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Assessment of the color modulation and stability of naturally copigmented anthocyanin-grape colorants with different levels of purification

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ABSTRACT

Grape skins or their by-products from wine production are rich sources of anthocyanins and various colorless phenolics, depending on the grape variety. Phenolics have strong antioxidant and anthocyanin stabilizing properties and help to produce functional anthocyanin colorants with improved stability. This study aimed to assess differences in color expression and stability of anthocyanin colorants from red grape varieties naturally copigmented and with different levels of purity and to compare them to synthetic FD&C Red No. 3. Model juice systems were prepared at pH 3.5 with anthocyanins and phenolic copigments extracted from four *Vitis vinifera* grape varieties ('Tempranillo', 'Syrah', 'C. Sauvignon', and 'Graciano') both crude and purified by C18 solid phase extraction. Attention was focused on differential colorimetry and phenolic composition related to the color. Degradation kinetics of total color were also studied during storage of 17 days in darkness at 25 °C. Grape variety significantly influenced pigment yield, proportion of acylation, and proportion of copigments:pigments ratios in crude extracts; purification modulated the copigment:pigment ratios. This proportion was related to perceptible color variability among colorants and to different stabilities. With the same pigment content, grape varieties richer in skin copigments and higher copigment/pigment ratios ('Syrah' and 'Tempranillo') produced more intensely colored crude extracts whose tonalities ranged from reddish ('Graciano') to red-bluish ('Syrah'), depending on the proportion of acylation. Increasing the purity of the pigments diminished the color variability due to variety, making them less vivid and visually more similar to one another and also to the synthetic colorant. Degradation kinetic studies showed that unpurified grape colorants had higher color stability over time, with the greatest stabilizing effects achieved with varieties richer in skin flavonols ('Tempranillo' and 'Syrah').

1. Introduction

Color additives are substances from natural or synthetic origin used to impart, restore, or standardize the color and appearance of foodstuffs making them more attractive to consumers (Pasias, Asimakopoulos, & Thomaidis, 2015). The use of synthetic colorants has unquestionable advantages for the food industry because they are comparatively easier and less expensive to produce than natural colors. From a technological perspective, they typically show higher chemical stability without imparting odor or flavor to products. However, one of the limiting factors of using synthetic colorants is evidence of their potential detrimental effects on human health depending on the dose used (Carocho, Morales, & Ferreira, 2015). Studies have shown that synthetic colorants are not themselves toxic, but when used in mixtures, there might be a synergistic effect (Amchova, Kotolova, & Ruda-Kucerova, 2015). Major international food safety authorities have restricted the use of some synthetic colorants to particular foods at the minimum possible dosage (Carocho et al., 2015). Besides regulations, consumers also show a higher preference for food products that use natural ingredients (including colors), which are in general perceived as healthy and safe because many of them have been found to be nutraceuticals (Bearth, Cousin, & Siegrist, 2014; Wrolstad & Culver, 2012).

All these circumstances have strongly influenced the food sector. The search for alternative natural pigments to replace the synthetic colorants is a current market trend, especially within premium foods and in products positioned for children (Carocho et al., 2015; Nielsen & Holst, 2002; Wrolstad & Culver, 2012). Red and yellow colorants account for ~90% of the total amount of colorants added to food (Potera,

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2010). Therefore, there is high interest for greater availability of natural red colorants which have increased stability in food matrices due to the continued restrictions of their synthetic counterparts (Giusti & Wrolstad, 2003; Rodríguez-Saona, Giusti, & Wrolstad, 1999).

One class of natural pigments traditionally used by the food industry that provide red colors is anthocyanins, a large group of flavonoids widely spread in nature (Sigurdson, Tang, & Giusti, 2017). Interest in anthocyanins is due to the many attractive colors they can produce and multiple health benefits associated with their consumption (He & Giusti, 2010). As they are water-soluble and innocuous pigments, anthocyanins have a great potential to color food products with added biofunctional value. However, color formulations based on anthocyanins have some limitations. Anthocyanins are sensitive to several different factors such as pH changes, exposure to heat, light, oxygen, temperature, metals, bleaching agents, etc. (Ioannou, Hafsa, Hamdi, Charbonnel, & Ghoul, 2012). The stabilization of anthocyanins is still a major challenge and constitutes an important topic for the food colorant industry (Cortez, Luna-Vital, Margulis, & Gonzalez de Mejia, 2016).

Grape skins, or their by-products from the wine industry, represent some of the main commercial sources of anthocyanins (classified as E163 number). The major pigments present in *Vitis vinifera* grape skin are delphinidin, cyanidin, petunidin, peonidin and malvidin 3-glucosides and their acylated derivatives with cinnamic acids (Narduzzi, Stanstrup, & Mattivi, 2015). Acylation improves the stability of anthocyanins by protection of the chromophore by intramolecular copigmentation (Giusti & Wrolstad, 2003; Zhao et al., 2017). Grape skins also contain colorless phenolics that can act as cofactors (the so-called copigments) of anthocyanins protecting them through intermolecular copigmentation phenomena; colorless copigments contribute to reduced degradative reactions (Narduzzi et al., 2015; Trouillas et al., 2016). The proportions and amounts of the different pigments and copigments in *Vitis vinifera* grapes are strongly dependent on the grape variety (Narduzzi et al., 2015). These factors have important impacts on the color properties and stability as natural anthocyanin colorants.

