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ARTICLE

Copigmentation effects and thermal degradation kinetics of purple sweet potato anthocyanins with metal ions and sugars

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Abstract The copigmentation effects of purple sweet potato anthocyanins (PSPAs) with metal ions and sugars were investigated in model solutions at pH 4. The thermal stability of PSPAs was also explored in the presence of sugars and 5-hydroxymethylfurfural (5-HMF). Copigmentation are characterized by hyperchromic effect and bathochromic shift. The hyperchromic effect of Fe^{3+} reached 25.15 % even at a very low concentration 0.005 mol L^{-1}, while the values of the other metal ions at 0.05 mol L^{-1} were ranked in the following ascending order: $K^+ < Ca^{2+} < Mg^{2+} < Zn^{2+} < Cu^{2+} < Fe^{2+} < Al^{3+}$. The bathochromic shift was not observed in all sugar reaction solutions; glucose showed the highest values of hyperchromic effect at the concentration range 150–300 g L^{-1}, followed by fructose and sucrose; konjac glucomannan showed the highest effect even at a much lower concentration among the macromolecular sugars. Especially, the konjac glucomannan exhibited a much better color enhancement than glucose. Small molecular sugars accelerated the thermal degradation of PSPAs, whereas macromolecular sugars showed a protective effect particularly at high temperatures. Moreover, 5-HMF resulted in the deterioration of the thermal stability of PSPAs and was suggested to be an important labile factor for copigmented PSPA solutions.

Keywords Copigmentation effects · 5-hydroxymethylfurfural · Metal ions · Purple sweet potato anthocyanins · Sugars · Thermal degradation kinetics

Introduction

Anthocyanins, bioactive compounds widely distributed in plant tissues (tuber, petal, leaf, flower, fruit, and seed), are a class of secondary metabolites responsible for a wide array of colors (Li et al. 2013; Malaj et al. 2013). Anthocyanins not only produce the attractive colors that determine customers' choice (Stintzing and Carle 2004), but also show health promoting benefits. Anthocyanins are studied and applied in the food and pharmaceutical industries. In an acidic to moderately acidic medium, four forms of anthocyanins, namely, flavylium cation, hemiketal, chalcone, and quinoidal base, are in equilibrium. Under extreme acidic conditions ($pH < 2$), the flavylium cation (red color) predominates; while at mildly acidic pH, colorless hemiketal becomes the dominant species together with neutral quinoidal base (purple color), with chalcone (yellow color) as a minor component (Limón et al. 2013; Nave et al. 2012; Teixeira et al. 2013).

The anthocyanin stability is comparatively low and can be affected by many factors, including molecular structure, pH, temperature, concentration, oxygen, light, enzyme, ascorbic acid, sugar, sulfite, and the composition of the food matrices (Cavalcanti et al. 2011). In consideration of these factors, a great deal of encapsulation techniques and four associated stabilization mechanisms (self-association, intramolecular copigmentation, intermolecular copigmentation, and metal complexation) were investigated and proposed (Awika 2008; González-Manzano et al. 2007; Schreiber et al. 2010; Yan et al. 2013). These methods are

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effective in enhancing the color and stability of anthocyanins. After copigmentation, the phenomena of hyperchromic effect (an increase in absorbance) and/or bathochromic shift (a positive increase of visible maximum absorption wavelength) in absorbance are always observed (Sun et al. 2010). In the case of intermolecular copigmentation, the main driving forces are suggested to be Van der Waals forces, hydrogen bonds, hydrophobic forces, and ionic interactions (Asen et al. 1972; Dangles et al. 1993).

Anthocyanins are glycosylated polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium cation (Fig. 1). Only the anthocyanins with more than one free hydroxyl group in the B ring (e.g., cyanidin, delphinidin, and petunidin) could generate metal complexation (Osawa 1982). Metal complexation maintains color stability in various anthocyanin-rich products, such as crowberry juice (Kallio et al. 1986) and blue creole corn (Schreiber et al. 2010). According to Kunsági-Máté et al. (2008), the oxidation state of iron ions plays a crucial role in the formation of copigmentation complexes, which is important for the winemaking procedure. Besides the man-made additions for chemical stabilization in the food medium, the complexation that often occurs between anthocyanins and metals is attributed to metal ion pollution, caused by antiquated manufacturing facilities and nonstandard or incorrect operating procedures.

Among the factors affecting the stability of anthocyanins, sugars are the most common components in food matrices. According to Tsai et al. (2005), sucrose exhibits a hyperchromic effect on mulberry extract anthocyanins before heating. The presence of sugars accelerates the degradation of anthocyanins, and the rate depends on the type of sugar present. Sugar at high concentrations (>20 %) show protective effect on anthocyanin content and color quality of berry extracts, such effects are presumably attributed to decreased water activity and the prevention of nucleophilic attack of water on the heterocyclic ring (Nikkhah et al. 2007). Besides the sugar itself, sugar degradation products and subsequent derivatives formed during heating, such as 5-hydroxymethylfurfural (5-HMF), could also affect the stability of anthocyanins (Debicki-Pospišil et al. 1983).

