



Copigmentation: An Important Feature of Anthocyanins

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Abstract

Copigmentation is a chemical phenomenon that occurs when a colorless organic molecule interacts with a pigment molecule to form a stable, intensely colored complex. It involves the interaction between colorless copigments and colored, water-soluble pigments, *i.e.*, anthocyanins. Examples of copigments include flavonols, polyphenols, organic acids, and alkaloids. The effect of copigments is predominant in weak acidic conditions. The process can occur in several ways, including self-association, intramolecular, and intermolecular interactions. Different types of shifts such as bathochromic, hyperchromic, and hypsochromic, determine the color quality and stability of these complexes. Understanding the mechanisms of copigmentation can lead to the development of strategies to enhance the color quality and stability of products in different food, cosmetics, and textile industries.

Keywords: Anthocyanins, Color stability, Copigmentation, Flavonols

Introduction

Copigmentation is a chemical phenomenon that occurs when a colorless organic molecule, known as a copigment, interacts with a pigment molecule to form a more stable, intensely colored complex. It is commonly observed in plant pigments, especially anthocyanins, which confers red, purple, and blue colors to different plant parts, especially flowers, leaves, and fruits. It is the modification in UV-visible adsorption of anthocyanins and still has not been observed in any other polyphenols. Some of the common copigments are flavonoids (rutin and quercetin), amino acids, polyphenols (sinapic acid and ferulic acid), organic acids, and alkaloids. The effect of copigments is predominant at pH 4-6 (weak acidic conditions) and it is an exothermic reaction. It is more preferred in anthocyanin 3,5-diglucosides than in the 3-mono glucosides. Fruit crops such as blueberries, blackberries, and strawberries have been shown to contain significant copigments, which contribute to their overall color stability. Recently, the primary cause of the color variation between red and pink-pulped guavas has been identified as cyanidin-3-*O*-sophoroside, which interacts with different copigments, including quercetin, myricitrin, and myricetin. The degree of copigmentation is influenced by the configuration of copigments and anthocyanins. More the hydroxyl groups (especially one situated at C-7), the stronger the copigmentation, whereas methoxyl groups of

the copigments decrease the copigmentation (Chen and Hrazdina, 1981). The phenomenon of copigmentation is important in many industries, particularly food, cosmetics, and textiles, and is crucial to a product's acceptance. Rosmarinic acid and sinapic acid as copigments can significantly enhance the color intensity in cranberry and lingoberry, and strawberry juices.

Anthocyanins

Anthocyanins are classified as polyphenols belonging to the subgroup of flavonoids. Because of their low toxicity and color along with water-soluble properties, anthocyanins are potential natural food colorants. They are derived from phenylalanine in the cytosol and are localized in vacuoles. In fruit crops, cyanidin-3-glucoside is the most prevalent anthocyanin (Figure 1). The structure of anthocyanins decides its various properties, including potential biological activity. Differences among the various anthocyanins are due to the hydroxyl group and methoxyl group attachments, and sugars attached to the molecule. Anthocyanins are pH-dependent, which means that their color can change depending on the pH of the medium.

Copigments

Copigments are colorless organic molecules and are typically classified into different groups based on their chemical

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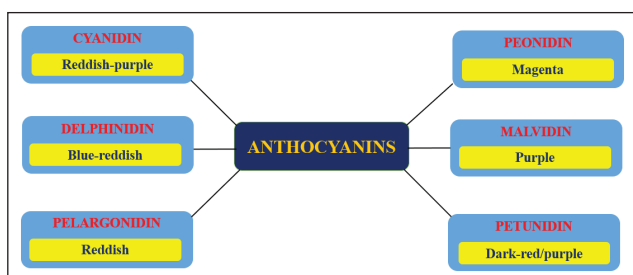


Figure 1: Types of anthocyanins and their color variations

structure and properties. Some of the common copigments are flavonoids, phenolic acids, amino acids, metal ions, and alkaloids. The copigments coexist with anthocyanins inside the vacuole of plant cells. The effectiveness of copigments depends on several factors such as structure, concentration, as well as pH, and temperature. Copigments can undergo π - π interactions, can form hydrogen bonds, or can be directly incorporated into the anthocyanin molecules. Copigments can also enhance the absorption of light in the visible spectrum by the anthocyanins, leading to a more intense and stable color. Flavanols are poor copigments than other flavonoids such as flavonols and hydroxycinnamic acids.

Types of Interactions

There are three ways in which copigmentation interactions can occur, *i.e.*, self-association, intramolecular, and intermolecular interaction, and thereby improve the color stability of anthocyanins significantly (Figure 2) (Brouillard *et al.*, 1989). Copigmentation doesn't produce any chemical bonds between anthocyanin and colorless copigments.

