Review Article

Pyranoanthocyanin Derived Pigments in Wine: Structure and Formation during Winemaking

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Received 9 November 2012; Revised 20 December 2012; Accepted 22 December 2012

Academic Editor: A. M. S. Silva

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In recent years many studies have been carried out on new pigments derived from anthocyanins that appear in wine during processing and aging. This paper aims to summarize the latest research on these compounds, focusing on the structure and the formation process. The main pyranoanthocyanins are formed from the reaction between the anthocyanins and some metabolites released during the yeast fermentation: carboxypyrananthocyanins or type A vitisins, formed upon the reaction between the enol form of the pyruvic acid and the anthocyanins; type B vitisins, formed by the cycloaddition of an acetaldehyde molecule on an anthocyanin; methylpyrananthocyanins, resulted from the reaction between acetone and anthocyanins; pinotins resulted from the covalent reaction between the hydroxycinnamic acids and anthocyanins; and finally flavanyl-pyranoanthocyanins. On the other hand, the second generation of compounds has also been reviewed, where the initial compound is a pyranoanthocyanin. This family includes oxovitisins, vinylpyrananthocyanins, pyrananthocyanins linked through a butadienylidene bridge, and pyrananthocyanin dimers.

1. Introduction

One of the main sensory attributes perceived by the consumer in red wines is the color. The major compounds responsible for this color in young wines are the anthocyanin pigments, which are directly extracted from grapes and then gradually disappear due to their degradation and transformation to other more complex and stable pigments that provide the color of aged wines [1].

Initially, it was thought that these pigments were formed by direct condensation between anthocyanins and flavanols [2] or through an acetaldehyde molecule [3–6]. Nevertheless, in recent years some authors have shown that anthocyanins can react with other low molecular weight compounds such as pyruvic acid [7–12], vinylphenol [13, 14], glyoxylic acid [15, 16], vinylcatechol [17], α -ketoglutaric acid [18], acetone [18–20], and 4-vinylguaiacol [20], obtaining a new anthocyanin-derived pigment family, namely, pyranoanthocyanins. This family includes a large number of compounds that can react again producing a new

generation of compounds, where the new precursors are the anthocyanin derivatives [21].

All these anthocyanin derivatives formed during wine aging contribute to the progressive shift of red-purple color of young wines to a more orangish color. However, the main interest of these pigments is that they have a greater color stability against pH changes [8] and bleaching by SO_2 than the anthocyanins monomer [8, 10, 22].

In recent years, various instrumental techniques have been used to confirm the structures and formation mechanisms of these anthocyanin derivatives. On the one hand, techniques to facilitate the compound separation such as solid phase extraction and high performance liquid chromatography [23–25], and on the other hand, techniques that allow a better identification of structures such as NMR (nuclear magnetic resonance) [26, 27] and mass spectrometry [28]: electrospray ionization mass spectrometry (ESI-MS) [29], matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) [30], matrix-assisted laser desorption/ionization time-of-flight

mass spectrometry (MALDI-TOF-MS) [31] and atmospheric pressure photoionization quadrupole time-of-flight mass spectrometry (APPI-QqTOF MS) [32].

Therefore, the aim of this work was to review the latest research on the structure of pyranoanthocyanins and the reaction mechanisms for the formation of these pigments during winemaking and aging of red wines.

2. Formation of Pyranoanthocyanin Derived Pigments in Wine

The pyranoanthocyanins are compounds that are produced in wines during the fermentation and aging processes. These compounds are responsible for a gradual change of the red-purple color towards orange hues since these adducts have a more reddish-orange color than their anthocyanin counterparts.

The pyranoanthocyanins resulting from condensation reactions on anthocyanins, which are modified to stable oligomers, result from substitutions on the C4 position, so the general structure includes an additional ring D formed between the group OH in C5 and the C4 of the anthocyanidin pyran ring [33], according to the mechanism shown in Figure 1. In these compounds, the positive charge is delocalized over the pyranoanthocyanin system (Figure 2).

