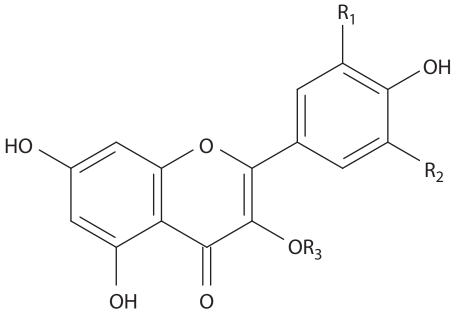
The presence of dihydroflavonols astilbin (dihydroquercetin-3-*O*-rhamnoside) and engeletin (dihydrokaempferol-3-*O*-rhamnoside) has been confirmed in grape and wine (Trousdale and Singleton 1983). Another compound from this group, dihydromyricetin-3-*O*-rhamnoside, has been reported in wine by Vitrac et al. (2001).

Nonflavonoids

The main nonflavonoid phenols in grape and wine that contain only one aromatic ring are derivatives of HCA and hydroxybenzoic acid. Stilbenes and stilbene glycosides are another class of nonflavonoids.

Hydroxybenzoic Acids

The commonly present hydrozybenzoic acids are gallic acid, gentisic acid, *p*-hydroxybenzoic acid, protocatechuic acid, salicylic acid, syringic acid, and vanillic acid, which are mainly found in conjugated forms as esters and glycosides in grapes (Figure 8.8). The free forms of these compounds mainly



Flavonols	**R_1**	**R_2**	**R_3**	**M_r**
Myricetin	OH	OH	H	318
Myricetin-3-glucoside	OH	OH	glucoside	480
Myricetin-3-glucuronide	OH	OH	glucuronide	494
Quercetin	OH	H	H	302
Quercetin-3-glucoside	OH	H	glucoside	464
Quercetin-3-glucuronide	OH	H	glucuronide	478
Kaempferol	H	H	H	286
Laricitrin-3-glucoside	OCH_3	OH	glucoside	494
Syringetin-3-glucoside	OCH_3	OCH_3	glucoside	508
Isorhamnetin	OCH_3	H	H	316
Isorhamnetin-3-glucoside	OCH_3	H	glucoside	498

FIGURE 8.7 Structures of flavonols.

prevail in the wine because of hydrolysis or heat breakdown reactions of the complex molecules, such as anthocyanins, that take place.

Gallic acid is the dominant hydroxybenzoic acid in wine. It originates from the grapes and also can be formed by hydrolysis of hydrolyzable and condensed tannins (i.e., the gallic acid esters of flavan-3-ols).

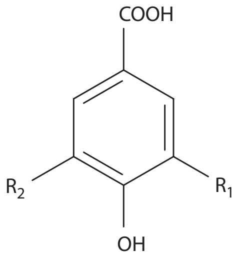
Hydroxycinnamic Acids and Derivatives

The main HCA present in the wine are caffeic acid, *p*-coumaric acid, ferulic acid, and sinapic acid (Figure 8.9a). They can be present in *cis*- and *trans*- forms, but the *trans* forms are more stable and therefore more prevalent (Häkkinen et al. 1998). In wine, HCA are predominant in a form of esters of *l*-(+)-tartaric acid and among the derivatives of HCA, caffeoyltartaric (caftaric) acid, *p*-coumaroyltartaric (*p*-coutaric) acid, and feruloyltartaric (fertaric) acid are main compounds present in grape and wine (Figure 8.9b). Caftaric and coutaric acids are the most abundant in the wine, which are highly oxidizible components that cause the browning of white must (Cheynier et al. 1989, 1990).