Therefore, different methods of extract preparation and purification may also influence the chemical composition of anthocyanin-based colorants. Purification is often necessary to remove other plant components that are simultaneously co-extracted with pigments and could have negative impacts on the sensorial attributes and stability of natural colorants. Conversely, the coexistence of pigments with colorless phenolics in anthocyanin extracts can improve their chemical stability through copigmentation (Jensen, Lopez-de-Dicastillo Bergamo, Payet, Liu, & Konczak, 2011). Moreover, these interactions can also increase the health-promoting properties of natural colorants through additive or synergistic effects, as reported by Seeram, Adams, Hardy, and Heber (2004).

Thus, the main aim of this study was to assess the colorimetric properties of anthocyanin-rich grape colorants according to variety and level of purity and compare them to synthetic FD&C Red No. 3. The kinetics of anthocyanin color degradation over time were also investigated from a colorimetric point of view, providing useful information to the food industry about the stabilization of these extracts as natural copigmented colorants.

2. Materials and methods

2.1. Plant material

Red grapes (*Vitis vinifera* sp.) used in this study were 'Tempranillo' (TE), 'Syrah' (SY), 'Cabernet Sauvignon' (CS), and 'Graciano' (GR) varieties. TE, SY, and CS varieties were grown in the Condado de Huelva Designation of Origin (Spain), while GR variety was grown in the Rioja Designation of Origin (Spain). Mature grapes of each variety (500 g) were harvested and stored at −20 °C until analyzed. Grapes were manually peeled, and the skins were freeze-dried (lyophilizer Cryodos-80, Telstar Varian DS 102, Terrasa, Spain) and pulverized to obtain a homogeneous powder.

2.2. Preparation of the crude and purified anthocyanin extracts from grape skin

Anthocyanins were extracted and purified according to the method of Rodriguez-Saona and Wrolstad (2001). One gram of the skin powder was extracted with 0.01% HCl acidified 70% aqueous acetone (v/v) until the skin powder had no coloration. Extraction was conducted in triplicate for each grape variety. The extracts were filtered through Whatman no. 4 paper (Whatman Inc., Florham, N.J., U.S.A.) and partitioned with 2 volumes of chloroform (Fisher Scientific) in a separatory funnel. The solution was gently mixed and left to stand overnight at 4 °C to ensure adequate separation. The aqueous layer containing anthocyanins was collected, and residual acetone in the samples was evaporated using a rotary evaporator at 30 °C.

The crude anthocyanin extracts (n = 12) were brought to 50 mL with acidified water (0.01% HCl), and a fraction of each sample was purified with Sep-Pak C18 cartridge (6 mL, 1 g sorbent; Waters Corp., Milford, MA) to obtain the respective purified anthocyanin extracts (n = 12). The cartridge was activated with methanol and washed with acidified water (0.01% HCl) before samples were loaded. Loaded cartridges were washed with acidified water (0.01% HCl) to remove sugars and organic acids and then with ethyl acetate to remove less polar phenolics. Then, anthocyanins were recovered with 0.01% HCl acidulated methanol, which was removed in a rotary evaporator at 35 °C under vacuum.

Model drink solutions were prepared dissolving the concentrated crude (TE_C, SY_C, CS_C, GR_C) and purified (TE_P, SY_P, CS_P, GR_P) extracts until 25 mL with McIlvaine's buffer (also known as citrate-phosphate buffer, pH 3.5) to a final anthocyanin concentration of 100 mg/L. All the samples (n = 24) were filtered through 0.45 μm Millipore membranes, stored in sterilized 20 mL capped vials, and allowed to equilibrate for 2 h at room temperature (25 °C ± 1) in the dark prior to chemical and colorimetric analysis.

Similarly, a solution of FD&C Red No. 3 (Noveon Hilton Davis, Inc., Cincinnati, OH, USA) was also prepared in McIlvaine's buffer (pH 3.5, 100 mg/L) to compare colorimetric characteristics against the natural grape skin colorants.

2.3. Total monomeric anthocyanin and total phenolic contents

The spectrophotometric determinations of total monomeric anthocyanin (TMA) and total phenolic (TP) contents were performed using a Shimadzu 2450 UV–visible spectrophotometer (Shimadzu, Columbia, MD, USA), using 10 mm path length glass cells and distilled water as reference.

Total monomeric anthocyanin (TMA) content was determined according to the pH differential method (Giusti & Wrolstad, 2001). Samples were diluted with aqueous buffers pH 1.0 and 4.5 (potassium chloride solution, 0.025 M, pH 1; sodium acetate buffer, 0.4 M, pH 4.5) and left standing for 15 min. Then, the absorbance measurements were recorded at 520 and 700 nm. Results of TMA were expressed in milligrams (as malvidin 3-glucoside equivalents) per 100 g of skin (dry and fresh matter: DM and FM, respectively), and in mg/L for model drink solutions, using the following equation:

$$\text{TMA (mg/L)} = [((A_{520} - A_{700})_{\text{pH 1}} - (A_{520} - A_{700})_{\text{pH 4.5}}) \times \text{DF} \times 1000 \times \text{MW}]/\varepsilon \times P$$

where DF is the dilution factor (15), MW is the molecular weight (493.2 for malvidin 3-glucoside), ε is the molar absorptivity coefficient (20,200 cm^{-1} mg^{-1} for malvidin 3-glucoside), and P is the cuvette path length.

The total phenolic (TP) content was determined using a