JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2008,** *56,* 10761–10766 **10761**

Composition and Thermal Stability of Anthocyanins from Chinese Purple Corn (*Zea mays* L.)

XIAOYAN ZHAO,*,†,§ MARGARITA CORRALES,‡ CHAO ZHANG,† XIAOSONG HU,§ YUE MA,† AND BERNHARD TAUSCHER‡

Beijing Academy of Agriculture and Forestry Sciences, P.O. Box 2443, 100097 Beijing; China, Max Rubner Institute, Haid-and-Neu Strasse 9, 76131 Karlsruhe, Germany; and College of Food Science and Nutritional Engineering, Chinese Agricultural University, 100094 Beijing, China

Chinese purple corn extracts (*Zea mays* L., Zhuozhou, Hebei, China) (EZPC) were selected among five Chinese purple corn hybrids due to their higher anthocyanin content, and their thermal stability was evaluated. The total anthocyanin content and total phenolic content of EZPC were 304.5 ± 16.32 mg of cyanidin-3-glucoside equiv/100 g of dry seeds and 489.8 ± 24.90 mg of gallic acid equiv/100 g of dry seeds, respectively. Moreover, the individual anthocyanins of EZPC were determined by HPLC-DAD/ESI-MS analysis. Seven main compounds were determined, including cyanidin-3-(malonylglucoside), cyanidin-3-*O*-glucoside-2-malonylglucoside, cyanidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, peonidin-3-(malonylglucoside), pelargonidin-3-(6″-malonylglucoside), and peonidin-3-(dimalonylglucoside). The thermal stability of EZPC was studied by differential scanning calorimetry. Thermodynamic analysis showed that the conversion of EZPC followed an Arrhenius relationship, where the delta enthalpy (*H*) and activation energy (E_a) were 97.0 J/g and 204 ± 2.72 kJ/mol, respectively. Furthermore, the relationships between the degree of conversion of EZPC and time or temperature were reported. This study demonstrated that the evaluated Chinese purple corn hybrids are a natural source of anthocyanins and are stable over a wide range of temperatures and times.

KEYWORDS: Purple corn; anthocyanin; mass spectrometer; thermodynamics; kinetic parameter; thermal stability

INTRODUCTION

Anthocyanins not only have vivid colors, ranging from pink through red and violet to dark blue, but also have potential beneficial health effects, reducing the risks of chronic diseases such as cardiovascular diseases or hyperglycemia (*1, 2*). Purple corn is an important source of anthocyanins with potential application as food natural colorants and antioxidants (*3*). The individual anthocyanins of Andean purple corn, leaves, cobs, and seeds have been characterized, including cyanidin-3-dimalonylglucoside, cyanidin-3-glucoside, pelargonidin-3-glucoside, peonidin-3-glucoside, and their respective malonated counterparts as their main anthocyanins (*3*−*5*). Likewise, a similar anthocyanin composition was also found in corn flowers (*6*). However, the literature is mainly focused on purple corn of Peruvian varieties or derived products. Only a limited number of works deal with Chinese varieties of purple corn (*7, 8*), whereas the anthocyanin composition of Chinese purple corn hybrids is still not well documented. Hence, the anthocyanin content and individual anthocyanin composition of five Chinese purple corn hybrids were determined for the first time in this work.

The stability of anthocyanins is one of the most important characteristics, which defines their feasibility as colorants. Previous studies showed that the stability of anthocyanins is mainly influenced by the pH, the temperature, and the presence of light, oxygen, or metallic ions (Fe^{2+}, Fe^{3+}, Ca^{2+}, and K^+, etc.) (*9*−*12*). However, the mechanism of anthocyanin conversion in purple corn extracts has not been yet reported. Thermodynamic analysis can give a deeper insight into anthocyanin conversion. For instance, thermodynamics analysis of the purple corn-cob anthocyanin (*8*), blackberry anthocyanin (*13*), and orange anthocyanin (*14*) had been reported and showed that the nature of the conversion follows a presumed first-order kinetic model. As a result, thermodynamics analysis of ethanol-extracted anthocyanins from Chinese purple corn (*Zea mays* L., Zhuozhou, Hebei, China) (EZPC) was studied by differential scanning calorimetry different from the former methods (*8, 13, 14*). By this means, the thermodynamics of EZPC were evaluated to show its nature by an Arrhenius relationship for the first time. Finally, the relationship between the degree of conversion of EZPC and the conversion time or temperature was further herein documented. This research provides an invaluable approach to

* Author to whom correspondence should be addressed (telephone +86-10-51503053; fax +86-10-88446286; e-mail zhaoxiaoyan@nercv.org).

† Beijing Academy of Agriculture and Forestry Sciences.

§ Chinese Agricultural University.

‡ Max Rubner Institute.

