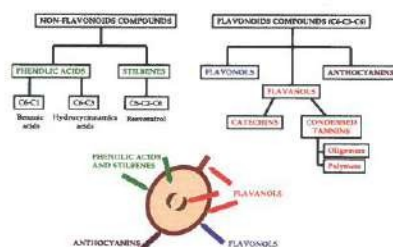


Phenolic Compounds

Types, Effects and Research



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NOVA

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Abstract

Wine is one of the most researched beverages due to its outstanding phenolic content. Phenolic compounds are responsible for the sensory attributes of wine, such as color and structure, and for health benefits derived from its consumption. The most studied substances are anthocyanins and flavones (related to color), tannins (causing astringency) and stilbenoids (antioxidants and cardiovascular protectors). Wine's phenolic compounds are important not only because of their high levels, but also their variability and heterogeneity, since they depend on geographic origin, grape variety and winemaking techniques. The different types of wine (white, red, rosé, naturally sweet, fortified, etc.) show different phenolic profiles in constant change during wine aging. Due to the various chemical equilibriums involved, even differences between vintages, oak or bottle storage can be observed in the phenolic content. In this chapter the main phenolic compounds present in wines are reviewed, in addition to their interactions and related effects, such as color stabilization, antioxidant capacity and wine structure.

1. Introduction

Phenolic compounds are secondary metabolites of grapes, whose biosynthesis seems to respond to specific needs of the vineyard [1]. For instance, wine content may depend on the sun exposure of grapes, given that some phenolics provide an effective protection against UV radiation [2]. Their phenolic content has been related with other factors such as the amount of nitrogen fertilizer applied to the vineyard [3], the water status of the vine [4] or the health status of the grape, given that some compounds may protect against fungal attacks [5].

Phenolic compounds are responsible for the typical sensory attributes of wine, such as color, bitterness and astringency. These substances have thus been traditionally described by winemakers as colored matter or tannic components [6]. For instance, anthocyanins are the phenolics that characterize the color of red wine, while tannins' capacity to coagulate proteins is responsible for its peculiar taste [7].

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Wine's phenolic composition largely depends on the raw material, normally grapes. Like any other plant product they are influenced by factors such as variety, ripeness at the time of harvest, cultivation techniques or climatic conditions, among other considerations [8]. Some phenolic compounds are produced during fermentation and others while the wine ages [9]. For instance, wine aged in oak contains substances coming from the wood. Due to this disparity of origins, wine phenolics are traditionally classified according to the primary source of the compound (Table 1).

Table 1. Wine phenolic composition according to the source, based on [10].

| |
|--|
| I. From grapes |
| Hydroxycinnamic acids (e.g. Caffeic acid) Hydroxybenzoic acids (e.g. Gallic acid) Anthocyanins (e.g. Malvidine-3-glucoside) Stilbenoids (e.g. Trans-resveratrol) Flavanols (e.g. Catechin) Flavonols (e.g. Quercetin) |
| II. Formed during fermentation |
| Non-flavonoid phenolic compounds (e.g. Tyrosol) |
| III. From the wood used for aging |
| Non-flavonoid phenolic compounds (e.g. P-vanillin) Hydrolyzable tannins |
| IV. Polymers formed during wine aging |
| Pyranoanthocyanins (e.g. Vitisins) Hydrolyzable tannins |

From a chemical point of view phenolic compounds are defined as structures characterized by a benzene ring together with one or more hydroxyl groups, and classified into two broad categories: flavonoids, which show a specific three-ring structure, and non-flavonoids [11]. Many compounds from the plant kingdom are flavonoids, a word coming from the Latin *flavus* meaning "yellow". This family includes several subgroups, such as anthocyanins, flavanols and flavonols. Non-Flavonoids are a highly heterogeneous group, as they include any other phenolic compound not considered flavonoid. Some non-flavonoid phenolic compounds are hydroxycinnamates, hydroxybenzoates and stilbenes. On the vine, non-flavonoid compounds are essentially located in the grape pulp, while flavonoids are in the skin, seeds and stalks. Therefore polyphenol extraction and final concentration largely depends on the winemaking technique and technology [12].

2. Phenolic compounds

Phenolic content is extremely variable due to its dependence on various external factors. A general idea of the phenolic levels in red and white wines is given in Table 2. Rosé wines are usually described with intermediate concentrations between red and white wines [13, 14].

Table 2. Range of phenolic compound concentrations in wine [15, 16].

| Compound (mg/l) | Red Wine | White Wine |
|--|------------------|----------------|
| <i>Non-Flavonoids</i> | <i>230-377</i> | <i>160-250</i> |
| -Hydroxybenzoic acids | 60-150 | 10-15 |
| i.e. Gallic acid | 55-126 | 4-11 |
| -Hydroxycinnamic acids | 60-165 | 130-154 |
| i.e. Caffeic acid | 4.7-12.8 | 1.3-3.6 |
| -Stilbenes | 0.2-7 | n.d.-0.5 |
| i.e. Resveratrol | 0.4-3.0 | n.d.-0.1 |
| -Hydrolyzable tannins | n.d.-250 | n.d.-100 |
| <i>Flavonoids</i> | <i>1300-1500</i> | <i>40-50</i> |
| -Flavanol monomers | 100-400 | 15-50 |
| i.e. Catechin | 120-390 | 16-46 |
| i.e. Epicatechin | 50-162 | 6-60 |
| -Flavonols | 10-100 | n.d. |
| i.e. Rutin | 2.4-31.7 | n.d. |
| i.e. Quercetin | 2.1-17.1 | n.d. |
| i.e. Myricetin | n.d.-17.9 | n.d. |
| -Anthocyanins | 90-400 | n.d. |
| i.e. Cyanidin-3-glycoside | n.d.-9.5 | n.d. |
| i.e. Malvidin-3-glycoside | n.d.-90.2 | n.d.-3.5 |
| -Proanthocyanidins and condensed tannins | 750-1000 | 20-30 |
| <i>Total phenols</i> | <i>1700-2000</i> | <i>200-300</i> |

Wine phenolic compounds are highly variable because of the differences in fruit sources (such as varietal characteristics or different ripening processes), as well as processing techniques (variations in maceration treatments or filtration processes). Red wine shows the highest phenolic content, due to such differences during winemaking. White wines are usually obtained avoiding contact with the grape skin, while red wines are made by fermenting the juice with skins and seeds, where many of the phenolic compounds are much more concentrated. Thus, red wines can be considered a purer fermented berry extract than white wines, which might thus be described as a product fermented almost exclusively from white grape juice. These differences mean red-wine phenolic compounds have been more extensively studied than those from white wines.

The main source of phenolic compounds in wine is the initial grape and its content depends greatly on its ripeness. However manufacturing and winemaking processes also affect the phenolic substances finally detected in wine. In fact, these compounds are in constant change during wine aging. Therefore the different techniques applied, such as aging in oak or prolonged contact with lees, also determine the final phenolic profile.

2.1. Non flavonoid compounds

This group includes diverse compounds often found in other foods derived from fruits and plants (Table 3). Phenolic acids are usually subclassified into benzoic and hydroxycinnamic acids. Stilbenes are considered phytoalexins appearing in the vineyard in response to external aggressive conditions. Phenolic alcohols are produced by yeasts during fermentation processes. Hydrolyzable tannins are present if wine has been in contact with wood during wine aging [17].

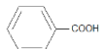
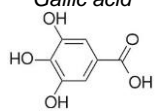
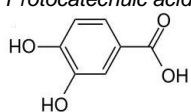
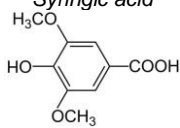
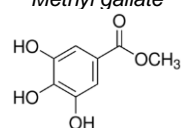
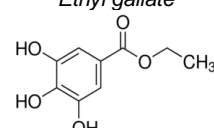
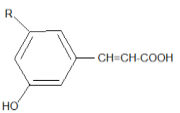
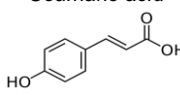
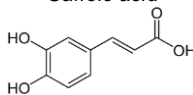
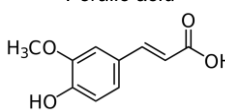
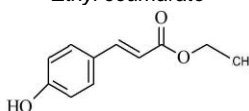
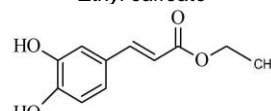
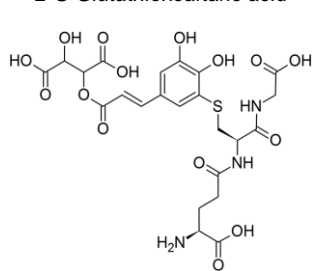
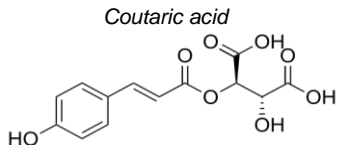
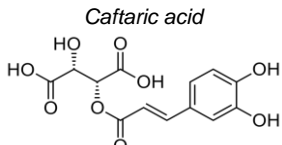
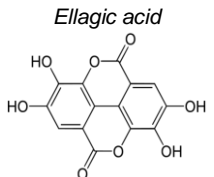
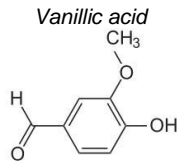
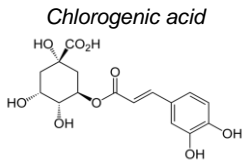
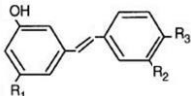
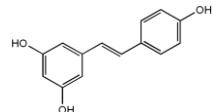
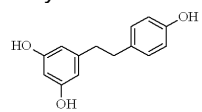
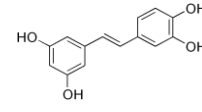
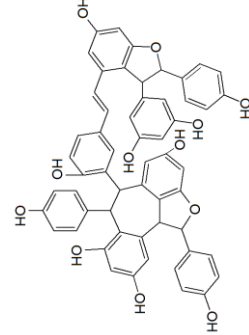
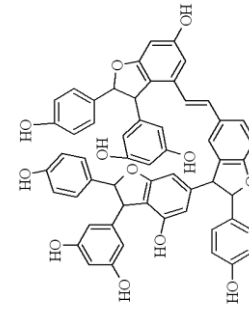
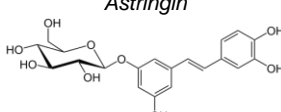
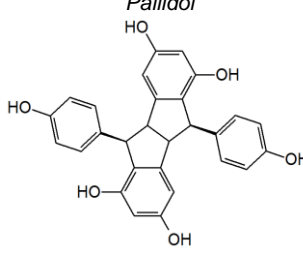
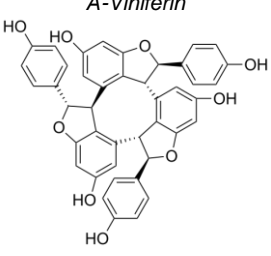
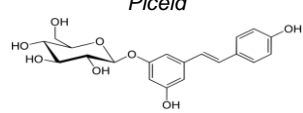
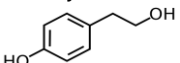
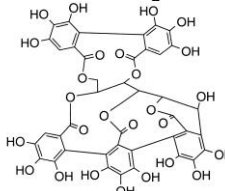
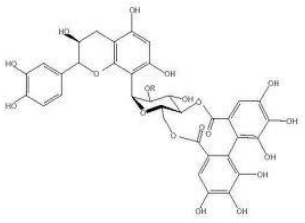
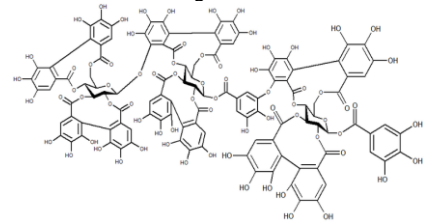
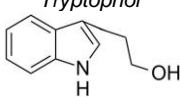
2.1.1. Phenolic acids

These compounds are characterized by a typical structure based on one benzene ring. Phenolic acids content is higher in red wines than white. They are mostly colorless, odorless and tasteless but during wine aging their oxidation products may produce yellow tonalities. Some phenolic acids become volatile phenols due to the action of microorganisms. These compounds can provoke undesired odors classified as wine defects [18]. Many phenolic acids can combine (normally esterified) with the organic acids and sugars present in wine.

