

PHENOLIC COMPOUNDS IN GRAPES

S. Ursu, PhD student

Technical University of Moldova

INTRODUCTION

Phenolic compounds play a major role in enology. They are responsible for all the differences between red and white wines, especially the color and flavor of red wines. They have interesting, healthful properties, responsible for the "French paradox". They have bactericide, antioxidant and vitamin properties that apparently protect consumers from cardiovascular disease [2].

These molecules come from various parts of grape bunches and are extracted during winemaking. Their structure varies a great deal when wine ages in the barrel or in the tank and in the bottle, according to the conditions, but these modifications have not yet been fully explained [1].

Indeed, even the latest chromatography techniques (HPTLC, LPLC, HPLC) still produce relatively limited results and are only capable of analyzing simple and little polymerized molecules.

Furthermore, physicochemical methods, focused on structural definition (NMR, mass spectrometry), are not very well-suited to the study of these types of molecules, although their applications are constantly being extended. Further complications are due to the interference of a colloidal state that does not involve covalent bonds. This interference definitely plays a role in the structure and, consequently, the properties of phenolic compounds in wine. The colloidal state is, however, difficult to study, as it is modified by any manipulation of these substances [4].

(yeasts in the genus *Brettanomyces* and bacteria). Ethyl phenols, with animal odors, and ethyl gaiacols are found in red wines. In white wines, vinyl phenols, with an odor reminiscent of gouache paint, are accompanied by vinyl gaiacols. It has been clearly established that these compounds result from the breakdown of *p*-coumaric acid and ferulic acid [5].

When wines are aged in new oak barrels, the toasting of the wood involved in barrel manufacture causes the breakdown of lignins and the formation of various components in the same family, with a variety of smoky, toasty and burnt smells (Figure 1): gaiacol, methyl gaiacol, propyl gaiacol, allyl gaiacol (isoeugenol), syringol and methyl syringol.


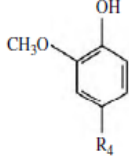
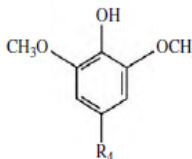
	R₄	Name	Origin
	CH ₂ -CH ₃	Ethyl phenol	Red wine
	CH=CH ₂	Vinyl phenol	White wine
	R₄	Name	Origin
	H	Gaiacol	Wood
	CH ₃	Methyl gaiacol	Wood
	CH ₂ -CH ₃	Ethyl gaiacol	Red wine
	CH=CH ₂	Vinyl gaiacol	White wine
	CH ₂ -CH ₂ -CH ₃	Propyl gaiacol	Wood
	R₄	Name	Origin
	H	Syringol	Wood
	CH ₃	Methyl Syringol	Wood

Figure 1. Volatile phenols in wine [1]

1. TYPES OF SUBSTANCES

1.2. Phenolic Acids

Grapes and wine contain benzoic and cinnamic acids. Concentrations are on the order of 100–200 mg/L in red wine and 10–20 mg/L in white wine.

Phenolic acids are colorless in a dilute alcohol solution, but they may become yellow due to oxidation. From an organoleptic standpoint, these compounds have no particular flavor or odor. They are, however, precursors of the volatile phenols produced by the action of certain microorganisms

Tyrosol (Figure B) or *p*-hydroxy-phenyl-ethyl alcohol may be included in this group of compounds. It is always present in both red and white wine (20–30 mg/L) and is formed during alcoholic fermentation from tyrosine (*p*-hydroxy phenylalanine), in turn synthesized by yeast. This compound, which remains at relatively constant concentrations throughout aging, is accompanied by other non-phenolic alcohols like tryptophol (0–1 mg/L) and phenyl-ethyl alcohol (10–75 mg/L).