10.1021/jf8025056 CCC: $40.75
Published on Web 10/25/2008

Table 1. TAC and TPC of the Five Purple Corn Hybrids[a]

sample	endosperm type	color	TAC	TPC
JHY	sinesis Kulesh	pink	12.74 ± 0.41 a	114.7 ± 7.87 a
BP	sinesis Kulesh	gray	29.22 ± 0.86 b	137.6 ± 7.39 b
SZ	amylacea Sturt	crimson red	149.3 ± 5.63 c	359.5 ± 22.45 c
JHN	sinesis Kulesh	dark purple	256.5 ± 11.21 d	477.6 ± 5.00 d
EZPC	amylacea Sturt	black	304.5 ± 16.32 e	489.8 ± 24.90 d

[a] TAC expressed as mg of cyanidin 3-glucoside per 100 g of dry seeds (means ± SD, $n = 3$); TPC expressed as mg of gallic acid per 100 g of dry seeds (means ± SD, $n = 3$). Data in the same column with different letters are significantly different ($P < 0.05$).

the stability of EZPCs over a wide range of temperatures and times, being useful for their application as colorants or antioxidants in foods.

MATERIALS AND METHODS

Extraction. Five purple corn hybrids, including Black Pearl from Guangdong province (BP), Jinheiyu from Shanxi province (JHY), Jingheinuo from Beijing (JHN), Shijiazhuang purple corn from Hebei province (SZ), and Zhuozhou purple corn from Hebei province (ZPC), were evaluated in this study. The crops were grown in Yanqing (Beijing, China, 2005), collected at maturity, air-dried, and ground.

The material (500 g) was stirred in 2500 mL of liquid [a solution of 60% (v/v) ethanol acidified with citric acid (1%, w/v)] at 60 °C for 120 min. The ethanol extracts were centrifuged at 9000 rpm and 20 °C for 10 min. The supernatants were evaporated to dryness at 40 °C with a rotary evaporator Büchi R-3000 (Büchi Labortechnic AG, Flawil, Switzerland).

Determination of the Total Anthocyanin Content (TAC). The TAC was determined according to the pH differential method described by Lee et al. (*15*). 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) at 510 and 700 nm, respectively. Absorbance was calculated as $\text{Abs} = (A_{510\text{nm}} - A_{700\text{nm}})\text{pH}_{1.0} - (A_{510\text{nm}} - A_{700\text{nm}})\text{pH}_{4.5}$ 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 seeds (eq 1). All TACs shown were made as cyanidin 3-glucoside equivalents.

$$\text{TAC (mg/100 g)} = \frac{\text{AB}}{eL} \times \text{MW} \times D \times \frac{V}{G} \times 100 \qquad (1)$$

where Ab is absorbance, e is cyanidin 3-glucoside molar absorbance (26900), 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 seed weight (mg).

Determination of the Total Phenolic Content (TPC). The TPC was determined according to the Folin−Ciocalteu method (*16*). Aliquots of 125 μL of the Folin−Ciocalteu reagent (10-fold diluted) were added to 125 μL of the sample. The mixture was at room temperature for 6 min, and then 1.25 mL of a 7% aqueous Na_2CO_3 solution was added. The final volume was adjusted to 3 mL with water. The absorbance was measured at 760 nm against water as a blank after 90 min. The amount of TPC was expressed as milligrams of gallic acid equivalents (GAE) per 100 g of dry seeds. A gallic acid hydrate solution (Roth, Karlsruhe, Germany) was used as a standard for the calibration curve, ranging from 20 to 500 μg/mL ($R^2 = 0.9969$).

HPLC-DAD/ESI-MS Analysis. The individual anthocyanins were separated by HPLC-DAD/ESI-MS (Agilent 1100 series, Palo Alto, CA). The absorbance of the elute from an Aqua column-RP 80 A column (250 × 1.6 mm i.d., particle size = 125 Å, Phenomenex, Aschaffenburg, Germany) was recorded with a DAD detector (G1315A, Agilent, Palo Alto, CA) at 280, 320, and 520 nm. Specifically, the column was eluted at a flow rate of 0.8 mL/min by solvent A (water/formic acid 99.9:0.1%, v/v) and solvent B (water/formic acid/acetonitrile 49.9:0.1:50%, v/v/v). A linear gradient of solvent B was used from 10 to 25% in 10 min, from 25 to 31% B in 5 min, from 31 to 40% in 5 min, from 40 to 45% in 10 min, from 45 to 100% in 10 min, and from 100 to 10% in 5 min, with an injection of 20 μL of the sample.

The ESI parameters were as follows: nebulizer, 30 psi; dry gas, 9 L/min; gas temperature, 350 °C; trap drive, 78.0; skimmer voltage, 40 V; capillary exit voltage, 94 V. The ion trap was operated in positive mode scanning from m/z 50 to 2200 at a scan resolution of 13000 amu/s. Trap ICC was 70000 units, and maximal accumulation time was 200 ms. The mass spectrometer (model G1946A) was operated at a fragmentation amplitude of 1.0 V, and threshold ABS was 50000 units. Data were evaluated by Agilent Chemstation Rev. A. 09.01 software.

Anthocyanin Quantification. The content of individual compounds was evaluated using the calibration curve of cyanidin-3-*O*-glucoside (Extrasynthese, Genay, France). Calibration of other anthocyanins was determined, including a pertinent molecular weight correction factor (*17*). Results were expressed as milligrams of cyanidin-3-*O*-glucoside equivalents per 100 g of dry seeds.