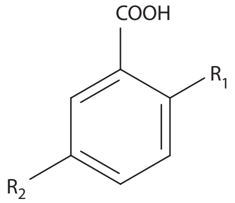
Another compound from the group of HCA, named 2-*S*-glutathionylcaffeoyltartaric acid (also, known as GRP or grape reaction product) has been identified. This compound is formed as a result of the nucleophilic addition of glutathione onto the caffeoyltartaric acid quinone that arises from enzymatic oxidation of coutaric and caftaric acid, catalyzed by polyphenoloxidases in musts (Singleton et al. 1985).

Stilbenes

Stilbenes, located in grape skin and pips, are a subclass of phenolic compounds in grape and wine that can be biosynthesized by grapevines as a defense response to stress, such as microbial infection and UV



Hydroxybenzoic Acids	**R_1**	**R_2**	M_r
Gallic acid	OH	OH	170
p-Hydroxybenzoic acid	H	H	138
Protocatechuic acid	OH	H	154
Syringic acid	OCH_3	OCH_3	198
Vanillic acid	H	OCH_3	168



	R_1	**R_2**	M_r
Gentistic acid	OH	OH	154
Salicylic acid	OH	H	138

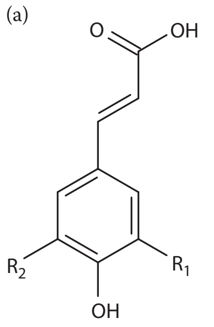
FIGURE 8.8 Structures of hydroxybenzoic acids.

irradiation. During the winemaking process, they are transferred from the grapes into the wine in very low quantities (Sun et al. 2006; Amira-Guebailia et al. 2006; Viñas et al. 2008).

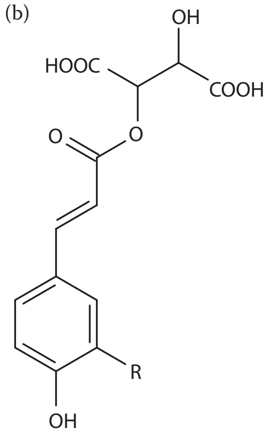
One of the most relevant and extensively studied stilbenes is *trans*-resveratrol (3,5,4′-trihydroxystilbene). In nature, resveratrol exists in two isomeric forms (*cis*- and *trans*-configurations) in the free, as well as in ß-gluconjugated forms. The 3-O-ß-D-glucosides of *cis*- and *trans*-resveratrol are called piceids (Figure 8.10). The levels of resveratrol depend on the grape variety; in general, red wines contain a higher concentration of resveratrol than white wines (Romero-Pérez et al. 1996).

Evolution of Polyphenols During Winemaking and Aging

For red wine production, studies are primary focused on the influence of maceration on the extraction of grape pigments and tannins. Anthocyanins are the first components to be extracted from the grape skins together with the skin tannins at the beginning of fermentation. According to the literature (Gil-Muñoz et al. 1997, 1999; Bautista-Ortín et al. 2004; Ivanova et al. 2009), anthocyanins reach the maximal values during the first days of vinification followed by decreasing till the end of the fermentation. Longer maceration time is very often accompanied by oxidative polymerization of monomeric anthocyanins and their complexation with other phenolics, whereupon oligomeric and polymeric pigments are formed that can precipitate and decrease the anthocyanin contents and the red color, enhancing the brown color of wine (Somers 1971). Tannin extraction from seeds, which is more dependent on ethanol content because of their lower solubility in water, starts toward the midpoint of alcoholic fermentation and continues until pressing during the postfermentation phase (Canals et al. 2005). In



Hydroxycinnamic Acids	R_1	R_2	M_r
Coumaric acid	H	H	164
Caffeic acid	OH	H	180
Ferulic acid	OCH_3	H	194
Sinapic acid	OCH_3	OCH_3	224



Derivatives of Hydroxycinnamic Acids	R_1	M_r
Coutaric acid	H	296
Caftaric acid	OH	312
Fertaric acid	OCH_3	326

FIGURE 8.9 (a) Structures of hydroxycinnamic acids, (b) structures of derivatives of hydroxycinnamic acids.

