New Family of Bluish Pyranoanthocyanins

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The use of anthocyanins has been investigated for the preparation of food and beverage natural colorants as they seem to have non-toxic effects. In this context, vinylpyranoanthocyanins were recently found to naturally occur in ageing red wine. This new family of anthocyanin-derived pigments may be obtained directly through the reaction between anthocyanin derivatives and other compounds. Some of these newly formed pigments have been found to exhibit a bluish color at acidic pH. The formation of bluish pigment was obtained through reaction between anthocyanin-pyruvic-acid adducts and flavanols in the presence of acetaldehyde. The formation of similar bluish pigments was attempted using other different precursors. The chromatic features of this kind of pigments bring promising expectations concerning the use of these naturally occurring blue pigments in the food industry.

VITIS VINIFERA ANTHOCYANINS AND DERIVATIVE PIGMENTS IN RED WINE

Anthocyanins are the most important group of water-soluble plant pigments visible to the human eye. These pigments are responsible for a great variety of colours of several fruits, vegetables, and plants. The colour of red wine is mainly due to the presence of these polyphenolic compounds extracted from grape berries during the wine-making process. The general structure of *Vitis vinifera* anthocyanidin monoglucosides is represented in Figure 1. These compounds differ in their hydroxylation and methoxylation patterns of ring B yielding a wide range of colours from orange-red to violet at very acidic pH [1]. The glucosyl moiety linked at the 3-O position of ring C may also be acylated with acetic acid, coumaric acid, or caffeic acid.

These compounds undergo chemical transformations during wine ageing yielding new pigments that become responsible for the changing colour and its longevity [2]. These new pigments were first thought to result mainly from condensation reactions between anthocyanins and flavanols directly or mediated by acetaldehyde [3, 4, 5, 6, 7, 8, 9]. Nevertheless, over the last decade, reactions involving anthocyanins with other compounds such as pyruvic acid [10, 11, 12, 13, 14, 15], vinylphenol [16, 17], vinlycatechol [18], α-ketoglutaric acid [19], acetone [19, 20, 21], and 4-vinylnluaiacol [21] have been demonstrated yielding new families of anthocyanin-derived pigments, namely, pyranoanthocyanins, with spectroscopic features that may somehow contribute to a more orange-red colour. This family of pyranoanthocyanins has been extensively investigated over the last years and several compounds have been recently evidenced in aged Port red wines [22, 23, 24] (see Table 1 and Figure 2).

It is interesting to notice that the anthocyanins-catechin pigments revealed the same $\lambda_{\text{max}}$ as the pyruvate derivatives, which is hypsochromically shifted from that of original anthocyanins. Strikingly, pigments 9 and 13 which contain a procyanidin dimer unit in their structure revealed an important bathochromic shift (9 nm) from that of their counterparts with a single flavanol monomeric unit ((+)-catechin or (−)-epicatechin). This outcome highlights the importance of the type of flavanol moiety on the color characteristics of the pigments (and it suggests that some kind of intramolecular copigmentation between the flavanol residue and the flavylium chromophore may somehow occur).

PORTISINS—A NEW GROUP OF VINYLPYRANOANTHOCYANINS DETECTED IN PORT WINE

More recently, two new pigments with unique spectroscopic features exhibiting a bluish colour in acidic solution were found to occur in aged Port red wines [25] (see Figure 3, pigments 22 and 23). Indeed, these two pigments with maximum absorption in the visible region at 583 nm were detected by HPLC in two-year-old Port wine samples. These newly formed pigment structures in which anthocyanins are linked to flavanols by a vinyl linkage were named as portisins. Likewise, similar pigments arising from different anthocyanins and flavanols were tentatively detected by LC-DAD-MS in Port wine samples (Table 2).
Furthermore, a portisin with a phenol group replacing the flavanol moiety (pigment 26) has also recently been found to occur in aged Port wine (Table 2) (N. Mateus et al, unpublished data). However, the maximum absorption of this pigment in the visible region (538 nm) was found to be quite hypsochromically shifted from that of portisins with a flavanol moiety (Figure 4). The small hydroxylation pattern of the phenol ring probably contributes to this hypsochromic shift more significantly compared to the phloroglucinol ring of flavanols. Effectively, a similar pigment with a phloroglucinol moiety replacing the phenol group resulting from the reaction between malvidin 3-O-glucoside and phloroglucinol in the presence of acetaldehyde (Figure 5) [26], or directly by reaction with $p$-vinylphenol. The last step of their formation is thought to include decarboxylation, dehydration, and oxidation yielding a structure with extended conjugation of the π electrons, which is likely to confer a higher stability of the molecule and is probably at the origin of its blue color. Similar vinylypyranoanthocyanins had previously been synthesized using starting chemicals not found in grapes or in the yeasts [27].