2.1.1.1. Hydroxybenzoic acids

These substances are relatively few in wine in comparison with other phenolic compounds. Hydroxybenzoic acids are mostly found esterified in grapes and as free acids in wine due to hydrolysis reactions. Their concentration is relatively low in newly fermented wines but increases with time and stabilizes after a few months [19]. In wine the most common is gallic acid, with a concentration about 70 mg/l in red wines [20]. Gallic acid and its dimer ellagic acid are also found in oak wood; therefore the amount of both compounds in wine is greater after oak aging.

Table 3. Non-flavonoid phenolic compounds

| | | | | | | | |
|----------------|---|---|--|--|---|---|---|
| NON-FLAVONOIDS | <p>Hydroxybenzoic acids</p>  | <p>Gallic acid</p>  | <p>Protocatechuic acid</p>  | <p>Syringic acid</p>  | <p>Methyl gallate</p>  | <p>Ethyl gallate</p>  | |
| | <p>Hydroxycinnamic acids</p>  | <p>Coumaric acid</p>  | <p>Caffeic acid</p>  | <p>Ferulic acid</p>  | <p>Ethyl coumarate</p>  | <p>Ethyl caffeate</p>  | <p>2-S-Glutathioncaftaric acid</p>  |
| | | <p>Coumaric acid</p>  | <p>Caftaric acid</p>  | <p>Ellagic acid</p>  | <p>Vanillic acid</p>  | <p>Chlorogenic acid</p>  | |
| PHENOLICS | <p>Stilbenoids</p>  | <p>Resveratrol</p>  | <p>Dihydro-resveratrol</p>  | <p>Piceatannol</p>  | <p>Vitisin A (resveratrol tetramer & pyranoanthocyanin)</p>  | <p>Vitisin B (resveratrol tetramer & pyranoanthocyanin)</p>  | |
| | | <p>Astringin</p>  | <p>Pallidol</p>  | <p>A-Viniferin</p>  | | | |
| | | <p>Piceid</p>  | | | | | |
| WINES | <p>Others (fermentation products, polymers from wood)</p> | <p>Tyrosol</p>  | <p>Vescalagin</p>  | <p>Gallotannins</p>  | <p>Ellagitannins</p>  | | |
| | | <p>Tryptophol</p>  | | | | | |

2.1.1.2. Hydroxycinnamic acids

These polyphenolics are among the most abundant in grapes. They are mainly present as tartaric acid esters, caftaric acid (caffeoyl tartaric) predominating, followed by coutaric (p-coumaroyl tartaric) and fertaric acids (feruloyl tartaric). These esters are naturally present in grapes and later hydrolyzed in the acidic aqueous conditions of wine. As a result, simple hydroxycinnamic acids such as caffeic and coumaric acids are detected within a few weeks after fermentation begins [21]. Free acids are also partially esterified in the ethanol of wines.

Hydroxycinnamic compounds are confined inside the vacuoles of grape-skin cells and in some cases also in the vacuoles of cells from the pulp. Hydroxycinnamic ester concentration decreases as the berry ripens but their final content reaches 60 mg/l in red wines. An important characteristic of *Vitis Vinifera* grapes is the high content of tartaric esters and the low concentration of quinic acid esters, known as chlorogenic acids [22].

2.1.2. Stilbenoids

These substances are present in wine at low concentrations. They are based on a two-benzene ring structure connected by two carbons and one double-bond [23]. Stilbenoids are produced in the vineyard in response to adverse external conditions [24] and are enhanced by viticulture practices such as foliar application of urea [25].

In wine, the main stilbenoid is resveratrol. This substance has been widely researched because of its beneficial health properties. Nevertheless resveratrol oligomers such as viniferins are the main compounds with antifungal activity [26]. Many oligomers have been described in wine, for instance dimers like pallidol or δ -viniferins [27], trimers: α -viniferin [28] or tetramers: hopeaphenol [29]. Red wine stilbenoid content is estimated around 7 mg/l [30], although high variability is commonly reported in the literature [31]. This heterogeneity is due to the impact of factors such as ripening [32], viticulture [33] or winemaking practices [34] on the natural biosynthesis of resveratrol in grapes. Its derivatives are found protecting the inner surface of the grape skin, thus they are much more concentrated in red wines where maceration with skins increases their final content.

2.1.3. Hydrolyzable tannins

The word tannin describes a substance used to tan animal hides into leather [35]. In enology this term refers to a complex mixture of high molecular-weight phenolic compounds. They are classified into condensed (flavonoid polymer) or hydrolyzable (gallic/ellagic acid based) tannin mixtures. Hydrolyzable tannins are oligomers linked by a gallic or ellagic acid esterification to sugars (mainly glucose) or alcohols. They are enhanced by interactions with wood, therefore wines stored in barrels for long periods show the highest concentrations. Red wines with more than two years of oak aging reach contents up to 250 mg/L [36].

The name hydrolyzable tannins refers to the type of ester linkage; they are thus further subdivided into gallotannins (those from gallic acid) and ellagitannins (from ellagic acid). Vescalagin and its isomer castalagin are the main compounds in this group [37].

Condensed tannins are also known as proanthocyanidins, and include both oligomeric and polymeric flavonoids composed of elementary flavan-3-ols units, linked by C–C bonds.

2.1.4. Fermentation products

In wine there are more non-flavonoids documented, but with a concentration substantially lower than the phenolic substances already described. For instance the phenolic alcohol tyrosol is produced by yeasts from tyrosine, similarly to how tryptophol is produced from tryptophan during the fermentation process. The concentration of these compounds decreases during wine aging until they eventually become undetectable [38].

2.2. Flavonoid compounds

These are polyphenolic compounds with multiple aromatic rings bonded to hydroxyl groups. They are characterized by a three-ring structure with a pyran-type oxygen in the central ring [39]. Flavonoids are water soluble and classified according to the degree of oxidation in the pyran ring (Ring C in Figure 1). The group known as flavonols is particularly well researched in white wines, whereas anthocyanins and flavanols or condensed tannins are the most studied families in red wine. Grapes and vines also contain other groups such as dihydroflavonols or flavanonols in grapes and flavones in vine leaves, but in wine these compounds are rarely detected [40].

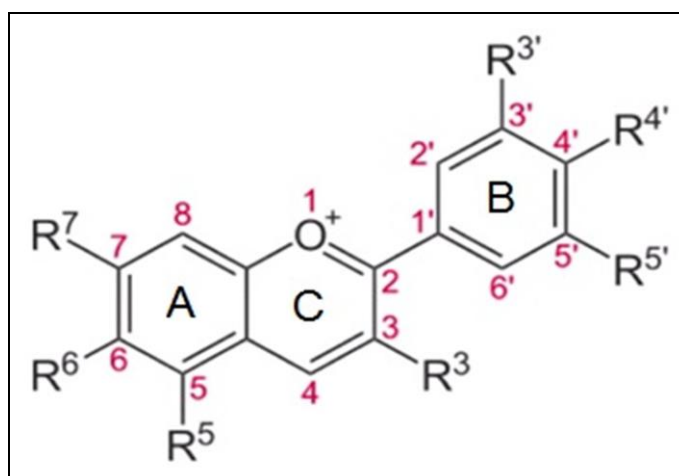


Figure 1. General structure of flavonoids

2.2.1. Anthocyanins

The term anthocyanin comes from the Greek *anthos* meaning "flower", and *kyanos* meaning "blue". These pigments are widely distributed in many plant-origin foods, from leaves to roots and flowers to fruits. Red and rosé wine anthocyanins come from the grape-skin, even though there are some rare grapes which also accumulate these

colored compounds in the pulp [41]. These molecules are responsible for the red, blue, and purple tones in grapes and wines. Their structure is based on two benzene rings linked through a heterocyclic ring. They show different colors that depend on their chemical state, including red-violet shades in acid media that become brighter as the acidity increases because the chemical equilibrium balances the flavylium ion. Anthocyanins change to blue or colorless compounds under neutral or weakly alkaline conditions turning yellow in alkaline media [42]. The eight double-conjugated bonds with positive charge on the oxygen in the heterocyclic ring C is responsible for the color spectrum exhibited by these molecules.

Anthocyanins are based on anthocyanidins, which are the polyhydroxy or methoxylated flavylium cation whose electron deficiency leads to high reactivity and instability. Anthocyanidins are the simplest flavonoid but they are found in only trace quantities in wine. The commonest chromophore is the 7-hydroxyflavylium ion, which due to its reactivity joins to sugars forming heterosides (glycosides). Therefore the term anthocyanin in fact implies a glycoside. Those sugars joined to the anthocyanidin can be esterified with organic acids. In fact, anthocyan pigments consist of two or three chemical units, i.e., an aglycone base or flavylium ring (anthocyanidin) linked with sugars and sometimes acylating groups [43].

Even though several anthocyanidins have been described in the laboratory, only six are found in foods: cyanidin, delphinidin, petunidin, peonidin, malvidin and pelargonidin. Pelargonidin is the only one of those six not present in wine. Each aglycone can be glycosylated and acylated by various sugars and aromatic or aliphatic acids. All these possibilities result in more than 600 anthocyanins identified in the plant kingdom [44].

The most common sugar attached to grape anthocyanins is glucose, although galactosides are also present, quantified in much lower concentrations [45]. Sugars bind to the 3 position of ring C or the 5 position of ring A, as in the schemain Figure 1. In *Vitis vinifera* the main acids esterifying sugar in the 6 position are acetic, p-coumaric and caffeic acid, the latter in a lesser proportion [46]. Diglycoside anthocyanins are only found in American and hybrid species [16].

