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Effect of cyclodextrins on the thermodynamic and kinetic properties of cyanidin-3-*O*-glucoside

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ABSTRACT

In this study, the effect of α and β-cyclodextrin on cyanidin-3-*O*-glucoside color was investigated by UV–visible absorption techniques. The equilibrium and kinetic constants of the network of chemical reactions taking place in cyanidin-3-*O*-glucoside were also studied in water at 25 °C by UV–visible absorption techniques. The results showed that the addition of β-cyclodextrin resulted in the fading of anthocyanin solution, and this fading effect was greater at higher pH. This anti-copigmentation effect is caused by the selective inclusion and stabilization of the anthocyanin colorless forms into the β-cyclodextrin cavity. Oppositely, no changes were observed in the cyanidin-3-*O*-glucoside absorption spectra with the addition of α-cyclodextrin. Direct pH jump, from thermally equilibrated solutions at pH = 1.0 (flavylium cation, **AH**$^+$), shows three kinetic processes: formation of the base **A**, hydration reaction to form the hemiketal **B** and the chalcone *cis–trans* isomerization (**Cc–Ct**). The results obtained clearly indicated that the equilibrium and kinetic constants of the network of chemical reactions taking place in cyanidin-3-*O*-glucoside were affected by the presence of β-cyclodextrin. Molecular inclusion in the β-cyclodextrin cavity resulted in the increase of the isomerization observed rate constant (k_{obs}) at pH 5.3 and in the increase of the hydration equilibrium constant K_h which is in agreement with the fading of the anthocyanin solution. For the macrocycle α-cyclodextrin, no significant changes were observed on the equilibrium and kinetic constants, which suggests that the inclusion of cyanidin-3-glucoside in the α-cyclodextrin's cavity is not favored.

1. Introduction

Color is one of the most important attributes in food products, as it affects the acceptability of products by consumers (Burin, Rossa, Ferreira-Lima, Hillmann, & Boirdignon-Luiz, 2011). Nowadays, as a consequence of perceived consumer preferences as well as legislative action, there is a worldwide trend towards the development of food colorants from natural sources, and the replacement of synthetic dyes by these natural colorants has been increasing (Idham, Muhamad, & Sarmidi, 2012). In this context, anthocyanins are attractive as food colorants since these natural pigments are responsible for the colors of flowers, fruits and vegetables, provide high colorant power and present a low toxicity and water solubility, which permit their incorporation in many food systems (Brouillard, Chassaing, Isorez, Kueny-Stotz, & Figueiredo, 2010; Ersus & Yurdagel, 2007). Moreover, several studies have suggested that these compounds are beneficial to human health and have been responsible for several positive therapeutic effects (Bridle & Timberlake, 1997; Clifford, 2000).

However, the use of these colorants in food products may face some problems due to their instability (Francis & Markakis, 1989). The color and stability of anthocyanins are highly dependent on pH, due to changes in the concentration of the four species present in acidic and neutral aqueous solutions: flavylium cation (**AH**$^+$), quinoidal base (**A**), hemiketal (**B**) and chalcone (**C**) (Fig. 1) (Brouillard, 1982; Brouillard & Delaporte, 1977; Pina, Melo, Laia, Parola, & Lima, 2012). Conversion of one species to another is typically accompanied by dramatic changes in color and stability (Burin et al., 2011; Xiong, Melton, Easteal, & Siew, 2006). To increase the stability of anthocyanins, molecular inclusion and encapsulation with several carbohydrates have been used (Burin et al., 2011; Cai & Corke, 2000; Ersus & Yurdagel, 2007; Idham et al., 2012; Selim, Khalil, Abdel-Bary, & Abdel-Azeim, 2008).

Cyclodextrins (CDs) have been used to stabilize anthocyanins (Chandra, Nair, & Iezzoni, 1993; Lewis, Walker, & Lancaster, 1995; Mourtzinos et al., 2008). These compounds are cyclic oligosaccharides composed of β-D-glucopyranosyl units linked by α-(1→4) bonds. The most common are α-, β- and γ-cyclodextrin which have six, seven and eight glucose units, respectively. The glucose monomers are orientated in a cyclic manner, forming a typical conical or truncated cone structure with a hydrophobic hollow cavity of specific diameter and volume and a relatively hydrophilic outer surface (Pegg & Shahidi,

