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Influence of Copigmentation on Stability of Anthocyanins from Purple Potato Peel in Both Liquid State and Solid State

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Copigmentation is an important way to improve the stability of anthocyanins. The effect of the copigmentation on the stability of the anthocyanin from the purple potato peel (PA) was evaluated from the view of the kinetics and thermodynamics in both the liquid state and the solid state for the first time. In the liquid state, the copigmentation of the ascorbic acid decreased the stability of PA by the activation-energy evaluation, while that of the citric-acid monohydrate and glucose increased the stability of PA. Moreover, the stability of the copigmented PA presented the positive correlation with its ratio to the ascorbic acid and citric-acid monohydrate individually. The copigmentation of the glucose at the different ratio did not affect the stability of PA significantly. In the solid state, the stability of PA was improved by the copigmentation with the ascorbic acid, citric-acid monohydrate, and glucose by the transition-temperature evaluation. Moreover, the stability of the copigmented PA showed the negative correlation with its ratio to the ascorbic acid, citric-acid monohydrate, and glucose individually.

KEYWORDS: Purple potato; anthocyanins; copigmentation; thermodynamics; kinetics

INTRODUCTION

The production of the potato in China reached 70 million tons in 2007, being the first place in the world for such a large potato production (*1*). The purple potato is originated in the Andes and cultivated for centuries in South America. Although the purple potato is relatively new to China, its production has boomed at 50% per year in the past five years in China. Hence, the application of the purple potato and its byproduct has become more and more urgent in China. Specifically, the disposal of the purple potato peel, that is the potato's byproduct of its starch and protein production, is a problem not only in China. Giusti et al. (*2*) reported that the anthocyanin content in the peel of the purple potato was 3−4 times higher than that in its tuber. Therefore, the anthocyanins from the purple potato peel (PA) seem to be a good source for the anthocyanin production.

Anthocyanins have been attributed with many biological functionalities, such as antioxidant, antimicrobial, antiobesity, antimutagenic, and anticarcinogenic capacities (*3*, *4*). However, their applications are still restricted as a pigment by their low stability. Copigmentation is an important way to improve the stability of anthocyanins (*5*). Gris et al. (*6*) reported that the copigmentation of the caffeic acid improved the stability of the grape anthocyanins in the yoghurt system. The polysaccharide copigmentation improved the stability of the anthocyanin from *Hibiscus sabdariffa* L. in the dry state (*7*). Furthermore, Bakowska et al. (*8*) reported that the stability of the anthocyanin showed the dose-dependent manner with the copigments. However, the effect of copigmentation on the stability of PA was a blank to our knowledge.

Consequently, the effect of the copigmentation on the stability of PA was evaluated from the view of its kinetics and thermodynamics. Specifically, the kinetics analysis of anthocyanins had been studied widely, including the anthocyanins from blackberry (*9*), orange juice (*10*), purple corn (*11*, *12*), and so on, which would help to explain their conversion mechanism. Moreover, the effect of the copigmentation on the thermodynamics of PA was determined by the shift of the exothermic peaks with the differential scanning calorimetry (DSC) analysis. Specifically, the effect of the copigments on the stability of PA was studied by the kinetics analysis of PA in the liquid state. The effect of copigments on the thermodynamics of PA was evaluated by DSC in the solid state, which was the novel way to reflect the stability variety and used for the first time in this area.

MATERIALS AND METHODS

Extraction. The purple potato was grown in Yanqing Farm (Beijing, China, 2008), collected maturely, and stored at 4 °C. The peel of the purple potato (500 g) was stirred in 2500 mL of 60% (v/v) ethanol acidified by the 1 mol/L hydrochloric acid (0.5%, v/v) at 60 °C for 120 min. The ethanol extract was centrifuged at 9000 rpm and 4 °C for 20 min. The supernatant was evaporated to about its 500 mL at 40 °C with a rotary evaporator Büchi R-3000 (Büchi Labortechnic AG, Switzerland), being similar to the early report (*12*). The concentrated solution was absorbed by XAD-7HP resin and eluted by 60% (v/v) ethanol acidified by the 1 mol/L hydrochloric acid (0.5%, v/v). The eluted was lyophilized and store at 4 °C without the light.