The pyranoanthocyanins have a maximum absorption wavelength between 495 and 520 nm, so these compounds present a hypsochromic shift in respect to the starting anthocyanins [34–36], in addition to an absorption peak in the 420 nm region, explaining the orange hues of these compounds [9]. The pyranoanthocyanins also present a higher color intensity and stability in a greater pH range than the anthocyanin counterparts, due to the different types of substituents directly joined to the C10 of the formed pyran ring D [8, 37, 38].

Moreover, the substitution at the anthocyanin C4 position in the ring D causes a steric hindrance which makes the pyranoanthocyanin molecule more stable to bleaching by SO₂ [8, 35, 39], to pH increases [10, 22, 35], to oxidative degradation [7], and even to temperature [40].

In the last few years, the pyranoanthocyanins have been described as derivatives not present in grapes of *Vitis vinifera*. However, recently these compounds have been found in skins from *Vitis amurensis* grapes [41]. Normally, the pyranoanthocyanins are formed in red wine during the alcoholic fermentation and the subsequent elaboration steps [7, 41]. Some of the most important pyranoanthocyanins result from the reaction between the original anthocyanin and yeast metabolites released during fermentation [33], such as pyruvic acid, acetoacetic acid, and acetaldehyde (Figure 3). In this regard, Morata et al. [42] have compared the production of pyranoanthocyanin by *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae*, and *Saccharomyces uvarum* during fermentation. They found that *S. pombe* produced more pyruvic acid than did either *Saccharomyces* species, and, as a consequence, it also formed more vitisin A-type pigments.

Other authors have found some pyranoanthocyanins in musts from raisins dried at a controlled temperature. These compounds have been synthesized with some metabolites obtained from enzymatic pathways [43, 44]. The drying process alters the permeability of grape membranes by the lipoxygenase activation effect (LOX), a switch to an anaerobic metabolism and the resulting triggering of the alcohol dehydrogenase enzyme (ADH). The activation of these and several other enzymes confirmed the occurrence of enzymatic transformations, and the formation of acetylvitisin A, the B vitisins of malvidin-3-glucoside, peonidin-3-glucoside, peonidin-3-acetylglucoside, and malvidin-3-acetylglucoside [43].

Their concentration in wines is much lower than other pigments, but since they are less sensitive to pH and bleaching by SO₂, almost all of these adducts are involved in color [45]. Furthermore, the pyranoanthocyanins are poorly adsorbed by the cell walls of the yeasts, because they are formed in the middle or at the end of the alcoholic fermentation, when the cell walls are already saturated by anthocyanins [46].

A study in model wines using red grape skin extracts, wine fermentation metabolites, and hydroxycinnamic acids has been developed focused on increasing the chromatographic (HPLC-DAD-ESI/MS) and spectroscopic (DAD-UV-Vis) database of some pyranoanthocyanin compounds formed in red wines [47].

3. Formation of Pyranoanthocyanin Adducts from Anthocyanins

3.1. Vitisins. The vitisins are the most studied pyranoanthocyanin family, and they are formed in the reaction between the anthocyanins with some metabolites released during the yeast fermentation, such as pyruvic acid, acetoacetic acid, and acetaldehyde [8, 9, 20], the latter of which can also be found in the wine as a result of the oxidation of ethanol. These metabolites are carbonyl compounds, commonly present in a keto-enol balance in hydroalcoholic solution. It is believed that the formation mechanism of the vitisins begins with the cycloaddition of these small metabolites at positions 4 (carbon) and 5 (hydroxyl group) of the anthocyanins, followed by a dehydration and a further oxidation obtaining the ring D [33].

3.1.1. Carboxypyrananthocyanins. In the vitisin group, the most important are the carboxypyrananthocyanins or type A vitisins, formed upon the reaction between the enol form of the pyruvic acid and the anthocyanins [8, 9]. Due to the formation of pyruvic acid during alcoholic fermentation, it is likely that the formation of these derivatives begins at this stage of winemaking.