Coumarins (Figure 2) may be considered derivatives of cinnamic acids, formed by the intramolecular esterification of a phenol OH into the α of the carbon chain. These molecules are

components of oak, either in glycosylated form (esculetin and scopoline) in green wood or in

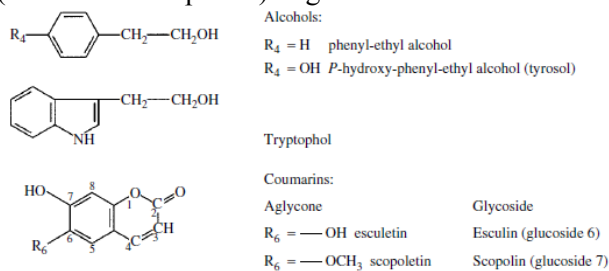


Figure 2. Phenolic alcohols and coumarins [1]

aglycone form (esculetin and scopoletin) in naturally seasoned wood. Although very small quantities (a few $\mu\text{g/l}$) of coumarins are found in wood-aged wine, they still affect its organoleptic characteristics, as glycosides are bitter and aglycones are acidic, with a detection threshold in red wine of 3 $\mu\text{g/L}$ [4].

Another family of more complex polyphenols is also present in grapes, wine and oak wood. Stilbens have two benzene cycles, generally bonded by an ethane, or possibly ethylene, chain. Among these *trans*-isomer compounds, resveratrol or 3, 5, 4- trihydroxystilben (Figure 3), is thought to be produced by vines in response to a fungal infection.

Resveratrol, located in the skins, is mainly extracted during the fermentation of red wines and seems to have some healthful properties. Concentrations are on the order of 1–3 mg/l. Recent research has identified many oligomers of resveratrol in *Vitis vinifera* [3].

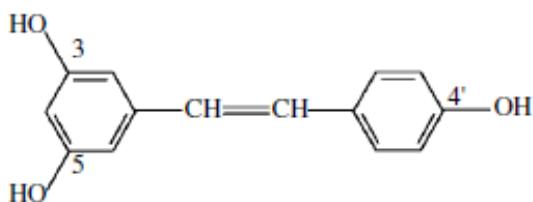


Figure 3. Trihydroxy-3, 5, 4-stilben [4]

2. FLAVONOIDS

These are more-or-less intense yellow pigments (Figure 4), with a structure characterized by two benzene cycles bonded by an oxygenated heterocycle, derived either from the 2-phenyl chromone nucleus (flavones and flavonols) or the 2-phenyl chromanone nucleus (flavanones and flavanonols).

The most widespread compounds are flavonols, yellow pigments in the skins of both red and white grapes and, to a lesser extent, flavanonols, which are much paler in color. In grapes, these molecules

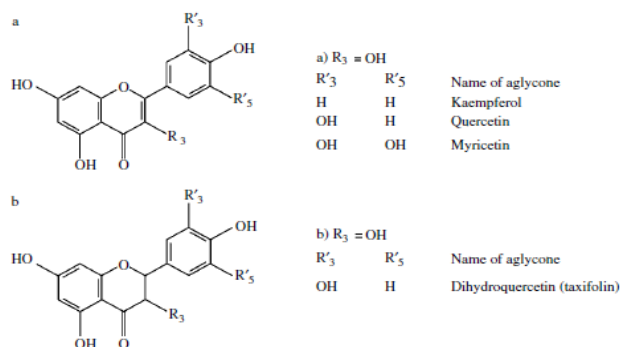


Figure 4. Flavonoids: a, flavone ($R_3 = H$) and flavonol ($R_3 = OH$); b, flavanone ($R_3 = H$) and flavanonol ($R_3 = OH$) [1]

are present in glycoside form (Figure 5), e.g. rhamnosylquercetin. They are differentiated by substitution of the lateral nucleus, producing kaempferol (1 OH), quercetin (2 OH) and myricetin (3 OH). All three pigments are present in red wine grapes, whereas white wine grapes only have the first two [2].

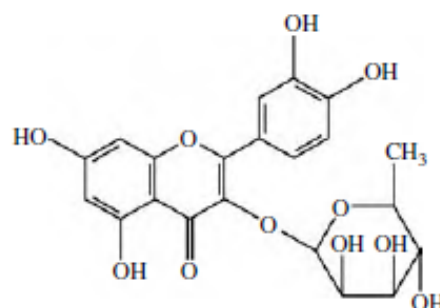


Figure 5. 3-*O*-Rhamnosylquercetin [1].

These compounds are present in red wine in aglycone form, as the glycosides are hydrolyzed during fermentation. Concentrations are in the region of 100 mg/l. In white wine, where fermentation takes place in the absence of grape solids, typical values are from 1 to 3 mg/l according to the grape variety. Pre-fermentation maceration in the aqueous phase has less impact on this concentration than settling.