The different forms of anthocyanins are in equilibrium as a mix of secondary structures such as flavylium ions, quinoidal bases, carbinol bases and chalcone pseudobases. Intermolecular and intramolecular copigmentation relationships lead to tertiary structures with different colored stabilization mechanisms [47].

Table 4. Anthocyanic flavonoid compounds in wine

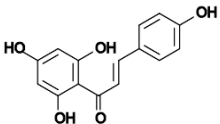
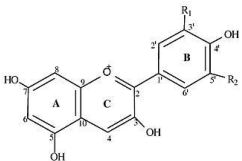
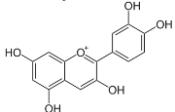
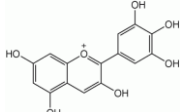
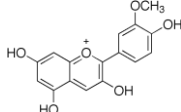
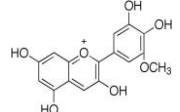
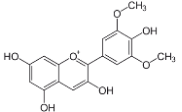
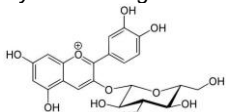
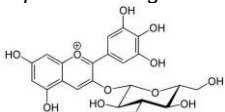
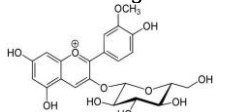
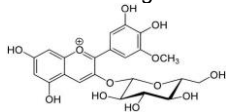
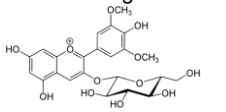
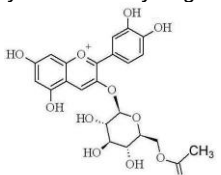
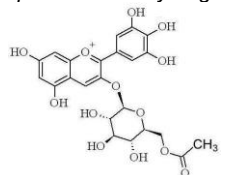
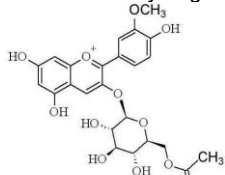
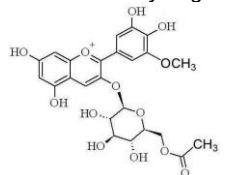
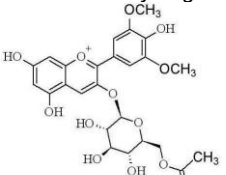
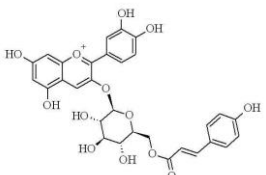
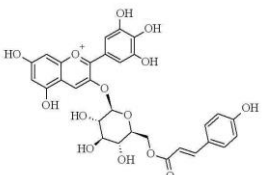
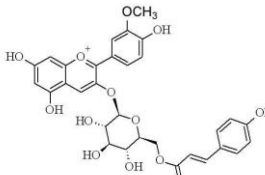
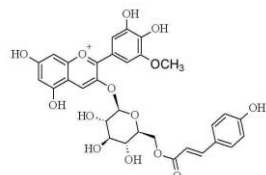
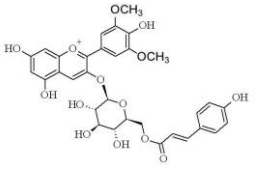
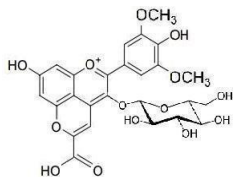
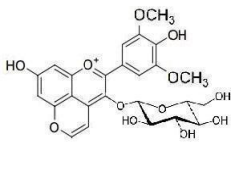
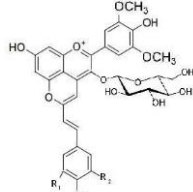
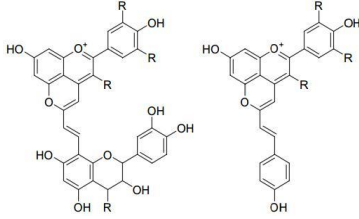
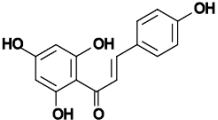
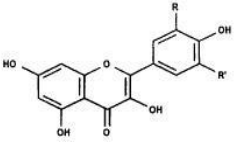
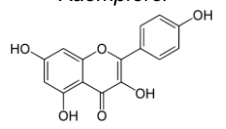
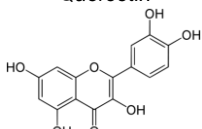
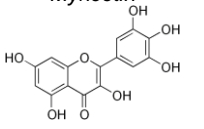
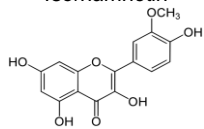
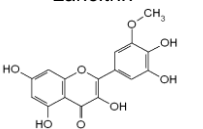
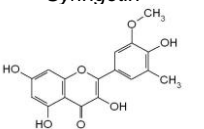
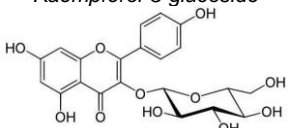
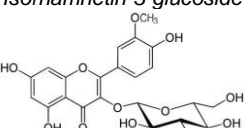
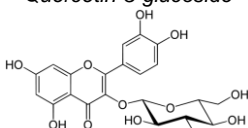
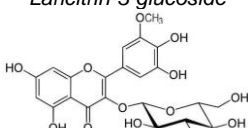
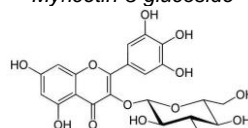
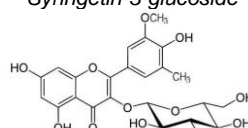
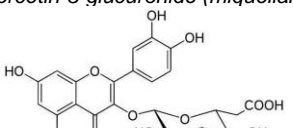
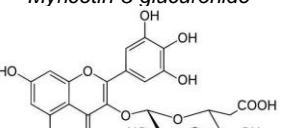
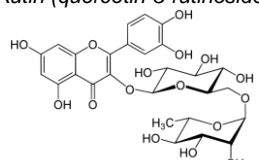
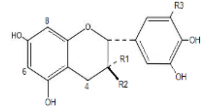
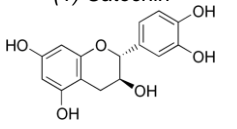
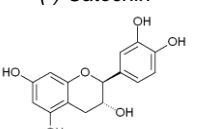
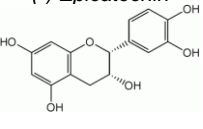
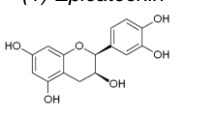
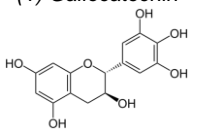
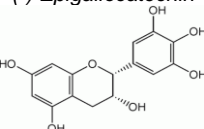
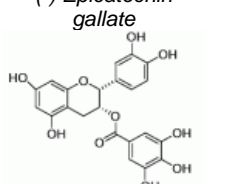
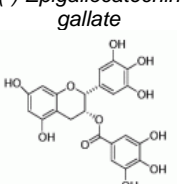
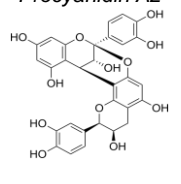
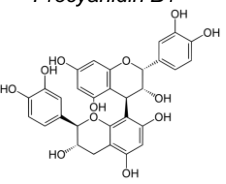
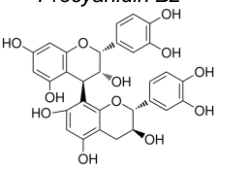
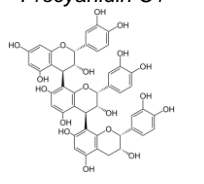
| | | | | | | |
|---|--|--|--|---|--|--|
| <p>Flavonoids</p>  | <p>Anthocyanins</p>  | <p><i>Cyanidin</i></p>  | <p><i>Delphinidin</i></p>  | <p><i>Anthocyanidins</i> <i>Peonidin</i></p>  | <p><i>Petunidin</i></p>  | <p><i>Malvidin</i></p>  |
| | | <p><i>Cyanidin-3-O-glucoside</i></p>  | <p><i>Delphinidin-3-O-glucoside</i></p>  | <p><i>Anthocyanins</i> <i>Peonidin-3-O-glucoside</i></p>  | <p><i>Petunidin-3-O-glucoside</i></p>  | <p><i>Malvidin-3-O-glucoside</i></p>  |
| | | <p><i>Cyanidin-6-acetyl-3-gluc.</i></p>  | <p><i>Delphinidin-6-acetyl-3-gluc.</i></p>  | <p><i>Acetyl Glucosides</i> <i>Peonidin-6-acetyl-3-gluc.</i></p>  | <p><i>Petunidin-6-acetyl-3-gluc.</i></p>  | <p><i>Malvidin-6-acetyl-3-gluc.</i></p>  |
| | | <p><i>Cyanidin-6-cumaroyl-3-gluc.</i></p>  | <p><i>Delphinidin-6-cumaroyl-3-gluc.</i></p>  | <p><i>Cumaroyl-3-glucosides</i> <i>Peonidin-6-cumaroyl-3-gluc.</i></p>  | <p><i>Petunidin-6-cumaroyl-3-gluc.</i></p>  | <p><i>Malvidin-6-cumaroyl-3-gluc.</i></p>  |
| | | <p><i>Pyranoanthocyanins (great number of compounds depending on chemical reactions during aging)</i></p> | | | | |
| <p><i>Vitisin examples</i></p> <p><i>Type A (with piruvic acid)</i></p>  | | <p><i>Type B (with ethanal)</i></p>  | | <p><i>Vinylphenol+anthocyanin+hydroxycinnamic examples</i></p>  | | <p><i>Portisin examples</i></p>  |
| | | | | | <p><i>R depends on the original hydroxycinnamic:</i></p> <p><i>Caffeic acid:</i> R₁: -OH R₂: -H <i>Malvidin-3-O-glucoside-4-vinylcatechol</i></p> <p><i>p-Coumaric acid:</i> R₁: -H R₂: -H <i>Malvidin-3-O-glucoside-4-vinylphenol</i></p> <p><i>Ferulic acid:</i> R₁: -OCH₃ R₂: -H <i>Malvidin-3-O-glucoside-4-vinylguaiacol</i></p> | |

Table 5. Non-anthocyanic flavonoid compounds in wine

| | | | | | | | |
|--|--|---|---|--|---|---|--|
| <p style="text-align: center;">Flavonoids</p>  | <p style="text-align: center;">Flavonols</p>  | <p style="text-align: center;"><i>Kaempferol</i></p>  | <p style="text-align: center;"><i>Quercetin</i></p>  | <p style="text-align: center;"><i>Myricetin</i></p>  | <p style="text-align: center;"><i>Isorhamnetin</i></p>  | <p style="text-align: center;"><i>Laricitrin</i></p>  | <p style="text-align: center;"><i>Syringetin</i></p>  |
| | | <p style="text-align: center;"><i>Kaempferol-3-glucoside</i></p>  <p style="text-align: center;"><i>Isorhamnetin-3-glucoside</i></p>  | <p style="text-align: center;">Glucosides</p> <p style="text-align: center;"><i>Quercetin-3-glucoside</i></p>  <p style="text-align: center;"><i>Laricitrin-3-glucoside</i></p>  | <p style="text-align: center;"><i>Myricetin-3-glucoside</i></p>  <p style="text-align: center;"><i>Syringetin-3-glucoside</i></p>  | | | |
| | | <p style="text-align: center;"><i>Quercetin-3-glucuronide (miquelianin)</i></p>  | <p style="text-align: center;">Other derivatives</p> <p style="text-align: center;"><i>Myricetin-3-glucuronide</i></p>  | <p style="text-align: center;"><i>Rutin (quercetin-3-rutinoside)</i></p>  | | | |
| | <p style="text-align: center;">Flavanols (solubles tannins)</p>  | <p style="text-align: center;">(+) Catechin</p>  | <p style="text-align: center;">(-) Catechin</p>  | <p style="text-align: center;">(-) Epicatechin</p>  | <p style="text-align: center;">(+) Epicatechin</p>  | <p style="text-align: center;">(+) Gallocatechin</p>  | <p style="text-align: center;">(-) Epigallocatechin</p>  |
| | <p style="text-align: center;">(-) Epicatechin gallate</p>  | <p style="text-align: center;">(-) Epigallocatechin gallate</p>  | <p style="text-align: center;">Procyanidin A2</p>  | <p style="text-align: center;">Procyanidin B1</p>  | <p style="text-align: center;">Procyanidin B2</p>  | <p style="text-align: center;">Procyanidin C1</p>  | |

2.2.2. Flavanols

Most grapevine flavanols are monomeric, although some are polymerized forms constituting condensed tannins also known as proanthocyanidins. Grape flavanols are glycosides located in seeds and skins, only trace levels being detected in the pulp. Wine flavanols are not found as glycosides [48].