The vitisin formed from malvidin-3-O-glucoside was called vitisin A by Bakker et al. [7], whose structure is shown in Figure 4(a). This vitisin has been found in the highest concentrations, due to that the malvidin-3-O-glucoside is the prevalent anthocyanin in *Vitis vinifera* [48]. The vitisin A is the main anthocyanin derivative detected by HPLC in Port wines after a year of aging, which clearly shows its importance in wine color [12, 49]. However, other studies

FIGURE 1: Pyranoanthocyanin formation by reaction between malvidin-3-O-glucoside and carbonyl compounds [9, 36].

FIGURE 2: General structures of pyranoanthocyanins derived from an anthocyanin-3-O-glucoside under the two possible ions flavylium [9, 60].

FIGURE 3: Yeast metabolites involved in anthocyanin transformations [33].

FIGURE 4: Chemical structures of vitisin A (a) and vitisin B (b) [8, 79].

with red table wines show different results, and the amounts of vitisin A were always lower than those of malvidin-3-*O*-glucoside [50, 51].

The maximum production of vitisin A is reached in the range between 10 and 15°C, whereas at higher temperatures (32°C) the formation of polymeric pigments is favored [10], since the temperature is an influential factor in the synthesis of these compounds.

Moreover, the vitisin A has a low rate of degradation [12, 52] and a high stability [49]. Some authors have determined that more than half of its initial content remains in wines after 15 years [17]. This is due to the high stability of the molecule to a nucleophilic attack and it is also possible to constantly generate these compounds during the life of the wine, while monomeric anthocyanins and pyruvic acid are available [36].

The monoglucoside and acetylglucoside anthocyanins seem to have the same reactivity towards pyruvic acid [10], although the vitisins formed from acetylated anthocyanins are less stable in wine than those formed from glucosylated anthocyanins [49].

3.1.2. Type B Vitisins. Another pyranoanthocyanins group, which is structurally closely related to the above compounds, is type B vitisins [7], which differs from carboxypyrananthocyanins lacking the carboxyl group in the C10 position of ring D. The type B vitisins are formed by the cycloaddition of an acetaldehyde molecule on an anthocyanin, giving rise to compounds with chemical structures as shown in Figure 4(b), which correspond to the type B vitisin derived from malvidin-3-*O*-glucoside [46]. In the formation of these vitisins, it must be considered that acetaldehyde reacts preferentially with acetylated anthocyanins, and less with coumaroylated anthocyanins [34].

During the alcoholic fermentation of wines, type A vitisins are formed more readily than type B vitisins, especially during the first days of the process, according to the concentrations of pyruvic acid and acetaldehyde in this stage of winemaking. In this respect, the maximum concentration of pyruvic acid excreted by the yeast is reached when about

50% of must sugar has been fermented, still being the medium rich in nutrients, also at this time the maximum rate of formation of the type A vitisins is achieved [46]. At the end of the fermentation, the medium is nutritionally depleted and the yeast starts to reuse part of the excreted pyruvate, thereby diminishing the rate of formation of this type of vitisin. Also, at that time the synthesis of type B vitisins begins [53], since the production of acetaldehyde is proportional to the amount of the fermented sugar and consequently is greater towards the end of the fermentation.

Figure 5 shows the formation of the oligomers catechin-(4-6/8)-vitisin A and catechin-(4-6/8)-vitisin B, which has been recently proposed [54]. These compounds result from the cycloaddition of the pyruvic acid (vitisin A) or acetaldehyde (vitisin B) on the anthocyanin moiety of the adducts formed between flavanols and (4-6/8)-anthocyanins in red wines [33].

3.1.3. Methylpyranoanthocyanins. Another pyranoanthocyanins group derived from the reaction between anthocyanins and yeast metabolites is methylpyranoanthocyanins, proposed as a result of the reaction between acetone and anthocyanins in red wines [18–20]. These compounds have been studied in Port wines, and they can be synthesized after the reaction of anthocyanins with acetoacetic acid using a cycloaddition mechanism similar to the formation of carboxypyrananthocyanins [55]. These derivatives show a yellow-orangish color as a result of the maximum wavelength of these pigments ($\lambda_{\max}$), which is set at 478 nm at acid pH.