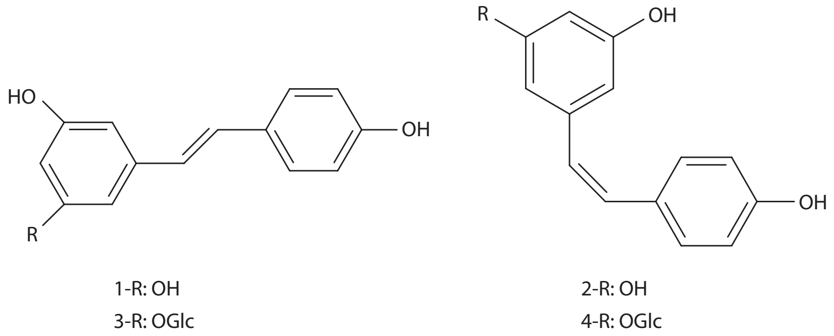


FIGURE 8.10 Structures of stilbenes: (1) *trans*-resveratrol, (2) *cis*- resveratrol, (3) *trans*-piceid, and (4) *cis*-piceid.

fact, flavan-3-ols in seeds are protected with a lipidic layer, which is disrupted when appropriate content of alcohol is formed, allowing their extraction from the seeds. Therefore, extraction of seed tannins occurs at later phases of vinification, and increasing of their concentrations during a longer maceration time is expected.

Sulphur dioxide is naturally present in wine, produced at concentrations up to 64 mg/L by the yeast metabolism (Larue et al. 1985), but most of the yeasts cannot produce more than 10 mg/L SO_2, so that contents of SO_2 higher than 30 mg/L usually are added during the vinification. The use of SO_2 in winemaking is due to its ability of an effective antioxidant, preventing the activity of oxidases. Also, it has significant activity as antimicrobial agent, as well as a potential for bleaching the pigments and elimination of unpleasant odors (as a result of oxidation). SO_2 can selectively act against the wild yeasts that come from the grape skin or equipment in the winery and can stop their activity, since yeasts are very sensitive to SO_2 (also, to other stress factors). Sulphur dioxide can be added in the form of a salt, potassium metabisulphite ($K_2S_2O_5$), which can be ionized in acid media, releasing gaseous SO_2. Higher doses of SO_2 in the must can lead to faster and more efficient precipitation, especially SO_2 in free form contributes to better extraction of phenolic components present in grape skins and seeds.

The potential of yeast to affect the extraction of grape phenolic compounds has already attracted the scientist's attention. The first study of the influence of yeast selection on the phenolic profile of Burgundy wine (Cuinier 1988) presented small effects on color intensity and total phenolics. Other studies focused on the effect of two yeast strains used for vinification of Pinot noir, as well as the Merlot wines, showed that no differences in total phenolics, anthocyanins, and color intensity were observed in the wines fermented with both yeasts (Gil-Muñoz et al. 1999; Girard et al. 2001). Recent studies showed that anthocyanins can be adsorbed at the yeast cell walls (Morata et al. 2003; Mazauric and Salmon 2005).

During maturation—aging and storage of wines, colored and noncolored phenolics have an important role on the color and taste of wine and they undergo a number of reactions during aging that result in changes in the sensory characteristics. Those changes are mostly due to the conversion of grape anthocyanins to derived pigments (Somers 1971) through reactions of anthocyanins with pyruvic acid (carboxy-pyrano-anthocyanins) and acetaldehyde (pyrano-anthocyanins) leading to pyranoanthocyanin formation (Timberlake and Bridle 1976; Glories 1984; Bakker and Timberlake 1986; Fulcrand et al. 1996; Remy et al. 2000). Another possible mechanism of the evolution of phenolics during wine aging that takes place spontaneously is the formation of direct condensation adducts between anthocyanins and flavonols (Timberlake and Bridle 1976), as well as, formation of ethyl linked pigments formed by acetaldehyde-mediated condensation between anthocyanins and flavan-3-ols. All of these formed adducts contribute to the color stability of wine and decrease in astringency.