Thermodynamics Analysis. Thermodynamic characteristics of the samples were measured by differential scanning calorimetry (Pyris 1, Perkin-Elmer Pyris, Boston, MA). Aliquots of 10 μg of the sample were sealed in a preweighed aluminum pan. The sample was heated from 25 to 150 °C at the rate of 5 °C/min in a nitrogen bath. The thermograph was analyzed by the scanning kinetics theory (Pyris 1, Perkin-Elmer, version 3.8). The thermodynamics parameter and character of the sample were studied. Especially, the degree of conversion (α) of the sample with time and its relationship with temperature were studied.

The degree of conversion (α) was assumed to follow eq 2 in the heating process

$$\frac{d\alpha}{dt} = k(1-\alpha)^n \qquad (2)$$

where α is the degree of conversion, n is the order of the conversion, k is the conversion rate, and $d\alpha/dt$ is the derivative with respect to time.

The conversion rate was generally assumed to have a temperature dependence as eq 3, known as an Arrhenius relationship.

$$k = Z\exp\left(\frac{E_a}{RT}\right) \qquad (3)$$

Z is the pre-exponential constant, E_a is the activation energy of the conversion, R is the universal gas constant, and T is the absolute temperature in degrees Kelvin. When eqs 2 and 3 were combined, eq 4 was formed.

$$\frac{d\alpha}{dt} = Z\exp\left(\frac{E_a}{RT}\right)(1-\alpha)^n \qquad (4)$$

Equation 4 had three degrees of freedom or three variables. To have a definite solution, one variable either might be held constant or might be related to another variable during the conversion process. Therefore, the conversion process was defined in this paper.

The resultant values of α, Z, E_a, and n now were calculated by eqs 5 and 6.

$$\alpha = \frac{\Delta H_{\text{partial}}}{\Delta H} \qquad (5)$$

The values of ΔH and $\Delta H_{\text{partial}}$were derived from the area under the thermograph. Because there were three unknowns, Z, E_a, and n, to be determined, a multilinear regression was done.

Equation 4 was reduced to a linear form by taking the natural logarithm of both sides of the equation to obtain eq 6.

$$\ln\left[\frac{d\alpha}{dt}\right] = \ln(Z) - \frac{E_a}{RT} + n \times \ln(1-\alpha) \qquad (6)$$

A multilinear regression was done using $\ln[d\alpha/dt]$, $-1/RT$, and $\ln(1-\alpha)$ as variables, which had been derived from the thermograph to solve for Z, E_a, and n.

After the kinetic parameters (Z, E_a, and n) had been calculated, they could be used to predict the behavior of a sample under isothermal conditions. Especially, the temperature was an absolute temperature in all of the equations.

The relationship between the time and temperature curve represented the time required to reach a given extent of α at a temperature T, calculted by eq 7.

$$t = \frac{1 - n(1-\alpha)^{(1-n)}}{Z(1-n)\exp(-E_a/RT)} \quad (7)$$

The plot displayed was based on the percent of sample reacted and the temperature (125−185 °C).

The relationship between α and time or temperature curve represented the percent of the sample reacted versus time at a given isothermal temperature or a given time (eq 8).

$$\alpha = 1 - [1 - (1-n)Zt\exp(-E_a/RT)]^{1/(1-n)} \quad (8)$$

The plot displayed was based on an isothermal temperature and two time limits or on the conversion time and two temperature limits.

Statistical Analysis. All data were 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 a criterion of $P < 0.05$.

RESULTS AND DISCUSSION

TAC and TPC of EZPC. The TAC, TPC, and variability of the five Chinese purple corn hybrids are summarized in **Table 1**. The TAC varied significantly among corn hybrids ($P < 0.05$). The TAC (304.5 mg/100 g) of EZPC with dark purple skin was nearly 30-fold higher than that of JHN and 10-fold higher than that of BP. Consistent with these results, Dykes et al. (*18*) also pointed out a higher anthocyanin content in dark pericarp corn hybrids. Furthermore, EZPC and JHN (known as dark corn hybrids) contained higher amounts of anthocyanins at 304.5 ± 11.21 and 256.5 ± 16.32 mg/100 g, respectively, than those reported for pigmented corns from Canada (127.7 mg/100 g) (*2*), Mexico (72.1 mg/100 g), and the United States (30.7 mg/100 g) (*19*). It was thus confirmed that variety and growth conditions of the purple corn also influence their anthocyanin content (*20*). To prove this, Moreno et al. (*21*) found that TAC of four Mexican blue corn varieties ranged from 54 to 115 mg/100 g. In summary, the TAC of EZPC was much higher than that of all mentioned hybrids.

The TPC of the five selected hybrids ranged from 114.7 to 489.8 mg of GAE/100 g (**Table 1**). The TPC of EZPC was the highest (489.8 mg of GAE/100 g), showing significant difference ($P < 0.05$) from that of JHY, BP, and SZ. Moreover, the TPC of Chinese hybrids was clearly higher than those of U.S. hybrids (ranging from 40 to 130 mg of GAE/100 g) (*19*). However, Andean purple corn extracts had the highest TPC (1756 mg/100 g) reported to date (*22*). These results demonstrated EZPC to have a high content of TAC and TPC and to be a good natural source of anthocyanins for marketability.

Individual Anthocyanin Composition of EZPC. The individual anthocyanins of EZPC were determined by HPLC-DAD/ESI-MS analysis. EZPC showed 11 peaks at Abs_{520nm} in HPLC analysis (**Figure 1**).