3.1.4. Other Pyranoanthocyanins. In addition to pyruvic acid, acetaldehyde, and acetone, other molecules can form pyranoanthocyanins, such as α -ketoglutaric acid [9, 11], glyoxylic acid [15, 16], and even acetoin and diacetyl [56]. The latter, in combination with anthocyanins, originate castavinols, which could act as a reserve of coloring matter [57].

FIGURE 5: Proposed reaction mechanism between acetaldehyde and catechin-malvidin-3-O-glucoside [54].

3.2. Pyranoanthocyanins Resulting from the Reaction between Anthocyanins and Vinyl Compounds

3.2.1. Pinotins. The hydroxycinnamic acids, acting by themselves (*p*-coumaric, caffeic, ferulic, or sinapic) or through their decarboxylation products (4-vinylphenols), can react covalently with anthocyanins, giving rise, pyranoanthocyanin pigments recently called pinotins [17, 58, 59]. At the wine pH, these pigments present a $\lambda_{\max}$ at 505–508 nm [60], showing reddish-orange colors [61–63].

The first pyranoanthocyanin identified in wine was pyranomalvidin-3-*O*-glucoside-phenol [13, 14]; this compound was synthesized in the reaction between malvidin-3-*O*-glucoside and vinylphenol, the latter formed by the decarboxylation of *p*-coumaric acid. The proposed formation mechanism involves a cycloaddition reaction between the vinyl group of vinylphenol and the groups in position 5 and 4 of the anthocyanin (hydroxyl and carbon, resp.), followed by an oxidation, leading to the aromatization of ring D (Figure 6(b)).

Subsequently, other structures were determined in wine with characteristics and color similar to the above, but with different substitution patterns in the phenol fraction such as catechol, syringol, or guaiacol [17, 64–66]. In this regard, in the Pinotage variety, the pyranomalvidin-3-*O*-glucoside-catechol was identified, which was denominated Pinotin A, and formed by the reaction between an anthocyanin and caffeic acid [17]. The mechanism was similar to that previously discussed, but with one additional decarboxylation (Figure 6(a)). Similarly, the same mechanism would take place for

the synthesis of the pyranoanthocyanins resulting from the reaction between anthocyanins and other cinnamic acids such as *p*-coumaric, ferulic, and sinapic acid, although it is believed that these reactions are slower.

At first it was thought that vinylphenols were formed via enzymatic decarboxylation of *p*-coumaric, caffeic, ferulic, and sinapic acids by *Saccharomyces cerevisiae*, and exclusively during fermentation [14]. However, Chatonnet et al. [67], studying the ability of different strains of *Saccharomyces cerevisiae* to decarboxylate the cinnamic acids identified that certain molecules such as catechin, epicatechin, and oligomeric procyanidins strongly inhibited the decarboxylase activity on *p*-coumaric acid, concluding that the cinnamate-decarboxylase activity would be hardly active during fermentation. Likewise, vinylphenols can also be produced by a chemical mechanism, from a slow hydrolysis of the corresponding tartaric esters of hydroxycinnamic acids [68], which would explain the constant increase of the pinotin concentration during wine storage.

In this regard, Schwarz et al. [17] found that the concentration of Pinotin A was 10-times higher in wines aged for 5 or 6 years than in young wines, possibly because these compounds are formed whenever there are free anthocyanins and hydroxycinnamic acids [59]. The fact that the formation of this type of pyranoanthocyanin in wine mainly occurs after several years in bottle [1] sometimes allows them to be used as markers for the aging time in wines.