The flavanonol most frequently identified in grapes and wine is dihydroquercetin, also known as taxifolin. The role played by these various compounds in the color of red and white wines will be discussed later in this chapter [3].

3. ANTHOCYANINS

Anthocyanins are the red pigments in grapes, located mainly in the skin and, more unusually, in the flesh (“teinturier” grape varieties).

They are also present in large quantities in the leaves, mainly at the end of the growing season.

Their structure, flavylum cation, includes two benzene rings bonded by an unsaturated cationic oxygenated heterocyclic, derived from the 2-phenyl-benzopyrylium nucleus. Five molecules have been identified in grapes and wines, with two or three substituent (OH and OCH₃) according to the substitution of the lateral nucleus (Figure 6).

These molecules are much more stable in glycoside (anthocyanin) than in aglycone (anthocyanidin) form. Only monoglucoside anthocyanins (Figure 7) and acylated monoglucoside anthocyanins have been identified in *Vitis vinifera* grapes and wines; acylation is made with *p*-coumaric (Figure 7), caffeic and acetic acids [3].

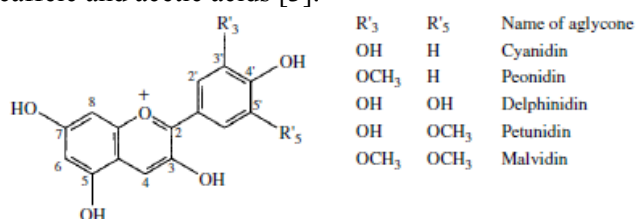
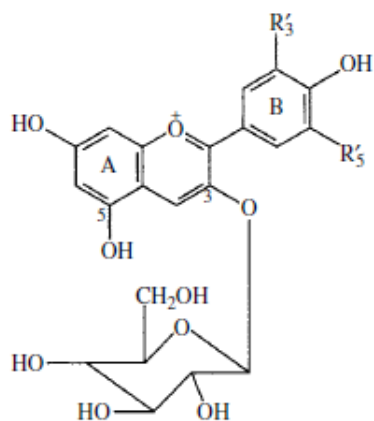


Figure 6. Structure of anthocyanidins in grapes and wine [3]

a



b

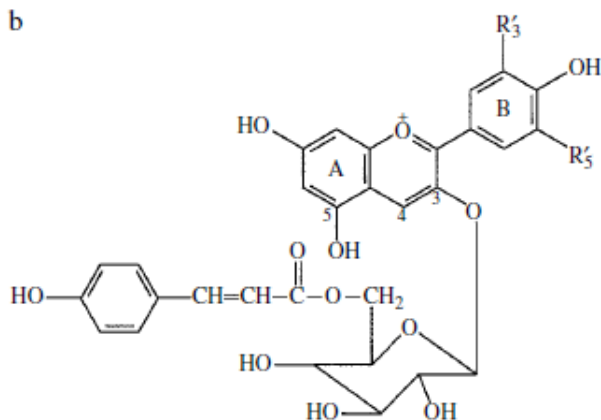


Figure 7. Structure of: (a) anthocyanin 3-monoglucosides, (b) anthocyanins 3-monoglucosides acylated by *p*-coumaric acid on position 5 of the glucose (R₃ and R₅) [1]

The presence of diglucoside anthocyanins (Figure 8) in large quantities is specific to certain species in the genus *Vitis* (*V. riparia* and *V. rupestris*). Traces have, however, been found in certain *V. vinifera* grapes. The “diglucoside” character is transmitted according to the laws of genetics, as a dominant characteristic. This means that a cross between a *vinifera* grape variety and an American species (*V. riparia* or *V. rupestris*) produces a population of first-generation hybrids that have all the diglucosides. On the other hand, results obtained with a new cross between a first-generation hybrid and a *V. vinifera* vine show that the recessive “absence of diglucoside” characteristic may be expressed in a second-generation hybrid.

These findings led to the development of the method for differentiating wines by chromatographic analysis of their coloring matter.

This played a major role in ensuring that traditional grape varieties were used in certain French appellations of origin, as well as in monitoring quality.