In wines, (+)catechin, (-)epicatechin and derivatives such as gallic esters are the most abundant substances from this group. Condensed tannins produced during wine-aging are oligomeric and polymeric proanthocyanidins formed by units of flavan-3-ol (catechins) or flavan-3-4-ol linked by C-C bonds [49].

Flavanols are considered responsible for astringency, one of the most characteristic sensory attributes of wine [50]. These substances are usually colorless but may form copigmentation complexes with anthocyanins. Flavanols acting as copigments contribute to color in red wines.

2.2.3. Flavonols

Flavonols are located in the grape-skin protecting the inside of the berry from UV radiation due to their absorbance at those wavelengths [51]. Accumulation in the vine is a response to treatments with UV and sunlight [52]. Therefore, grape flavonol biosynthesis depends on the degree and intensity of illumination. Berries located in the same bunch of grapes but on the more sun-exposed side reach up to six times the amount of flavonols than those located in the shaded part. According to some researchers, the synthesis of anthocyanins and tannins is less affected by this [53]. More expensive wines have been described to present higher levels of flavonols because usually these wines come from lower yielding vines with greater sun-exposure of the fruit. This fact suggests that the level of flavonols may indicate grape sun exposure and potential wine quality [54].

The flavonol content is similar among vine varieties (10-100 mg/kg of grapes) even though different profiles have been described [55]. In wine the most concentrated is quercetin, followed by kaempferol and myricetin. Traditionally, isorhamnetol glycosides are considered exclusive to red wines [17], but minor levels have been detected in white wines [56].

Wine flavonol content ranges from none detected to 100 mg/L, either free or conjugated. Between 20-50% of the wine flavonols are in their free state, giving a yellow tonality masked in red wines by the purplish red of anthocyanins, but noticeable also in white wines [57].

Most grape flavonols are glycosylated as 3-O-glycosides. However, free aglycones in wines are produced by acid hydrolysis. Glucose is the common sugar bonded to the C3 position of kaempferol, quercetin, myricetin and isorhamnetin. Glucuronic acid has also been identified as a possible agent bonded to non-methoxylated flavonols, such as kaempferol, quercetin and myricetin [58]. Grapes also contain diflavonols like quercetin-3-O-rhamnosyl-glucoside, also known as rutin, but tri-substituted methoxylated flavonols (laricitrin and syringetin) and their 3-O-glucosides derivatives are less common [59].

Flavonols are the main copigments involved in wine copigmentation [60]. They are also important antioxidant compounds in white wines in particular, because the other phenolic compounds more abundant in red wines, such as flavanols or anthocyanins, do not contribute significantly to the final antioxidant capacity [61].

2.3. Wine aging: polymers and reactions

Grape phenolic compounds react together and form polymers during wine aging. The following interactions are the most common:

- Tannins-proteins. These complexes lead to instability as they precipitate, causing a loss of wine astringency and structure [62].
- Tannins-polysaccharides or tannins-peptides. These reactions partially reduce wine astringency but do not involve a great loss of body [63].
- Tannins-anthocyanins. The interactions between these two groups stabilize red wine color and reduce astringency [64].
- Polymerization. High molecular weight tannins precipitate out, reducing wine astringency and bitterness. Tannins with an average molecular weight reduce bitterness but increase astringency [65].
- Anthocyanin oxidation. This reaction causes color losses as the bright red from young red wines becomes partially browned [66].
- Hydroxycinnamate oxidation. This reaction is particularly important in white wines as it involves browning effects. Oxidation takes place by excessive aeration or by polyphenol oxidase enzymes released when grapes are crushed. Oxidation processes are enhanced by high temperatures or light. Hydroxycinnamates rapidly evolve to quinones, which further react with flavonols to form brown pigments [67].
- Formation of pyranoanthocyanins or vitisins. These compounds stabilize red wine color but also increase the brown hues [68].

3. Main effects of phenolics in wine

The phenolic compounds presented in the previous section are responsible for specific attributes of wine. They influence characteristics such as color, taste, mouth-feel and health properties. Anthocyanins and tannins are the flavonoids directly related to wine color and mouth-feel, while non-flavonoids like stilbenoids or phenolic acids contribute to flavor and enhance preservation because of their antioxidant capacity [69].

Color greatly affects consumer preferences and thus plays a key role for winemakers [70]. Wine's visual characteristics depend on the numerous factors influencing the balance of anthocyanins. Due to this, wine color continuously evolves over time and is recognized as an indicator of age and quality [71].

Wine has widely been used as a form of medication. Indeed, Sumerian tablets from 2200 BC detail the medicinal uses of wine, making it one of the oldest medicines prepared by mankind [72]. Moderate red wine consumption has been linked to health benefits. Wine's effects are related to the anti-aging properties derived from the phenolic compounds present. These compounds are responsible for wine's outstanding antioxidant capacity [73].

Wine consumption is based on the pleasurable feelings deriving from its flavor and other sensory or organoleptic qualities. The sensations, satisfaction or "fullness" perceived as wine passes over the palate are largely due to its phenolic structure and the relationships between components such as acids, sugars, ethanol or glycerol. Tannin interactions make up the wine's "body" and some phenolic compounds are involved in wine's *bouquet* and aromas [74].

3.1. Color

Wine color depends on the phenolic compounds and their evolution during aging. Oxidation, condensation and polymerization reactions are responsible for visual changes in wine.

Yellow tonalities of white wines are due to phenolic acids, chalcones and flavonols. Phenolic compounds from white wines are affected by browning reactions which may be of varying types, enzymatic in musts and non-enzymatic in wines. White wine color is unstable because of these browning reactions. Young white wines show an initial pale yellow typical of less oxidized wines, which progressively evolves to the brown hues of highly oxidized white wines. Browning reactions depend on specific substrates and conditions known in the industry as *browning potential*. For instance, inappropriate handling and treatment of grapes and musts enhance browning reactions by the action of enzymes such as polyphenol oxidase. This enzyme especially affects the tartaric esters of hydroxycinnamic acids. The main factors increasing phenolic oxidation and consequent brown polymers in white wines are redox potential, oxygen content and reaction with other substances like sugars [75].

Anthocyanins are red and rosé wine pigments but many other phenolic compounds affect these colors. Young red wines show a maximum spectral peak at 520 nm with

large yellow (420 nm) and blue (620 nm) components. They are therefore characterized by intense red colors with violet hues. During wine aging this red component decreases while the yellow increases. After 10-20 years, the red component is minimal and the yellow is relatively high, giving brown hues [76]. Indeed, the wine's phenolic profile results in a greater or lesser impact on color over time [77].

Traditionally, a high content of phenolic compounds in wine is considered to mean a strong wine color. Nevertheless wines with a high content of orthophenolic compounds are easily oxidized, more rapidly developing those straw and brown colors in the case of white wines and orange and russet colors in rosé and red wines respectively.

As mentioned, the oxidation products from some flavonoids provide brown or yellow hues in aged white wines. Effects from these flavonoids are limited to anthocyanin copigments in red wine. For instance colorless flavanols do not directly determine red wine color, but act as copigments and form new pigments by polymerization with anthocyanins [78]. Other compounds such as the flavonols also act as copigments exhibiting a yellow color in their free form.

The high quantity of various pigments in red wine makes for a heterogeneous palette of colorimetric nuances. Young wines generally show red colors with some purple hues, but the pigments' resonant behavior makes them highly reactive and unstable. Thus, the color of wine anthocyanins varies according to their methylations, acylations and glycosylations [79]. Given that anthocyanins found in grape skins are mainly responsible for red wine color, the maceration process must include the skins to ensure sufficient presence of these molecules in the final wine.

Reactions involving acetaldehyde are enhanced as wine ages. Acetaldehyde is produced by ethanol oxidation through microoxygenation processes. Acetaldehyde-derived reactions originate new substances such as polymeric pigments or pyranoanthocyanins. These compounds gradually modify the wine's appearance. Free anthocyanins progressively disappear and new complex pigments prevail in the color during wine aging.

The effect of polyphenolic compounds on color depends on various factors, such as pH [80], temperature [81], SO₂ levels, vintage [82], winemaking technique [83] or reactions with other compounds present in wine. The influence of these factors is discussed in the following sections.

3.1.1. pH

The balance between anthocyanins is highly influenced by pH. These substances are usually defined as red pigments. However, they show different colorations depending on pH and the equilibrium between the different chemical species [84].

As mentioned, the red-colored flavylium cation is the main form of anthocyanin in acid conditions and its color is due to the location of the positive charge. As pH increases, the cation becomes a purple quinone base (pKa 4.7) and a colorless carbinol (Figure 2). The equilibrium towards carbinol involves the introduction of a water molecule in the structure and the consequent release of a proton. The hydroxyl group derived from this water introduction neutralizes the cation making the anthocyanin color tend to

disappear. This flavylum hydration is what really leads to the anthocyanin losing its color. A similar mechanism is involved when the bisulfite anion from SO_2 , routinely used as wine preservative, neutralizes the flavylum cation [85].

Furthermore, carbinol is in equilibrium with a group of yellow molecules known as chalcones (cis and trans) by a reaction which strongly shifts at high temperatures. This involves an irreparable loss in wine color. Trans-chalcones can be irreversibly oxidized to phenolic acids, which often happens when wine is stored at high temperatures [86].