3.2.2. Flavanyl-Pyranoanthocyanins. The flavanyl-pyranoanthocyanins are anthocyanin derivatives in which one

FIGURE 6: Formation reaction of pinotins from hydroxycinnamic acids (a) and formation of pyranomalvidin-3-O-glucoside-phenol (b) [59, 90].

pyranoanthocyanin molecule has been directly joined to a flavanol. These compounds were firstly proposed by Francia-Aricha et al. [22] after a study in model solutions. Then, these compounds were confirmed in experimental wines [69] and in commercial red wines [70–72].

These pigments present a hypsochromic shift of λ_{max} to values of 490–511 nm, showing a more orangish color than the starting anthocyanins [60].

A mechanism similar to the vinylphenols was proposed for this group of pyranoanthocyanins, where the compounds would result from the cycloaddition reaction between vinylflavanols and anthocyanins. The vinylflavanols are produced from the depolymerization of flavanol polymers (unions between flavanols mediated by acetaldehyde) or the hydrolysis of flavanol-ethyl-anthocyanin condensations. Specifically, Cruz et al. [73] found that vinylcatechin readily reacts with anthocyanins producing these pyranoanthocyanins (Figure 7). The vinylflavanols are not naturally synthesized in grapes. They may result from the dehydration of flavanol-ethanol adducts, or by the decomposition of flavanol

adducts linked by a methyl-methine bridge, although, in both cases, the starting compounds result from the reaction of flavanols with acetaldehyde [73, 74].

Cruz et al. [75] found that the pyranomalvidin-3-O-glucoside-flavanol pigments have a greater resistance to discoloration in comparison with the starting anthocyanins. According to these authors, this fact as for the carboxypyranomalvidin-3-O-glucoside is attributable to their structural properties, characterized by a substitution at C4 of the anthocyanin molecule, thereby protecting the colored forms of the compound against the nucleophilic attack of water, which normally occurs at positions 2 and 4 of the chromophore. Thus, the equilibrium of the pyranoanthocyanins in aqueous solutions according to the pH changes in the medium could correspond only to proton-transfer reactions, in which the pyranoflavylum leads to the formation of their quinonoidal bases.

Malvidin-3-O-glucoside can react with 8-vinylcatechin to produce dimers of pyranoanthocyanins-flavanol and pyranoanthocyanin-flavanol with more polymerized structures,

FIGURE 7: Formation reaction of pyranomalvidin-3-O-glucoside-catechin in red wines [73].

FIGURE 8: Formation reaction of pyranone-anthocyanins from vitisin A [78].

FIGURE 9: Proposed mechanism for the formation of Portisin A [26].

FIGURE 10: Proposed mechanisms for the formation of type B portisins (a) and the formation of vinylpyranomalvidin-3-O-glucoside-phenol (b) [83].

which have been identified in red wines [62, 76, 77], although they are shown in trace levels [73].

4. The Second Generation: Formation of Adducts from Pyranoanthocyanin

4.1. Oxovitisins. He et al. [78] have demonstrated that the type A vitisins react with water leading to neutral pyranone-anthocyanins, called oxovitisins, which show a yellowish color in acidic medium with $\lambda_{\text{max}} = 373 \text{ nm}$, at pH 2. These authors proposed that the pyranone-anthocyanin A may arise from the nucleophilic attack of water to the electrophilic C10

of the carboxypyrananthocyanin, leading to hemiacetal formation (Figure 8). The decarboxylation of this intermediate under mild conditions and further oxidation of the hydroxyl group of the hemiacetal to the pyran-2-one results in the formation of the final product, a stabilized neutral pyranone-anthocyanin derivative.

Some authors have shown that vitisins B are not in equilibrium with the hemiacetal forms resulting from the nucleophilic attack by water [79]. These results show that the nucleophilic attack may occur very slowly and that this should be the first step for the irreversible change of carbonium vitisins to the formation of the neutral pyranone-anthocyanins [78].

FIGURE 11: Proposed mechanism for the formation of pyranomalvidin-butadienyldene-sinapyl from the reaction of methylpyranomalvidin-3-O-glucoside with sinapaldehyde [85].

