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Stabilization of grape skin anthocyanins by copigmentation with enzymatically modified isoquercitrin (EMIQ) as a copigment

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ABSTRACT

The thermal and light stability of grape skin anthocyanins with enzymatically modified isoquercitrin (EMIQ) as a copigment was investigated at different pH levels of 3, 4 and 5. The ratios of anthocyanins to EMIQ were 2:1, 1:1, and 1:2 (w/w), respectively, in the thermal experiments at 90 °C, and EMIQ concentrations (0.25, 0.5, and 1%, w/w) were evaluated respectively in the light experiments. Results revealed that the degradation of anthocyanins copigmented with EMIQ followed first-order reaction kinetics. The half life of anthocyanins extended significantly with the increase of EMIQ concentration ($p<0.05$), moreover, the color stability increased due to the addition of EMIQ as the total color difference values ΔE* were smaller for the copigmented anthocyanins. The magnitude of the bathochromic (λ_{max}) shifted to the longest wavelength absorption band with the increasing copigment concentration for all pH levels. Results demonstrated that EMIQ was an effective copigment to stabilize grape skin anthocyanins.

1. Introduction

Color is one of the most important characteristics of fruit and vegetable products significantly determining customers' choice (Stintzing & Carle, 2004). To improve the visual appearance and restore initial color shades, synthetic colorants have commonly been applied in food processing. However, toxic effects have been described, resulting in a ban of some of these synthetics. A recent study demonstrated that synthetic colorants were shown to increase hyperactivity in children (attention-deficit hyperactivity disorder — ADHD) syndrome (McCann et al., 2007).For these reasons, synthetic food additives are increasingly rejected by consumers. So far, natural pigments, such as anthocyanins, have not been broadly used in foods and beverages since they are not as stable as synthetic colorants. Therefore, great attentions have been paid to the intrinsic and extrinsic factors affecting pigment stability and color shade, such as temperature, the presence of light, pH value (Baranac, Petranović, & Dimitrić-Marković, 1996) and thermal processing, such as pasteurization, sterilization or concentration particularly causing the degradation and color loss (Sadilova, Stintzing, Kammerer, & Carle, 2009).

Copigmentation is a natural phenomenon which plays a major role in the expression of a wide range of colors provided by anthocyanins in plants, fruits, vegetables, and food products. The study carried out by Mazza (1993) showed that the molecular complexation of anthocyanins with other phenols named copigments is the main color-stabilizing mechanism in plants. The color of the solution can be greatly enhanced when the copigment was added. The copigments promote the stabilization of the colored structural forms of the anthocyanins and consequently enhance the retention of their color (Liao, Cai, & Haslam, 1992).The copigments may be one of flavonoids, alkaloids, amino acids, organic acids, nucleotides, polysaccharides, metals, and anthocyanins themselves (Mazza & Brouillard, 1990). The complexation of a copigment with an anthocyanin causes a bathochromic shift and hyperchromic effect which means an increase in color intensity (Mazzaracchio, Pifferi, Kindt, Munyaneza, & Barbiroli, 2004).

Several studies have evaluated the stability of anthocyanins present in juices, wines and fruit products due to the interaction between pigments and phenols. The presence and amount of hydroxyl and/or glycosylated groups on the copigment structure had a great influence on the copigmentation as well as on anthocyanins stabilization (Cavalcanti, Santos, & Meireles, 2011). González-Manzano, Dueñas, Rivas-Gonzalo, Escribano-Bailón, and Santos-Buelga (2009) testified the importance of the qualitative phenolic composition, determined in the wine by the type of grape and winemaking practices, to the production of an effective copigmentation process. Meanwhile, Pacheco-Palencia and Talcott (2010) investigated the influence of polyphenolic copigments on the phytochemical and color stability of anthocyanins in açai fruit and the results revealed that flavone-C-glycoside is a stabilizing agent. The results were in accordance with previous study carried out by Chen and Hrazdina (1981) which showed that flavonoids that contained many hydroxyl groups are the best copigments.

Enzymatically modified isoquercitrin(EMIQ) which was approved as generally regarded as safe (GRAS) by FDA in 2003, was the world's

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first commercial water-soluble flavonol glycoside obtained from rutin manufactured by transglycosylation with cyclodextrin glucanotransferase (CGTase) (Akiyama, Washino, Yamada, Koda, & Maitani, 2000). The glycosylated groups were important for EMIQ as a copigment and the solubility of isoquercitrin was also improved by adding glycosyl instead of rhamnose. EMIQ has been used in various beverages and foods to prevent the deterioration of flavor and color caused by sunlight or fluorescent light irradiations in stores. The structure of EMIQ was shown in Fig. 1.

There are two main methods to stabilize anthocyanin: encapsulation and copigmentation. Although the stability of anthocyanins could be increased by encapsulation, it had some drawbacks as the requirement for sophisticated equipments and high percentage of losses obtained in the encapsulation process (Cavalcanti et al., 2011). However, as a copigment, EMIQ was available and could be used easily in food industry. For the high antioxidant ability of EMIQ, furthermore, the positive effect of EMIQ was not only related to the stability of anthocyanins, but also to the functionality and possible improvement of the antioxidant capacity of food products. The objective of the present work was to study the effect of EMIQ as a new copigment on the stability of grape skin anthocyanins during thermal process and light exposure.

2. Materials and methods

2.1. Materials

Grape skin anthocyanin solution (AC 3 WS colorant) was obtained from Chr. Hansen (Hørsholm, Demark). EMIQ was provided by San-Ei Gen. (Osaka, Japan). The content of EMIQ was 20% (w/w). All the reagents (HPLC grade) were obtained from Merck (Shanghai, China). Other chemicals and solvents (analytical grade) used were purchased from Beijing Chemical Co. (Beijing, China).