Seven compounds were determined by the mass identification that was simplified using a combination of the retention time, peak spectra, mass-to-charge ratio, and MS^2 and MS^3 fragmentation (**Table 2**). Specifically, compounds **3** and **5** had the same molecular weight and MS^2 and MS^3 fragmentations with different retention times. This indicated the presence of two

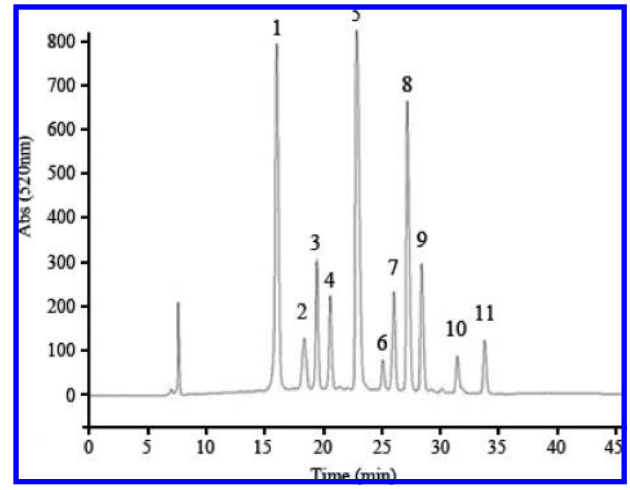

Figure 1. HPLC profile of EZPC with absorbance at 520 nm.

Table 2. HPLC-DAD/ESI-MS Results of Individual Anthocyanins Detected in EZPC

compd	RT (min)	λ_{max} (nm)	major ions (m/z)	MS^2	MS^3	compd presumed
1	16.02	517	449	287	212	cyanidin-3-*O*-glucoside[a]
2	18.47	518	371			unknown 1
3	19.55	516	535	287	137	cyanidin-3-(malonylglucoside)
4	21.78	516	463	301		peonidin-3-*O*-glucoside
5	23.05	516	535	287	137	cyanidin-3-(malonylglucoside)
6	25.31	518	549	301	287	peonidin-3-(malonylglucoside)
7	26.25	506	519	271		pelargonidin-3-(6″-malonylglucoside)
8	27.32	516	621	287		cyanidin-3-*O*-glucoside-2-malonlyglucoside
9	28.53	518	549	301	287	peonidin-3-(malonylglucoside)
10	31.60	519	605	271	253	unknown 2
11	33.44	520	635	301	287	peonidin-3-(dimalonylglucoside)

[a] This compound is confirmed by comparison of the combination of the retention time, peak spectra, mass-to-charge ratio, and MS^2 and MS^3 fragmentation of the standard (cyanidin-3-*O*-glucoside, Extrasynthese, Genay, France).

isomers of malonylated cyanidin 3-glucosides as previously reported by Aoki et al. (*3*). Likewise, compounds **6** and **9** were also isomers, being cyanidin-3-glucoside acylated with malonic acid. On the other hand, compounds **2** and **10** could not be discerned.

The individual anthocyanins of EZPC were similar to those of JHN and different from those of JHY, BP, and SZ (**Table 3**). Specifically, peonidin-3-(malonylglucoside) or peonidin-3-(dimalonylglucoside) was not detected in JHY, BP, and SZ or in JHY and BP, respectively, compared with EZPC. Moreover, the individual anthocyanins of EZPC showed remarkable differences with that reported by Aoki et al. (*3*) or by Moreno et al. (*21*) because cyanidin-3-glucoside was the principal anthocyanin in EZPC, whereas pelargonidin-3-*O*-glucoside was not found. Specifically, the contents of cyanidin-3-*O*-glucoside, cyanidin-3-(malonylglucoside), cyanidin-3-*O*-glucoside-2-malonylglucoside, peonidin-3-*O*-glucoside, peonidin-3-(malonylglucoside), peonidin-3-(dimalonylglucoside), and pelargonidin-3-(6″-malonylglucoside) of EZPC were 91.6 ± 11.67, 97.9 ± 8.28, 35.33 ± 6.56, 18.84 ± 1.24, 21.34 ± 2.58, 6.62 ± 0.05, and 15.41 ± 2.30 mg/100 g, respectively.

The cyanidin derivatives were the predominant compounds in the five hybrids (**Table 3**). Cyanidin derivatives constituted 73−77% of TAC in JHY, SZ, JHN, and EZPC, whereas they were up to 97.14% in BP hybrids. Cyanidin-3-(malonylglucoside) was the predominant cyanidin derivative and constituted >55% (average) of the cyanidin derivatives in the five hybrids. Specifically, cyanidin-3-(malonylglucoside) was also the predominant compound in EZPC. Consequently, cyanidin deriva-

