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The improved thermal stability of anthocyanins at pH 5.0 by gum arabic

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ABSTRACT

Gum arabic (GA) was studied to improve the thermal stability of anthocyanins. Solutions with 0.51 mg/mL anthocyanins at pH 5.0 were heated at 80 and 126 °C up to 80 min with and without 10 mg/mL GA. The half-life of thermal degradation of anthocyanins at 80 and 126 °C was increased by 2.0 and 1.8 times, respectively, after adding GA. The residual concentration of anthocyanins with GA was 1.02 and 1.35 times higher than that without GA after 30-min heating at 80 and 126 °C, respectively. The DPPH and ABTS free-radical scavenging capacities and ferric reducing power after heating anthocyanin solutions with GA at 126 °C for 30 min were significantly higher than those without GA. Fluorescence spectroscopy data suggested the formation of complexes between anthocyanins and GA. Zeta-potential data suggested the formation of complexes by hydrophobic attraction. Stable particle size and zeta-potential of GA with and without anthocyanins were observed after heating. This study indicated that the assembled GA nanostructures significantly reduced the thermal degradation of anthocyanins at pH 5.0.

1. Introduction

Anthocyanins are water-soluble pigments delivering a clear color that changes from salmon pink, red, violet, to dark blue as pH increases (Cavalcanti, Santos, & Meireles, 2011). Anthocyanins are present at high concentrations in various food products (Santos & Meireles, 2009) and have been studied for their potential biological activities improving human health such as reduced risks of chronic diseases (Cavalcanti et al., 2011) and coronary heart disease (Colantuoni, Bertuglia, Magistretti, & Donato, 1991). Anthocyanins have also shown good antioxidant (Cavalcanti et al., 2011), anti-inflammatory (J. Wang & Mazza, 2002), antimutagenic (Peterson & Dwyer, 1998), and chemo preventive activities (Zhao, Giusti, Malik, Moyer, & Magnuson, 2004). Despite these findings, anthocyanins have not been used as therapeutic and health-promoting agents (Cavalcanti et al., 2011).

One of the major challenges limiting the application of anthocyanins is their instability during storage and processing. The degradation of anthocyanins involves the hydrolysis of the double bond of ring C of flavylium cation via intramolecular charge transfer, which is more significant above pH 4.5 (Brouillard & Delaporte, 1977). The degradation of anthocyanins during thermal processing and storage can be accelerated by oxygen (Odriozola-Serrano, Soliva-Fortuny, & Martín-Belloso, 2010) and light (Maier, Fromm, Schieber, Kammerer, & Carle, 2009).

Approaches studied for stabilizing anthocyanins include encapsulation, self-association, co-pigmentation, and adoption of metallic ions (Cavalcanti et al., 2011). Among these methods, encapsulation has been a rapidly expanding area because immobilization of bioactive compounds in particles can protect them against degradation (Oidtmann et al., 2012). Technologies of encapsulating anthocyanins have been studied for spray drying (Main, Clydesdale, & Francis, 1978), lyophilization, thermal gelation, and ionic gelation (Cavalcanti et al., 2011). Encapsulation materials have been studied for maltodextrin (Ersus & Yurdagel, 2007), glucan (Xiong, Melton, Easteal, & Siew, 2006), pectin, sodium alginate, gurdlan (Cavalcanti et al., 2011) and gum arabic (GA) (Valduga, Lima, Prado, Padilha, & Treichel, 2008), with some studies reporting the improved heat stability of anthocyanins after encapsulation. Recently, we showed that mannoproteins extracted from the yeast cell wall formed complexes with and improved thermal stability of anthocyanins at pH 7.0 (Wu, Guan, & Zhong, 2015). For clear beverage products, water-soluble biopolymers giving a low viscosity are to be studied.

GA is a commonly-used ingredient with a high water solubility and low solution viscosity (Gomes et al., 2010). GA is known for its

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ability to form stable emulsions over a wide range of acidity and ionic conditions (Gomes et al., 2010; Jayme, Dunstan, & Gee, 1999). GA is a glycoprotein with polysaccharide chains composed of six types of monomers (galactopyranose, arabinopyranose, arabinofuranose, rhamnopyranose, glucuropyranosyluronic acid and 4-O-methylglucuro-pyranosyluronicacid) and a small fraction of protein (Jayme et al., 1999). The periphery of GA has a protein:carbohydrate molar ratio of about 1:40, while this ratio is 1:11 at the galactan core (Islam, Phillips, Sljivo, Snowden, & Williams, 1997). Both acidic amino acid residues of the protein portion and carbonyl groups of carbohydrates contribute to negative surface charges of GA (Islam et al., 1997). Besides electrostatic repulsion, the steric hindrance originating from polysaccharides after the polypeptide moiety of GA adsorbing to hydrophobic surfaces contributes to the excellent stabilization function of GA in various colloidal systems (Buffo, Reineccius, & Oehlert, 2001; Williams, Gold, Holoman, Ehrman, & Wilson Jr, 2006). In our recent study, GA was observed to form complexes with water-soluble pigment norbixin, and the complexes improved the thermal stability of norbixin at pH 3.0–5.0 (Guan & Zhong, 2014). Currently, there is no study about the heat stability of anthocyanins above pH 4.5 when they are co-dissolved with GA.