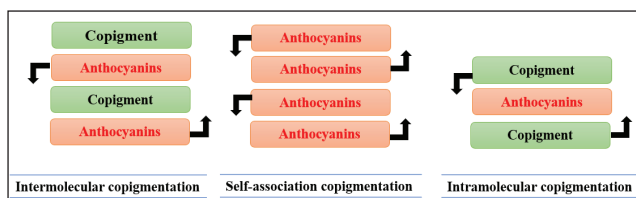


Figure 2: Sandwich configuration of copigmentation complexes

Self-Association

In self-association, π -stacking interactions take place, which usually happens during wine aging and might contribute to the color of matured wines. Self-association occurs when anthocyanin concentration exceeds 0.1 mM, *i.e.*, a higher concentration of anthocyanins.

Intramolecular Interactions

In intramolecular interaction, copigment is part of the anthocyanins itself. Compared to the intermolecular interactions, it is more efficient in stabilizing the color of anthocyanins, likely due to the strength of the bond. It occurs with anthocyanins with aromatic acylation. The structure, number and position of acyl groups attached to glycosyl residues, as well as the structure and quantity of the saccharides, can influence this type of interaction. It usually occurs in anthocyanins with a long and branched structure. "Sandwich stacking" occurs in such types of interactions.

Intermolecular Interactions

In intermolecular copigmentation, colored anthocyanins interact with colorless copigments. The primary driving forces behind this interaction are hydrogen bonding and hydrophobic interactions. The copigment doesn't bind covalently to anthocyanin molecules and these reactions are specific in nature. Vertical stacking occurs in aromatic residues. It shields the flavylium chromophore from water and nucleophilic species. This type of interaction is more common in fruits and berries that lack acylated anthocyanins. These reactions are important for the formation of Metallo-anthocyanin complexes.

Metal Interactions

Metal complexation is another type of interaction where metallic ions, such as iron ($\text{Fe}^{2+}/\text{Fe}^{3+}$), aluminium (Al^{3+}), magnesium (Mg^{2+}), sodium (Na^+), and Zinc (Zn^{2+}), form complexes with anthocyanins. In this, metallic cations modify the absorption spectra of the pyrylium rings. Anthocyanin with multiple free hydroxyl groups in B-ring (such as petunidin, cyanidin, and delphinidin) have the ability to chelate metals. Such types of interactions are important for the blue coloration. More intense effects are observed with metal ions that are highly positively charged. Ternary complexes can also be formed when the metal links to both anthocyanins and copigment.

Color and Chemical Stability

There are two types of stability in anthocyanin copigmentation, *i.e.*, color stability and chemical stability (Trouillas *et al.*, 2016). Chemical stability refers to the ability of the anthocyanin complex to resist chemical degradation, such as hydrolysis or oxidation. Color stability, in contrast, pertains to the capability of the anthocyanin complex to sustain its color intensity over time. These effects can be produced by organic copigments or metallic ions. Both types of stability, namely chemical and color stability, are significantly impacted by various factors, including concentration of anthocyanins, pH and temperature, exposure to sunlight *etc.* Changes in pH have a profound effect on the structure and color of anthocyanins, with flavylium cation appearing as red at pH 1-3, chalcone and carbinol pseudo base as yellow and colorless, respectively at pH 4-5, and quinoidal anhydrobase as blue at pH greater than 5 (Koh *et al.*, 2020). Copigmentation is primarily influenced by Van der Waals interactions through vertical stacking and hydrophobic effects via dispersive stacking.

Bathochromic and Hyperchromic Shifts

Two types of phenomena occur in the copigmentation, *i.e.*, bathochromic shift ($\Delta\lambda_{\text{max}}$) and hyperchromic shift (ΔA). In bathochromic shift ($\Delta\lambda_{\text{max}}$), there is a shift of the λ_{max} toward a higher wavelength, resulting in a decrease in color's perceived energy. This shift occurs due to the change in the electronic structure of the molecule, resulting in the molecule's absorption of light at a longer wavelength. The bluing effect arises when copigmentation causes a shift in the color of anthocyanin

from red towards blue hues (Chatham *et al.*, 2020). Copigmentation leads to the enhancement of the intensity of anthocyanin color (A) in the hyperchromic shift (ΔA), which refers to the increase in maximum absorbance. It is a shift towards higher absorbance values, resulting in the stabilization of the molecule's excited state leading to an increased intensity of color. Another type of shift, hypsochromic shift, refers to a shift towards the lower wavelength.

Conclusion

Copigmentation occurs when a colorless organic molecule interacts with a pigment molecule. Anthocyanins are potential natural food colorants, in addition to their health-promoting properties, *i.e.*, anti-oxidative and anti-inflammatory. However, commercial application is limited due to low thermal and chemical stability. It can be enhanced by the phenomenon of copigmentation to form stable complexes, thereby increasing their color intensity and stability. Anthocyanins as coloring agents are mainly used in carbonated beverages and fruit juices and their utilization is expected to be increased in the future as a healthy alternative to synthetic food dyes. Therefore, copigmentation is an effective strategy to increase the stability of anthocyanins and to be widely use them as natural colorants.

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