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# Stabilization of natural colors and nutraceuticals: Inhibition of anthocyanin degradation in model beverages using polyphenols

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ABSTRACT

Anthocyanins are widely used as natural colorants in foods, but they are highly susceptible to chemical degradation during storage leading to color fading. This study examined the potential of natural quillaja saponin and polyphenols (vanillin, epigallocatechin gallate, green tea extract, and protocatechualdehyde) at inhibiting color fading of anthocyanins in model beverages. The purple carrot anthocyanin (0.025%) in model beverages (citric acid, pH 3.0) containing L-ascorbic acid (0.050%) degraded with a first-order reaction rate during storage (40 °C/7 days in light). The addition of polyphenols (0.2%) delayed color fading, with the most notable improvement observed with green tea extract addition. The half-life for anthocyanin color fading increased from 2.9 to 6.7 days with green tea extract. Fluorescence quenching measurements showed that the green tea extract contained components that interacted with anthocyanins probably through hydrophobic interactions. Overall, this study provides valuable information about enhancing the stability of anthocyanins in beverage systems using polyphenols.



## 1. Introduction

Natural colorants are widely used in food and beverage products due to increasing consumer demand for clean labels and natural ingredients (Katz & Williams, 2011). Anthocyanins are one of the most commonly utilized water-soluble natural colorants because they exhibit vibrant colors that range from red to blue (Cheynier, 2012; Mercadante & Bobbio, 2007). This class of molecules are polyphenols (flavonoids) that are typically extracted from the red and blue parts of certain plants, including fruits, vegetables, flowers, and leaves (Cheynier, 2012; Mercadante & Bobbio, 2007). The color of anthocyanins is strongly dependent on the pH of the surrounding aqueous phase (Cheynier, 2012). Besides their vibrant colors, anthocyanins also have anti-oxidant and bioactive properties linked to certain health benefits *e.g.*, anti-diabetic, anti-inflammatory, and anti-cancer effects (Benn et al., 2014; Kuntz et al., 2014; Signorelli et al., 2015).

Chemically, anthocyanins consist of two aromatic benzyl rings attached to a flavylium core in the center (Bueno et al., 2012; Castaneda-Ovando, Pacheco-Hernandez, Paez-Hernandez, Rodriguez, & Galan-Vidal, 2009; Mercadante & Bobbio, 2007). The intensity of the anthocyanin color is attributed to the resonant structure of the flavylium ion (Wrolstad, Durst, & Lee, 2005). There are more than five hundred different types of anthocyanins, which are differentiated by the types, numbers, and positions of sugar or acylating groups attached to the anthocyanidin moiety (Bueno et al., 2012; Castaneda-Ovando et al., 2009; Mercadante & Bobbio, 2007).

Anthocyanins isolated from their natural environment are typically highly susceptible to chemical degradation, which leads to color fading and loss of bioactivity. The rate of degradation is affected by many factors including pH, light, temperature, oxygen, enzymes, and ingredient interactions (Bordenave, Hamaker, & Ferruzzi, 2014; Castaneda-Ovando et al., 2009; Mercadante & Bobbio, 2007; West & Mauer, 2013). Color fading and off-flavor formation limits the shelf life of commercial products, and restricts anthocyanin utilization for certain applications. For this reason, many investigations have been carried out to improve the stability of anthocyanins so they can be used more widely in food and beverage products. Previous studies have shown that anthocyanin stability can be improved by adding certain types of food-grade components (*e.g.*, pectin, rutin, organic acids, and metal ions) that interact with anthocyanin molecules (Brenes, del Pozo-Insfran, & Talcott, 2005; Castaneda-Ovando et al., 2009; Cheynier, 2012; Hernandez-Herrero & Frutos, 2015).

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In previous studies, we have shown the potential of whey protein and gum arabic for improving the color stability of anthocyanins in model beverage systems (Chung, Rojanasasithara, Mutilangi, & McClements, 2015a, 2015b). In these systems, the color fading of anthocyanin was promoted by the presence of ascorbic acid, which is often included in foods as a source of vitamin C (Hernandez-Herrero & Frutos, 2015; Mercadante & Bobbio, 2007; Poei-Langston & Wrolstad, 1981). In the current study, we investigated the potential of a number of phytochemicals at inhibiting color fading of anthocyanins in model beverages: a saponin (quillaja saponin) and four polyphenols (vanillin, epigallocatechin gallate, green tea extract, and protocatechualdehyde). Quillaja saponin, a glycoside extracted from the bark of a tree, was used because it is a commercially available food emulsifier, which has also been reported to have antioxidant properties (Güçlü-Üstündağ & Mazza, 2007). The polyphenols were also tested because they are known to have antioxidants properties (Azevedo et al., 2014; Chowdhury, Sarkar, Chakraborti, Pramanik, & Chakraborti, 2016; Colon & Nerín, 2016; Park, 2011). Consequently, these natural components may be able to inhibit the chemical degradation of the anthocyanins and prolong their color stability. The addition of these phytochemicals may also add nutritional value to beverage products due to their ability to act as nutraceuticals with anti-oxidant and other health benefits. The color stability of anthocyanins was determined by absorbance, tristimulus color, and visual appearance of the model beverage systems during storage at elevated temperature in the presence of light. The mechanism involved in stabilizing anthocyanins was studied using a fluorescence quenching method.