**INTEREST AND POSSIBLE APPLICATION OF PORTISINS IN THE FOOD INDUSTRY**

The chromatic features of this kind of pigments bring promising expectations concerning the use of these naturally occurring blue pigments in the food industry.
Indeed, despite the extensive colour palette available in nature, pigments exhibiting blue colours are very scarce. For instance, the blue colours displayed by some flowers are mainly due to copigmentation phenomena [28, 29, 30, 31, 32]. Moreover, bluish hues may be obtained by the presence of quinonoidal forms of anthocyanins in high pH media [33, 34].

Therefore, the food industry has been searching for new alternative ways to produce products (foodstuffs and beverages) with bluish colours. Bearing this in mind, the production of bluish pigments was attempted in the laboratory using different precursors. Firstly, the formation of such pigments requires anthocyanins, which can be obtained using several red fruit extracts. Sweet cherry, bilberry, red apple, plum, blackberry, and elderberry extracts were used as anthocyanin sources for the synthesis of anthocyanin-derived pigments. Following this, the formation of the anthocyanin-pyruvic-acid
Table 2. Portisins detected in Port wine fractions. (Mv = malvidin; Pn = peonidin; Pt = petunidin; gluc = glucoside; cat = catechin; PC = procyanidin dimer.)

| Pigment | Portisin                                      | \( \lambda_{\text{max}} \) (nm) | Structural elucidation   |
|---------|-----------------------------------------------|-----------------------------------|--------------------------|
| 22      | VinylpyranoMv-3-gluc-PC                       | 583                               | NMR, MS, UV-Vis          |
| 23      | VinylpyranoMv-3-coumaroylgluc-PC              | 583                               | NMR, MS, UV-Vis          |
| 24      | VinylpyranoMv-3-gluc-cat                      | 572                               | MS, NMR, UV-Vis          |
| 25      | VinylpyranoMv-3-coumaroylgluc-cat             | 577                               | NMR, MS, UV-Vis          |
| 26      | VinylpyranoMv-3-phenol                        | 538                               | MS, NMR, UV-Vis          |
| 27      | VinylpyranoPt-3-gluc-cat                      | 570                               | MS                       |
| 28      | VinylpyranoPn-3-gluc-cat                      | 569                               | MS                       |
| 29      | VinylpyranoMv-3-acetylg gluc-cat              | 577                               | MS                       |

Table 3. Some anthocyanins respective pyruvic acid adducts and portisins obtained from different red fruit extracts. (Cy = cyanidin; Mv = malvidin; py = pyruvic acid derivative; gluc = glucoside; samb = sambubiose; ara = arabinose; rut = rutinose; cat = catechin.)

| Source    | Anthocyanin | \( \lambda_{\text{max}} \) (nm) | Pyruvic acid adduct | \( \lambda_{\text{max}} \) (nm) | Portisin                                      | \( \lambda_{\text{max}} \) (nm) |
|-----------|-------------|-----------------------------------|---------------------|-----------------------------------|-----------------------------------------------|-----------------------------------|
| Blackberries | Cy-3-gluc   | 516                               | Cy-3-gluc-py        | 505                               | VinylpyranoCy-3-gluc-cat                      | 567                              |
| Sweet cherries | Cy-3-rut    | 516                               | Cy-3-rut-py         | 505                               | VinylpyranoCy-3-rut-cat                      | 567                              |
| Elderberries | Cy-3-samb   | 516                               | Cy-3-samb-py        | 505                               | VinylpyranoCy-3-samb-cat                     | 567                              |
| Red apple  | Cy-3-gal    | 516                               | Cy-3-gal-py         | 505                               | VinylpyranoCy-3-gal-cat                      | 567                              |
| Bilberries | Cy-3-ara    | 516                               | Cy-3-ara-py         | 505                               | VinylpyranoCy-3-ara-cat                      | 567                              |
| Bilberries | Mv-3-ara    | 528                               | Mv-3-ara-py         | 511                               | VinylpyranoMv-3-ara-cat                      | 572                              |
| Bilberries | Mv-3-gluc   | 528                               | Mv-3-gluc-py        | 511                               | VinylpyranoMv-3-gluc-cat                     | 572                              |
| Grape berries | Mv-3-coumaroylgluc | 532                               | Mv-3-coumaroylgluc-py | 516                               | VinylpyranoMv-3-coumaroylgluc-cat          | 578                              |