4.2. Vinylpyranoanthocyanins. Mateus et al. [80] identified a new class of pigments derived from anthocyanins in Port wines after 2 years of aging. The structure of these new compounds is a pyranoanthocyanin linked to a flavanol or phenol unit through a vinyl bridge, and, due to the kind of wine where they were firstly identified, they were named portisins [26]. Studies revealed that these vinylpyranoanthocyanins had a blue color under acidic conditions with a $\lambda_{\max}$ close to 570 nm; the extended electron conjugation would possibly be responsible for the blue color so rare in acidic conditions [81].

Some studies carried out in model solutions revealed that these portisins pigments were derived from the reaction between type A vitisins and flavanols in the presence of acetaldehyde [26]. The first to be identified was the compound resulting from the reaction of vitisin A with a vinyl-flavanol moiety. The last one derived from the rupture of ethyl-linked flavanol oligomers or the dehydration of the flavanol-ethanol adducts formed in reactions of flavanol with acetaldehyde (Figure 9). The compound showed an absorption $\lambda_{\max}$ at 575 nm, and, although this compound was in very small amounts, due to its stability, it would be likely to contribute to the color change of the wines during aging [81].

Other portisins derived from type A vitisins have been identified in Port wines, including the catechin-vinylpyrano derivatives of the anthocyanins petunidin, peonidin and malvidin-3-O-glucoside, peonidin and malvidin-3-O-acetylglucoside, and malvidin-3-O-coumaroylglucoside [82]. Furthermore, Mateus et al. [65] identified the vinylpyranomalvidin-3-O-glucoside-phenol, which was the reaction product between the vitisin A and a vinyl-phenol moiety from the decarboxylation of *p*-coumaric acid (Figure 10(b)). This new compound had a $\lambda_{\max}$ at 535 nm, purple hues, and high stability and could play a crucial role as a precursor of other new pigments during the color development.

Oliveira et al. [83] identified three compounds similar to vinylpyranomalvidin-3-O-glucoside-phenol, but with other

phenolic moieties (catechol, syringol, or guaiacol). The proposed mechanism for the formation of these compounds, which are called type B portisins begins with a nucleophilic attack of a hydroxycinnamic acid olefinic double bond on the C10 position of the anthocyanin-pyruvic acid adduct, followed by the loss of a molecule of formic acid and a decarboxylation, according to the mechanism shown in Figure 10(a).

According to Carvalho et al. [84], the type B portisins show a bathochromic shift of the absorption $\lambda_{\max}$ to values close to 540 nm in respect to the starting anthocyanin-pyruvic acid adduct ($\lambda_{\max}$ 511 nm), due to the extended conjugation of the Π electrons in the ring D. Interestingly, the color of these anthocyanin derivatives changes to blue hues when they are frozen in water, which is explained by a reversible physicochemical change due to the electronic and vibrational properties. The more ordered crystalline phase can potentially induce stronger interactions between water and the solvatable hydroxyl groups, with the consequent increase of the vibrational frequencies associated with the Θ and Γ torsional modes. This increase of the vibrational frequency can induce the increase of the ground state energy, consistent with the observed color change. On the other hand, the deviation from the planarity, associated with a reducing of the electronic delocalization which induces the decrease of $\lambda_{\max}$, had been confirmed when the solution was frozen.

The characterization of portisins revealed that they were more resistant to discoloration by a nucleophilic attack of water and SO_2 than the starting anthocyanins. However, the resistance to the discoloration of type B portisins was less than type A, because the hydroxycinnamic group does not protect against the nucleophilic attack at the C2 position [83].

4.3. Pyranoanthocyanins Linked through a Butadienyldene Bridge. Recently, a new pyranoanthocyanins-derived pigment with a bluish color has been obtained from the reaction

FIGURE 12: Proposed pathways for the formation of pyranoanthocyanin dimers [60].

of methylpyranomalvidin-3-glucoside with a cinnamic aldehyde [85]. The structure of this compound is similar to the portisin reported in the literature yielded from the reaction of a carboxypyrananthocyanin with sinapic acid [86]. The difference in this new pigment is that the binding between the pyrananthocyanin and the syringol moieties is not by a vinyl linkage, but through two conjugated vinyl groups (butadienylidene group). The formation mechanism involves a charge-transfer reaction pathway (Figure 11).