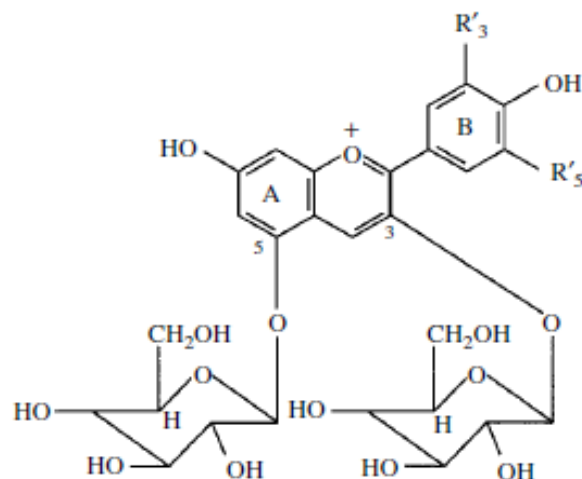


Figure 8. Structure of anthocyanin 3,5-diglucosides (R₃ and R₅ see Figure 7) [1]

The color of these pigments depends on conditions in the medium (pH, SO₂), as well as the molecular structure and the environment. On the one hand, substitution of the lateral cycle leads to a bath chrome shift of the maximum absorption wavelength (towards violet). On the other hand, glucose fixation and acylation shift the color in the opposite direction, i.e. towards orange. These molecules are mainly located in the skin cells, with a concentration gradient from the inside towards the outside of the grape.

Pigment molecules are in solution in the vacuolar juice in the presence of other polyphenols (phenolic acids, flavonoids, etc.) likely to affect their color. Copigmentation generally gives wines a

violet tinge. These factors explain the different colors of red grapes. All grape varieties have the same basic anthocyanidin structures, but there are a few small variations in composition. Indeed, among the five anthocyanins, malvidin is the dominant molecule in all grape varieties, varying from 90% (Grenache), 50% (Sangiovese). Malvidin monoglucoside (malvine) may be considered to form the basis of the color of red grapes and, by extension, red wine [1].

On the other hand, the quantity of acylated monoglucosides is highly variable according to the grape variety. In *vitis vinifera* wines, the presence of ethanol works against copigmentation (Section 6.3.8), and the acylated anthocyanins disappear rapidly a few months after fermentation, so it is not reasonable to use them to identify grape varieties.

This leaves only the five monoglucosides, predominantly malvidin. Concentrations vary a great deal according to the age of the wines and the grape varieties. Starting at levels of 100 mg/l (Pinot Noir) to 1500 mg/l (Syrah, Cabernet Sauvignon, etc.) after fermentation, they decrease rapidly in the first few years, during barrel and bottle aging, until they reach a minimum value on the order of 0–50 mg/l. In fact, this concentration was determined by a free anthocyanin assay, using chemical and chromatographic methods. In fact, the majority of these pigments combines and condenses with tannins in wine to form another, more stable, class of color molecules that are not detected by current assay methods. These complex combined anthocyanins are responsible for color in wine but cannot be identified by standard analyses. Another relatively small fraction of the anthocyanins, however, disappears, either broken down by external factors (temperature, light, oxygen, etc.) or precipitated in colloidal coloring matter. The elimination of these pigments is particularly detrimental to the quality of the wine, as it leads to loss of color [4].

Another recently demonstrated property of anthocyanins involves their reaction with compounds containing an α -dicarbonylated group, such as diacetyl ($\text{CH}_3\text{—CO—CO—CH}_3$). This reaction gives rise to castavinols (Figure 9), not present in grapes but formed spontaneously in wine. These colorless compounds are capable of regenerating colored anthocyanins in an acid medium, by a process called the Bate–Smith

reaction, which converts procyanidins into cyanidin. However, in the case of castavinols, this reaction does not require very high temperatures and acidity as it occurs spontaneously and gradually in wine during aging. The color of the anthocyanin is stabilized by substitution of the molecule in carbon