Determination of the Total Anthocyanin Content. The total anthocyanin content (TAC) was determined by the pH differential method

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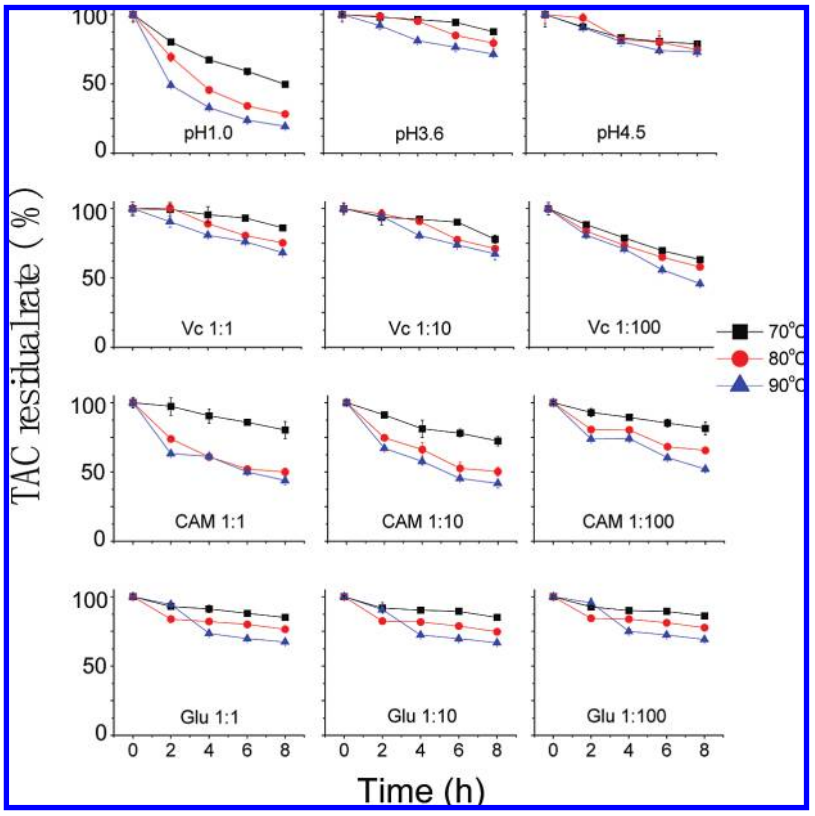

Figure 1. Effect of the pH, temperature, and copigments on the TAC residual rate of PA in the liquid state.

described early (*12*). An aliquot of the sample (1 mg) was placed into a 25 mL volumetric flask and made up to the final volume with pH 1.0 buffer. Another 1 mg of the sample was also placed into a 25 mL volumetric flask, made up to a final volume with pH 4.5 buffer. Absorbance was measured by a spectrophotometer (UV-1601, Shimadzu, Japan) at 510 and 700 nm, respectively. Absorbance was calculated as Abs = $(A_{510\ nm} - A_{700\ nm})_{pH_{1.0}} - (A_{510\ nm} - A_{700\ nm})_{pH_{4.5}}$ with a molar extinction coefficient for cyanidin 3-glucoside of 26900. TAC was calculated using the following equation and expressed as milligrams of cyanidin 3-glucoside equivalents per 100 g dry material (eq 1).

$$\mathrm{TAC}(\%) = \frac{\mathrm{Abs}}{eL} \times \mathrm{MW} \times D \times \frac{V}{G} \times 100 \quad (1)$$

where Abs is absorbance, e is cyanidin 3-glucoside molar absorbance [26900 mL/(mmol·cm)], L is the cell path length (1 cm), MW is the molecular weight of anthocyanin (449.2 Da), D is a dilution factor, V is the final volume (mL), and G is the dry material (mg).

Kinetics Analysis in the Liquid State. The sample was dissolved in the pH 1.0, 3.6, and 4.5 buffers at content of 1 mg/mL, respectively. The solution was heated at 70, 80, and 90 °C, respectively, and measured the corresponding TAC at 2 h intervals. Previous studies showed that the conversion of anthocyanins followed the first-order conversion kinetic (*13*, *14*), expressed by Arrhenius equation (eqs 2−4).