## 2. Materials and methods

### 2.1. Materials

Anthocyanins (AC) from purple carrot extracts were provided by PepsiCo (Valhalla, NY, USA). L-ascorbic acid (AA), calcium chloride, sodium citrate, and sodium benzoate were purchased from the Sigma-Aldrich Company (St Louis, MO, USA). Citric acid was purchased from Fischer Scientific (Waltham, MA, USA). The quillaja saponins (Q-Naturale 200) were kindly donated by Ingredion (Bridgewater, NJ, USA). Vanillin (98%) (Fluka) was purchased from Fisher Scientific (Pittsburgh, PA, USA). Epigallocatechin gallate (EGCG) and 3,4-dihydroxybenzaldehyde (protocatechualdehyde) were purchased from Sigma Aldrich Company (St Louis, MO, USA). Green tea extract (NuSci) containing ~50% EGCG was purchased from Herb Store USA (Walnut, CA, USA).

### 2.2. Methods

All concentrations are reported on a percentage weight/weight basis unless otherwise stated, and are presented as "%" for the sake of concision.

#### 2.2.1. Preparation of plant extract/polyphenol solutions

Weighed amounts (0.4%) of each phytochemical (quillaja saponin, vanillin, EGCG, green tea extract, or protocatechualdehyde) were dissolved in deionized water and stirred until fully hydrated. The pH of the solutions was then adjusted to pH 3.0 using 1 M citric acid.

#### 2.2.2. Preparation of anthocyanin and calcium chloride solutions

Solutions containing anthocyanin (0.05%) and calcium chloride (0.02% $Ca^{2+}$) were prepared at pH 3.0. Initially, a weighed amount of anthocyanin powder was dissolved in 20 mM citric acid buffer solution (containing citric acid, sodium citrate, sodium benzoate, pH 3.0). After stirring for 10 min, a weighed amount of calcium chloride solution (from 200 mM stock solution) was added into the anthocyanin-containing buffer solutions to reach a final concentration of 0.02% $Ca^{2+}$ and stirred for 10 min. The pH of the solutions was adjusted to pH 3.0 if required. The composition of the aqueous phase was designed to model those in a typical soft drink. In particular, sodium benzoate was added as a preservative, while citric acid and sodium citrate were added for their buffering capacity at pH 3.0.

#### 2.2.3. Preparation of anthocyanin-extract mixed systems

Appropriate amounts of each extract solution and anthocyanin/$Ca^{2+}$ stock solution were mixed together for 30 min to produce mixed systems containing 0.2% phytochemical (quillaja saponin, vanillin, EGCG, green tea extract or protocatechualdehyde), 0.025% anthocyanin, and 0.01% $Ca^{2+}$. The pH of the mixed systems was adjusted back to pH 3.0 if required.

#### 2.2.4. Preparation of ascorbic acid-anthocyanin-extract mixed systems

A weighed amount of L-ascorbic acid was added to the different anthocyanin-phenol mixed systems and stirred until dissolved. The final content of the systems had 0.2% plant extract/polyphenol, 0.025% anthocyanin, 0.05% ascorbic acid, and 0.01% $Ca^{2+}$. The pH of the mixed systems was adjusted back to pH 3.0 if required.

#### 2.2.5. Accelerated storage study

All solutions were stored at ~40 °C from 0 to 7 days with exposure to light to accelerate the potential degradation of the anthocyanin.

### 2.3. Measurement of color fading

#### 2.3.1. Absorbance

The chemical stability of the anthocyanin in the solutions over seven day storage was determined by measuring the decrease in their absorbance at a wavelength of 523 nm using a UV–visible spectrophotometer (Cary 100, Agilent Technologies, Santa Clara, CA, USA). This wavelength was selected because it was where the maximum absorbance of anthocyanin solutions was observed in the visible range.

#### 2.3.2. Colorimetry

Changes in the color of the anthocyanin solutions over seven day storage was monitored using a colorimeter (HunterLab, Reston, Virginia, USA) with a tristimulus absorption filter to measure the tristimulus coordinates: $L^*$ (lightness), $a^*$ (red to green), and $b^*$ (yellow to blue). These values were then used to calculate the overall change in color ($\Delta E^*$) throughout storage: $\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$. Here, $\Delta L^*$, $\Delta a^*$, and $\Delta b$, represent the change in the color coordinates compared to the initial values.

#### 2.3.3. Visual appearance

Digital photographs of the different systems were captured after each storage time using a digital camera.

#### 2.3.4. Kinetic reaction analysis

Anthocyanin degradation kinetics were analyzed using zero-order and first-order models, as it has previously been reported to typically follow one of these kinetic orders depending on solution and environmental conditions (Fernandez-Lopez, Angosto, Gimenez, & Leon, 2013; Maskan, 2006; Remini et al., 2015; Summen & Erge, 2014; Verbeyst, Oey, Van der Plancken, Hendrickx, & Van Loey, 2010).

The zero order reaction is expressed by Eq. (1):

$$C_t = C_0 - kt \quad (1)$$