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Thermal Degradation of Anthocyanins from Purple Potato (Cv. Purple Majesty) and Impact on Antioxidant Capacity

Balunkeswar Nayak,[†] Jose De J. Berrios,[§] Joseph R. Powers,[‡] and Juming Tang*[,†]

[†]Department of Biological Systems Engineering, Washington State University, Pullman, Washington 99163, United States

[§]Processed Foods Research Unit, WRRC, ARS, U.S. Department of Agriculture, 800 Buchanan Street, Albany, California 94710, United States

[‡]School of Food Science, Washington State University, Pullman, Washington 99163, United States

ABSTRACT: Degradation parameters of purified anthocyanins from purple-fleshed potato (cv. Purple Majesty) heated at high temperatures (100−150 °C) were determined. Purified anthocyanins, prepared by removing salts, sugars, and colorless nonanthocyanin phenolics from the crude extract, were monitored and quantified using HPLC and spectrophotometry for heat-induced degradation products. Separation of colorless phenolics from the anthocyanins was confirmed using HPLC at two wavelengths, 280 and 520 nm. The degradation kinetics of purified anthocyanins followed a first-order reaction with reaction rate constants (k values) of 0.0262−0.2855 min^{-1}, an activation energy of 72.89 kJ/mol, thermal death times (D values) of 8.06−8789 min, and a z value of 47.84 °C over the temperature range of 100−150 °C. The enthalpy and entropy of activation were 59.97 kJ/mol and −116.46 J/mol·K, respectively. The antioxidant capacity in the purified anthocyanins, measured by DPPH and ABTS assays, was increased after the thermal treatment, indicating antioxidant activities of degradation products in the samples.

KEYWORDS: purple potato, thermal treatment, thermal degradation, kinetics, anthocyanins, antioxidant capacity

INTRODUCTION

Anthocyanins are one of the most important groups of water-soluble plant pigments and are largely responsible for the cyanic colors of flowers, fruits, vegetables, and grains, accumulating in the vacuoles of epidermal or subepidermal cells.[1] Recently, anthocyanins have gained increasing attention as functional compounds for natural color replacing synthetic dyes.[2] In addition, these compounds have health-related beneficial antioxidative[3] and anticarcinogenic properties.[5] Mishra et al.[6] studied the degradation of anthocyanins above 100 °C and hypothesized that anthocyanins could be used as food colorants in high-temperature processes such as for extruded snacks or baked cakes. However, successful use of anthocyanins either as natural colors or nutraceuticals depends on their physical and chemical stability during different processing conditions.

Commercial production of colored potatoes such as the 'Purple Majesty' variety has attracted consumers' attention due to their taste and appearance. An added attribute of colored potatoes is their potential health benefit to consumers, due to their antioxidant capacity,[4] mainly due to the presence of polyphenols including anthocyanins. Stushnoff et al.[8] reported five petunidin glucosides and a single glucoside of malvidin, peonidin, and delphidin aglycones in 'Purple Majesty' potato. Various mechanisms of stability of anthocyanins such as association between pigments and cofactors (polyphenols, metal ions, other anthocyanins) have been proposed in scientific papers.[9,10] Acylation of aromatic acids to the structure and side-chain double bond has also been attributed to the stability of anthocyanins.[11]

Besides their chemical structure, the stability of anthocyanins depends on temperature, light, enzymes, metal ions, sugars, ascorbic acid, oxygen, and the presence of other phenolic compounds.[12] Numerous studies have reported the degradation of anthocyanins in fruits and vegetables during processing and storage.[13,14] The investigators assumed in their papers first-order kinetics for anthocyanin degradation in selected fruits and vegetables.[14−16] Those studies were carried out at temperatures below 100 °C and/or in whole pulp puree/extracts from whole pulp of fruits and vegetables or their powders. Because pulp puree/extracts were not purified, they would contain anthocyanins with other compounds such as salts, sugars, and other colorless nonanthocyanin phenolics that could affect the stability or degradation kinetics and antioxidant capacity of anthocyanins. Little information is available regarding the thermal kinetics of purified anthocyanins and antioxidant potencies of degradation products. For example, Harbourne et al.[17] studied the degradation kinetics of anthocyanins in a model juice (black currant) system using isothermal and nonisothermal methods. Stintzing et al.[3] reported color, visual detection thresholds, hydration constants, and ORAC (Oxygen Radical Absorbance Capacity) antioxidant activities of purified cyanidin-based anthocyanins. The stability and antioxidant activities of several purified anthocyanins are affected by the B-ring structure and glycosylation.[11,13] In a previous study, we demonstrated that 30−40% of anthocyanins' antioxidant capacity was retained in extrudates formulated with 'Purple Majesty' potato and dry pea flours.[18] We hypothesized that at high temperatures, such as those used in extrusion cooking, the degradation products of anthocyanins could be responsible for the overall antioxidant activity determined in the extrudates. In the present study, anthocyanins purified from 'Purple Majesty' potato were used to evaluate the thermal kinetics parameters over the temperature