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

$$k = k_0 e^{-E_0/RT} \quad (3)$$

$$t_{1/2} = 0.693/k \quad (4)$$

where C_0 is the initial TAC, C_t is the TAC after time t (h), $t_{1/2}$ is the half-life time, k is the rate constant (min^{-1}), k_0 is the frequency factor (min^{-1}), E_a is the activation energy (kJ/mol), R is the universal gas constant (8.314 J/mol·K), and T is the absolute temperature (Kelvin).

Moreover, the effect of copigmentation on the stability of PA was evaluated. The kinetics analysis of PA with copigments, including ascorbic acid (Vc), glucose (Glu), and citric-acid monohydrate (CAM), was evaluated following eqs 23−4 in pH 3.6 buffer. The effect of the ratio of PA to copigments was evaluated at 1:1, 1:10, and 1:100 levels, respectively.

Determination of the Transition Temperature in the Solid State. The transition temperature of the sample was evaluated by DSC (DSC Q200, TA Instruments Inc., New Castle, DE). Aliquots of 1.0 mg of the sample were sealed in a preweighed aluminum pan. The sample was at equilibrium at 35 °C for 5 min and heated to 300 °C at the rate of 5 °C/min in a nitrogen bath (40 mL/min). The transition temperature of the sample was the exothermic peaks analyzed by TA Instruments, Universal Analysis 200 (version 4.5A).

The sample was the colyophilized powder of the copigmented PA. Specifically, PA (1.0 mg) was dissolved with the copigments, including Vc, CAM, and Glu individually, at the ratio of 1:1, 1:10, and 1:100 (w/w) in 1.0 mL of ddH_2O. The mixture was shaken for 30 min at room temperature and lyophilized, followed by DSC analysis.

Statistical Analysis. All data was expressed as the mean value ± standard deviation ($n \geq 3$). All statistical analyses were done with the Super ANOVA (Version 1.11, Abacus Concepts Inc., Berkeley, CA). One way ANOVA and multiple comparisons (Fisher's least-significant difference test) were used to evaluate the significant differences of data at criterion of $P < 0.05$.

RESULTS AND DISCUSSION

Effect of the pH Value and Temperature on the Stability of PA in the Liquid State. The stability of anthocyanins was affected by a few factors in the liquid state, including the pH value, temperature, ion, ion intensity, sunshine, and copigments, etc. (*15*). Among these factors, the pH value, temperature, and copigments were the main factors for the anthocyanins stability. Hence, the effect of the pH value, temperature, and copigments on the stability of PA was evaluated from the view of the kinetics by comparing with the TAC residual rate and E_a of each sample. Specifically, the TAC residual rate reflected the stability of PA with respect to time and temperature. E_a helped to understand the possibility of the anthocyanins conversion (*9*).