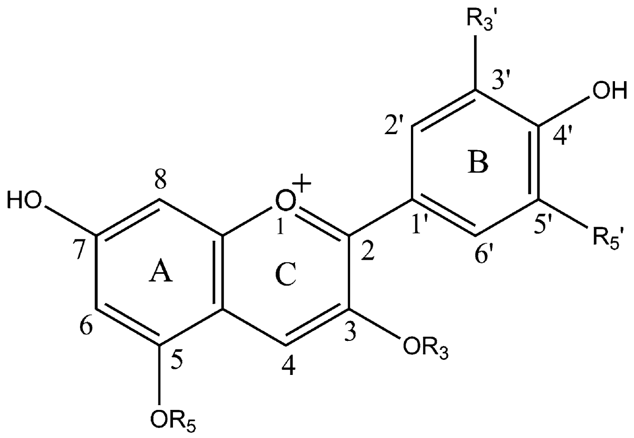

Fig. 1 Basic structure of flavylium cation

Purple sweet potato (*Ipomoea batatas* L.), an annual herbaceous plant of Convolvulaceae, contains high amounts of mono- and di-acylated anthocyanins in storage tubers. The main anthocyanins are 3-sophoroside-5-glucoside from cyanidin and peonidin, acylated with *p*-hydroxybenzoic acid, ferulic acid, or caffeic acid (Li et al. 2013; Lee et al. 2013). In spite of numerous and well-documented studies on the copigmentation with anthocyanins, information is lacking on the copigmentation effects of purple sweet potato anthocyanins (PSPAs) with metals and sugars. Furthermore, numerous studies have reported that the spectroscopic properties of copigmentation are observed more strongly at pH 3 and 4 (Brouillard et al. 1989; Dimitrić-Marković et al. 2000; Mazza and Brouillard 1990; Sun et al. 2010). Therefore, in this dissertation, the copigmentation effects of PSPAs with 8 metal ions and 6 sugars were investigated at pH 4 using hyperchromic effect and bathochromic shift as parameters. The paper aimed to highlight the importance of such phenomena in the color expression in food media. Meanwhile, the thermal degradation of PSPAs in the presence of sugars was explored at pH 4 at temperatures of 80, 90, and 100 °C. The effect of 5-HMF on the thermal stability of PSPAs was also investigated and discussed. The copigmentation effects and thermal degradation studies of PSPAs can be useful in establishing appropriate processing guidelines.

Materials and methods

Materials and solutions

Commercial PSPA powder (color value: 60; purity: 14.67 %; main anthocyanins: 42.84 % peonidin 3-caffeoyl sophoroside-5-glucoside, 18.70 % peonidin 3-caffeoyl-*p*-hydroxybenzoyl sophoroside-5-glucoside, 12.69 % cyanidin 3-(6″-caffeoyl sophoroside)-5-glucoside and cyanidin 3-caffeoyl-*p*-hydroxybenzoyl sophoroside-5-glucoside, 9.11 % peonidin 3-(6″-caffeoyl-6‴-feruloyl sophoroside)-5-glucoside), which was produced by acidic ethanol extraction, macroporous resin purification, and spray drying, was generously supplied by Zixin Biological Technology Co., Ltd. (Huangshi City, Hubei Province, China). 5-HMF was purchased from Sigma-Aldrich Co. LLC. (Shanghai, China). The pH 1 and 4.5 buffers used for determining the total monomeric anthocyanin content of

PSPA powder were prepared according to the method described by Lee et al. (2005). The pH 4 acetate buffer was prepared by mixing 0.2 M $CH_3CO_2Na{\cdot}3H_2O$ and 0.2 M glacial acetic acid, using a pH meter (FE20, Mettler-Toledo, Shanghai, China). Original solutions of metal ions (0.2 M) were obtained by dissolving certain amounts of KCl, $CaCl_2$, $MgCl_2$, $AlCl_3$, $ZnCl_2$, $FeSO_4$, $FeCl_3$, or $CuSO_4$ in pH 4 acetate buffer (0.2 M). Original sugar solutions, including solutions of 3 small molecular sugars (glucose, sucrose, and fructose, 400 g L^{-1}) and 3 macromolecular sugars (maltodextrin, konjac glucomannan, and sodium carboxymethyl cellulose, 10 g L^{-1}), were obtained by dissolving corresponding amounts of sugars in pH 4 acetate buffer (0.2 M). Original 5-HMF solution (2.4×10^{-4} M) was also prepared with pH 4 acetate buffer (0.2 M) as solvent. All chemicals were of analytical reagent grade, except 5-HMF and methanol, which were of high performance liquid chromatography (HPLC) grade.