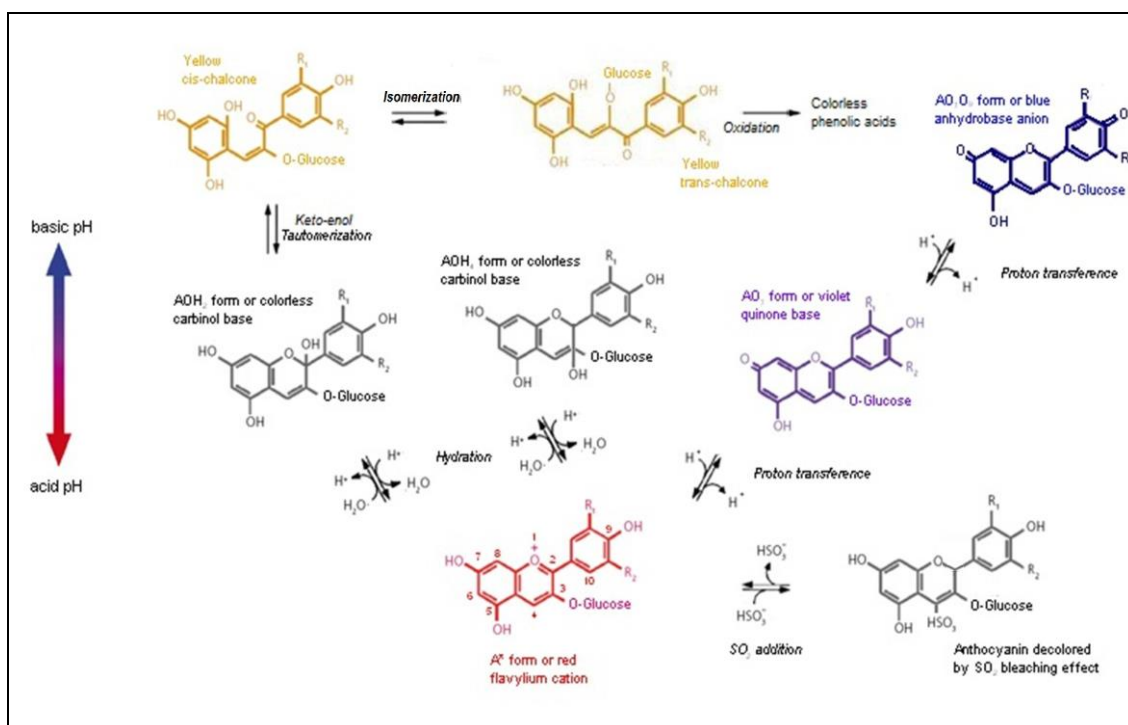


Figure 2. Stable forms of anthocyanin according to pH

If pH were the only factor affecting anthocyanin equilibria, red wine would be characterized by low intensity bluish hues at its normal pH (pH 3.2~3.9). However, wines show red hues because of the influence of many other factors, such as copigmentation, interaction with other molecules, and generation of new pigments and equilibria [87].

3.1.2. Copigmentation

Copigmentation phenomenon occurs when a monomer anthocyanin pigment associates with itself or with another substance known as a copigment. This association prevents the hydration of the flavylum ion that takes place in aqueous medium. In wine, copigments are mainly phenolic compounds known as cofactors.

Copigmentation consists of a 1:1 stoichiometry complex between a pigment and a usually colorless copigment. This complex forms a stacked sandwich structure (Figure 3). The aromatic rings of these molecules are linked by weak interactions, such as Van der Waals or hydrophobic forces [88]. Such hydrophobic associations prevent nucleophilic attack by water. Thus, the amount of hydrated colorless base (carbinol) is reduced as the equilibrium towards the colored flavylum cation is enhanced. Consequently, a greater percentage of anthocyanins contribute to color than those that would correspond to the pH value, as long as enough suitable copigments are present.

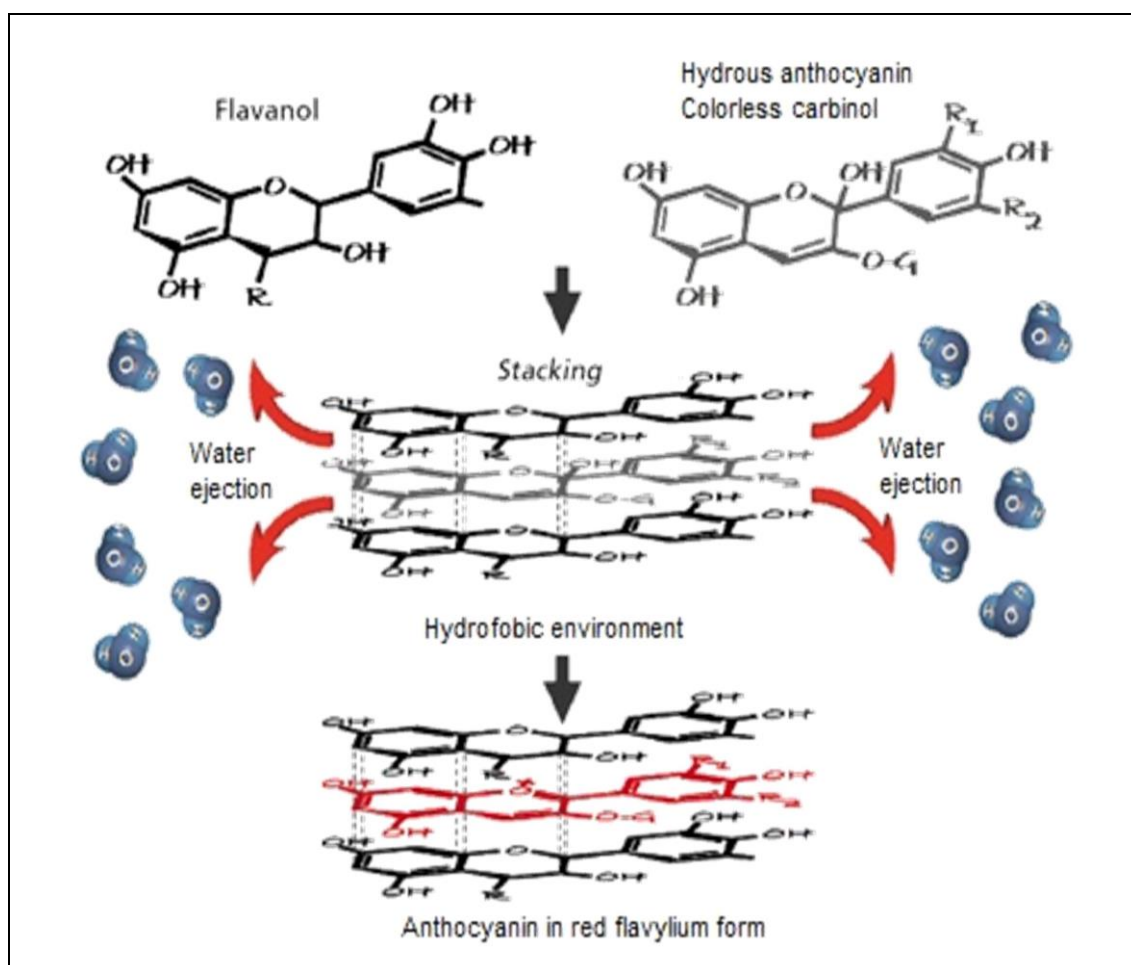


Figure 3. Schema illustrating copigmentation

Copigmentation processes are classified according to the copigment involved (Figure 4):

1. Autoassociation, when the copigment is another anthocyanin.
2. Intramolecular, when the copigment is part of the anthocyanin itself. For example the coumaroyl substituent from a coumaryl anthocyanin.
3. Intermolecular, when the copigment is a different molecule. For example a flavonol or a phenolic acid.

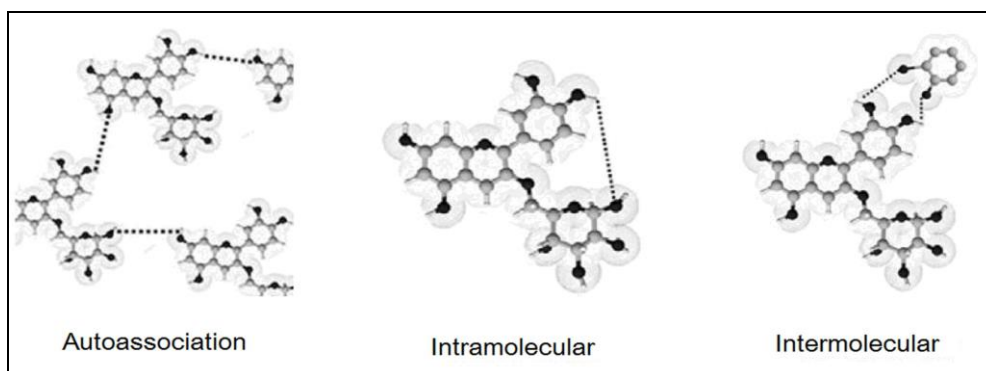


Figure 4. Copigmentation mechanisms

Different molecules can act as copigments, from anthocyanins to phenolic acids, flavonoids, amino acids, nucleotides or polysaccharides [60]. Flavonols and hydroxycinnamic acids are considered suitable wine copigments [89]. Copigmentation complexes are easily formed but also dissociated by a simple dilution with certain wine cosolvents such as ethanol or acetic acid [90].

The degree of copigmentation depends on factors such as the content of compounds and their chemical structures [91], pigment/copigment molar ratio [92], or temperature [93]. For example, malvidin is more prone to copigmentation than cyanidin [94] and the effects are more pronounced in diglucoside anthocyanins than in monoglucosides [95]. Similarly, in model solutions pigments with aromatic acyl groups form more stable copigmentation complexes than non-acylated anthocyanins [96].

Copigmentation enhances wine color by a hyperchromic effect consisting of a red intensity increase and produces hue changes to bluish tonalities due to bathochromic shifts [97]. Thus, red wines exhibit different colors according to their phenolic composition because copigmentation plays a key role in anthocyanin stabilization processes.

3.1.3. Phenolic reactions

Red wine pH and copigmentation are the most important factors for anthocyanin color. However, wine color is not stable as gradual losses are observed during aging [98]. This has led to aging being considered as a color stabilization process in which anthocyanins react to create new pigments (Figure 5). Such reactions are classified into two groups, either producing pyranoanthocyanins or polymeric pigments. Both groups exhibit greater resistance to degradation than monomer anthocyanins. Pyranoanthocyanins are orange-red pigments insensitive to bisulfite bleaching because the flavylum cation is protected by the pyran ring. These compounds are more stable towards pH and temperature variations, most of them remaining dissolved, in contrast to polymeric pigments [99].