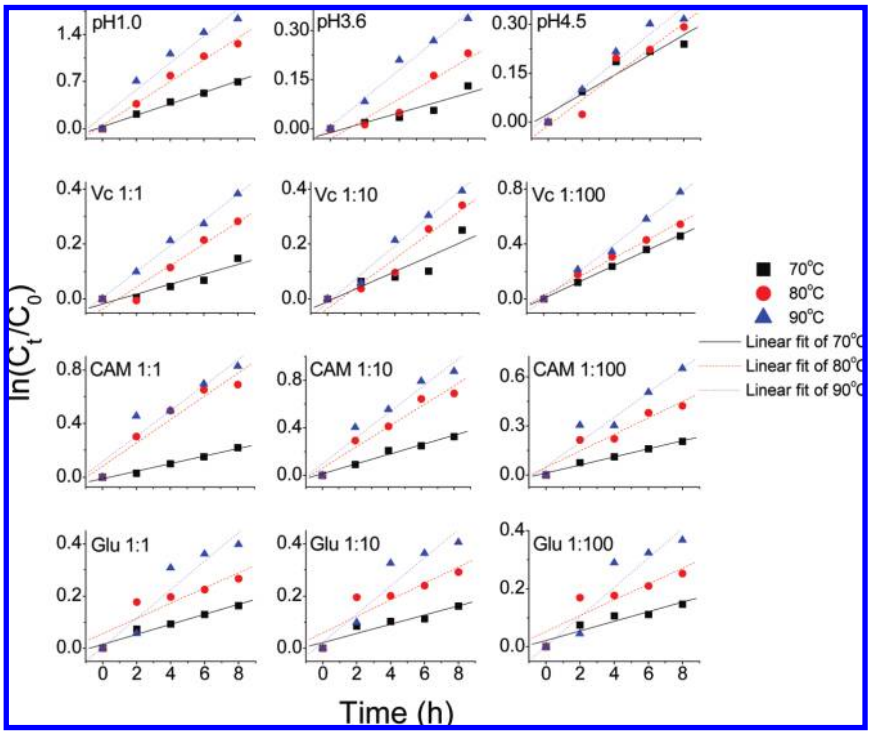

Figure 2. Plot of $\ln(C_t/C_0)$ vs t of the copigmented PA.

From the view of the TAC residual rate, the stability of PA was evaluated. The effect of the thermal treatment on the TAC residual rate of PA is shown in **Figure 1**. The stability of PA was influenced by the pH value and temperature, similar to the early results (*16*, *17*). Specifically, the TAC residual rate of PA was (19.4 ± 0.564), (71.4 ± 1.39), and (73.0 ± 0.2)% at pH 1.0, 3.6, and 4.5 after the thermal treatment at 90 °C for 8 h, respectively. Moreover, the same phenomenon also presented at 70 and 80 °C. The TAC residual rate of PA at pH 3.6 was similar to that at pH 1.0 and was different significantly to that at pH 4.5 after the thermal treatment at 90 °C for 8 h. Meschter (*16*) reported that the predominant structural forms of anthocyanins transformed with the pH varieties. The flavilium cation was the main and stable form of anthocyanins at pH 2−3, when quinonoidal base and chalcone were the main form at pH 4.5 (*18*). The quinonoidal base and chalcone form of anthocyanins were hydrolyzed easily at pH 4.5 or higher. Moreover, the stability of flavilium cation form was also decreased when pH decreased to about pH 1.0 (*19*), similar to our results. On the other hand, the stability of PA in the acid buffers was higher than that in the alkaline buffers because of the transforming conformation (*20*, *21*). Therefore, the stability of PA in the pH 3.6 buffer was similar to that in the pH 1.0 buffer and was higher than that in the pH 4.5 buffer. The stability of PA was also influenced by the temperature (*17*). The TAC residual rate of PA was (71.4 ± 1.39), (79.3 ± 4.04), and (87.7 ± 1.23)% at 90, 80, and 70 °C after the thermal treatment in pH 3.6 buffer for 8 h, respectively (**Figure 1**). Consequently, the stability of PA showed the negative correlation with the temperature, being consistent to the former result (*17*). The similar results also presented in the pH 1.0 and 4.5 buffers.

From the view of the kinetics analysis, the stability of PA was also evaluated. The effect of the pH value and temperature on the stability of PA was evaluated following eqs 23−4. The plot of $\ln(C_t/C_0)$ vs t of each sample is shown in **Figure 2**. The linear correlation of the plot proved that the conversion of PA followed the first-order reaction kinetics, being consistent to the previous results (*9*−*11*, *22*). Specifically, k of PA was 0.0151, 0.0306, and 0.0431 h^{-1} at 70, 80, and 90 °C in the pH 3.6 buffer, respectively. The k of PA showed the positive correlation with the temperature in the pH 3.6 buffer, which also presented in pH 1.0 and 4.5 buffers. Hence, the stability of PA was decreased with respect to the temperature (**Table 1**), being consistent with the former results (*9*, *10*, *23*).

