Food Chemistry 268 (2018) 416–423

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Impact of location, type, and number of glycosidic substitutions on the color expression of *o*-dihydroxylated anthocyanidins

Gregory T. Sigurdson[a], Rebecca J. Robbins[b], Thomas M. Collins[b], M. Mónica Giusti[a,*]

[a] *The Ohio State University, Dept. of Food Science and Technology, 2015 Fyffe Ct., Columbus, OH 43210-1007, United States*
[b] *Science Group, Mars Wrigley Confectionery Inc., 800 High St., Hackettstown, NJ 07840, United States*

ARTICLE INFO

Keywords:
Anthocyanin
Cyanidin
Luteolinidin
Spectral distribution
Glycosylation
Natural colors

ABSTRACT

Anthocyanins express many hues depending on environmental factors and structural aspects, of which aglycone structure and acylation have been considered most impactful. Effects of different glycosylations on anthocyanins' colors are less known. Twelve cyanidin and 3-deoxy-cyanidin (luteolinidin) derivatives were isolated from red cabbage and black carrot hydrolysates and from black sorghum, diluted in pH 1–9, and analyzed by spectrophotometry and colorimetry. Location, number, and structure of glycosylations affected λ_{max} and spectral shape of *o*-dihydroxylated anthocyanins, playing important roles on color expression. Generally, glycosylation of cyanidin decreased its λ_{max} (≤ 27 nm), greatest decreases by 3-monoglycosylation. All cyanidin-glycosides appeared red in pH 1–3 and paled in pH 4–6. However, cyanidin-3-glycosides did not decolor completely like 3,5-glycosides. In alkaline pH, glycosylation patterns affected color more greatly: Cy-3-glycosides expressed maroon-purple hues (300–20°), 5-glycosides were green (100–115°), and only 3,5-glycosides expressed blue (240–250°). Luteolinidin derivatives shifted from yellow to red–purple hues with increasing pH.

1. Introduction

Synthetic food colorants are popular in the food industry due to their low production cost, high tinctorial strength, and chemical stability. However, consumer demand has been driving for the replacement of artificial colorants with naturally derived pigments. Colorants can be derived from many natural sources including plants, microorganisms, animals, and also minerals (Sigurdson, Tang, & Giusti, 2017).

Of plant sourced pigments, anthocyanins compose the largest group of water soluble naturally occurring pigments; more than 700 unique structures have been identified (Andersen & Jordheim, 2014). This abundant class of natural pigments is responsible for a range colors from yellow – red – purple – blue of many fruits, vegetables, and flowers. Anthocyanins are increasingly gaining popularity in the food industry as alternatives for synthetic pigments, due to the many hues they express and their potential health promoting properties (He & Giusti, 2010). This subclass of polyphenolic compounds is uniquely distinguished by their ability to express different hues as result of structural changes due to environmental factors and chemical substitutions that include chromophore methoxylation, hydroxylation, glycosylation, and acylation. The 6 most commonly encountered anthocyanidins (anthocyanins lacking glycosyl attachment) in edible produce differ in hydroxylation or methoxylation on the B-ring, and increasing degree of substitution results in bathochromic shifts on the absorption spectra, resulting in slightly bluer colors (Ananga, Georgiev, Ochieng, Phills, & Tsolova, 2013).

Anthocyanidins are rarely found in nature due to their instability; glycosylation, attachment of sugar moieties to the anthocyanidin through *O*-linkages, improves the stability of the pigment and its water solubility (He & Giusti, 2010). Although the positive charge of anthocyanins is delocalized across the whole system, it is principally located at C2 and C4 of the C-ring. Glycosylation of the pigments stabilizes them through various mechanisms including intramolecular H-bonding and conferring different molecular shapes (providing steric hindrances) (Borkowski, Szymusiak, Gliszczynska-Swiglo, & Tyrakowska, 2005; Zhao et al., 2014). These effects can slow rate of hydrolysis (reducing degradation to phenolic acids and aldehydes) and raise energy of activation for fading by displacing hydration equilibrium (Zhao et al., 2014).