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range of 100−150 °C and the antioxidant potencies of degradation products from the anthocyanins.

MATERIALS AND METHODS

Chemicals. Folin−Ciocalteu reagents, potassium chloride, sodium acetate, gallic acid, trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), 2,2-diphenyl-1-picrylhydrazyl (DPPH), acetonitrile, 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), α-cyano-4-hydroxycinnamic acid (CHCA), ethyl acetate, and potassium persulfate were purchased from Sigma-Aldrich (St. Louis, MO). Laboratory grade methanol was used in the extraction and preparation of samples.

Materials. Purple potatoes (cv. Purple Majesty) were obtained from Colorado State University. Potatoes were washed with tap water and stored at 4 °C. To prepare flakes, potatoes were peeled using a mechanical abrasive peeler, sliced to 6 mm thickness, and steam blanched for 8 min to inactivate polyphenolic oxidase (PPO), similar to the procedures reported for peroxidase inactivation.[15] The puree was then dehydrated using a 15.24 cm × 20.32 cm pilot-scale counter-rotating twin-drum dryer (Blaw Knox Food and Chemical Equipment Co., Buffalo, NY). Potato flakes were cooled at room temperature and stored at −20 °C for further analysis. The moisture content of the flakes was determined using the standard procedure of AACC moisture−air−oven method 44-45A,[19] and the data were expressed on a dry weight (DW) basis.

Extraction of Anthocyanins. Purple potato flakes were ground using a food processer and passed thorough a sieve US 35 (0.5 mm). Fifty grams of the flakes was homogenized in 500 mL of extraction solvent (1.5 M HCl/methanol/water; 10:70:20 v/v/v) using a high-speed homogenizer. The homogenate was filtered with a double layer of cheesecloth after the mixture had been kept for 90 min at 4 °C. The residue was again homogenized twice in 250 mL of extraction solvent at room temperature and filtered. All of the filtrates were pooled and stored at −20 °C for further analyses.

Purification of Anthocyanins. Extracts from the potatoes were centrifuged (23000*g*, 4 °C, 15 min), and 10 mL of clear supernatant was loaded to a Sep-Pak C18 column (part WAT023635, Waters, Milford, MA) previously activated with HPLC grade methanol followed by 0.01% aqueous HCl. Anthocyanins and polyphenolics were adsorbed onto the column (silica-based bonded phase with strong hydrophobicity), whereas sugars, acids, and other water-soluble compounds were removed by washing the column with 20 mL of 0.01% aqueous HCl. The column was further washed with 20 mL of ethyl acetate to remove colorless nonanthocyanin phenolics. Anthocyanins from the extract were collected by washing the column with 40 mL of 0.01% HCl in methanol. The anthocyanin-rich extract was dried under vacuum at 30 °C and resuspended in 10 mL of deionized water. The pH values of the aqueous anthocyanin extract were in the range of 5.9−6.0. These samples were stored at −20 °C for thermal kinetics study. The purity of anthocyanins was checked with HPLC at a wavelength of 280 nm.