REFERENCES

Alcalde-Eon, C., Escribano-Bailón, M. T., Santos-Buelga, C., and Rivas-Gonzalo, J. C. 2006. Changes in the detailed pigment composition of red wine during maturity and ageing. A comprehensive study. *Anal. Chim. Acta* 563:238–54.

Amico, V., Napoli, E. M., Renda, A., Ruberto, G., Spatafora, C., and Tringali, C. 2004. Constituents of grape pomace form the Sicilian cultivar "Nerello Mascalese." *Food Chem.* 88:599–607.

Amira-Guebailia, H., Chira, K., Richard, T., Mabrouk, T., Furiga, A., Vitrac, X., Monti, J. P., Delaunay, J. C., and Mérillon, J. M. 2006. Hopeaphenol: The first resveratrol tetramer in wines from North Africa. *J. Agric. Food Chem.* 54:9559–64.

Arnold, R. A., Noble, A. C., and Singleton, V. L. 1980. Bitterness and astringency of phenolic fractions in wine. *J. Agric. Food Chem.* 28:675–8.

Arozarena, I., Casp, A., Marín, R., and Navarro, M. 2000. Multivariate differentiation of Spanish red wines according to region and variety. *J. Sci. Food Agric.*70:1909–17.

Bakker, J., Bridle, P., Honda, T., Kuwano, H., Saito, N., Terahara, N., and Timberlake, C. F. 1997. Identification of an anthocyanin occurring in some red wines. *Phytochemistry* 44:1375–82.

Bakker, J., and Timberlake, C. F. 1986. The mechanism of color changes in aging port wine. *Am. J. Enol. Vitic.* 37:288–92.

Baldi, A., Romani, A., Mulinacci, N., Vincieri, F. F., and Casetta, B. 1995. HPLC/MS application to anthocyanins of Vitis-vinifera L. *J. Agric. Food Chem.* 43:2104–9.

Bate-Smith, E. C. 1954. Leuco-anthocyanins. 1. Detection and identification of anthocyanidins formed from leuco-anthocyanins in plant tissues. *Biochem. J.* 58:122–5.

Bautista-Ortín, A. B., Fernádez-Fernádez, J. I., López-Roca, J. M., and Gómez-Plaza, E. 2004. Wine-making of high coloured wines: Extended pomace contact and run-off of juice prior to fermentation. *Food Sci. Technol. Int.* 10:287–95.

Berg, H. W., and Akiyoshi, M. A. 1975. On the nature of reactions responsible for color behavior in red wine: A hypothesis. *Am. J. Enol. Vitic.* 26:134–43.

Bourzeix, M., Weyland, D., and Heredia, N. 1986. Etude des catechines et des procyanidols de la grape de raisin, du vin et d'autres derives de la vigne. *Bull OIV* 59:1171–254.

Brouillard, R., Delaporte, B., and Dubois, J. E. 1978. Chemistry of anthocyanin pigments. 3. Relaxation amplitudes in pH-jump experiments. *J. Am. Chem. Soc.* 100:6202–5.

Brouillard, R., and Dubois, J. E. 1977. Mechanism of structural transformations of anthocyanins in acidic media. *J. Am. Chem. Soc.* 99:1359–64.

Burns, J., Gardner, P. T., O'Neil, J., Crawford, S., Morecroft, I., Mc Phail, D. B., Lister, C., et al. 2000. Relationship among antioxidant activity, vasodilation capacity, and phenolic content of red wines. *J. Agric. Food Chem.* 48:220–30.

Canals, R., Llaudy, M. C., Valls, J., and Canals, J. M. 2005. Influence of ethanol concentration on the extraction of color and phenolic compounds from the skin and seeds of Tempranillo grapes at different stages of ripening. *J. Agric. Food Chem.* 53:4019–25.