Many sugars have been reported as glycosyl moieties for anthocyanins, including mono-, di-, and tri-saccharides (Andersen & Jordheim, 2006). The most common site of glycosylation is C3 followed by C5; other less common attachments at C7, C3′, C4′, and C5′ have been reported, see Fig. 1 (Schwartz, von Elbe, & Giusti, 2008). Glucosylation of cyanidin at C3 intensified and stabilized color of the

---

[*] Corresponding author.
E-mail address: Giusti.6@osu.edu (M.M. Giusti).

https://doi.org/10.1016/j.foodchem.2018.06.079
Received 7 February 2018; Received in revised form 15 June 2018; Accepted 18 June 2018

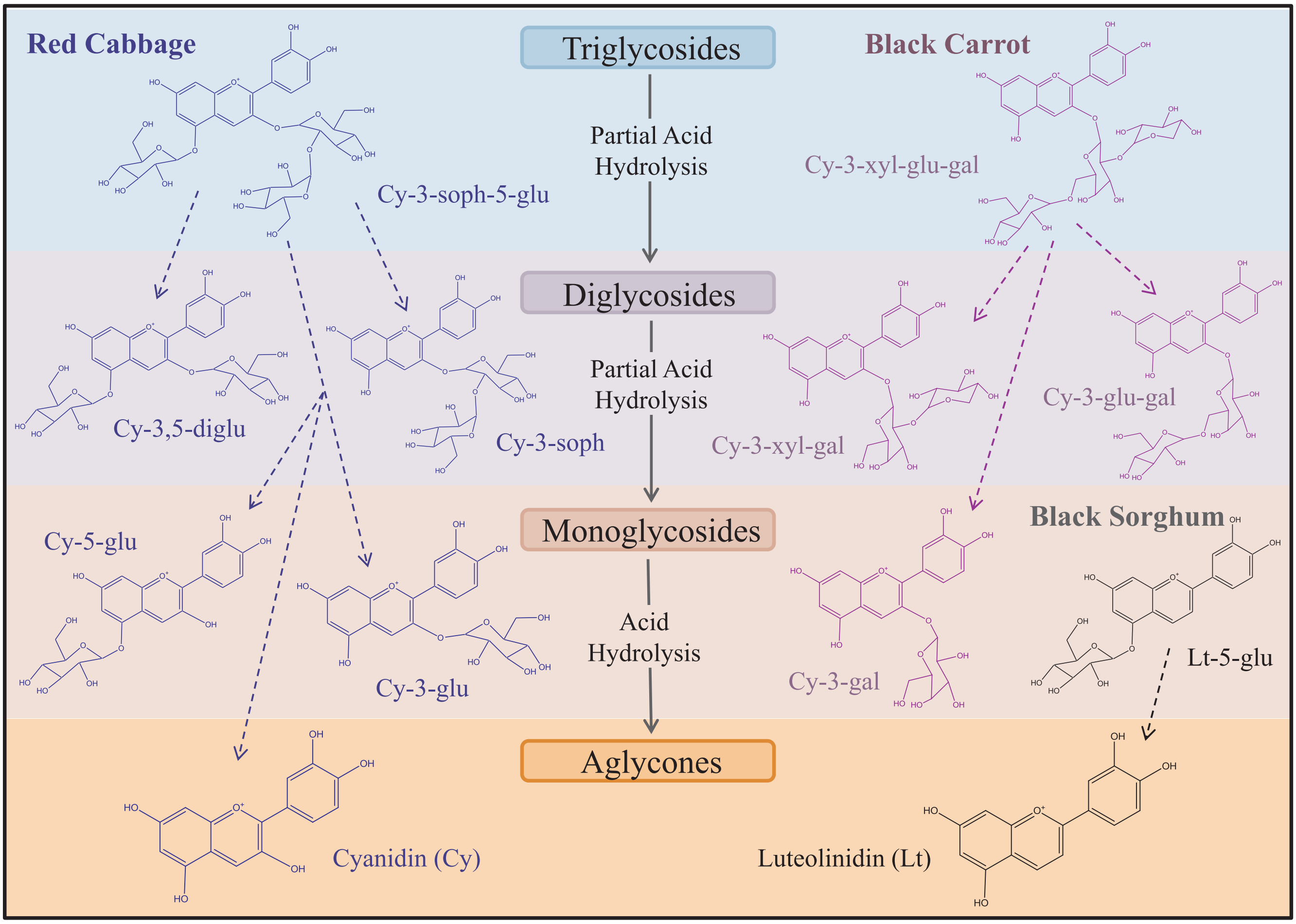

Fig. 1. Structures of *o*-dihydroxylated anthocyanins extracted from black sorghum or produced from hydrolysis of red cabbage or black carrot anthocyanins.

anthocyanidin in pH 2 and 4 but not in pH 7 (Rakic et al., 2015). Increasing glycosylation has been thought to impart greater stability to anthocyanins; however glycosylation of cyanidin at C3 and C5 has been reported to decrease pK_h and color stability (Fossen, Cabrita, & Andersen, 1998; Mazza & Brouillard, 1987). This pattern was also reported to shift hues of delphinidin derivatives toward more purple-blue tones with acylation in pH ≤ 6 compared to C3 glycosylated delphinidin (Stintzing, Stintzing, Carle, Frei, & Wrolstad, 2002; Torskangerpoll & Andersen, 2005). Cyanidin-3-sophoroside-5-glucoside was observed to express blue hues in pH 8–9 despite lacking acylation; while chokeberry anthocyanins, containing mainly cyanidin-3-glycosides, never expressed blue hues in any pH (Sigurdson & Giusti, 2014; Sigurdson, Robbins, Collins, & Giusti, 2016, 2017). Increasing number of sugars at C3 has been reported to impact colorimetric properties of anthocyanins but unpredictably (Stintzing et al., 2002).