Heat Treatment of Anthocyanins. Specially designed thermal kinetics test (TKT) cells (Figure 1) were used in an oil bath using silicon oil for heat treatment to reduce the come-up time (CUT). Stored sample was thawed to room temperature before 1 mL of the sample was placed into the TKT cells. Duplicate samples were considered from preliminary results that showed little variation in total anthocyanin content data for treatment experiments. The total anthocyanin content in the purified extracts was measured over the temperature range of 100−150 °C. The temperatures of the samples were measured using a precalibrated type T copper−constantan thermocouple with a diameter of 0.1 mm and recorded at 0.5 s intervals with a temperature data logger (TracerDAQ Pro, Measurement Computing, Norton, MA). The CUT, for example, the time to reach 0.5 °C below the set temperature, was 60−135 s. Treatment time zero was considered at the end of the CUT. Details for the experimental design of thermal treatment are given in Table 1. The samples were cooled immediately in ice-water after treatments to reduce the thermal shock. The samples were collected in 2 mL centrifuge tube and stored at −20 °C for further quantification and HPLC and mass spectrometry analyses.

Table 1. Experimental Design for Heat Treatments of Purified Anthocyanins from 'Purple Majesty' Potato[a]

temperature (°C)	heating time (min)				
100	0	5	15	30	60
110	0	5	15	30	60
120	0	5	15	30	60
130	0	5	15	30	45
140	0	5	10	15	30
150	0	3	5	10	20

[a] Purified anthocyanins in specially designed thermal kinetic test (TKT) cells were heat treated in an oil bath. Time = 0 min was considered as the come-up time, that is, the time to reach ±0.5 °C of the samples at the center of the cells (n = 2).

HPLC Analyses. An analytical reverse phase HPLC technique was used for the identification and separation of anthocyanins. Stored samples (control and heated) were thawed at room temperature, and each sample was passed through a Whatman 0.45 μm NYL filter to vials before they were applied to HPLC. The experiments were carried out using a Varian Star HPLC solvent delivery and a control system with automatic sample injector and a variable Varian UV−vis detector. A Varian Microsorb-MV 100-5 C18 250 × 4.6 mm column was fitted with a 10 × 3 mm Varian Chromo guard column maintained at room temperature. Twenty microliters of samples was injected. The two elution solvents used were 10% (v/v) acetic acid (A) and 50% (v/v) aqueous acetonitrile (B). The flow rate of the elution solvents was maintained at 1 mL/min with a linear 30 min gradient from 0 to 30% B followed by a 5 min hold at 30% B. The column was then washed with 50% B for 5 min and then returned to 0% B. The column was re-equilibrated for 10 min before the next analysis. The eluted compounds were monitored at 280 nm for phenolics and at 520 nm for anthocyanins. Standards of gallic, protocatechuic, chlorogenic, caffeic, *p*-coumaric, and ferulic acid with residence times of 3.336, 4.699, 8.083, 9.664, 15.893, and 20.643 min, respectively, were used to determine the presence of these colorless phenolic acids in the anthocyanin extracts (Figure 2).

Matrix-Assisted Laser Desorption Ionization (MALDI) Mass Spectroscopy. MALDI-TOF-MS is a rapid technique to identify a group of anthocyanins with different masses. The mass spectrometry analyses were performed using a 4800 Plus MALDI TOF/TOF analyzer (Applied Biosystems, Framington, MA). The positive ion reflector mode was used. The instrument was equipped with a pulsed nitrogen laser (337 nm, 3 ns pulse duration, 3 Hz frequency). All spectra were obtained by averaging 25 laser shots. The matrix used in this study was α-cyano-4-hydroxycinnamic acid (CHCA). Five milligrams of CHCA was solubilized in 1 mL of an acetonitrile/water mixture (1:1 v/v) for preparing matrix stock solution. The sample to be analyzed was prepared by mixing purified anthocyanin extracts (control or heat treated) with the matrix (1:1 v/v). The samples mixtures were applied to the plate and analyzed.

Measurement of Anthocyanins. Determination of total anthocyanin was based on the pH differential method following the procedures of Giusti and Wrolstad.[20] Absorbance readings were taken at maximum wavelengths (λ_{max}) of 535 and 700 nm to correct for turbidity[21] in a UV−visible spectrophotometer, previously blanked with distilled water. Total anthocyanins were quantified and expressed in milligram equivalent malvidin-3-glucoside[22−24] according to the