The stability of PA resulted from its inherent E_a. The kinetics analysis showed that E_a of PA was 22.7, 27.8, and 8.66 kJ/mol in pH 1.0, 3.6, and 4.5 buffers, respectively, being similar to the results from the view of the TAC residual rate analysis. E_a of PA in the pH 3.6 buffer was lower that that of purple-flesh potato anthocyanin (72.49 kJ/mol in pH 3.0) (*23*), resulting from both the pH value and the variety of the purple potato. Moreover, E_a of PA at pH 3.6 was higher than that of the purple corn anthocyanin (18.3 kJ/mol at pH 4.0) (*11*), and was lower than that of the red-flesh potato anthocyanin (66.7 kJ/mol at pH 3.0) (*23*), the blackberry anthocyanin (59.0 kJ/mol at pH 2.89) (*9*), and the black carrot anthocyanin in orange juices (61.0 kJ/mol at pH 3.38) (*24*).

Effect of the Copigmentation on the Stability of PA in the Liquid State. The effect of the copigmentation on the stability of PA was evaluated only in the pH 3.6 buffer to simplify the analysis.

From the view of the TAC residual rate, the effect of the copigmentation on the stability of PA was also evaluated (**Figure 1**). The TAC residual rate of the copigmented PA showed the negative correlation with both the time and temperature, being similar to the conversion phenomenon of PA during the thermal treatment.

From the view of the kinetics analysis, the effect of the copigmentation on the stability of PA was also evaluated. The linear correlation of the plot (**Figure 2**) proved that the conversion of copigmented PA followed the first-order reaction kinetics, being consistent to the results of PA and previous studies (*9*−*11*, *22*). Moreover, the $t_{1/2}$ value of copigmented PA presented the negative correlation with the temperature, being similar to that of PA. Nevertheless, the effect of the copigmentation of PA with

Table 1. Kinetics Analysis of PA and the Copigmented PA

pH	copigment	temp (°C)	k (h^{-1})	$t_{1/2}$ (h)	E_a (kJ/mol)
1.0	no	70	0.0852	7.50	22.7
		80	0.162	3.95	
		90	0.200	3.20	
4.5	no	70	0.0300	21.3	8.66
		80	0.0391	16.4	
		90	0.0415	15.4	
3.6	no	70	0.0151	42.5	27.8
		80	0.0306	20.9	
		90	0.0431	14.8	
3.6	Vc 1:1	70	0.0180	35.6	25.7
		80	0.0393	16.3	
		90	0.0470	13.6	
3.6	Vc 1:10	70	0.0270	23.7	17.3
		80	0.0450	14.2	
		90	0.0516	12.4	
3.6	Vc 1:100	70	0.0577	11.1	13.1
		80	0.0670	9.54	
		90	0.0961	6.65	
3.6	CAM 1:1	70	0.0281	22.7	32.7
		80	0.0866	7.38	
		90	0.0944	6.77	
3.6	CAM 1:10	70	0.0404	15.8	30.9
		80	0.0863	7.41	
		90	0.1067	5.99	
3.6	CAM 1:100	70	0.0247	25.9	29.3
		80	0.0505	12.6	
		90	0.0752	8.50	
3.6	Glu 1:1	70	0.0192	33.3	28.2
		80	0.0291	22.0	
		90	0.0549	11.6	
3.6	Glu 1:10	70	0.0175	36.5	29.2
		80	0.0314	20.4	
		90	0.0537	11.9	
3.6	Glu 1:100	70	0.0165	38.6	29.0
		80	0.0273	23.4	
		90	0.0507	12.6	

Vc, CAM, and Glu on their corresponding E_a was different (**Figure 2**).

Specifically, the copigmentation of Vc lowered E_a of PA (**Table 1**). Moreover, E_a of PA decreased when the ratio of PA/Vc was from 1:1 to 1:100. Hence, the copigmentation of Vc decreased the stability of PA, and the stability of copigmented PA showed the positive correlation with its ratio to Vc. In the previous study, the similar results had also been described (*24*−*26*). Four main reasons accounted for this stability variety. First, Vc and its degradation products accelerated the conversion of anthocyanins (*27*). The second reason was that Vc was condensed with C4 of anthocyanins to form a colorless compound, resulting in the anthocyanins conversion (*26*). Third, Vc also followed the first-order reaction kinetics in pH 3.5 solution with E_a of about 21 kJ/mol (*28*, *29*), which was lower than that of PA. Lastly, Vc accelerated the degradation of the polymeric anthocyanin instead of the monomeric form at the low level of Vc (*25*). However, the monomer of anthocyanins was main reason for its color, and showed high positive correlation with its TAC. Consequently, the low level of Vc degraded the polymer of PA without affecting its TAC distinctly, while the high level of Vc would accelerate the degradation of all forms of PA, resulting in TAC reduction of PA.