Table 3. Anthocyanin Composition of Five Chinese Purple Corn Hybrids (Milligrams per 100 g of Dry Seeds)[a]

anthocyanin	JHY	BP	SZ	JHN	EZPC
cyanidin-3-*O*-glucoside	1.21 ± 0.02	1.83 ± 0.03	21.2 ± 3.42	41.45 ± 4.47	91.6 ± 11.67
cyanidin-3-(malonylglucoside)	5.50 ± 0.15	11.91 ± 1.27	82.37 ± 2.78	110.03 ± 12.31	97.9 ± 8.28
cyanidin-3-*O*-glucoside-2-malonylglucoside	2.00 ± 0.12	11.80 ± 0.93	5.97 ± 0.89	62.84 ± 4.82	35.33 ± 6.56
peonidin-3-*O*-glucoside	0.01	0.34 ± 0.03	5.19 ± 0.08	6.46 ± 0.12	18.84 ± 1.24
peonidin-3-(malonylglucoside)	nd	nd	nd	19.71 ± 3.23	21.34 ± 2.58
peonidin-3-(dimalonylglucoside)	nd	nd	9.18 ± 1.20	7.21 ± 1.43	6.62 ± 0.05
pelargonidin-3-(6″-malonylglucoside)	2.50 ± 0.13	0.34 ± 0.01	14.49 ± 2.74	17.64 ± 1.23	15.41 ± 2.30
unknown 1	0.63 ± 0.03	0.07 ± 0.01	3.61 ± 0.63	6.35 ± 0.58	11.31 ± 1.45
unknown 2	0.04 ± 0.01	nd	5.34 ± 0.51	4.6 ± 0.08	5.62 ± 0.21
cyanidin derivatives	8.71 ± 0.27 (73.33%)	25.54 ± 1.56 (97.14%)	109.54 ± 4.23 (74.32%)	214.32 ± 13.25 (77.53%)	224.83 ± 10.83 (73.96%)
peonidin derivatives	0.01 (2.86%)	0.34 ± 0.03 (1.2%)	14.37 ± 2.23 (9.75%)	33.38 ± 3.14 (11.96%)	46.8 ± 2.34 (15.13%)
pelargonidin derivatives	2.50 ± 0.13 (21.04%)	0.34 ± 0.01 (1.2%)	14.49 ± 2.74 (9.74%)	17.64 ± 1.23 (6.32%)	15.41 ± 2.30 (5.08%)

[a] Data are expressed as a means ± SD ($n = 3$). nd, not detected or lower than 0.01. Data in parentheses represent the percentage of the individual anthocyanins to the total content.

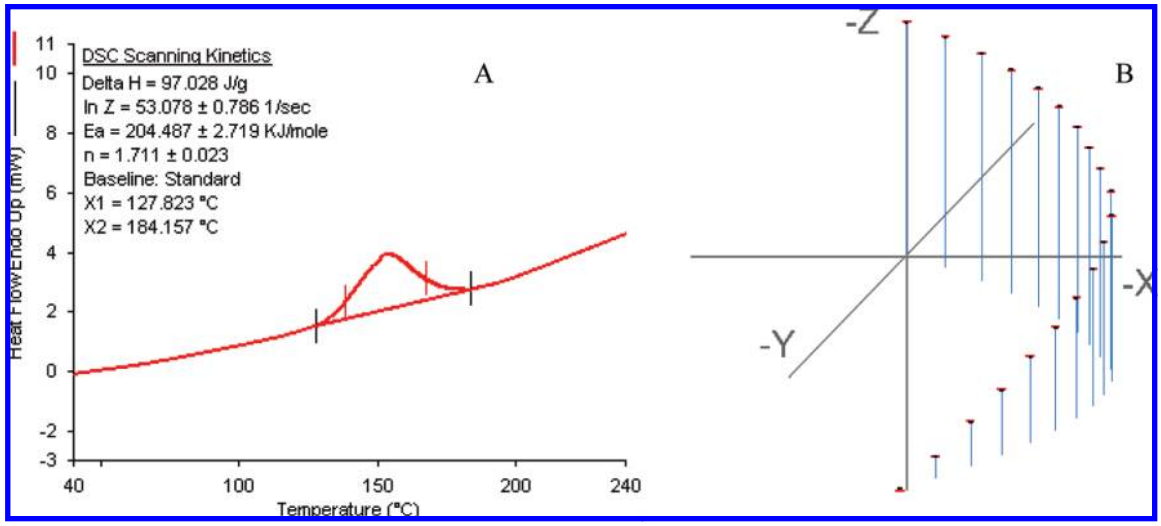

Figure 2. Determination of the kinetic parameters (Z, E_a, and n) of EZPC: (**A**) scanning kinetics thermograph of EZPC; **B**) multilinear regression plot. Axes X, Y, and Z are $\ln[d\alpha/dt]$, $-1/RT$, and $\ln(1-\alpha)$, respectively.

tives were 73.96% (w/w) of the total anthocyanins, differing from the content reported by other authors (*3, 21*). The difference may be due to the different varieties and growing conditions (*6, 20*). The contents of cyanidin derivatives, peonidin derivatives, and pelargonidin derivatives of EZPC were 73.96, 15.13, and 5.08% (w/w), respectively.

Kinetic Parameters of EZPC. The kinetic parameters of EZPC were estimated following eqs 3−6, where the delta H was 97.0 J/g, ln Z was 53.1 ± 0.786 1/s, E_a was 204 ± 2.72 kJ/mol, and n was 1.71 ± 0.023 (**Figure 2A**).