Copigmentation effects of PSPAs with metal ions and sugars

According to the PSPA powder purity determined based on the pH differential method outlined by Lee et al. (2005), 1 g L^{-1} PSPA solution was prepared in pH 4 acetate buffer (0.2 M). A certain volume of original metal ion or sugar solutions was withdrawn, mixed with 1 mL pigment solution, and diluted to 10 mL with pH 4 buffer to produce the copigments at the desired working concentrations. The pigment/copigment solutions were shaken well and left to settle in dark for 2 h. After equilibrium, the UV–Vis spectra were acquired using a spectrophotometer (UV-1750, SHIMADZU, Kyoto, Japan) versus distilled water at room temperature from 450 to 700 nm. The values of maximum absorption wavelength and corresponding absorbance of each curve were recorded.

Thermal stability of PSPAs in the presence of sugars

Solutions of glucose, sucrose, and fructose at 50 and 200 g L^{-1}; maltodextrin at 8 g L^{-1}; konjac glucomannan at 1.6 g L^{-1}; and sodium carboxymethyl cellulose at 4 g L^{-1} were prepared in pH 4 acetate buffer for thermal degradation studies at temperatures of 80, 90, and 100 °C. The concentrations of sugars used were relative to the amounts needed for copigmentation. All sugar solutions contained 0.1 g L^{-1} PSPAs. After equilibrium for 2 h in dark, the reaction solutions were transferred into test tubes, which were capped tightly to avoid evaporation and placed in a thermostatic water bath preheated to a given temperature. Samples were withdrawn at regular time intervals, rapidly cooled by running water, and subjected to spectrophotometry. The anthocyanin retention rate (%) was calculated as A_t/A_0, where A_t is the absorbance of reaction solutions at the maximum absorption wavelength in the visible region after t hours of heating at a given temperature, and A_0 is the initial absorbance of reaction solutions. Numerous previous studies reported that the degradation of anthocyanins under isothermal heating followed the first-order kinetics (Hillmann et al. 2011; Kirca et al. 2006; Li et al. 2013; Wang and Xu 2007). The dependence of ln (A_t/A_0) on the heat treatment time t yielded a line with determination coefficient (R^2) by linear fitting. The time required to degrade 50 % anthocyanins (half-life, $t_{1/2}$) and the dependence of the degradation on temperature (activation energy, E_a) was determined using the following equations:

$$\ln(A_t/A_0) = -k \times t, \tag{1}$$

$$t_{1/2} = -\ln 0.5 \times k^{-1}, \tag{2}$$

$$\ln k = \ln k_0 - E_a/RT, \tag{3}$$

where $t_{1/2}$ is the half-life time; k is the first-order kinetic rate constant (h^{-1}); k_0 is the frequency factor (h^{-1}); E_a is the activation energy (kJ mol^{-1}); R is the universal gas constant (8.314 J mol^{-1} K); and T is the absolute temperature (K).

Effect of 5-HMF on the thermal stability of PSPAs

Different concentrations of 5-HMF solutions, which contained 0.1 g L^{-1} PSPAs, were prepared using pH 4 buffer. After equilibrium, each solution was incubated in a water bath at 90 °C for 2 and 4 h, respectively, cooled rapidly by running water, and subjected to spectrophotometry for retention rate (A_t/A_0) determination.

Determination of 5-HMF generated from sugars during heating

Different concentrations of glucose, sucrose, fructose, maltodextrin, konjac glucomannan, and sodium carboxymethyl cellulose were prepared with distilled water. After incubating in a water bath at 90 °C for a predetermined time, the samples were cooled to room temperature and filtered through a 0.22-μm filter before 5-HMF content determination. The measurements were performed using an HPLC system (LC-20, SHIMADZU, Kyoto, Japan) equipped with a UV detector. Chromatographic separation was conducted on a ZORBAX Eclipse plus-C18 column (5 μm, 250 mm × 4.6 mm i.d., Agilent, Santa Clara, USA). Two solvents were used for elution, as follows: A ultrapure water and B methanol. The elution profile consisted of an isocratic elution (15 % B) for 15 min. The analysis was conducted at a flow rate of 0.6 mL min^{-1} and at a detection wavelength of 284 nm. Column temperature was set at 35 °C, injection volume was 20 μL.

Statistical analysis

In the model system, all the measurements were carried out in triplicate. Multiple comparison analysis was conducted for statistical calculation using the software SPSS 16.0 (SPSS Inc., Chicago, USA).

Results and discussion

Effect of metal ions on copigmentation

Among the 8 metal ions investigated in the present study, 1 monovalent ion, 5 divalent ions, and 2 trivalent ions, which were the most common in food medium, were selected. The concentration of PSPAs was kept constant (0.1 g L^{-1}), and the copigmentation effects were examined at pH 4 with increasing amounts of metal ions. To evaluate the magnitude of the hyperchromic effect, reported as $(A - A_0)/A_0$, the absorbance of the pigment/copigment mixtures was registered consecutively at the visible maximum absorption wavelength, whereas the bathochromic shift ($\Delta\lambda_{max}$) was monitored by registering the spectra in the visible range of 450–700 nm. The hyperchromic and bathochromic values after statistical calculation for all the reactions are summarized in Table 1.