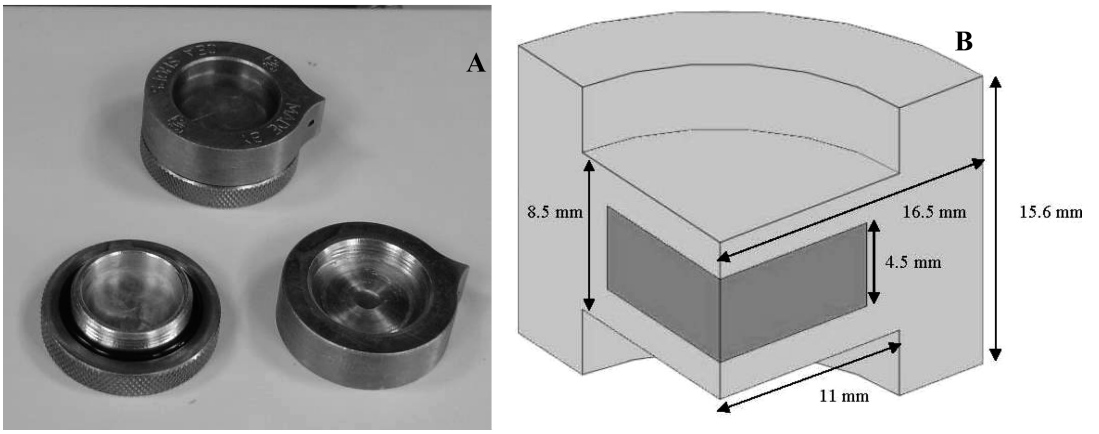
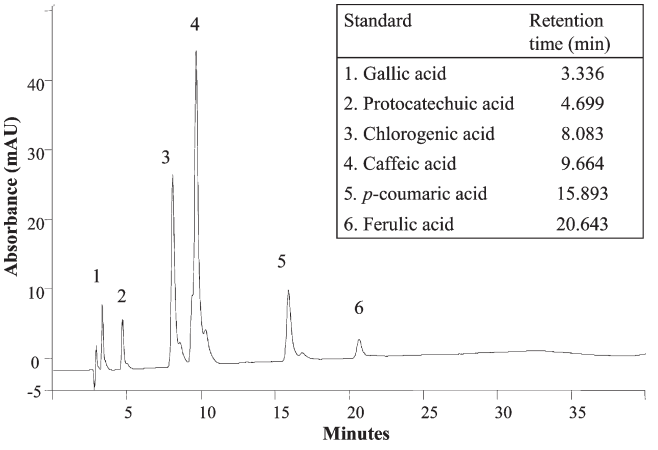


Figure 1. (A) Specially designed thermal kinetics test (TKT) cells used for heat treatment of purified anthocyanins (darker area) from 'Purple Majesty' potato over the temperature range of 100−150 °C for 0−60 min. Purified anthocyanins were heat treated in an oil bath. (B) TKT cells with detail dimensions.



Standard	Retention time (min)
1. Gallic acid	3.336
2. Protocatechuic acid	4.699
3. Chlorogenic acid	8.083
4. Caffeic acid	9.664
5. *p*-coumaric acid	15.893
6. Ferulic acid	20.643

Figure 2. Chromatogram of standards as detected at 280 nm using a reverse phase HPLC. The two elution solvents used were 10% (v/v) acetic acid (A) and 50% (v/v) aqueous acetonitrile (B). The flow rate of the elution solvents was maintained at 1 mL/min with a linear 30 min gradient from 0 to 30% B followed by a 5 min hold at 30% B. The column was then washed with 50% B for 5 min and then returned to 0% B.

formula

$$C\ (mg/L) = \frac{A \times \mathrm{MW} \times \mathrm{DF}}{e \times d} \quad (1)$$

where A = absorbance of the sample, MW = molecular weight of malvidin-3-glucoside (718.5 g/mol), DF = dilution factor, d = path length of the cuvette (1 cm), and e = molar extinction coefficient of malvidin-3-glucoside (30200 L/cm·mol). The absorbances of the samples were calculated as $A = (A_{\lambda\max} - A_{700})_{\mathrm{pH1.0}} - (A_{\lambda\max} - A_{700})_{\mathrm{pH4.5}}$.

Measurement of Antioxidant Capacity. The antioxidant capacities of the samples were measured using DPPH and ABTS assays.

DPPH Assay. DPPH, a stable radical deep purple in color, is reduced in the presence of antioxidants. The loss of color results in a decrease in the absorbance intensity, thus providing a basis for measurement of the antioxidant capacities in the extracts. The assay was based on the procedures of Brandwilliams et al.[25] Control or heat-treated sample (0.05 mL) was added to 1.95 mL of 6×10^{-5} M DPPH solution in a cuvette, and the absorbance reading at 515 nm was taken after 2 h of equilibration time using an Ultraspec 4000 UV−visible spectrophotometer (Pharmacia Biotech, Cambridge, U.K.).