2.2. Thermal stability test of anthocyanins with EMIQ

An aliquot of 5 ml grape skin anthocyanins solution was diluted with McIlvaine buffers (citric acid/disodium hydrogen phosphate) to 250 ml at pH 3, 4, and 5, respectively. After 1 h equilibrium at room temperature before heating, solutions with and without copigment (8 ml) in tubes were placed in a water bath at 90 °C. Samples were collected with an interval of 30 min, and then cooled in ice bath for 3 min. 1 ml of the treated solution was collected and diluted to a volume of 10 ml. The total anthocyanin content of the solution was determined by the pH-differential method. Each sample was used for one measurement, in order to minimize the effect of oxygen.

2.3. Light stability test of anthocyanins with EMIQ

Light stability test was involved to evaluate the effect of EMIQ on the stability of anthocyanins exposed to light and elevated temperatures. An aliquot of 5 ml grape skin anthocyanins solution was diluted with McIlvaine buffers (citric acid/disodium hydrogen phosphate) to 250 ml at pH 3, 4, and 5, respectively. The concentration of EMIQ added was 0.25%, 0.5% and 1% (w/w) of anthocyanins, respectively. Each colored solution was transferred to an IWAKI flask (70 ml, Tokyo, Japan) and exposed to accelerated light in a controlled light cabinet (SUNTEST CPS+, 450 W/m^2, 45 °C) at 30 min interval.

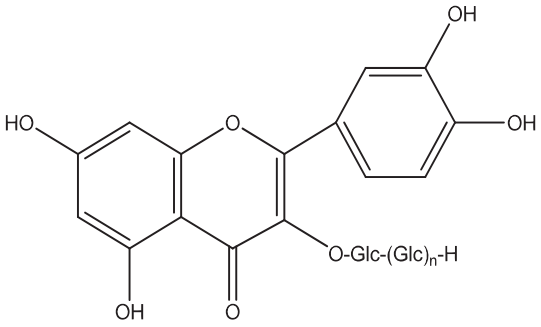

Fig. 1. The structure of EMIQ.

2.4. Spectrophotometric measurements of anthocyanins

All the spectrophotometric measurements were performed by SHIMADZU UV-1800 UV–vis spectrophotometer, using 1 cm path length glass cuvettes. After 1 h of equilibration at room temperature, visible spectra from 450 to 700 nm with a 1 nm bandwidth were recorded for the co-pigmentation reactions and colorimetric calculations.

2.5. HPLC-DAD-ESI/MS/MS analysis of anthocyanins

Analysis of anthocyanins by HPLC–MS/MS was carried out according to the methods described by Wu and Prior (2005) with some modifications. Chromatographic analyses were performed on an Agilent 1100 series HPLC equipped with diode array detector (DAD). A Zorbax Stablebond Analytical SB-C_{18} column (4.6×250 mm, 5 μm, Agilent Technologies, Rising Sun, MD) was used for separation. Elution was performed using mobile phase A (aqueous 5% formic acid solution) and mobile phase B (methanol).The flow rate was 1 ml/min, and detection was at 520 nm. Gradient was used as follows: 0–1 min, 5% B; 1–15 min, 5–25% B; 15–20 min, 25% B; 20–22 min, 25–27% B; 22–30 min, 27–33% B; 30–36 min, 33% B; 36–43 min, 33–40% B; 43–45 min, 47% B; 45–51 min, 47–50% B; and 51–60 min, 50–5% B. Low-resolution electrospray mass spectrometry was performed with an Esquire 3000 on trap mass spectrometer (MS) (Bruker Daltoniks, Billerica, MA). The experimental conditions were as follows: ESI interface, positive ion, nebulizer pressure, 35 psi; dry gas, nitrogen 11.0 psi; dry temperature, 350 °C; collision gas, helium; and MS/MS scan from m/z 100 to 1000.

2.6. Analysis of the total anthocyanin content

The total anthocyanin content (TAC) was determined by using the pH-differential method described by Lee, Durst, and Wrolstad (2005) with modification, using two buffer systems: potassium chloride buffer (0.025 M, pH 1.0), and sodium acetate buffer (0.4 M, pH 4.5). Briefly, aliquots of 1 ml of the samples were mixed with 9 ml of the pH buffer solution, and the absorbance was measured at 520 and 700 nm. Total anthocyanin content was calculated using the following equation:

$$\text{Total anthocyanin (mg/L)} = A \times M_W \times DF \times 1000/(\varepsilon \times 1) \quad (1)$$

where $A = (A_{520}-A_{700})_{pH=1.0} - (A_{520}-A_{700})_{pH=4.5}$, M_W = molecular weight, DF = dilution factor, 1 = path length (1 cm), Pigment contents were calculated as malvidin-3-O-glucoside using an extinction coefficient ε of 28,000 L/mol/cm and a molecular weight of 493.2 g/mol, 1000 = conversion from g to mg.

2.7. Thermal degradation kinetics of anthocyanins with EMIQ

Previous study showed that the thermal degradation of anthocyanins in aqueous solutions followed the first order kinetics (Mourtzinos et al., 2008). The reaction rate constants (k) and half lives ($t_{1/2}$, the time needed for 50% degradation of anthocyanins) were calculated by the following equations:

$$Ln\,(C_t/C_0) = -kt \quad (2)$$

$$t_{1/2} = 0.693/k \quad (3)$$