As shown in Table 1, the magnitude of hyperchromic effect was strongly affected by the concentrations and species of metal ions. Among these metal ions, K^+ was the only one that failed to exhibit significant hyperchromic effect even when its concentration reached 0.1 mol L^{-1}. The other metal ions all showed increasing degrees of hyperchromic effect with increasing concentrations, except for Fe^{3+}, which exhibited significant ($p < 0.001$) hypochromic effect even when its concentration decreased to 0.0001 mol L^{-1}. The hyperchromic effect of Fe^{3+} reached 25.15 % even at a very low concentration (0.005 mol L^{-1}), whereas the magnitudes of hyperchromic effect of the other seven metal ions at 0.05 mol L^{-1} were ranked in the following ascending order: $K^+ < Ca^{2+} < Mg^{2+} < Zn^{2+} < Cu^{2+} < Fe^{2+} < Al^{3+}$. The metal ions which take more charge are probably more easily to combine with the free hydroxyl groups in the B ring of the anthocyanin and thereby enhance the color intensity. Further studies are required to determine whether high valence state of metal ion results in high magnitude of hyperchromic effect or does not follow the abovementioned ascending order. All anthocyanins possessing a catechol structure in the B ring, that is, all derivatives of cyanidin, delphinidin, and petunidin, are known to have the capacity of complex formation with several small divalent and trivalent metal cations (Osawa 1982; Goto and Kondo 1991). The result that the anthocyanin-containing solutions copigmented with K^+ did not exhibit remarkable hyperchromic effect and bathochromic shift was in agreement with the results obtained by them. Meanwhile, peonidin and cyanidin were the main anthocyanin aglycones in purple sweet potato, which met the demand for metal complexation.

In addition of the hyperchromic effect, increasing the concentration of metal ions also resulted in a different magnitude of bathochromic shift of the maximum absorption wavelength. Reactions with K^+ and Fe^{2+} failed to result in bathochromic shift. With increasing concentrations in the studied ranges, Ca^{2+}, Mg^{2+}, Zn^{2+}, and Cu^{2+}, Al^{3+} gradually expressed slight hypsochromic and bathochromic shifts, respectively. Among these metal ions, 0.05 mol L^{-1} Al^{3+} showed the highest bathochromic shift (4 nm) followed by 0.05 mol L^{-1} Cu^{2+} (3 nm). Fe^{3+} exhibited bathochromic shifts at low concentrations, but hypsochromic shifts occurred at high concentrations. The colors of reaction solutions resulting from the mixing of PSPAs with Cu^{2+} and Fe^{3+} were bluish violet and reddish-brown, respectively. As reported, the metal complexation has been demonstrated to be at the origin of the blue color in some flowers (Goto and Kondo 1991; Kondo et al. 1994a, b), and those metals most commonly found in the formation of such metalloanthocyanins are Fe^{3+}, Mg^{2+}, and Al^{3+}. However, in a food system, where PSPAs were selected as colorants and red was the color preferred by consumers, manufacturers should seriously consider the sensory change caused by these special metal species.

Effect of sugars on copigmentation

Six saccharides, including glucose, sucrose, fructose, maltodextrin, konjac glucomannan, and sodium carboxymethyl cellulose, were involved in the copigmentation study, where the concentration of PSPAs was kept constant at 0.1 g L^{-1}. The extents of hyperchromic effect and bathochromic shift were examined. However, no bathochromic shift was observed. The magnitudes of hyperchromic effect for all reactions are summarized in Table 2.

Given that the hyperchromic effect of three small molecular sugars, it is concluded that when a high amount of copigment for complex formation was added, the hyperchromic effect becomes more significant compared with that in the PSPA solution with no sugar added. The small molecular sugars induced significant hyperchromic effect until their concentrations reached 100 g L^{-1}. At the concentration range from 150 to 300 g L^{-1}, glucose showed the highest values of hyperchromic effect followed by fructose and sucrose, but fructose exhibited the best at 100 g L^{-1}. The hyperchromic effect values of glucose and

Table 1 The spectroscopic parameters including hyperchromic effect (expressed as $(A - A_0)/A_0$) and bathochromic shift ($\Delta\lambda_{max}$) of PSPAs ($0.1\ g\ L^{-1}$) on complex formation with metal ions in pH 4 buffer