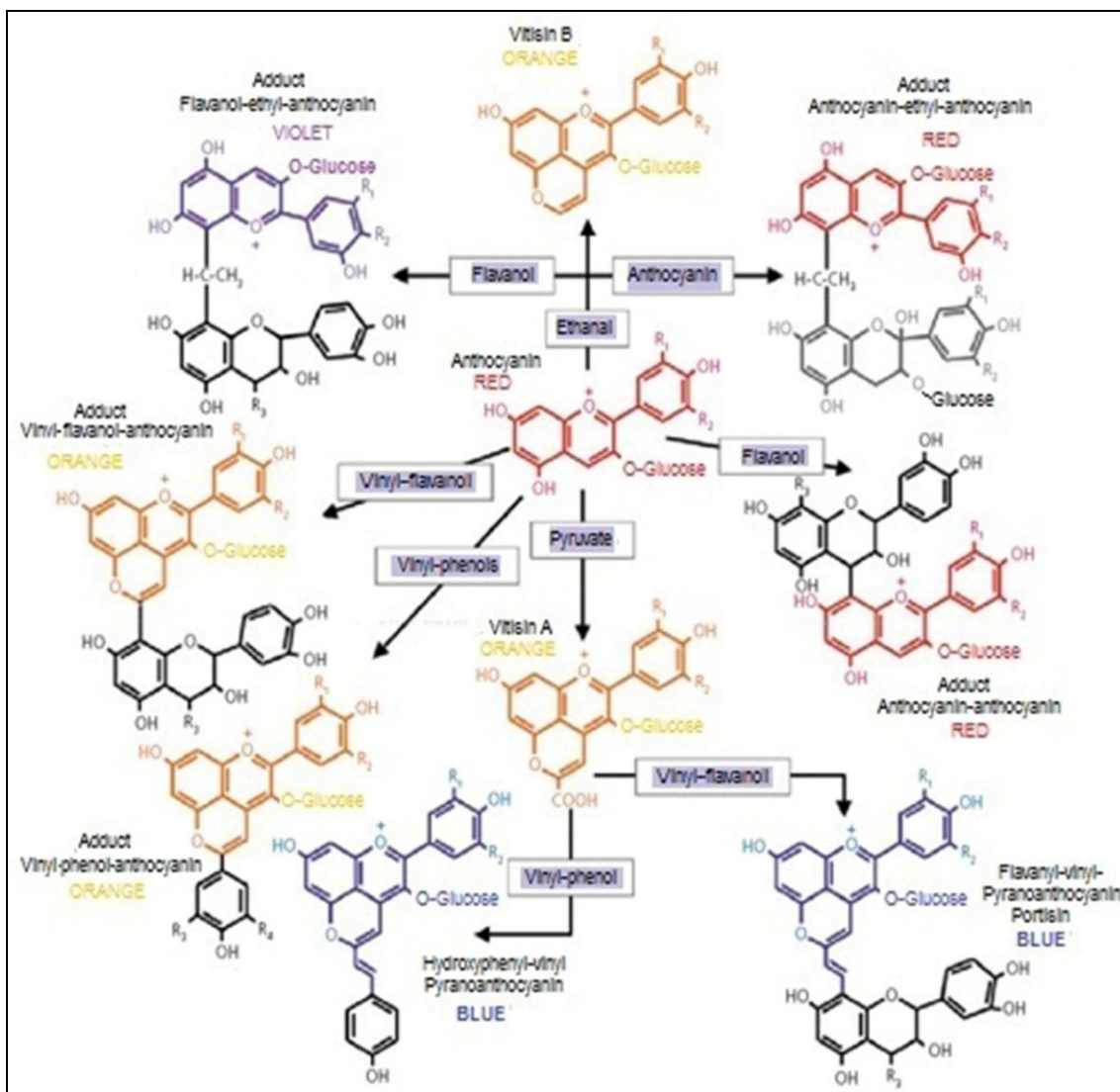


Figure 5: Some color significant anthocyanin reactions described in wine

3.1.3.1. Pyranoanthocyanins

Pyranoanthocyanins are products of cycloaddition characterized by a D-pyran ring between the OH group of the C-5 and C-4 from the original flavylum cation (Figure 6). The term derives from the inclusion of this fourth pyran ring in the initial anthocyanin [100]. These compounds are formed during alcoholic fermentation and subsequent processing steps, therefore they are not detected in grapes [101]. Their concentrations are low and they are insensitive to pH changes, SO₂ attack and oxidative degradation, since the pyran ring causes a steric hindrance [102]. Thus, most of these compounds participate in wine color. Pyranoanthocyanins produce a hyperchromic and hypsochromic displacement in comparison to the initial anthocyanin colors, therefore they lead to red and orange hues.

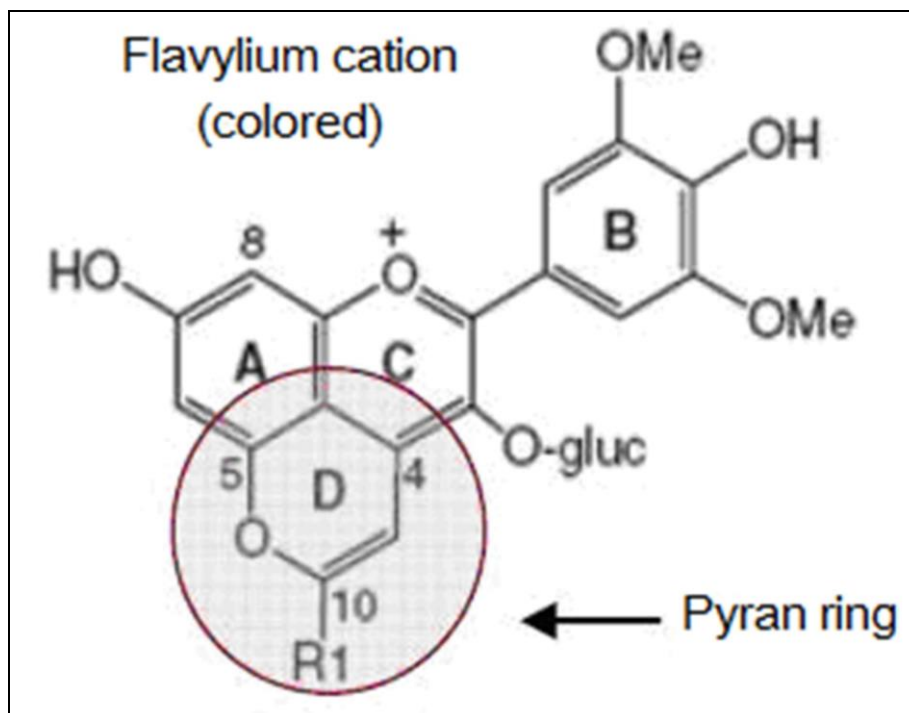


Figure 6. Pyranoanthocyanin structure

Pyranoanthocyanins are classified in families according to the compound in position 10 of the pyran ring D [103]. Some of them are products of the reaction between anthocyanins and ethanal, resulting in an orange pigment called vitisin B. Vitisin A is another type of pyranoanthocyanin produced by the reaction between anthocyanins and pyruvic acid. For this reason, vitisin B and vitisin A are generally known as anthocyanin-acetaldehyde and anthocyanin-pyruvic acid adducts respectively. Vitisins appear during fermentation, so they are characteristic from the early stages of winemaking. Vitisins A are produced faster than vitisins B [104]. Both groups are able to form oligomeric vitisins by cycloaddition with wine flavanols such as catechin [105].

The first compound of this group identified was produced by reaction between malvidin-3-glucoside and pyruvic acid, therefore it was a vitisin A pyranoanthocyanin [106]. Pinotin A was first described in a study with Pinot Noir wines where the same anthocyanin interacted with caffeic acid [107]. Pinotins are also known as pyranoanthocyanin-vinylphenol adducts because they are produced by the reaction between these pigments and hydroxycinnamic acids or their decarboxylation products (4-vinylphenols). Pinotins are considered aged wine markers since they form slowly, appearing after long periods of aging [108]. Vitisins A may react with vinylphenols or vinylflavanols, resulting in blue pigments called vinylpyranoanthocyanin-flavanols, commonly known as portisins because they were identified for the first time in Port wines [109].

Under certain conditions, vitisins exposed to the slow but irreversible nucleophilic attack of water become neutral pyranone-anthocyanins, known as oxovitisins [110]. These compounds exhibit a yellowish or colorless hue in acid medium. Other pyranoanthocyanins generated by the reaction between anthocyanins and vinyl flavanols are orange/red pigments. In these compounds anthocyanins bind to flavanols

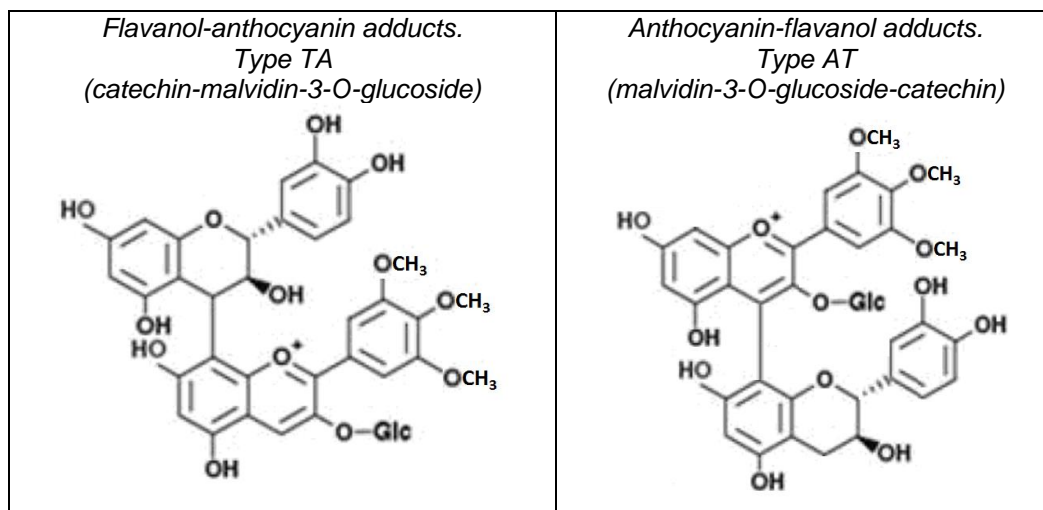
through the intermediation of acetaldehyde and consequently are known as anthocyanin-vinylflavanol adducts [111]. In addition to acetaldehyde, pyruvic acid, phenolic acids and vinylphenols other compounds are capable of joining anthocyanins to form pyranoanthocyanins, for example α -ketoglutaric acid, glyoxylic acid, acetoin, acetone, diacetyl or diacetic acid [112-114]. The cycloaddition of anthocyanins with diacetic acid produces yellow-orange pigments known as methylpyranoanthocyanins [115].

3.1.3.2. Polymeric pigments

Polymeric pigments are phenolic compounds formed by the reaction of monomeric anthocyanins with flavanols (tannins). These compounds are formed by condensation processes where anthocyanins (A) and tannins (T) either directly interact or either via other intermediate substances such as acetaldehyde [116]. The anthocyanin can be located at the initial position of the polymer (AT type) or at the end (TA type).