ABTS Assay. The total antioxidant capacity of the samples was also quantified using the ABTS radical cation decolorization assay.[26] Working solution for the assay was prepared by mixing stock solutions of 7 mM ABTS and 2.4 mM potassium persulfate in equal volumes and allowing them to react for 12 h at room temperature in the dark. One milliliter of $ABTS^{\bullet+}$ solution was diluted with 60 mL of methanol to obtain an absorbance of 0.70 ± 0.01 unit at 734 nm. Fresh $ABTS^{\bullet+}$ solution was prepared for each assay. One milliliter of sample was added to 1 mL of diluted $ABTS^{\bullet+}$ solution and allowed to equilibrate for 7 min before the absorbance was measured at 734 nm.

In both antioxidant assays, the spectrophotometer was blanked with methanol. Percentage inhibition of DPPH and ABTS was calculated as

$$\text{inhibition}\ (\%) = \left(1 - \frac{\mathrm{Abs}_{\mathrm{sample}}}{\mathrm{Abs}_{\mathrm{control}}}\right) \times 100 \quad (2)$$

where $\mathrm{Abs}_{\mathrm{control}}$ is the absorbance of DPPH or ABTS radical with methanol and $\mathrm{Abs}_{\mathrm{sample}}$ is the absorbance of DPPH or ABTS radical with sample extract or standard. The total antioxidant capacity was quantified from a trolox standard curve and expressed as trolox equivalent per gram of dry weight sample (μg TE/g DW) ± SD for duplicate samples.

Determination of Thermal Kinetics Parameters. The order of the reaction in thermal degradation of anthocyanins was predicted using the model

$$\frac{dC}{dt} = -k(C)^n \quad (3)$$

where k is the rate constant, n is the reaction order, C is the concentration of total anthocyanins, and t is the reaction time.

The order of reaction was determined by graphical analysis, where exponent n in eq 3 was set to zero, half, one, and two to compare the coefficients of determination among zero-, half-, first-, and second-order reactions, respectively. The integrated forms of zero-, half-, first-, and second-order kinetic models are given in eqs 4−7.

$$\text{zero-order}: \quad C_t = C_0 - kt \quad (4)$$

$$\text{half-order}: \quad 2\sqrt{C_t} - \sqrt{C_0} = kt \quad (5)$$

$$\text{first-order}: \quad \ln\frac{C_t}{C_0} = -kt \quad (6)$$

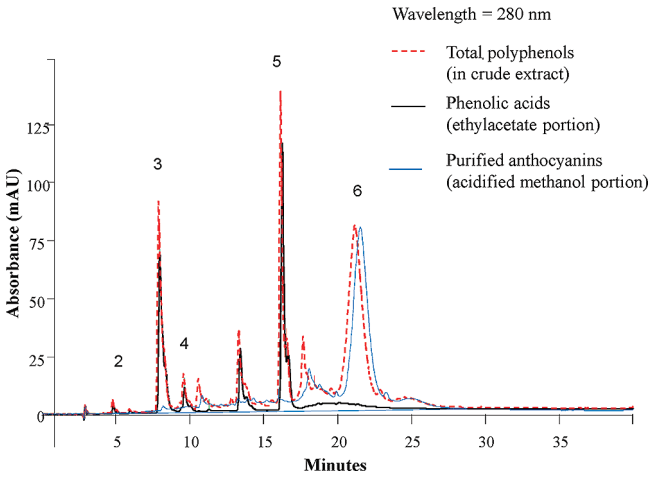


Figure 3. HPLC-DAD profile of total polyphenols (in crude extract), phenolic acids (in ethyl acetate portion), and purified anthocyanins (in HCl/methanol portion) from 'Purple Majesty' potato purified using a Sep-Pak C18 column as detected at 280 nm using a reverse phase HPLC. Peaks: 2, protocatechuic acid; 3, chlorogenic acid; 4, caffeic acid; 5, *p*-coumaric acid; 6, ferulic acid.