Metal ions	Concentration (mol L^{-1})	Hyperchromic effect (%)	Bathochromic shift (nm)	Metal ions	Concentration (mol L^{-1})	Hyperchromic effect (%)	Bathochromic shift (nm)
K^+	0.002	−1.21 ± 0.26[a]	0	Ca^{2+}	0.002	−1.61 ± 0.22	0
	0.01	−0.60 ± 0.49	0		0.01	0.60 ± 0.27	0
	0.0 5	0 ± 0.48	0		0.05	3.42 ± 0.25*	−1
	0.1	0 ± 0.63	0		0.1	9.45 ± 0.91***	−1
Mg^{2+}	0.002	−2.41 ± 0.36	0	Zn^{2+}	0.002	0.20 ± 0.11	0
	0.01	−1.81 ± 0.17	0		0.01	2.99 ± 0.32*	0
	0.05	4.43 ± 0.55**	−1		0.05	8.58 ± 0.97***	0
	0.1	8.65 ± 0.87***	−1		0.1	16.77 ± 1.03***	−1
Cu^{2+}	0.001	−0.88 ± 0.49	0	Fe^{2+}	0.001	0.26 ± 0.17	0
	0.005	0.88 ± 0.36	0		0.005	4.98 ± 0.78*	0
	0.025	7.65 ± 0.83***	1		0.025	9.17 ± 0.92***	0
	0.05	13.46 ± 0.94***	3		0.05	18.16 ± 1.07***	0
Al^{3+}	0.001	2.99 ± 0.83*	2	Fe^{3+}	0.0001	−4.59 ± 0.89**	1
	0.005	5.39 ± 0.97**	4		0.0005	−2.59 ± 0.53	1
	0.025	15.37 ± 1.28***	4		0.0025	8.78 ± 0.95***	−1
	0.05	25.95 ± 1.56***	4		0.005	25.15 ± 1.39***	−3

[a] Negative values mean hypochromic effect or hypsochromic shift

*, **, *** Significance levels of $p < 0.05$, 0.01, 0.001, respectively, by comparing the absorbance (A) of copigmented PSPAs solution with the value (A_0) of corresponding solution without adding metal ions by multiple comparison analysis

its isomer fructose indicated that even a small difference in the molecular structure could result in different magnitudes of hyperchromic effect. Moreover, both monosaccharides gave a higher contribution to color enhancement compared with disaccharide sucrose.

The hyperchromic effects of three macromolecular sugars were investigated at different concentration ranges. Sodium carboxymethyl cellulose failed to exhibit significant hyperchromic effect in the range 0–4 $g\ L^{-1}$, whereas maltodextrin at 8 $g\ L^{-1}$ expressed a significant contribution ($p < 0.05$) to color enhancement. Among the three macromolecular sugars, konjac glucomannan showed the highest magnitude of hyperchromic effect (5.66–16.64 %) even at a low concentration range of 0.4–1.6 $g\ L^{-1}$, thereby indicating that konjac glucomannan is a satisfactory choice for color enhancement of PSPAs in food media. The promising copigmentation effect between PSPAs and konjac glucomannan probably resulted from the abundant OH groups in the molecules of glucomannan. Since there are no literatures found about the copigmentation between anthocyanin and glucomannan, the mechanism of such phenomenon is hard to explain and further studies are demanded for filling this research gaps. At the same time, the results above indicated that konjac glucomannan was a much better copigment than glucose for PSPAs.

Thermal degradation kinetics of PSPAs with sugars

With the addition of different amounts of sugars in reaction solutions, stability of PSPAs with reference to control ln (A_t/A_0) as a function of heat treatment time t (h) at pH 4 from 80 to 100 °C were investigated (Fig. 2). The control sample was 0.1 $g\ L^{-1}$ PSPA solution with no sugar added. The thermal degradation of PSPAs followed a first-order reaction kinetics model, with a coefficient of determination of $R^2 > 0.97$ (Table 3). Moreover, an increase in the degradation rate constant (k) and a corresponding decline in the half-life ($t_{1/2}$) values was observed with increasing heating temperature in all reaction solutions.

Given the thermal degradation kinetics parameters of three small molecular sugars, the species and concentrations of sugars significantly affected the thermal stability of PSPAs. With increasing sugar concentrations from 50 to 200 $g\ L^{-1}$, the degradation rate constant k increased at all temperatures, except with fructose at 90 and 100 °C. Comparing the k values with the control, all sugars stimulated the thermal degradation of PSPAs to some extent. Among these sugars, glucose at 50 $g\ L^{-1}$ showed the lowest and most negligible effect. The half-life values of 27.3–8.2 h and 18.7 h–8.3 h from 80 to 100 °C were obtained in 50 and 200 $g\ L^{-1}$ glucose reaction solutions,

Table 2 The spectroscopic parameters of hyperchromic effect (expressed as $(A - A_0)/A_0$) of PSPAs (0.1 g L^{-1}) on complex formation with sugars in pH 4 buffer