The copigmentation of CAM increased E_a of PA. Specifically, E_a of PA copigmented with CAM at ratio of 1:1 was the highest from the ratio 1:1 to 1:100, showing the positive correlation with its ratio to CAM. Hence, the copigmentation of CAM showed the best capacity to improve the stability of PA at ratio of 1:1. This improvement maybe resulted from the acetylation of PA with CAM.

The copigmentation of Glu increased E_a of PA at all tested ratios. E_a of PA copigmented with Glu was 28.2, 29.2, and 29.0 kJ/mol at the ratios of 1:1, 1:10, and 1:100, respectively. One reason is that PA was glycosylated with Glu (*30*). The other reason was that Glu lowered the water activity resulting in the improvement of the anthocyanin stability (*7*, *31*).

Effect of the Copigmentation on the Stability of PA in the Solid State. The effects of the copigmentation on the thermodynamics of copigmented PA are evaluated by their transition temperature in the solid state (**Figure 3**). The transition temperature represented the conversion of PA, determining by the shift of the exothermic peaks in DSC profiles.

Two main transition temperatures of PA presented at 136 and 170 °C in the solid state (**Figure 3**A).

The effect of the copigmentation of Vc to PA on the transition temperature is shown in **Figure 3**B. Two main transition temperatures presented during the thermal treatment of the copigmented PA-Vc. Specifically, the transition temperatures were 160 and 176 °C at a ratio of 1:1, 167 and 192 °C at a ratio of 1:10, and 172 and 210 °C at a ratio of 1:100, which were higher than those of PA, respectively. Moreover, the transition temperature of the copigmented PA-Vc was improved with respect to the level of Vc. On the other hand, the transition temperature of Vc was 195 °C (data was not shown), being different to any conversion temperatures of copigmented PA-Vc. Consequently, PA was proved to polymerize with Vc. The copigmentation of Vc improved the stability of PA at a dose-dependent manner in the solid state, being similar to the results in the liquid state. These results may be mainly the result from the interaction of Vc with the flavilium cation form of PA (*32*).

The effect of the copigmentation of CAM to PA on the transition temperature presents in **Figure 3**C. The transition temperature of the copigmented PA-CAM was improved by the CAM copigmentation. Three main transition temperatures of the copigmented PA-CAM presented during the thermal treatment being different to that of PA. CAM lost its bound water at 128 °C (data was not shown), showing the similar profiles of the exothermic peaks to that of the conversion temperature of 115, 123, and 131 °C in copigmented PA-CAM at ratio of 1:1, 1:10, and 1:100, respectively. Hence, the transition temperature of 115, 123, and 131 °C of CAM resulted from losing its bound water. The transition temperatures related with the conversion of the copigmented PA-CAM were 168 and 202 °C at the ratio of 1:1, 187 and 201 °C at the ratio of 1:10, and 187 and 201 °C at the ratio of 1:100, which were higher than those of PA. Moreover, the transition temperature of the copigmented PA-CAM was improved with the raising ratio of CAM. Hence, CAM, as a copigment, improved the stability of PA at a dose-dependent manner in the solid state, being different to the corresponding results in the liquid state.