Castillo-Muñoz, N., Gómez-Alonso, S., García-Romero, E., and Gutiérrez I. H. 2007. Flavonol profiles of *Vitis vinifera* red grapes and their single-cultivar wines. *J. Agric. Food Chem.* 55:992–1002.

Cheynier, V., Basire, N., and Rigaud, J. 1989. Mechanism of trans-caffeoyltartaric acid and catechin oxidation in model solutions containing grape polyphenoloxidase. *J. Agric. Food Chem.* 37:1069–71.

Cheynier, V., and Rigaud, J. 1986. HPLC separation and characterization of flavonols in the skins of Vitis vinifera var. Cinsault. *Am. J. Enol. Vitic.* 37:248–52.

Cheynier, V., Rigaud, J., Souquet, J. M., Duprat, F., and Moutounet, M. 1990. Must browning in relation to the behavior of phenolic compounds during oxidation. *Am. J. Enol. Vitic.* 41:346–9.

Cuinier, C. 1988. Influence des levures sur les composés phénoliques du vin. *Bull. OIV* 689–690:596–601.

Czochanska, Z. Y., Newman, R. H., Porter, L. J., Thomas, W. A., and Jones, W. T. 1979. Direct proof of a homogeneous polyflavan-3-ol structure for polymeric proanthocyanidins. *J. Chem. Soc. Chem. Commun.* 8:375–7.

Da Silva, J. M. R., Bourzeix, M., Cheynier, V., and Moutounet, M. 1991a. Procyanidin composition of Chardonnay, Mauzac and Grenache blanc grapes. *Vitis* 30:245–52.

Da Silva, J. M. R., Rigaud, J., Cheynier, V., Cheminat, A., and Moutounet, M. 1991b. Procyanidin dimers and trimers from grape seeds. *Phytochemistry* 30:1259–64.

Favretto, D., and Flamini, R. 2000. Application of electrospray ionization mass spectrometry to the study of grape anthocyanins. *Am. J. Enol. Vitic.* 51:55–64.

Foo, L. Y., McGraw, G. W., and Hemingway, R. W. 1983. Condensed tannins: Preferential substitution at the interflavanoid bond by sulphite ion. *J. Chem. Soc. Chem. Commun.* 12:672–3.

Fulcrand, H., Benabdeljalil, C., Rigaud, J., Cheynier, V., and Mountounet, M. 1998. A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins. *Phytochemistry* 47:1401–7.

Fulcrand, H., Doco, T., Es-Safi, N. E., Cheynier, V., and Moutounet, M. 1996. Study of the acetaldehyde induced polymerisation of flavan-3-ols by liquid chromatography-ion spray mass spectrometry. *J. Chromatogr. A* 752:85–91.

Girard, B., Yuksel, D., Cliff, M. A., Delaquis, P., and Reynolds, A. G. 2001. Vinification effects on the sensory, colour, and GC profiles of Pinot noir wines from British Colombia. *Food Res. Int.* 34:483–99.

Giusti, M. M., Rodriguez-Saona, L. E., Griffin, D., and Wrolstad, R. E. 1999. Electrospray and tandem mass spectroscopy as tools for anthocyanin characterization. *J. Agric. Food Chem.* 47:4657–64.

Gil-Muñoz, R., Gómez-Plaza, E., Martínez, A., and López-Roca, J. M. 1997. Evolution of the CIELAB and other spectrophotometric parameters during wine fermentation. Influence of some pre and postfermentative factors. *Food Res. Int.* 30:699–705.

Gil-Muñoz, R., Gómez-Plaza, E., Martínez, A., and López-Roca, J. M. 1999. Evolution of phenolic compounds during wine fermentation and post-fermentation: Influence of grape temperature. *J. Food Compos. Anal.* 12:259–72.