Direct associations between anthocyanins and flavanols result in stable red/orange pigments. Anthocyanin-flavanol adducts (AT) are formed by addition as the electrophile flavylium anthocyanin joins a nucleophile flavanol molecule [117]. Flavanol-anthocyanin adducts (TA) are generated by direct condensation between anthocyanins and proanthocyanidins, traditionally known as condensed tannins. In this case, the reaction is based on the formation of a carbocation arising from the flavylium junction rupture and proanthocyanidins in acidic medium [118].

Table 6. Direct associations between anthocyanins and flavanols



Reactions between anthocyanins and tannins mediated by acetaldehyde give rise to purple pigments and are common in aged red wines. Acetaldehyde is a natural compound with an oxidative microbial origin, which can be increased by winemaking practices like microoxygenation or oak aging [119]. Acetaldehyde links anthocyanins and tannins by an ethyl bridge producing violet pigments known as flavanol-ethyl-

anthocyanin adducts. When anthocyanin molecules are joined between themselves by the mediation of acetaldehyde the resultant polymers are red. Acetaldehyde mediation accelerates the formation of polymeric anthocyan pigments with bluish-red shades and violet hues. This color is due to intermolecular copigmentation phenomena [120].

Phenolic polymeric pigments are responsible for color losses during wine aging because some are insoluble and precipitate out. These pigments lower the levels of free monomeric anthocyanins and therefore the amount of copigmentation decreases [97]. Traditionally, polymeric pigments are considered to be more stable pigments than free monomeric anthocyanins, because their color equilibrium is not as sensitive to pH and only the TA type is sensitive to bisulfite discoloration.

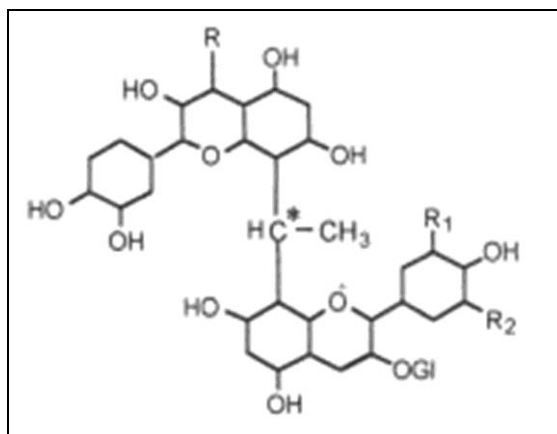


Figure 7. Anthocyanin-tannin association mediated by acetaldehyde

3.2. Antioxidant capacity

Substances that are easily oxidized are potentially highly valuable antioxidants for mankind. Many of wine's phenolic compounds possess a catechol group (1,2 dihydroxybenzene) which can be easily oxidized because the resulting phenoxyl radical is stabilized by the adjacent oxygen anion. There have been many studies of wine's antioxidant capacity and its preventive role in diseases associated with oxidative stress [121-125]. The antioxidant capacity of herbal extracts [126], teas [127], or onions [128] is also directly related with their phenolic content.

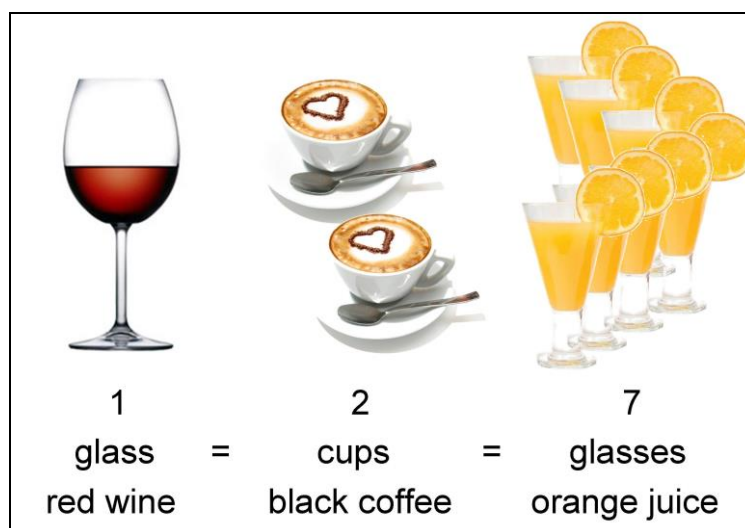


Figure 8. Comparison between beverages rich in antioxidant capacity, based on [129].

Phenolic compounds act as antioxidants by capturing free radicals and inhibiting the generation of oxidative chain reactions [130]. Therefore this antioxidant capacity largely depends on their structure. Specifically, its effectiveness depends on the number and position of their hydroxyl or sugar groups, the degree of polymerization, and the solubility [15]. For instance polymeric compounds are considered more powerful antioxidants than monomeric phenolics [131].

Tocopherols [14], flavonoids [132], anthocyanins [133], carotenoids [134], and phenolic acids [135] are well known antioxidants present in foods. Flavonoids and phenolic acids are particularly important wine compounds. Some of them stand out because of their high antioxidant capacity, such as caffeic acid [136], flavanols like epicatechin and catechin [137], gallic acid [138], anthocyanins such as cyanidin or malvidine-3-glucoside [139], flavonols such as rutin, myricetin or quercetin, and resveratrol [140]. The concentration of these substances in wine depends on factors such as grape variety, soil, climate, agricultural practices, aging or winemaking processes among others [141].

Wine antioxidant capacity can be evaluated by applying different methods. Values are greatly dependent on the analytic procedure followed [142]. The absence of a reference method implies that only values obtained applying the same methodology

can be reliably compared. The DPPH technique is one of the most frequently used to evaluate the antioxidant capacity of foods and beverages [143].

As wine's antioxidant capacity is directly related to its phenolic content, those wines with a greater phenolic concentration show the highest values. Therefore red wine capacity is greater than rosé wines, which in turn is greater than white wines [13]. Red wines' average antioxidant capacity is equivalent to 10-12 mM Trolox [144], while rosé wines show values around 4-5 mM Trolox and white wines 1-2 mM Trolox, expressed as Trolox Equivalent Antioxidant Capacity (TEAC) [145]. Nevertheless wine's antioxidant capacity shows great variability depending on varieties, winemaking techniques and climatic areas [55].

The antioxidant capacity of phenolic compounds has been extensively analyzed in model solutions. The values for the most important phenolic compounds present in wine are summarized in Table 7 [146]. Studies in vitro reveal that epicatechin gallate exerts the highest antioxidant capacity, but its concentration in wine is much lower than other phenolic compounds such as gallic acid or quercetin. The important role of synergistic and antagonistic effects among such phenolic compounds has been highlighted [147].

Table 7. Hierarchy of antioxidant capacities of wine polyphenolic compounds

| Compound | Phenolic Group | TEAC (mM) |
|--------------------------|----------------------|-------------|
| Epicatechin gallate | Flavanol | 4.90 ± 0.02 |
| Epigallocatechin gallate | Flavanol | 4.80 ± 0.06 |
| Quercetin | Flavanol | 4.70 ± 0.10 |
| Delphinidin | Anthocyanidin | 4.44 ± 0.11 |
| Cyanidin | Anthocyanidin | 4.42 ± 0.12 |
| Epigallocatechin | Flavanol | 3.80 ± 0.06 |
| Myricetin | Flavonol | 3.72 ± 0.28 |
| Gallic acid | Hydroxybenzoic acid | 3.01 ± 0.05 |
| Epicatechin | Flavanol | 2.50 ± 0.02 |
| Gallic acid methyl ester | Hydroxybenzoate | 2.44 ± 0.03 |
| Catechin | Flavanol | 2.40 ± 0.05 |
| Rutin | Flavonol | 2.42 ± 0.12 |
| Peonidin | Anthocyanidin | 2.22 ± 0.20 |
| P-coumaric acid | Hydroxycinnamic acid | 2.22 ± 0.06 |
| Malvidin | Anthocyanidin | 2.06 ± 0.10 |
| Resveratrol | Stilbenoid | 2.00 ± 0.06 |
| Ferulic acid | Hydroxycinnamic acid | 1.90 ± 0.02 |
| Oenin | Anthocyanin | 1.78 ± 0.02 |
| Syringic acid | Hydroxybenzoic acid | 1.36 ± 0.01 |
| Kaempferol | Flavonol | 1.34 ± 0.06 |
| Caffeic acid | Hydroxycinnamic acid | 1.26 ± 0.01 |
| Protocatechuic acid | Hydroxybenzoic acid | 1.19 ± 0.03 |

3.3. Flavor

The term flavor refers to the set of perceptions that the brain associates with the union of different aromas, tastes and tactile sensations perceived jointly while consuming any food or beverage. Wine flavor is a highly intricate issue because many substances are involved and it is greatly influenced by the winemaking techniques used. Polyphenolic compounds are one of the groups affecting wine flavor. Tannins are responsible for the tactile sensations perceived in the mouth and volatile phenols contribute to wine *bouquet*, as they have a distinct aroma ranging from “sweaty saddles” to cloves [148]. The potential impact of these phenols is high, even though they are not as concentrated as other compounds, due to their very low sensory thresholds.

3.3.1. Astringency and structure

Polyphenolic compounds lend vital unique characteristics to the wine. They are responsible for many quality aspects considered essential by producers and consumers. In particular, tannins are one of the main compounds affecting taste and flavors in wines. As detailed in section 2.3; tannin interactions modify wine astringency and structure.

The word astringency comes from the Latin *stringere* meaning “to bind” [149]. Astringency is a tactile quality caused by tannins commonly associated with a sensation of dryness on the lips and the side of the mouth [150]. The term is defined by the American Society for Testing and Materials as “the complex of sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as alums or tannins” [151]. Therefore, astringency is not directly detected by the taste buds or the olfactory epithelium. In fact its effects are perceived in the mouth tactilely and not in a specific place like other taste sensations. Astringency stimulates receptors located on the tongue, cheeks, palate and gingival area. It is a cumulative feeling that takes time to disappear and its perception depends on the content and profile of tannins.

Wine structure is associated with the overall characteristics perceived in the mouth. A wine presents a suitable structure when feelings, aromas and flavors are powerful but at the same time balanced [152]. Tannins are the phenolic compounds that have the greatest impact on wine structure and aging potential.

Drying and puckering sensations related to wine astringency are due to associations between tannins and proteins. Drying is due to the affinity between tannins and saliva proteins. Tannins bind to proteins causing their precipitation, thus saliva loses its lubricity and wetness. Puckering is related to a roughness perception produced by the interaction of tannins with proteins from the oral mucosa (Figure 9). The association of bitterness and astringency sensations with tannins, catechins and glycosylated flavonols is based on these tannin-protein interactions [153].