The effect of the copigmentation of Glu to PA at the different ratio on the transition temperature is shown in **Figure 3**D.The transition temperature of PA was improved by the Glu copigmentation. Three main transition temperatures of the copigmented PA-CAM presented during the thermal treatment being different to that of PA. Specifically, the transition temperature was 144, 161, and 192 °C at a ratio of 1:1, 165, 196, and 214 °C at a ratio of 1:10, and 176, 205, and 221 °C at a ratio of 1:100, which were higher than that of PA. On the other hand, the transition temperature of Glu was 82.8 °C (data was not shown), showing a slight difference to that of 92 °C reported by Dan et al. (*33*). However, the copigmented PA did not show any transition temperature between 82.8 and 92 °C. Hence, PA was proved to

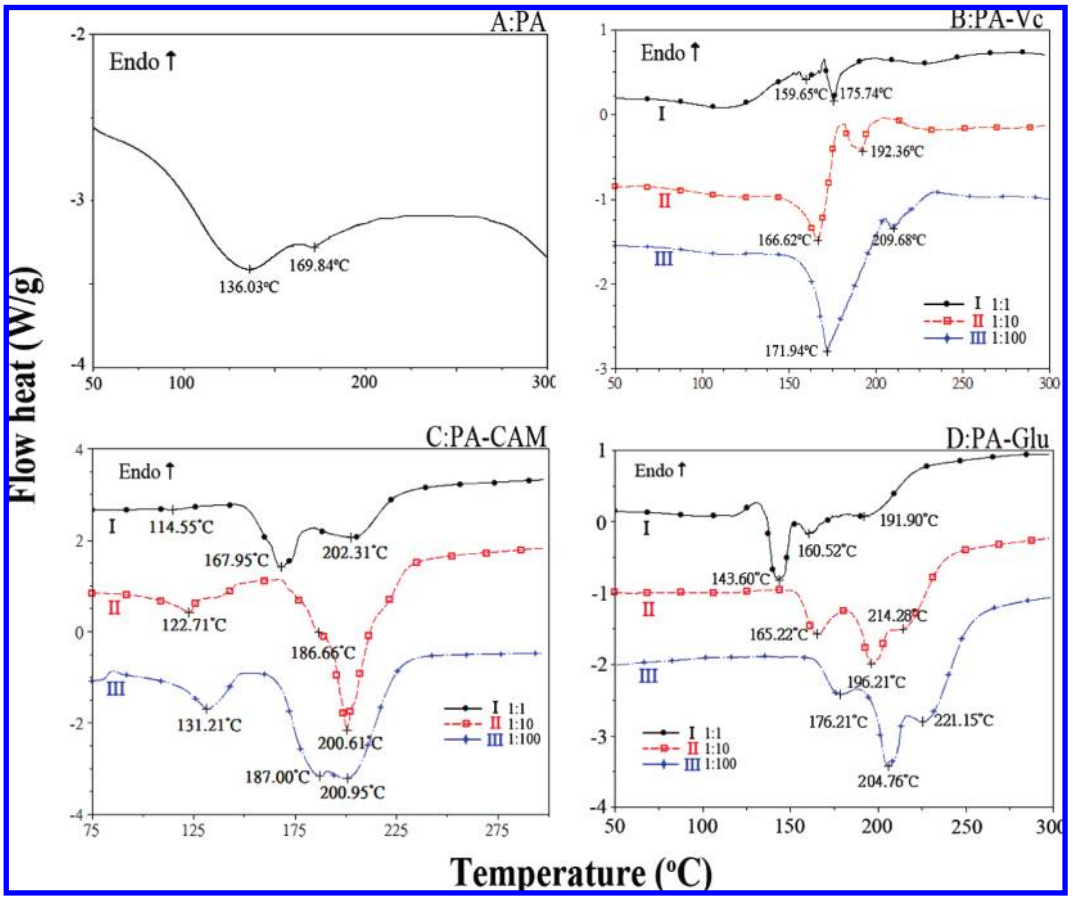

Figure 3. DSC profiles of PA and copigmented PA in the solid state.

glycosylate with Glu. Remarkably, the transition temperature of the copigmented PA-Glu was improved with the ratio of Glu, being similar to the dose-dependent manner of the PA-Vc or PA-CAM.

ABBREVIATIONS USED

CAM, citric acid monohydrate; DSC, differential scanning calorimetry; Glu, glucose; PA, anthocyanin from the purple potato peel; TAC, total anthocyanin content; Vc, ascorbic acid.

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