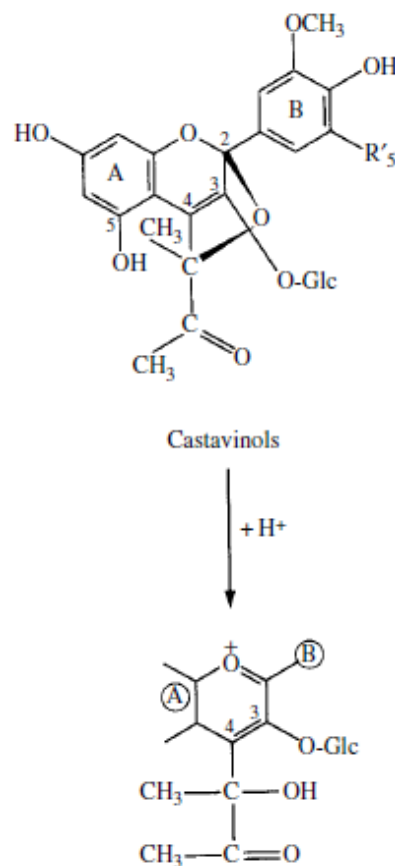


Figure 9. Structure of castavinols resulting from fixing diacetyl on carbons 2 and 4 of the anthocyanin and their transformation into flavylium substituted in 4 and colored by heating in an acid medium [1]

4. According to several authors, the concentration of castavinols in wine is on the order of a few mg/l. Nevertheless, these substances are likely to play a role as reserves of coloring matter [3].

4. TANINNS

Tannins are, by definition, substances capable of producing stable combinations with proteins and other plant polymers such as polysaccharides. The transformation of animal skins into rot proof leather results from this property, as doe's astringency, fining and enzyme inhibition. Tannins react with proteins in each instance:

collagen in tanning, glycoproteins in saliva and proline-rich proteins (PRP) for astringency, protein-based fining agents in fining wines and the protein fraction of enzymes. In chemical terms, tannins are relatively bulky phenol molecules, produced by the polymerization of elementary molecules with phenolic functions. Their configuration affects their reactivity. They must be sufficiently bulky to produce stable combinations with proteins, but if they are too bulky, they are likely to be too far from the active protein sites. The molecular weights of active tannins range approximately from 600 to 3500. Condensed or catechic tannins are distinguished from complex or mixed tannins by the type of elementary molecules. Hydrolyzable tannins include gallotannins and ellagitannins that release gallic acid and ellagic acid, respectively (Figure 10 a, b), after acid hydrolysis. They also contain a glucose molecule. The two main ellagitannin isomers in oak used for cooperage are vescalagin and castalagin ($M = 934$), as well as two less important compounds, grandinin and roburin (Figure 10). These molecules include a hexahydroxydiphenic and a nonahydroxydiphenic acid, esterified by a non-cyclic glucose [1].

The partial hydrolysis of vescalagin and castalagin, involving the loss of hexahydroxydiphenic acid, produces vescalin and castalin ($M = 632$) (Figure J c). The various molecules are water soluble and dissolve rapidly in dilute alcohol media such as wines and brandies. They play a considerable role in the aging of red and white wines in oak barrels, due to their oxidizability and flavor properties. The ellagitannin composition of extracts from the duramen depends on the species of oak. All four monomeric and four dimeric (roburin A, B, C and D) ellagitannins are present in the three species of European oak, while the American species have practically no dimmers. Hydrolyzable tannins are not naturally found in grapes. On the other hand, they are the main commercial tannins legally authorized as wine additives. Ellagic acid in wine originates either from wooden containers or from the addition of enological tannins. On the other hand, gallic acid from the skins and seeds is always present in wine. Condensed tannins in grapes and wine are more or less complex polymers of flavan-3-ols or catechins. The basic structural units are (+) - catechin and (-) - epicatechin (Figure 10). Heating these polymers in