The thermograph of EZPC was a smooth peak (**Figure 2A**), showing a cumulate heat flow of individual anthocyanins. The multilinear regression plot was done with $\ln[d\alpha/dt]$, $-1/RT$, and $\ln(1-\alpha)$ as axes X, Y, and Z, respectively, which was derived from the thermograph to solve for Z, E_a, and n (**Figure 2B**). Specifically, n of EZPC was done by the multilinear regression plot. Before this multilinear regression plot, studies on the degradation of purple corn-cob anthocyanin (*8*), blackberry anthocyanin (*13*), and orange anthocyanin (*14*), etc., in aqueous solutions have been reported to follow a first-order kinetic model based on prediction. Likewise, the degradation of anthocyanins from *Hibiscus sabdariffa* L. in the solid state was reported to follow a first-order reaction (*23*). By our multilinear regression plot, n of EZPC was 1.71 ± 0.023, higher than the value predicted before. This result showed that the kinetics of EZPC's conversion in the solid state was more complex than known. Copigmentation or self-association of anthocyanins might explain this kinetics, although it has not been yet demonstrated (*24*).

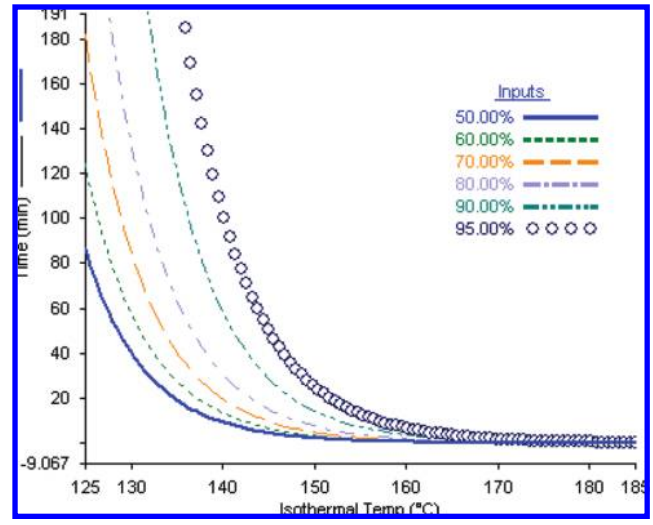

Figure 3. Isothermal conversion time of EZPC.

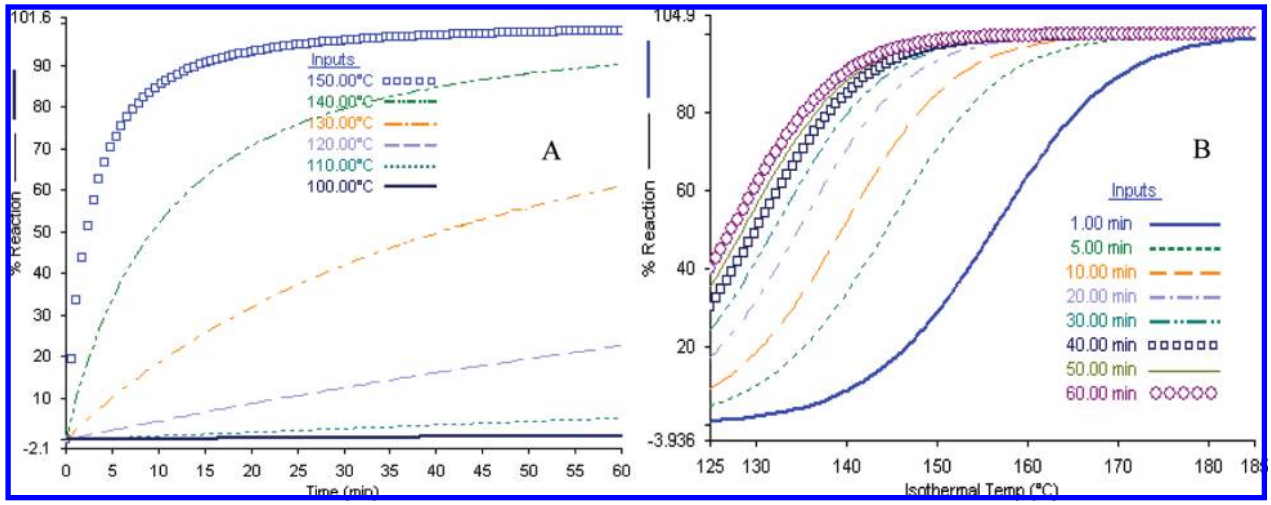

Figure 4. Degree of conversion versus time (**A**) or temperature (**B**).

The E_a of EZPC conversion was 204 ± 2.72 kJ/mol, higher than those reported for purple corn-cob anthocyanins (18.3 kJ/mol) (*8*), blackberry anthocyanins (73 ± 2 kJ/mol) (*13*), orange anthocyanins (73−89 kJ/mol) (*14*), and *H. sabdariffa* L. (62.8 kJ/mol) (*23*). These results indicate that the thermal stability of EZPC was higher than those of mentioned anthocyanins from the view of thermodynamic theory.

Relationship between the Degree of Conversion and Time or Temperature of EZPC. The isothermal conversion time is shown in **Figure 3**. The 50% conversion time of EZPC was >3 days at 100 °C and was 84 min at 125 °C (data not shown). Hence, EZPC is expected to be stable at ultraheat sterilization conditions.