4.4. Pyranoanthocyanins Dimers. Recently, two new families of anthocyanin-derived pigments have been detected in a

9-year-old red Port wine and the respective lees, displaying unusual spectroscopic features [21]. One group of these newly formed pigments displayed a $\lambda_{\max}$ at 676 nm in the UV-Vis spectrum at acidic and neutral pH, with an unusually attractive turquoise blue color. These compounds were detected in higher levels in wine lees probably because of their lower solubility in 20% aqueous ethanol. Their structure was found to correspond to a double pyrananthocyanin arrangement linked by a methine bridge. These pigments may arise from the reaction of carboxypyrananthocyanins with vinylphenolics and mainly with other pyrananthocyanins occurring in the wine, such as methylpyrananthocyanins.

FIGURE 13: Hypothetic general structure for dimers detected in wine and lees [21].

De Freitas and Mateus [33] suggested that these pigments arising from the reaction between methylpyranoanthocyanins and carboxypyrananthocyanins, and two reaction pathways have been proposed (Figure 12). The first involves the deprotonation of the methyl group of the methylpyranoanthocyanin with the formation of a methylene group at carbon C10. These new pigments may result from the nucleophilic attack of the double bond of this methylene group to the electrophilic carbon C10 of the carboxypyrananthocyanin molecule. The last step should involve the loss of a formic acid molecule, leading to the formation of a structure with two pyranoanthocyanin moieties linked through a methine group.

The second pathway involves the formation of a charge-transfer complex between the two precursors that is stabilized by the π -interaction of the aromatic rings. Further condensation between both occurs through an ionic or radical reaction, and the last step involves the loss of a formic acid molecule and the formation of the dimer. Although there are still no clear conclusions about which mechanism actually occurs, the formation process of charge-transfer complex seems to be the most likely [21, 33, 87].

Another group of these newly formed pigments was also detected in both Port wine and lees, with a λ_{max} at 730 nm in the UV-Vis spectrum. The LC-MS data of these compounds also suggested that they are likely to be characterized by a double anthocyanin-derived arrangement (Figure 13). The difference between both families of compounds seems to be

an unsaturated carbon involved in the conjugation system, which would also explain the higher λ_{max} [21]. Since most of these pigments were found to occur in wine lees, probably because of their low solubility, their contribution to the overall color of red wine is thought to be negligible.

Recently, the pyranomalvidin-3-glucoside dimer linked through a methyl-methine bridge has been synthesized for the first time in a hydroalcoholic model solution through the reaction of the carboxypyranomalvidin-3-glucoside with ethylpyranomalvidin-3-glucoside [88]. This compound displays a blue/green color in solution and the condensation reaction of carboxypyranomalvidin-3-glucoside with ethylpyranomalvidin-3-glucoside to form it may start with a charge-transfer reaction between the two pyranoflavylum moieties through π - π stacking [88]. Then ionic or radical reactions may occur, leading to the formation of the pyranomalvidin-3-glucoside methyl-methine dimer pigment (Figure 14). This dimer presents its pyranoflavylum cationic form in equilibrium with the respective neutral quinoidal form in an aqueous solution at pH 4 [89].

After portisins, pyranoanthocyanin dimers constitute a second group found in wines belonging to the second generation of anthocyanin-derived pigments in which grape anthocyanins are no longer involved directly in their formation. However, the knowledge of their formation mechanisms would establish new chemical pathways involving other wine pigments which could contribute indirectly to the color evolution of red wines.

FIGURE 14: Proposed mechanism for the formation of pyranomalvidin-3-O-glucoside methyldimethine dimer [88].

Acknowledgments

The authors gratefully acknowledge the financial support from the Spanish Government and the Minister of Education (FPU scholarship of A. Marquez) for the realization of this work.

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