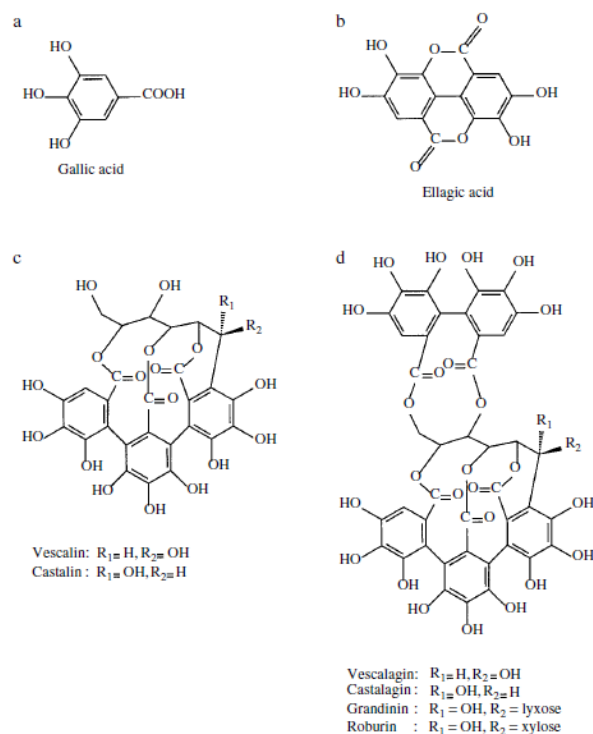


Figure 10. Structure of phenolic acids (a and b) and ellagitannins (c and d) in extracts from the duramen of oak and chestnut wood [1]

solution in an acid medium releases highly unstable carbocations that are converted into brown condensation products, mainly red cyanidin, which explains why these compounds are known as “procyanidins”, replacing the formerly used term “leucocyanidin”. Analysis of these molecules is particularly complex, due to the great structural diversity resulting from the number of hydroxyl groups, their position on the aromatic nuclei, the stereochemistry of the asymmetrical carbons in the pyran cycle, as well as the number and type of bonds between the basic units. In spite of the progress made in liquid chromatography, mass spectrometry and NMR, all of the structures have not been analyzed: only the procyanidin dimers and some of the trimers have been completely identified. This diversity explains the existence of tannins with different properties, especially as regards flavor, in various types of grapes and wine.

Tannin content should not be the only factor considered, as structure and colloidal status also affect the impression tannins give on tasting [3]. It is possible to isolate and fractionate the following constituents of grapes and wine: (+) - catechin, gallocatechin, (-) - epicatechin, epigallocatechin,

and epicatechin-3-0-gallate. There are also dimeric, trimeric, oligomeric, and condensed procyanidins. Basic “catechin” units may not be considered as tannins, as their molecular weight is too low and they have very restricted properties in relation to proteins. They only have a high enough molecular weight in dimeric form to bond stably with proteins. Catechins (Figure 11) have two benzene cycles bonded by a saturated oxygenated heterocyclic (phenyl-2 chromane nucleus). This structure has two asymmetrical carbons (C2 and C3) that are the origin of the four isomers. The more stable forms are (+) - catechin and (–) - epicatechin.

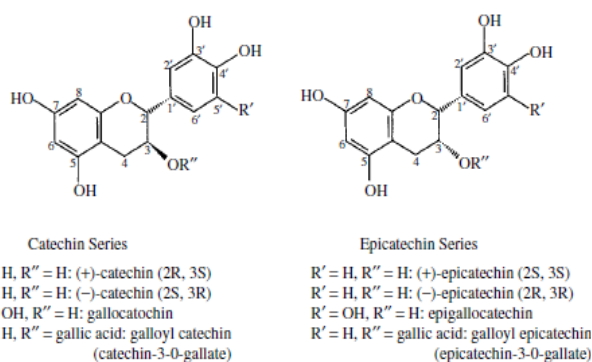


Figure 11. Structure of flavan-3-ol precursors of procyanidins and tannins [3]

Dimeric procyanidins may be divided into two categories, identified by a letter of the alphabet and a number:

1. Type-B procyanidins (C₃₀H₂₆O₁₂) are dimers resulting from the condensation of two units of flavan-3-ols linked by a C4–C8 (B1 to B4) or C4–C6 (B5 to B8) bond. As there are five different types of monomers and two types of intermonomeric bonds, there may be $2 \times 5^2 = 50$ dimers in wine. The eight procyanidins presented have been identified as the most common ones in wine.

2. Type-A procyanidins (C₃₀H₂₄O₁₂) are dimers that, in addition to the C4–C8 or C4–C6 interflavan bond, also have an ether bond between the C5 or C7 carbons of the terminal unit and the C2 carbon of the upper unit. Procyanidin A2 has been identified in wine Form B can change to form A via a radical process [4].

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