Glories, Y. 1984. La couleur des vins rouges. Mesure, origine et interprétation. Partie I. *Connaiss. Vigne Vin* 18:195–217.

González-San José, M. L., Barrón, L. J. R., and Díez, C. 1990. Evolution of anthocyanins during maturation of Tempranillo grape variety (Vitis vinifera) using polynomial regression models. *J. Sci. Food Agric.* 51:337–43.

Häkkinen, H., Kärenlampi, S. O., Heinonen, I. M., Mykkänen, H. M., and Törroönen, A. R. 1998. HPLC method for screening of flavonoids and phenolic acids in berries. *J. Sci. Food Agric.* 77:543–51.

Ivanova, V., Stefova, M., and Vojnoski, B. 2009. Assay of the phenolic profile of Merlot wines from Macedonia: Effect of maceration time, storage, SO_2 and temperature of storage. *Maced. J. Chem. Chem. Eng.* 28:141–9.

Kennedy, J. A., and Jones, G. P. 2001. Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *J. Agric. Food Chem.* 49:1740–6.

Koupai-Abyazani, M. R., McCallum, J., and Bohm, B. A. 1992. Identification of the constituent flavonoid units in sainfoin proanthocyanidins by reversed-phase high-performance liquid chromatography. *J. Chromatogr.* 594:117–23.

Larue, F., Park, M. K., and Caruana, C. 1985. Quelques observations sur les conditions de la formation d'anhydride sulfureux en vinification. *Connaiss. Vigne Vin* 19:241–8.

Lee, C. Y., and Jaworski, A. W. 1987. Phenolic compounds in white grapes grown in New York. *Am. J. Enol. Vitic.* 38:277–81.

Mazauric, J. P., and Salmon, J. M. 2005. Interactions between yeast lees and wine polyphenols during simulation of wine aging: I. Analysis of remnant polyphenolic compounds in the resulting wines. *J. Agric. Food Chem.* 53:5647–53.

Morata, A., Gómez-Corovés, M. C., Suberviola, J., and Bartolomé, B. 2003. Adsorption of anthocyanins by yeast cell walls during the fermentation of red wines. *J. Agric. Food Chem.* 51:4084–8.

Nagel, C. W., and Wulf, L. W. 1979. Changes in the anthocyanins, flavonoids and hydroxycinnamic acid esters during fermentation and aging of Merlot and Cabernet Sauvignon. *Am. J. Enol. Vitic*. 30:111–6.

Núñez, V., Monagas, M., Gómez-Cordovés, C., and Bartolom, B. 2004. *Vitis vinifera* L. cv. Graciano grapes characterized by its anthocyanin profile. *Postharvest Biol. Technol.* 31:69–79.

Piretti, M. V., Ghedini, M., and Serrazanetti, G. 1976. Isolation and identification of the polyphenolic and terpenoid constituents of *Vitis vinifera*. v. Trebbiano variety. *Annali di Chimica* 66:429–37.

Porter, L. J., Hirtstich, L. N., and Chang, B. G. 1986. The conversion of procyanidins and prodelphinidins to cyanidins and delphinidins. *Phytochemistry* 25:223–30.

Prieur, C., Rigaud, J., and Cheynier, V. 1994. Oligomeric and polymeric procyanidins from grape seeds. *Phytochemistry* 36:781–4.

Remy, S., Fulcrand, H., Labarbe, B., Cheynier, V., and Moutounet, M. 2000. First confirmation in red wine of products resulting from direct anthocyanin-tannin reactions. *J. Sci. Food Agric.* 80:745–51.

Ribereau-Gayon, P. 1965. La couleur des vins. *Aliment Vie* 53:232–48.

Rigaud, J., Perezilzarbe, J., Da Silva, J. M. R., and Cheynier, V. 1991. Micro method for the identification of proanthocyanidin using thiolysis monitored by high-performance liquid-chromatography. *J. Chromatogr.* 540:401–5.