Small molecular sugars	Glucose		Sucrose		Fructose	
	Concentration (g L^{-1})	Hyperchromic effect (%)	Concentration (g L^{-1})	Hyperchromic effect (%)	Concentration (g L^{-1})	Hyperchromic effect (%)
	10	−0.27 ± 0.47[a]	10	−0.67 ± 0.28	10	−0.37 ± 0.27
	50	1.40 ± 0.42	50	−0.07 ± 0.19	50	2.53 ± 0.40
	100	3.44 ± 0.14*	100	2.40 ± 0.81*	100	5.40 ± 1.03**
	150	5.61 ± 0.90***	150	2.84 ± 0.42*	150	5.40 ± 1.18**
	200	9.21 ± 1.06***	200	3.64 ± 0.99*	200	5.03 ± 0.42**
	250	10.68 ± 0.99***	250	6.84 ± 0.71***	250	8.33 ± 1.26***
	300	11.25 ± 1.24***	300	8.54 ± 0.57***	300	8.93 ± 1.35***
Macromolecular sugars	Maltodextrin		Konjac glucomannan		Sodium carboxymethyl cellulose	
	Concentration (g L^{-1})	Hyperchromic effect (%)	Concentration (g L^{-1})	Hyperchromic effect (%)	Concentration (g L^{-1})	Hyperchromic effect (%)
	0.1	−0.27 ± 0.64	0.1	1.24 ± 0.54	0.1	−0.51 ± 0.79
	0.5	−0.12 ± 0.45	0.2	1.44 ± 0.12	0.5	−0.59 ± 0.60
	1	0.48 ± 0.55	0.4	5.66 ± 0.83**	1	−1.46 ± 0.35
	2	−0.27 ± 0.64	0.8	6.76 ± 0.24**	2	−0.84 ± 0.09
	4	1.99 ± 0.25	1.2	10.56 ± 1.27***	3	−0.68 ± 0.45
	8	2.44 ± 0.38*	1.6	16.64 ± 1.12***	4	0.08 ± 0.37

[a] Negative values mean hypochromic effect

*, **, *** Significance levels of $p < 0.05, 0.01, 0.001$, respectively, by comparing the absorbance (A) of copigmented PSPAs solution with the value (A_0) of corresponding solution without adding sugars by multiple comparison analysis

respectively. The degradation of PSPAs was slightly inhibited at a glucose concentration of 200 g L^{-1} at 100 °C, compared with that of 50 g L^{-1} glucose at the same temperature. This phenomenon was more obvious in the fructose reaction solutions, in which the thermal degradation of PSPAs was lower at 200 g L^{-1} fructose than at 50 g L^{-1} even at 90 °C. With the addition of small molecular sugars, the thermal degradation of PSPAs increased from 80 to 100 °C. However, at higher temperature, the degradation was reduced with more amounts of glucose or fructose than the corresponding diluted sugar reactions. During heat treatment, the sugars and their derivatives jointly accelerated the degradation of PSPAs. Whereas, at higher temperature, with more sugars and increasing heat treatment time the content of sugar derivatives achieved the saturated condition for reacting with PSPAs. Thus, the remaining undegraded sugar may decrease water activity and then prevent the nucleophilic attack of water on heterocyclic ring of anthocyanins. So at higher temperatures, these extra sugars, instead, produced a protective effect on PSPAs. But the thermal stability of PSPAs copigmented with small molecular sugars was still lower than the control. By comparing the activation energy values of all reaction solutions at the same sugar concentrations, the thermal stability of these three PSPAs/sugar complexes can be ranked in the following ascending order: fructose < sucrose < glucose. Hou et al. (2013) reported that a substrate in a reaction with high-activation energy was more susceptible to a low temperature change during heating and was less stable. Therefore, the temperature of a glucose-containing food system which was colored using PSPAs should be kept as stable as possible to preserve the anthocyanins during heat treatment, such as pasteurization or high temperature sterilization.

The thermal stability of PSPAs copigmented with three macromolecular sugars, including maltodextrin, konjac glucomannan, and sodium carboxymethyl cellulose, was also investigated, and the relevant parameters are summarized in Table 3. As shown in table, a significant decline in the k values and a corresponding increase in the $t_{1/2}$ and E_a values of the 8 g L^{-1} maltodextrin reaction solutions occurred in comparison with the control, thereby suggesting that maltodextrin enhanced the thermal stability of PSPAs at 80–100 °C. However, at 80 °C, the reaction solutions containing 1.6 g L^{-1} konjac glucomannan and 4 g L^{-1} sodium carboxymethyl cellulose accelerated the