$$\text{second-order}: \quad \frac{1}{C_t} - \frac{1}{C_0} = kt \tag{7}$$

Using the experimental anthocyanins data, the coefficient of determination was observed to be minimum for $n = 1$, predicting a first-order reaction. According to the activated complex theory for chemical reaction rates, for first order, the Arrhenius equation relates the reaction rate constants to the absolute temperature[27]

$$\ln k = \ln A - \frac{E_a}{RT} \tag{8}$$

where E_a is the activation energy (kJ/mol), A is a pre-exponential factor/frequency factor (1/s), T is the absolute temperature (K), and R is the gas constant (8.31 J/mol·K). The reaction rate constant k and the activation energy, E_a were determined graphically from a plot of $\ln(C/C_0)$ versus time and $\ln k$ versus $1/T$, respectively.

The thermal death time method ($D-z$ model) was used to estimate the decimal reduction time (D value), that is, the heating time required to reduce the anthocyanin concentration by 90%, and the z value, that is, the temperature change necessary to alter the thermal death time by one log cycle[27] with the relationships

$$D = \frac{\ln 10}{k} \tag{9}$$

$$\log(D/D_{ref}) = -(T - T_{ref})/z \tag{10}$$

where D_{ref} is the D value at temperature T_{ref}. The half-lives ($t_{1/2}$) of the anthocyanins were calculated as

$$t_{1/2} = \frac{\ln 2}{k} \tag{11}$$

The enthalpy of activation (ΔH) and entropy of activation (ΔS) were estimated using the Eyring–Polanyi model based on the transition state theory

$$k = \frac{k_b}{h} T \times e^{-\Delta H - T\Delta S/RT} \tag{12}$$

where T is the absolute temperature (K), k_b is the Boltzmann constant (1.381×10^{-23} J/K), h is the Planck constant (6.626×10^{-34} Js), and R is the gas constant (8.31 J/mol.K).

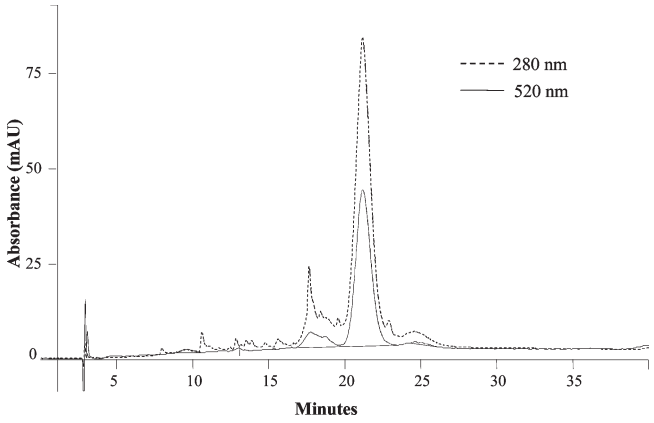


Figure 4. HPLC-DAD profile of control unheated purified anthocyanins from 'Purple Majesty' potato purified using a Sep-Pak C18 column. The peaks were detected at 280 and 520 nm using a reverse phase HPLC.

RESULTS

Purification of Anthocyanins. Aqueous anthocyanin extracts were analyzed for phenolic acids using HPLC. The analysis showed the presence of chlorogenic, caffeic, *p*-coumaric, ferulic, and protocatechuic acids. Other unknown phenolic compounds were also detected at 280 nm (Figure 3). After the separation of salts, sugars, and colorless phenolics from the aqueous anthocyanin extracts, purified anthocyanins as detected at 280 nm did not show any peak coincident with phenolic acid standards other than ferulic acid. When the purified anthocyanins were detected at 520 nm (Figure 4), it was observed that the peak for the major anthocyanin eluted at the same time as the ferulic acid peak. The absence of individual ferulic acid mass (m/z 176.2) in MALDI data (Figure 5A) showed no contamination of phenolic acids in the purification of anthocyanins. Comparison of the spectra of the purified anthocyanins from 'Purple Majesty' potato with that of the pure anthocyanin[28] revealed acylation of a phenolic acid to the major anthocyanin. The MALDI data (Figure 5A) showed the presence of petunidin glucosides (m/z 479.1046), petunidin glucosides acylated with ferulic acid (m/z 963.1570), and also petunidin glucosides acylated with coumaric acid (m/z 933.2020) in the purified anthocyanins.[8,29−31] The latter mass (m/z 933.2020) agreed with the observations of Stushnoff et al.[8] on the presence of petunidin-3-rutinoside-5-glucoside acylated with coumaric acid as the major anthocyanin in 'Purple Majesty' potato. The investigators also reported having petunidin-3-rutinoside-5-glucoside acylated with ferulic acid in the 'Purple Majesty' potato along with delphidin, malvidin, and peonidin aglycones with single glucosides. Although one or two smaller and broader peaks were also observed in the HPLC chromatogram (Figure 4), no other individual anthocyanin was characterized.