The 50% conversion time of EZPC was >12 years at 70−90 °C. Yang et al. (*8*) reported that the 50% conversion times of the purple corn-cob anthocyanin were 11.6, 9.0, and 7.5 h at 70, 80, and 90 °C, respectively. Wang et al. (*13*) reported the 50% conversion times of blackberry anthocyanin were 8.8, 4.7, and 2.9 h at 70, 80, and 90 °C, respectively. Kirca et al. (*14*) showed that the 50% conversion time of anthocyanins was from 0.4 to 6.3 h at 70−90 °C in 11.2, 45, and 69,°Brix in blood orange juice. Hence, it was clear that the 50% conversion time of EZPC was much longer than that of purple corn-cob anthocyanin (*8*), blackberry anthocyanin (*13*), and orange anthocyanin (*14*) at 70−90 °C. These achievements may be explained by the nature of the EZPC (solid state) differing from the nature of the samples in the other studies (liquid state). The kinetic results of EZPC's conversion followed an Arrhenius relationship, whereas the other results followed an Arrhenius relationship with the first-order kinetic presumptions. Consistent with our results, Gradinaru et al. (*23*) studied the thermal stability of *H. sabdariffa* L. anthocyanins in solid state. The 50% conversion time of *H. sabdariffa* L. anthocyanins degradation was <1 year at 40 °C, much shorter than that of EZPC (271 years) at 40 °C. Therefore, the thermal stability of EZPC was stronger than those of all studied anthocyanins.

The stability of anthocyanin is dependent on several factors, including chemical structure, pH, temperature, light intensity, presence of copigments, metallic ions, enzymes, oxygen, ascorbic acid, sugars and their degradation products, and sulfur dioxide, etc. (*25, 26*). From all these factors, it has been demonstrated that pH and temperature mainly affect the stability of anthocyanins (*25*). As a result, the relationship between α and time or temperature was investigated and is shown in **Figure 4**. The degree of conversion increased 736-fold in the limits of 100−150 °C (**Figure 4A**). Specifically, the degree of conversion was 0.1, 8.6, and 73.6% in 5 min at 100, 130, and 150 °C, respectively, consistent with the results of Yang et al. (*8*). These results have a great relevance because food processes, such as blanching, pasteurization, and baking, require high temperatures and do not involve any change of pH. Therefore, the temperature was the most important factor influencing the stability of EZPC.

EZPC was stable below 157 °C at 1 min with an α below 50% (**Figure 4B**). On the other hand, the degree of conversion of EZPC showed an increase at 140−150 °C, remarkably, resulting from the conversion at 127−184 °C shown in **Figure 2A**. Furthermore, the degree of conversion of EZPC had a linear region with the isothermal temperature at any conversion time. Consequently, the degree of conversion of EZPC can be predicted and controlled manually over a wide range of temperatures and times by the thermodynamics calculation. For example, Plocharsk et al. (*27*) required a year to acquire the anthocyanin losses in black chokeberry. In contrast, the thermodynamics calculation reported in this study showed that the 50% conversion time of EZPC was 271 years with the isothermal temperature of 40 °C, which is difficult to achieved by traditional analysis. Finally, the thermodynamics calculation may provide valuable information to evaluate the stability of EZPC when applied in processes such as blanching, cooking, pasteurization, and baking.

ABBREVIATIONS USED

BP, black pearl from Guangdong province; EZPC, ethanol extracted from Chinese purple corn (Zhuozhou, Hebei province, China); GAE, gallic acid equivalents; HPLC-DAD/ESI-MS, high-performance liquid chromatography−diode array detector/electrospray ionization−mass spectrometry; JHN, Jingheinuo from Beijing; JHY, Jinheiyu from Shanxi province, China; SZ, Shijiazhuang purple corn from Shijiazhuang, Hebei province, China; TAC, total anthocyanin content; TPC, total phenolic content; ZPC, Zhuozhou purple corn from Zhuozhou, Hebei province, China.

LITERATURE CITED

(1) Tsuda, T.; Horio, F.; Uchida, K.; Aoki, H.; Osawa, T. Dietary cyanidin 3-*O*-β-D-glucoside-rich purple corn color prevents obesity and ameliorates hyperglycemia in mice. *J. Nutr.* **2003**, *133*, 125–130.