Romero-Pérez, A. I., Lamuela-Raventós, R. M., Buxaderas, S., and de la Torre-Boronat, M. C. 1996. Resveratrol and piceid as varietal markers of white wines. *J. Agric. Food Chem.* 44:1975–8.

Sarni-Manchado, P., Cheynier, V., and Moutounet, M. 1999. Intereaction of grape seed tannins with salivary proteins. *J. Agric. Food Chem.* 47:42–7.

Shen, Z., Haslam, E., Falshaw, C. P., and Begley, M. J. 1986. Procyanidins and polyphenols of *Larix gmelini* bark. *Phytochemistry* 25:2629–35.

Singleton, V. L., Salgues, M., Zaya, J., and Trousdale, E. 1985. Caftaric acid disappearance and conversion to products of enzymic oxidation in grape must and wine. *Am. J. Enol. Vitic.* 36:50–6.

Somers, T. C. 1971. The polymeric nature of wine pigments. *Phytochemistry* 10:2175–86.

Slinkard, K. W., and Singleton, V. L. 1984. Phenol content of grape skins and the loss of ability to make anthocyanins by mutation. *Vitis* 23:175–8.

Souquet, J. M., Cheynier, V., and Brossaud, F. 1996. Polymeric proanthocyanidins from grape skins. *Phytochemistry* 43:509–12.

Souquet, J. M., Labarbe, B., Le Guernevé, C., Cheynier, V., and Moutounet, M. 2000. Phenolic composition of grape stems. *J. Agric. Food Chem.* 48:1076–80.

Spayd, S. E., Tarara, J. M., Mee, D. L., and Ferguson, J. C. 2002. Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv Merlot berries. *Am. J. Enol. Vitic.* 53:171–82.

Su, C. T., and Singleton, V. L. 1969. Identification of three flavan-3-ols from grapes. *Phytochemistry* 8:1553–8.

Sun, B., Ribes, A. M., Leandro, M. C., Belchior, A. P., and Spranger, M. I. 2006. Stilbenes: Quantitative extraction from grape skins, contribution of grape solids to wine and variation during wine maturation. *Anal. Chim. Acta* 563:382–90.

Thompson, R. S., Jacques, D., Haslam, E., and Tanner, R. J. N. 1972. Plant proanthocyanidins. Part I. Introduction; the isolation, structure, and distribution in nature of plant procyanidins. *J. Chem. Soc. Perkin I* 1387–99.

Timberlake, C. F., and Bridle, P. 1976. Interactions between anthocyanins, phenolic compounds, and acetaldehyde and their significance in red wines. *Am. J. Enol. Vitic.* 27:97–105.

Trousdale, E. K., and Singleton, V. L. 1983. Astilbin and engeletin in grapes and wine. *Phytochemistry* 22:619–20.

Viñas, P., Campillo, N., Hernández-Pérez, M., and Hernández-Córdoba, M. 2008. A comparison of solid-phase microextraction and stir bar sorptive extraction coupled to liquid chromatography for the rapid analysis of resveratrol isomers in wines, musts and fruit juices. *Anal. Chim. Acta* 611:119–25.

Vitrac, X., Castagnino, C., Waffo-Téguo, P., Delaunay, J. C., Vercauteren, J., Monti, J. P., Deffieux, G., and Mérillon, J. M. 2001. Polyphenols newly extracted in red wine from southwestern France by centrifugal partition chromatography. *J. Agric. Food Chem.* 49:5934–8.

Wang, H., Race, E. J., and Shrikhande, A. J. 2003. Anthocyanin transformation in Cabernet Sauvignon wine during aging. *J. Agric. Food Chem.* 51:7989–94.

Wulf, L. W., and Nagel, C. W. 1978. High-pressure liquid chromatography separation of anthocyanins of *Vitis Vinifera. Am. J. Enol. Vitic.* 29:42–9.