Degradation of Anthocyanins. The total anthocyanin contents (TA) in the purified anthocyanins determined before and after heat treatments are summarized in Table 2. The TA of the control unheated extract was 0.487 ± 0.03 mg malvidin-3-glucoside/g of DW sample. Extract heated at 100 °C showed degradation of anthocyanins as observed by a reduction in the peaks detected at 520 nm (Figure 6A). A similar trend was observed when using 280 nm for detection, with a major peak with an elution time of 20.643 min (Figure 6B).

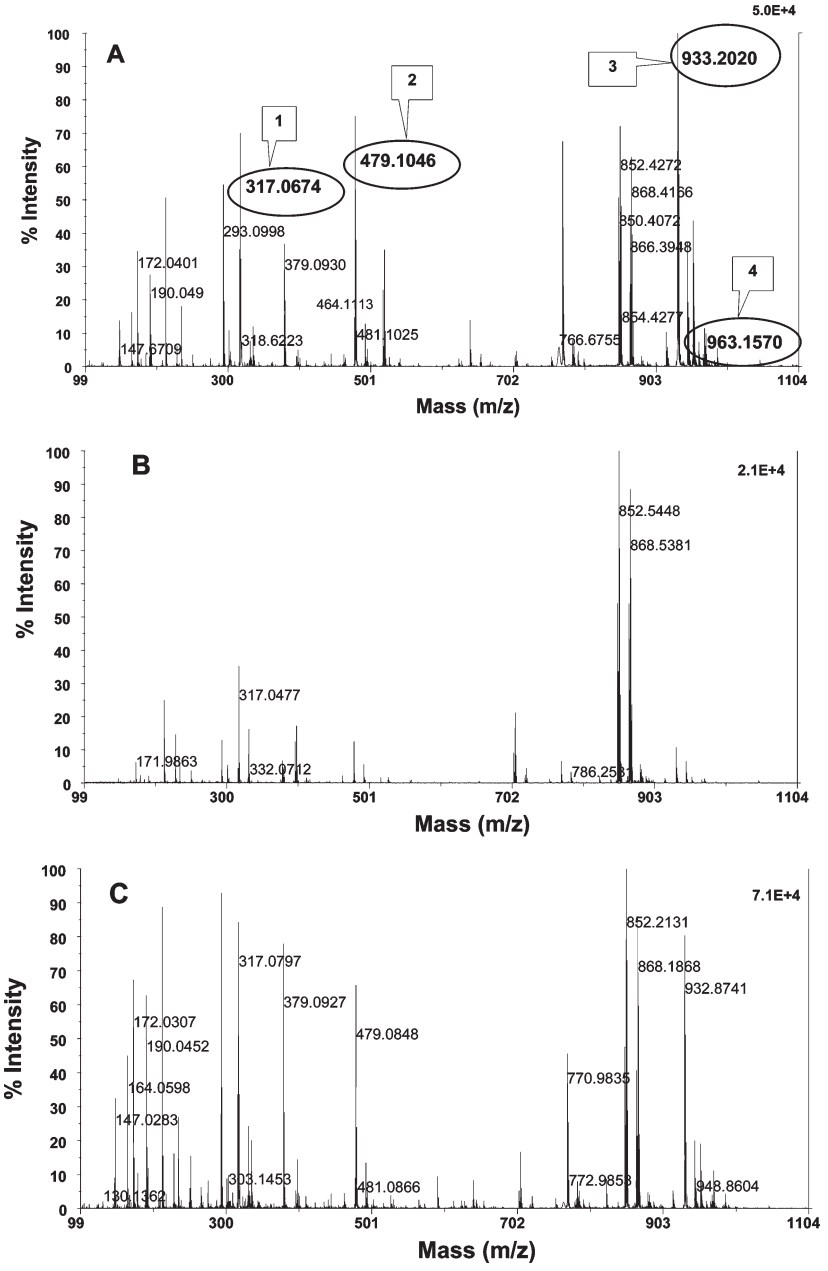


Figure 5. MALDI mass spectra of the pigments from purified anthocyanins of 'Purple Majesty' potato. (A) Spectra of control purified anthocyanins samples. Peaks: 1, petunidin; 2, petunidin monoglucoside; 3, petunidin-3-rutinoside-5-glucoside acylated with coumaric acid; 4, petunidin-3-rutinoside-5-glucoside acylated with ferulic acid. (B) Spectra of heat-treated samples at 100 °C for 30 min. (C) Spectra of heat-treated samples at 100 °C for 60 min. Note: Scales of spectra A, B, and C are different.

The order of the thermal degradation was estimated by examining the coefficient of determination (r^2) from the plots of TA versus treatment time over the temperature range of 100−150 °C (Table 3). On the basis of the mean r^2, the thermal degradation of anthocyanins tended to follow first-order kinetics (r^2 = 0.98). The temperature-dependent rate constants, k values, from first order over 100−150 °C as calculated from a plot of $\ln(C/C_0)$ versus treatment time (Figure 7) were 0.0262−0.2855 min^{-1}. The reaction rate increased almost 10 times and the D values decreased 11 times (8.06−87.89 min) as the heating temperatures increased from 100 to 150 °C (Table 4). The activation energy, E_a, and z value of the degradation reaction, calculated from the Arrhenius plot (Figure 8), were 72.89 kJ/mol and 47.9 °C, respectively. Additionally, the activation enthalpy (ΔH) and entropy (ΔS) estimated from transition state theory were 59.97 kJ/mol and −116.46 J/mol·K, respectively (Table 4).