(2) Abdel-Aal, E. M.; Young, J. C.; Rabalski, I. Anthocyanin composition in black, blue, pink, purple, and red cereal grains. *J. Agric. Food Chem.* **2006**, *54*, 4696–4704.
(3) Aoki, H.; Kuze, N.; Kato, Y. Anthocyanins isolated from purple corn (*Zea mays* L.). *J. Foods Food Ingred.* **2002**, *199*, 41–45.
(4) Pascual-Teresa, S.; Santos-Buelga, C.; Rivas-Gonzalo, J. C. LC-MS analysis of anthocyanins from purple corn cob. *J. Sci. Food Agric.* **2002**, *82*, 1003–1006.
(5) Harborne, J. B.; Self, R. Malonated cyanidin 3-glucosides in *Zea mays* and other grasses. *Phytochemistry* **1997**, *26*, 2417–2418.
(6) Fossen, T.; Slimestad, R.; Andersen, Ø. M. Anthocyanins from maize (*Zea mays*) and reed canarygrass (*Phalaris arundinacea*). *J. Agric. Food Chem.* **2001**, *49*, 2318–2321.
(7) Zhang, X.; Mu, Z.; Gong, Z. Study and utilization of black corn germ plasm resources in China. *Crops Resour.* **1998**, *4*, 12–14.
(8) Yang, Z.; Han, Y.; Gu, Z.; Fan, G.; Chen, Z. Thermal degradation kinetics of aqueous anthocyanins and visual color of purple corn (*Zea mays* L.) cob. *Innovative Food Sci. Emerging Technol.* **2008**, *9*, 341–345.
(9) Mollov, P.; Mihalev, K.; Shikov, V.; Yoncheva, N.; Karagyozov, V. Colour stability improvement of strawberry beverage by fortification with polyphenolic copigments naturally occurring in rose petals. *Innovative Food Sci. Emerging Technol.* **2007**, *8* (3), 318–321.
(10) de Rosso, V. V.; Mercadante, A. Z. Evaluation of colour and stability of anthocyanins from tropical fruits in an isotonic soft drink system. *Innovative Food Sci. Emerging Technol.* **2007**, *8* (3), 347–352.
(11) Bordignon-Luiz, M. T.; Gauche, C.; Gris, E. F.; Falcao, L. D. Colour stability of anthocyanins from Isabel grapes (*Vitis labrusca* L.) in model systems. *LWT−Food Sci. Technol.* **2007**, *40* (4), 594–599.
(12) Veigas, J. M.; Narayan, M. S.; Laxman, P. M.; Neelwarne, B. Chemical nature, stability and bioefficacies of anthocyanins from fruit peel of *Syzygium cumini* Skeels. *Food Chem.* **2007**, *105* (2), 619–627.
(13) Wang, W. D.; Xu, S. Y. Degradation kinetics of anthocyanins in blackberry juice and concentration. *J. Food Eng.* **2007**, *82*, 271–275.
(14) Kirca, A.; Cemeroglu, B. Degradation kinetics of anthocyanins in blood orange juice and concentrate. *Food Chem.* **2003**, *81* (4), 583–587.
(15) Lee, J.; Durst, R. W.; Wrolstad, R. E. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. *J. AOAC Int.* **2005**, *88*, 1269–1278.
(16) Chun, O. K.; Kim, D. O.; Smith, N.; Schroeder, D.; Han, J.; Lee, C. Y. Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. *J. Sci. Food Agric.* **2005**, *85*, 1715–1724.
(17) Chandra, A.; Rana, J.; Li, Y. Separation, identification, quantification, and method validation of anthocyanins in botanical supplement raw materials by HPLC and HPLC-MS. *J. Agric. Food Chem.* **2001**, *49*, 3515–3521.
(18) Dykes, L.; Rooney, L. W. Sorghum and millet phenols and antioxidants. *J. Cereal Sci.* **2006**, *44* (3), 236–251.
(19) Del Pozo-Insfran, D.; Brenes, C. H.; Serna Saldivar, S. O.; Talcott, S. T. Polyphenolic and antioxidant content of white and blue corn (*Zea mays* L.) products. *Food Res. Int.* **2006**, *39* (6), 696–703.
(20) Ferreyra, R. M.; Vina, S. Z.; Mugridge, A.; Chaves, A. R. Growth and ripening season effects on antioxidant capacity of strawberry cultivar Selva. *Sci. Hortic.* **2007**, *112* (1), 27–32.
(21) Moreno, Y. S.; Sánchez, G. S.; Hernández, D. R.; Lobato, N. R. Characterization of anthocyanin extracts from maize kernels. *J. Chromatogr. Sci.* **2005**, *43*, 483–487.
(22) Cevallos-Casals, B. A.; Cisneros-Zevallos, L. Stoichiometric and kinetic studies of phenolic antioxidants from Andean purple corn and red-fleshed sweet potato. *J. Agric. Food Chem.* **2003**, *51*, 3313–3319.
(23) Gradinaru, G.; Biliaderis, C. G.; Kallithraka, S.; Kefalas, P.; Garcia-Viguera, C. Thermal stability of *Hibiscus sabdariffa* L. anthocyanins in solution and in solid state: effects of copigmentation and glass transition. *Food Chem.* **2003**, *83* (3), 423–436.
(24) Boulton, R. The copigmentation of anthocyanins and its role in the color of red wine: a critical review. *Am. J. Enol. Vitic.* **2001**, *52* (2), 67–87.
(25) Cevallos-Casals, B. A.; Cisneros-Zevallos, L. Stability of anthocyanin-based aqueous extracts of Andean purple corn and red-fleshed sweet potato compared to synthetic and natural colorants. *Food Chem.* **2004**, *86* (1), 69–77.
(26) Francis, F. Food colourants: anthocyanins. *Crit. Rev. Food Sci. Nutr.* **1989**, *28*, 273–314.
(27) Plocharski, W.; Zbroszcyzk, J. Aronia fruit (*Aronia melanocarpa*, Elliot) as a natural source for anthocyanin colorants. *Fruit Process.* **1992**, *2*, 85–89.

Received for review May 14, 2008. Revised manuscript received September 25, 2008. Accepted September 26, 2008.

JF8025056