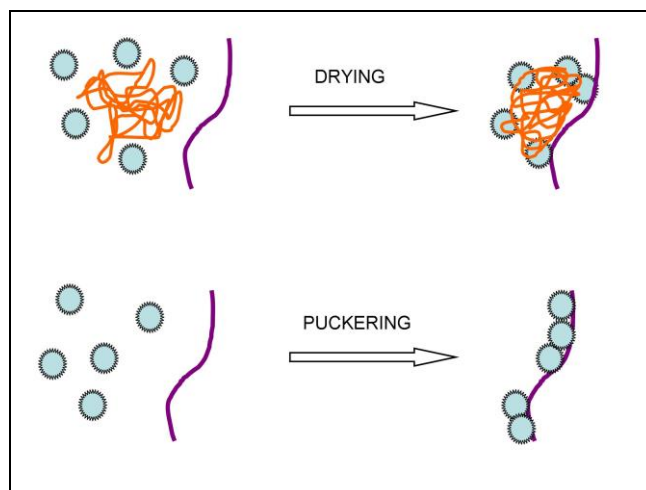


Figure 9. Astringency-related drying and puckering mechanisms

Grape skins and pulp contain few tannins whereas seeds and stems contain the greatest part, giving them a bitter taste and harsh sensation. The phenolic content of pulp, skin and seeds is in continuous evolution during grape ripening. On one hand, skin proanthocyanidins are greatly polymerized and their amounts increase while ripening. On the other, seed proanthocyanidins are less polymerized and their contents decrease while ripening, this is why the seed color changes from green to brown in a process known as lignification, where plant material becomes woody [154]. Therefore, if grape ripeness is adequate at harvest time, the contribution of tannins to wine comes mostly from the skin and only in a small proportion from the seeds. This means a lower final astringency and refines the global perceptions of the wine [155].

Winemaking practices also affect wine astringency. Aspects such as maceration times, fermentation temperatures or the use of enzymes can enhance or reduce the extraction of tannins from grapes into the must. Furthermore, wines are sometimes aged in oak casks. This aging technique adds further tannins from the wood in the form of ellagic derivatives but also enables tannins to soften because of the gentle oxygen exchange through the wood. As red wine ages in oak casks, tannins develop softer and more complex flavors while the roughest tannins form solids and precipitate out [119].

Astringent polyphenolic compounds have molecular weights between 500 and 3000 Da [156], but smaller compounds may also produce astringency [50]. An increase in the degree of polymerization of an astringent compound usually involves a greater ability to precipitate proteins [157]. Nevertheless, the astringency of aging red wines is reported to decrease as polyphenol polymerization increases [158]. The reason might be that low molecular weight tannins are small in size, and therefore a greater reactive surface contacts the oral mucosa than polymerized tannins, which have lower contact surfaces (Figure 10). In fact, polymerization decreases their reactive surface and thus the “rough” feeling in the mouth. Given that astringency is a subtle tactile sensation mainly residing on the surface of the molecules, wine-tasters perceive more highly polymerized polyphenolics tannins as softer and more harmonious.

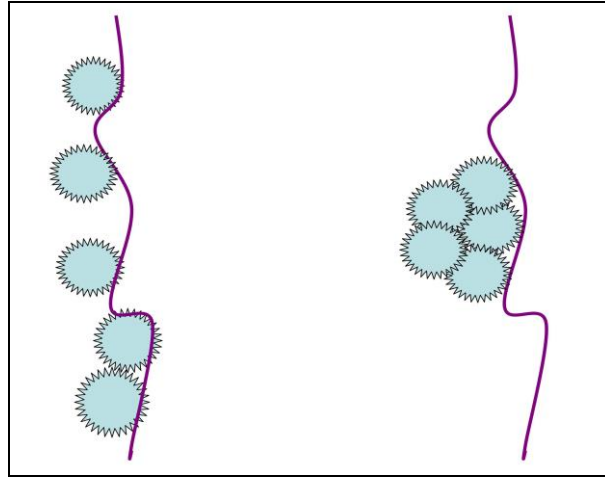


Figure 10. Puckering phenomena due to monomeric and polymeric polyphenolic compounds

Wine aging affects red wine's astringency due to the polymerization phenomenon detailed above. Young or recently fermented red wines contain small condensed tannins and a few polymers (mostly dimers or trimers) and therefore wines are perceived as astringent and bitter. After some aging most wines reach a peak in their organoleptic quality when condensed tannins polymerize into units of 8 to 14 monomers. At this stage, astringency is less pronounced despite the size increase in these polymerized molecules. This is partly due to the sensory integration of other bitter and acid components. Wines continue evolving and when tannins are highly polymerized they no longer cause bitterness in the taste buds or astringency elsewhere in the mouth, since they do not bind proteins on being too large. Some polymeric tannin molecules might reach such a size they even precipitate out.

Bitterness and astringency deriving mostly from tannins are complemented by wine acidity, since tannins and acidity work in opposite directions when it comes to sensorial characters [159]. A low-acidity wine can bear a higher tannin concentration but the other way round, a high-acidity wine coupled with a high tannin concentration would be overly harsh and astringent [12]. Wine astringency is mostly associated with small condensed tannins, which are not considered responsible for bitterness [160]. Low molecular weight phenolics such as phenolic acids or flavonols are also involved in wine astringency but these compounds do contribute to bitterness [161]. Proanthocyanidins (mostly dimers and trimers) and flavanols are considered to contribute to wine bitterness as well [153].

Multiple interactions affect the final astringency. For instance, bitterness is reduced by the sweetness coming from sugars or glycerol [162] but enhanced by ethanol [163]. Compounds such as polysaccharides or polymeric pigments modulate the astringency perception [164] and even volatile compounds have been described to affect wine astringency and bitterness [165]. The great number of factors affecting wine astringency and bitterness has forced researchers to use advanced mathematical models to achieve a better understanding of the phenomenon [166]. Recently, wine astringency was modeled on the basis of proanthocyanidins, polymeric pigment and hydroxycinnamic acid concentrations, with bitterness related to the content of procyanidins and specific flavonols [152].

3.3.2. Aroma

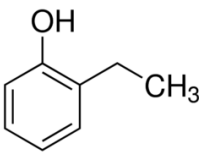
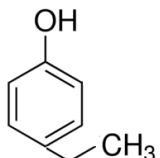
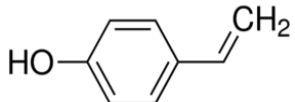
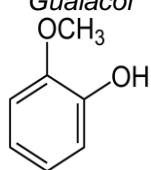
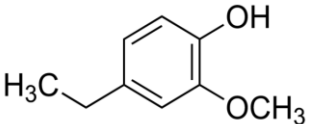
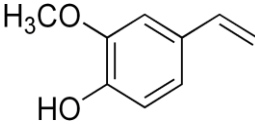
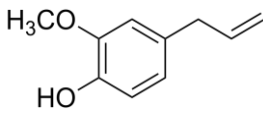
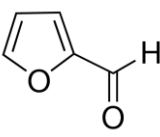
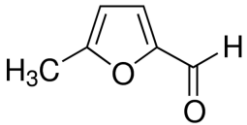
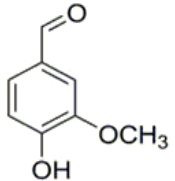
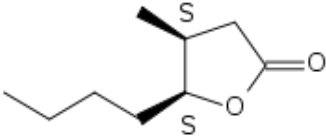
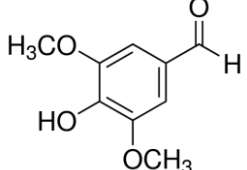
Wine aroma is not a plain uncomplicated topic. It is such an intricate issue that aroma cannot be simply reconstituted by mixing the chemical components previously identified in it [167]. The complexity derives from the balance between more than 800 components identified in the volatile fraction of wines [168].

The contribution of phenolic compounds to wine aroma is due to a small group known as volatile phenols. These phenols have no single identified origin in wines. They can be produced by microorganisms (yeast or bacteria), by hydrolysis of higher phenols or by aging in oak casks. Those related to microorganisms and hydrolysis are essentially 2-ethylphenol, 4-ethylphenol, 4-vinylphenol, guaiacol, 4-ethylguaiacol and 4-vinylguaiacol [169, 170]. The volatiles derived from oak aging are eugenol, furfural, 5-methylfurfural, vanillin, syringaldehyde and oak lactones [171, 172]. They can be considered olfactory attributes or defects, as pleasant perceptions that evoke flowers or gradually pass into displeasing smells of lacquer and pharmaceuticals. 4-ethylphenol and 4-ethylguaiacol are produced when ferulic and p-coumaric acids are decarboxylated by the action of the yeast *Saccharomyces cerevisiae*. Hydroxycinnamate decarboxylase is an endocellular enzyme only active during alcoholic fermentation. Therefore, vinylphenols do not increase during wine aging or during maturation on the lees [12].

Vinylphenol concentrations are lower in red wines than in white because some polyphenolic compounds, such as procyanidins and catechins exert inhibitory effects on hydroxycinnamate decarboxylase activity. Significant amounts of vinylphenols have been described in red wines with particular “animal” or “farm” odors. Vinylphenols are in fact produced by contaminating microorganisms such as lactic bacteria or *Brettanomyces/Dekkera* yeasts, whose activity is not inhibited by red wine's polyphenolic compounds [148]. Vinylphenols are particularly critical when these microorganisms contaminate wood, and similarly undesirable ethylphenols are formed in wine aged in reused oak barrels, conferring unpleasant odors [173].

Wine compounds extracted from oak normally contribute to the woody, smoky and spicy hints of wine aromas rather than conferring undesirable odors [174]. Many of these pleasant compounds are volatile phenols extractable from oak barrels, whose content depends on factors such as the species of oak [175], seasoning of the staves [176], toasting [177] or age of the barrel [178], aging time [179] and wine composition [180]. These are directly related to the wine's geographical origin [181]. Once extracted, such compounds undergo various chemical transformations during wine aging [182]. The main phenolic compounds passing from oak wood to wine are furfural, 5-methyl furfural, eugenol and cis/trans oak lactones [183]. Oak lactones are considered markers of the wood used during aging since the ratio between the cis and trans form in wine is directly indicative of the type of oak [184].

Table 8. Phenolic compounds involved in wine aroma

| | | |
|---|---|--|
| <p><i>2-ethylphenol</i></p>  | <p><i>4-ethylphenol</i></p>  | <p><i>4-vinylphenol</i></p>  |
| <p><i>Guaiacol</i></p>  | <p><i>4-ethylguaiacol</i></p>  | <p><i>4-vinylguaiacol</i></p>  |
| <p><i>Eugenol</i></p>  | <p><i>Furfural</i></p>  | <p><i>5-methylfurfural</i></p>  |
| <p><i>Vanillin</i></p>  | <p><i>Oak lactones</i></p>  | <p><i>Syringaldehyde</i></p>  |

4. Conclusion

Phenolic compounds play a key role in wines because of their organoleptic properties and wide diversity. The detailed study of these compounds allows us to identify the origin, varieties, winemaking techniques and even vintages due to their different evolution during aging. The interactions between them and how to enhance or reduce their concentration remains a current research issue. Analysis of wine phenolics and the complete knowledge of their actual consequences in the commercial product and in human health is a wide scientific field which still needs to be further studied in depth during the coming years and decades.

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