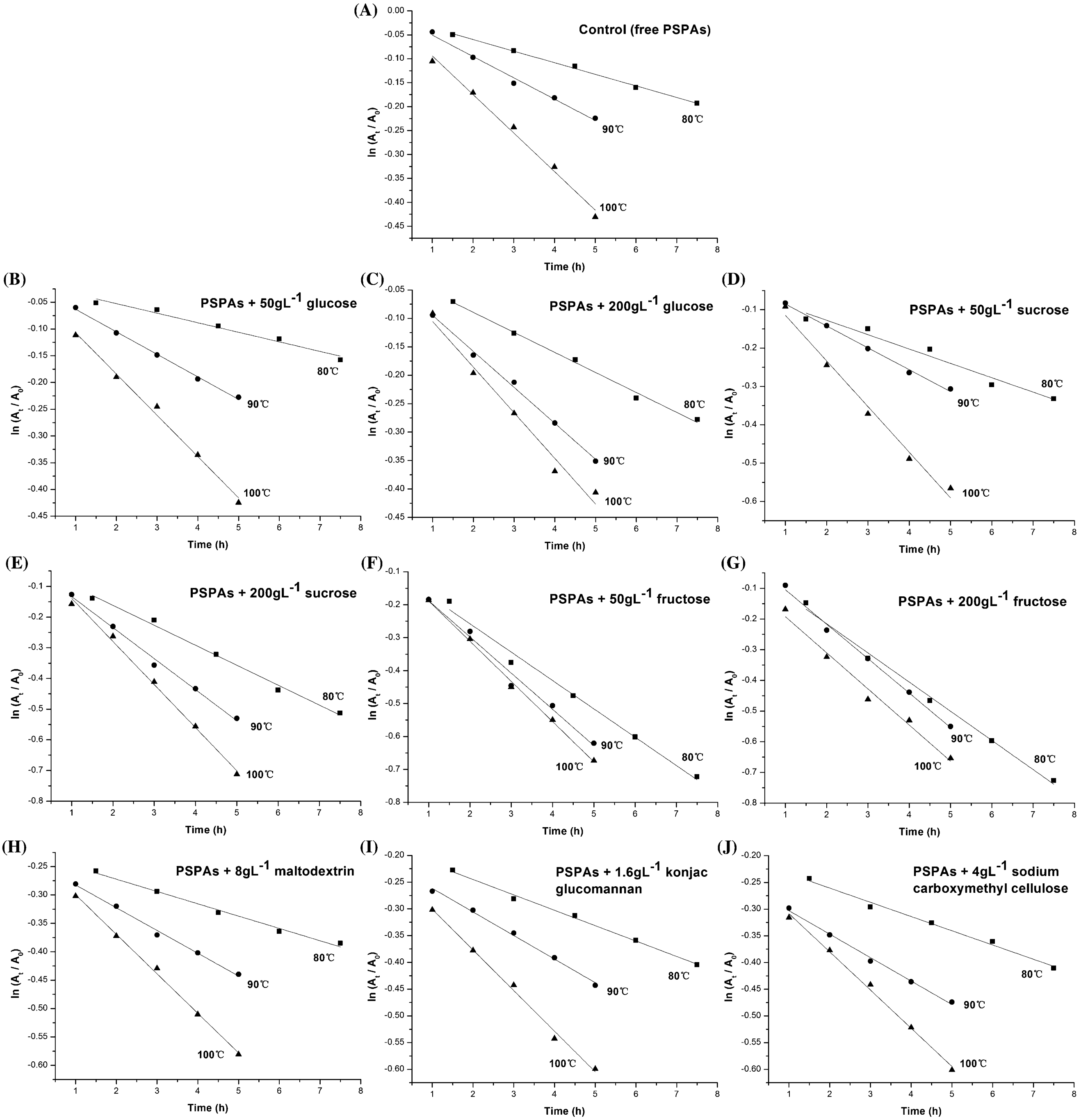

Fig. 2 Relative changes of the absorbance of PSPAs with or without sugars at pH 4 during heating

degradation of PSPAs. Conversely, at 90 and 100 °C, the degradation was inhibited to some extent. However, the activation energy E_a of these two sugars was still lower than that of control. Overall, at high temperatures, these three macromolecular sugars showed protective effects on the thermal stability of PSPAs. Hence, in the process of high temperature sterilization of colored food products using PSPAs, such as fruit juice beverages, little konjac glucomannan could have a good protective effect.

Effect of 5-HMF on the thermal stability of PSPAs

5-HMF is an organic compound that is generally derived under acidic conditions from dehydration of ketohexoses, or by the transformation of ascorbic acid or polyuronic acids (Debicki-Pospišil et al. 1983). 5-HMF consists of a furan ring containing both aldehyde and alcohol functional groups and is always used as an unwanted marker for heat treatment or for long-term storage of foods rich in sugar. In

Table 3 Kinetic parameters for the thermal degradation of PSPAs with sugars at pH 4

Sugar addition	Concentration (g L^{-1})	$k \times 10^{-3}$ (h^{-1})			$t_{1/2}$ (h)			E_a (kJ mol^{-1})
		80 °C	90 °C	100 °C	80 °C	90 °C	100 °C	
Control		25.3 (0.9960)[a]	45.4 (0.9938)	82.5 (0.9944)	27.4	15.3	8.4	64.73
Glucose	50	25.5 (0.9891)	45.6 (0.9928)	82.7 (0.9929)	27.3	15.2	8.2	64.38
	200	37.0 (0.9941)	67.8 (0.9925)	83.9 (0.9876)	18.7	10.2	8.3	45.03
Sucrose	50	42.5 (0.9791)	61.1 (0.9928)	118.4 (0.9929)	16.3	11.3	5.9	55.96
	200	68.1 (0.9936)	105.7 (0.9946)	140.3 (0.9976)	10.2	6.6	4.9	39.66
Fructose	50	94.3 (0.9791)	121.0 (0.9928)	131.5 (0.9929)	7.4	5.7	5.3	18.29
	200	97.5 (0.9965)	111.1 (0.9970)	128.4 (0.9833)	7.1	6.2	5.4	15.07
Maltodextrin	8	21.0 (0.9903)	40.1 (0.9948)	69.5 (0.9976)	33.0	17.3	10.0	65.59
Konjac glucomannan	1.6	28.8 (0.9951)	44.1 (0.9954)	76.0 (0.9944)	24.1	15.7	9.1	53.08
Sodium carboxymethyl cellulose	4	26.6 (0.9905)	43.9 (0.9952)	71.5 (0.9959)	26.1	15.7	9.7	54.16