The interaction of anthocyanins with light (therefore color) can be altered by structural distortions of the aglycone (stretching, bending, or torsion) that modify the π-delocalization of the chromophore by molecular substitution (Malcıoğlu, Calzolari, Gebauer, Varsano, & Baroni, 2011). In the case of cyanidin-3-glucoside in aqueous solution, glycosylation constrains the dynamics of the molecule (a geometric effect) (Malcıoğlu et al., 2011); however the effects of additional glycosylations require further investigation. With increasing interest in anthocyanins as food colorants, there is a need to better understand how differing glycosylation patterns impact the color of these pigments in a wide pH range representative of different foods, particularly *o*-dihydroxylated anthocyanins like cyanidin which is the most prevalent in nature. Therefore, the objective of this study was to evaluate the impact of degree, location, and structure of glycosylations on the color expression of *o*-dihydroxylated anthocyanins.

2. Materials & methods

2.1. Materials

Cyanidin rich anthocyanin extracts were prepared from dried red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*) and black carrot (*Daucus carota*) juice samples were provided by Mars Wrigley Confectionery (Hackettstown, NJ, USA). 3-deoxyanthocyanin extracts were prepared from black sorghum bran provided by Nu Life Market LLC (Scott City, KS, USA).

Tris(hydroxymethyl)aminomethane, 99% was purchased from Alfa Aesar (Ward Hill, MA, USA). All other chemicals and solvents were ACS or HPLC grade and purchased from Fisher Scientific (Fair Lawn, NJ, USA).

2.2. Methods

2.2.1. Anthocyanin preparation for pigment isolation

3-deoxyanthocyanins were extracted from black sorghum bran with acidified aqueous acetone, phase partitioned with chloroform and purified by solid phase extraction with acidified water and ethyl acetate following procedures described by Rodríguez-Saona and Wrolstad (2001).

To obtain rich glycosylated anthocyanin extracts, approximately 1 g aliquots of either red cabbage or black carrot juice powder were dissolved in 10 mL of 10% KOH for 10 min to saponify the ester bonds between the acylating and glycosylating moieties of the anthocyanins,