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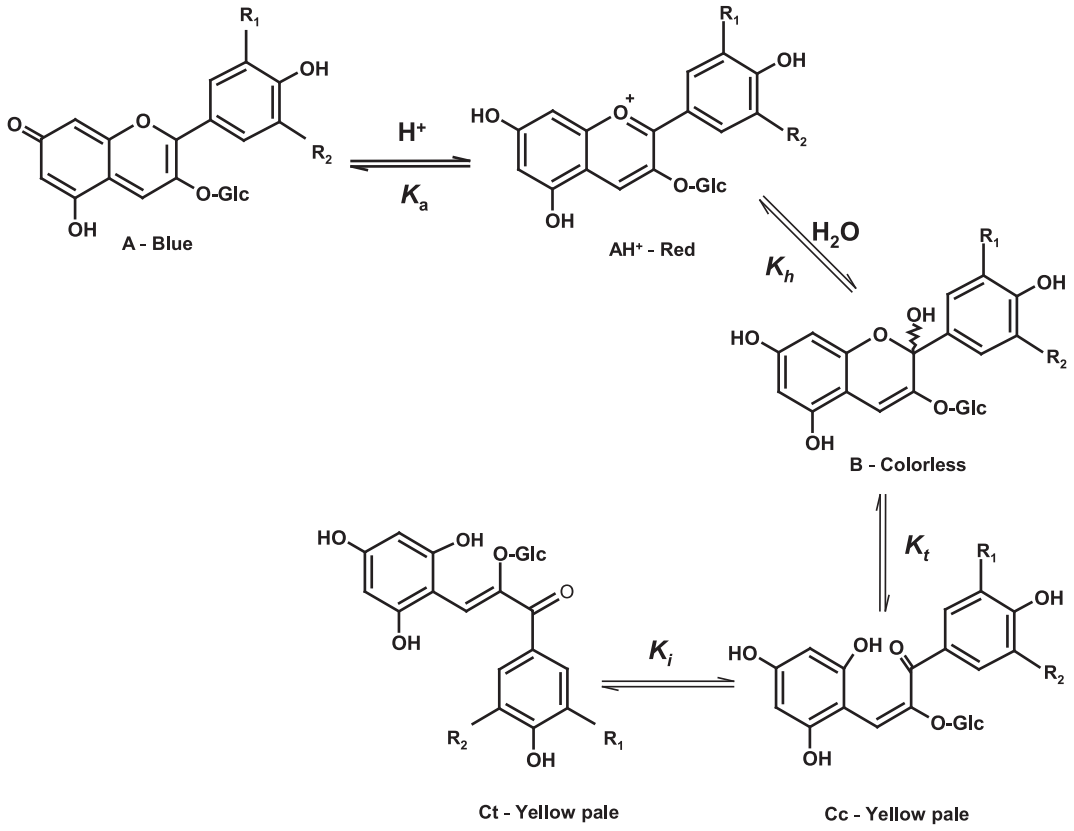

Fig. 1. Structural transformations of flavylium cation in strongly acidic to alkaline aqueous media (Brouillard, 1982; Brouillard & Delaporte, 1977).

2007). The hollow molecular shape gives cyclodextrins their unique ability to form reversible inclusion complexes with a wide variety of organic compounds (often phenolic substances) yielding supramolecules (Hendy & Breslin, 2011). Formation of the host–guest complex can be easily monitored using techniques such as nuclear magnetic resonance (NMR) (Cai et al., 1990; Ishizu, Kintsu, & Yamamoto, 1999; Ishizuka et al., 2002), UV–visible spectroscopy (UV–vis) (Divakar, 1993), fluorescence spectroscopy (Divakar & Maheswaran, 1997), IR spectroscopy, electrochemical approaches and solubility measurements (Hendy & Breslin, 2011).

Concerning anthocyanins, they include in their structure hydrophobic aromatic moieties and hydrophilic polar groups like hydroxyl groups. This amphiphilic character makes anthocyanins a very good candidate for molecular inclusion with cyclodextrins (Dangles, Stoeckel, Wigand, & Brouillard, 1992). Several studies have reported the formation of inclusion complexes of anthocyanins with cyclodextrin macrocycles (Dangles & Brouillard, 1992; Dangles, Stoeckel, et al., 1992; Dangles, Wigand, & Brouillard, 1992; Lewis et al., 1995; Mourtzinos et al., 2008; Nagatomo, 1985; Tamura, Takada, Yamagami, & Shiomi, 1998; Yamada, Komiya, & Akaki, 1980).

However, interaction between cyclodextrins and some anthocyanins was found to promote the anthocyanin discoloration and lead to an anti-copigmentation effect (Dangles & Brouillard, 1992; Dangles, Stoeckel, et al., 1992; Lewis et al., 1995; Yamada et al., 1980).

The main goal of this study was to promote the use of anthocyanins as food colorants and to overcome the limitations of the application of these natural pigments in food systems, enhancing its stability. In this study, the effect of cyclodextrins (α and β) on the color of cyanidin-3-*O*-glucoside was evaluated. The effect of these two cyclodextrins on the equilibrium and kinetic constants of the network of chemical reactions taking place in cyanidin-3-*O*-glucoside was also evaluated by UV–visible absorption techniques.

2. Materials and methods

2.1. Materials

β-Cyclodextrin (β-CD) and α-cyclodextrin (α-CD) were purchased from Sigma-Aldrich® (Madrid, Spain). All aqueous solutions were prepared with distilled water. Cyanidin-3-*O*-glucoside (cy3glc) was extracted and purified in the laboratory from blackberries (*Rubus fruticosus*) by semipreparative HPLC with C_{18} reversed phase column, as described elsewhere (Azevedo et al., 2010).

A universal buffer of Theorell Stenhagen was made by dissolving 2.25 mL of phosphoric acid (85% w/w), 7.00 g of monohydrated citric acid, 3.54 g of boric acid and 343 mL of 1 M NaOH solution in water (until 1 L completion) (Küster & Thiel, 1982).

2.2. pH measurements

All pH measurements were made in a pH meter WTW pH 320 fitted with a Crison electrode. The calibration was made with standard buffers pH 7.00 and pH 4.00 purchased from Crison.

2.3. Effect of cyclodextrins on the color of cyanidin-3-O-glucoside

A stock solution of cy3glc (1.50×10^{-4} M) was prepared using a solution of 0.1 M HCl. The low pH value of the stock solution ensured that most of the cy3glc was in the flavylium form and thus prevented pigment degradation. The solution composed of 1.0 mL of cy3glc stock solution, 1.0 mL of NaOH solution (0.1 M) and 0.5 mL of universal buffer solution at pH 1.0 and 2.0 was allowed to equilibrate for 2 h. To these anthocyanin solutions β-CD was added at various concentrations (6.00×10^{-4}; 1.80×10^{-3} and 7.50×10^{-3} M) and the solutions were left to equilibrate for 2 h. The absorbance (260–700 nm) was