[a] Numbers in parentheses are the determination coefficients obtained from ln (A_t/A_0) versus heat treatment time t

the present study, the effect of 5-HMF on PSPAs during heating was investigated. As shown in Fig. 3, the anthocyanin retention rate decreased from 75.57 to 69.72 % and from 72.38 to 64.79 % at a 5-HMF concentration range of 0–27 mg L^{-1} after heating at 90 °C for 2 and 4 h, respectively. The thermal instability of PSPAs was significantly increased with increasing heat treatment time and with additional amounts of 5-HMF. This phenomenon was in agreement with results of previous well-documented studies (Daravingas and Cain 1968; Meschter 1953; Tinsley and Bockian 1960).

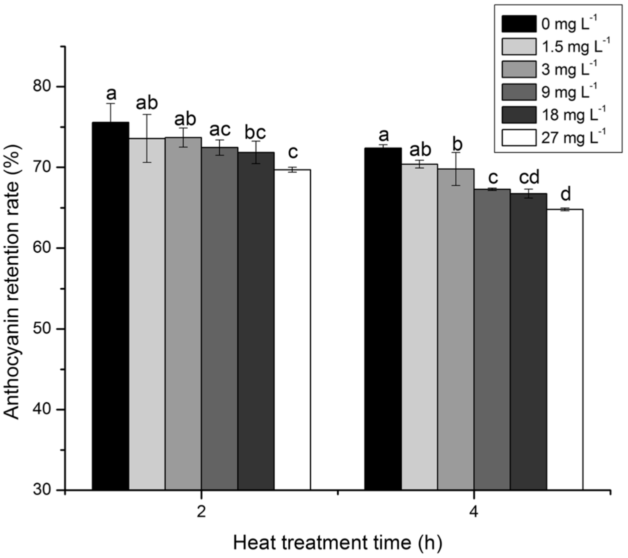

Fig. 3 The retention rate of PSPAs induced by different concentrations of 5-HMF at pH 4.0 after heating for 2 and 4 h, respectively. Columns within each group marked by the same letter are not significantly different ($p < 0.05$)

Sadilove et al. (2009) studied the generation of 5-HMF in sugar model solutions (50 g L^{-1}) after 4 h of heating at 90 °C, at pH levels of 1, 2, and 3.5. They found that 5-HMF was easier to generate under high acidic conditions than under low acidic conditions, and the generated amounts of 5-HMF for fructose, sucrose, and glucose at pH 3.5 were 7.66, 1.87, and 0 mg L^{-1}, respectively. We examined the generation of 5-HMF in different concentrations of sugar model solutions at 90 °C in a neutral environment, which is the same as the environment in most food systems. The generated amounts of 5-HMF from small molecular sugars at the same concentrations were in the following descending order: fructose > glucose > sucrose. This ranking was different from the data obtained at pH 3.5, as reported by Sadilova et al. However, the results of Sadilova et al. were in agreement with the effect of thermal degradation on PSPAs when these three small molecular sugars were added as copigments. Thus, 5-HMF derived from sugars under acidic conditions was a relatively important labile factor for the thermal degradation of PSPAs. As expected, the generated amounts of 5-HMF from macromolecular sugars were remarkably low (<0.1 mg L^{-1}), and 5-HMF was not detected in the sodium carboxymethyl cellulose reaction solutions after 5 h of heating at 90 °C. The protective effect of macromolecular sugars on PSPAs at high temperatures was exhibited via unknown mechanisms.

As a summary, the findings from the present study provided detailed spectroscopic information, including the hyperchromic effect and bathochromic shift of the copigmentation between PSPAs and metal ions. Data on sugars (in relation to color enhancement) and on the thermal degradation kinetics of PSPAs/sugar complexes, as well as on the effect of 5-HMF, were obtained. The above results allow us to conclude that the types and concentrations of metal ions or sugars had a significant influence on the copigmentation of the PSPAs solution. Therefore, the industrial processes which intend to use this phenomenon for the improvement of the color or the thermal stability of their products should seriously consider the strong dependence of the copigmentation reactions on the above factors in all stages of the product cycle, including processing, transportation, commercialization, and storage (Malaj et al. 2013).

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