The first objective of the present study was to evaluate thermal degradation of anthocyanins at 80 and 126 °C as improved by GA at pH 5.0. These two temperatures were chosen because of the relevance to pasteurization (Berk, 2008) and sterilization (targeting spores of *Bacillus stearothermophilus* ATCC 7953) (Marques, Narita, Costa, & Rezende, 2010). The pH 5.0 was chosen because anthocyanin solutions are degraded to be colorless after thermal treatments at 70, 80 and 90 °C (Xu, Liu, Yan, Yuan, & Gao, 2015). The second objective was to understand physicochemical properties of anthocyanin-GA mixture solutions before and after heating at 80 and 126 °C for 30 min. The third objective was to evaluate impacts of thermal processing on antioxidation properties of anthocyanins as improved by GA.

2. Materials and methods

2.1. Chemicals

The anthocyanin sample (ColorFruit Violet 100WS, 540 mg/mL) was obtained from Chr. Hansen (Hørsholm, Denmark). GA, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), potassium ferricyanide, and ferric chloride were purchased from Fisher Scientific (Pittsburgh, PA). 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, and trichloroacetic acid were purchased from Sigma–Aldrich Corp. (St. Louis, MO).

2.2. Preparation of samples

Anthocyanins were dissolved in distilled water, followed by dissolving GA powder to a final concentration of 0.51 mg/mL anthocyanin and 10 mg/mL GA. After stirring for 30 min, the mixture was adjusted to pH 5.0 using 1 mol/L HCl and 1 mol/L NaOH. Solutions with and without GA were heated at 80 and 126 °C for up to 80 min to determine degradation kinetics, while those heated for 30 min were used to characterize other properties. All samples were placed at 4 °C in dark before further tests within 48 h.

2.3. Color and concentration of anthocyanins

The effects of GA on the color stability of anthocyanin solutions were evaluated for L^*, a^*, and b^* values. Five milliliters of a sample was measured at room temperature (21 °C) using a MiniScan XE Plus Hunter colorimeter (Hunter Associates Laboratory, Inc., Reston, VA), following the protocol in a previous study (Zhang & Zhong, 2013).

The absorbance of samples before and after heating was measured at 570 nm to determine the anthocyanin concentration using an Evolution 201 UV–Vis spectrophotometer (Thermo Scientific, Waltham, MA). Two calibration curves were established for samples with and without GA using standard solutions with various anthocyanin concentrations with and without 10 mg/mL GA adjusted to pH 5.0.

2.4. Thermal degradation kinetics of anthocyanins

In this set of samples, the above anthocyanin solutions at pH 5.0 were heated at 80 or 126 °C for 0, 10, 20, 30, 40, 60 and 80 min. After cooling to room temperature (21 °C) in a water bath, the absorbance at 570 nm was tested, and the determined anthocyanin concentrations were fit to Eq. (1) based on the first order degradation kinetics (Kırca, Özkan, & Cemeroğlu, 2007; W.-D. Wang & Xu, 2007). The half-life ($t_{1/2}$), the duration corresponding to anthocyanin concentration reducing to one-half of the starting concentration, was calculated according to Eq. (2) (Gris, Ferreira, Falcão, & Bordignon-Luiz, 2007).

$$c_t = c_0 e^{-kt} \tag{1}$$

$$t_{1/2} = -\ln(0.5)\cdot k^{-1} \tag{2}$$

where c_0 and c_t are anthocyanin concentrations before and after heating at 80 or 126 °C for a duration of t (min), and k is the degradation rate constant at 80 or 126 °C (min^{-1}).

2.5. Fluorescence spectroscopy

Fluorescence spectra of GA-anthocyanin solutions were determined according to the method in the literature (Zhang & Zhong, 2012) with a slight modification. The instrument was a model RF-1501 spectrofluorometer (Shimadzu Corp., Tokyo, Japan). The excitation wavelength was set at 330 nm, and the emission spectra were recorded from 220 to 600 nm with background fluorescence calibrated using distilled water.

2.6. Particle size and zeta-potential measurements

Samples were diluted 10 times using distilled water and adjusted to pH 5.0. Samples were analyzed for hydrodynamic diameters using a Delsa™ Nano-Zeta Potential and Submicron Particle Size Analyzer (Beckman Coulter Inc., Brea, CA) at room temperature (21 °C). Samples at pH 5.0 without dilution were tested for zeta-potential (Zetasizer Nano ZS, Malvern Instruments Ltd, Worcestershire, UK).

2.7. Atomic force microscopy (AFM)

AFM assay of structures before and after heating followed the protocol in our previous study (Pan & Zhong, 2013). Samples were diluted 100 times in distilled water and readjusted to pH 5.0. Five microliters of the diluted sample was dropped onto a freshly-cleaved mica sheet with an area of ~1.8 cm^2 and dried at room temperature (21 °C) overnight in a fume hood. Topography images were collected at the tapping mode using a nano-probe cantilever tip (Bruker Corp., Santa Barbara, CA) driven at a frequency from 50 to 100 kHz on a Multimode VIII microscope (Bruker Corporation, Billerica, MA). Images were analyzed using the AMF instrument software (Nanoscope Analysis version 1.50, Bruker Corporation, Billerica, MA).