A pyruvic acid adduct was achieved through a reaction with pyruvic acid, as previously developed for grape malvidin 3-O-glucoside-pyruvic-acid adduct. The different anthocyanins from the red fruit extracts yielded pyruvic acid adducts with a \( \lambda_{\text{max}} \) hypsochromically shifted from that of genuine anthocyanins, some of which are indicated in Table 3. Consequently, the colour of all the extracts turned to a more orange-like hue. These anthocyanin-pyruvic acid adducts were used as precursors for the formation of portisins, which was attempted using (+)-catechin in the presence of acetaldehyde.

As an example, Figure 6 shows the anthocyanin profile of an elderberry extract (Sambucus nigra) after two days of reaction with pyruvic acid. The anthocyanins of elderberries are two cyanidin monoglucosides (2) (cyanidin 3-O-glucoside and cyanidin 3-O-sambubioside) and a cyanidin 3,5-diglucoside (1) (3-O-sambubioside, 5-O-glucoside). This latter is not likely to react with pyruvic acid as position 5-O of the anthocyanin must be free from any substitution. Therefore, the only two pigments formed are the pyruvic acid adducts of the cyanidin monoglucosides ((3) and (4)), as seen from the respective HPLC chromatogram recorded at 520 nm (Figure 6a). Moreover, the HPLC chromatogram recorded at 570 nm of the purified pyruvate extract further treated with catechin in the presence of acetaldehyde is shown in Figure 6b. This portisin profile of the elderberry extract was obtained when practically all the pyruvic acid derivatives had reacted. The two portisins obtained correspond to the vinylpyranoanthocyanins of cyanidin 3-O-glucoside (5) and cyanidin 3-O-sambubioside (6), as confirmed by LC-DAD-MS (data not shown).

Overall, the malvidin monoglucosides and derivatives appeared to be the anthocyanins with the highest \( \lambda_{\text{max}} \) in the UV-Vis spectrum when compared with cyanidin.
mono- or diglucosides, as seen from Table 3. The type of sugar moiety and the presence of a mono- or disaccharide in the anthocyanin structure did not seem to induce any influence on its $\lambda_{\text{max}}$. This behaviour was also observed with regard to the anthocyanin-pyruvic-acid adducts and the respective portisins.

Additionally, acylation of the sugar moiety of malvidin monoglucosides with $p$-coumaric acid yielded in a $\lambda_{\text{max}}$ higher than its nonacylated counterpart. It has already been reported that, in the case of anthocyanins, acylation of the sugar moiety with hydroxycinnamic acids induces a bathochromic shift, as well as an intensification and stabilization of the colour, probably through intramolecular copigmentation phenomena, as reported elsewhere [35]. This bathochromic shift arising from the acylation of the sugar moiety was also observed for the portisins reported in aged Port red wine (Table 3).

**CONCLUSION**

The search for new natural food colourings has attracted the interest of several manufacturers over the last years. From the organoleptic point of view and considering the available colours widespread in nature, it can be seen that blue pigments are rare. Therefore, the production of new natural blue colourings for the food industry appears to be a priority. Concerning the food quality and safety, the natural colourings present significant benefits compared to the synthetic ones, even if it may be due to psychological concerns of the consumer. In fact, nature-derived pigments are easily accepted as being healthy and are thus a major issue for the food industry.

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