Antioxidant Capacity of the Degradation Products. DPPH and ABTS antioxidant assays were performed to evaluate the total antioxidant capacity (TAC) of the purified anthocyanins, before and after heating. The TACs of the unheated sample using DPPH and ABTS assays were 1237 ± 14 and 1546 ± 5 μg TE/g DW sample, respectively. For each heating temperature, the TAC determined on the heated samples was either increased or remained unchanged ($P > 0.05$) compared to the control unheated samples. However, ABTS antioxidant assay of degradation products for samples treated at 110 and 140 °C showed some decrease in

Table 2. Quantity of Total Anthocyanins from 'Purple Majesty' Potato Extract upon Heating at 100−150 °C[a]

	total anthocyanins (mg mal-3-glu/g dry weight sample)					
heating time (min)	100 °C	110 °C	120 °C	130 °C	140 °C	150 °C
control	0.487 ± 0.030a	0.487 ± 0.030a	0.487 ± 0.030a	0.487 ± 0.030a	0.487 ± 0.030a	0.487 ± 0.030a
0	0.467 ± 0.009a	0.420 ± 0.005b	0.436 ± 0.002b	0.402 ± 0.018b	0.368 ± 0.017b	0.364 ± 0.007b
3						0.098 ± 0.012c
5	0.410 ± 0.013b	0.344 ± 0.017c	0.285 ± 0.020c	0.202 ± 0.004c	0.084 ± 0.008c	0.044 ± 0.005 cd
10					0.030 ± 0.005 cd	0.011 ± 0.008e
15	0.339 ± 0.007c	0.216 ± 0.009d	0.138 ± 0.017d	0.059 ± 0.005d	0.007 ± 0.002e	
20						0.001 ± 0.000f
30	0.186 ± 0.005d	0.082 ± 0.014e	0.042 ± 0.006e	0.010 ± 0.005e	0.001 ± 0.001f	
45				0.003 ± 0.004f		
60	0.101 ± 0.003e	0.034 ± 0.007f	0.006 ± 0.003f			

[a] Purified anthocyanins in specially designed thermal kinetic test (TKT) cells were heat treated in an oil bath. Time = 0 min was considered at the come-up time, that is, the time to reach ±0.5 °C of the samples at center of the cells (mean ± SD, $n = 2$). Significant differences within the values in the same column are indicated by different letters ($p < 0.05$, Tukey's pairwise comparison test).

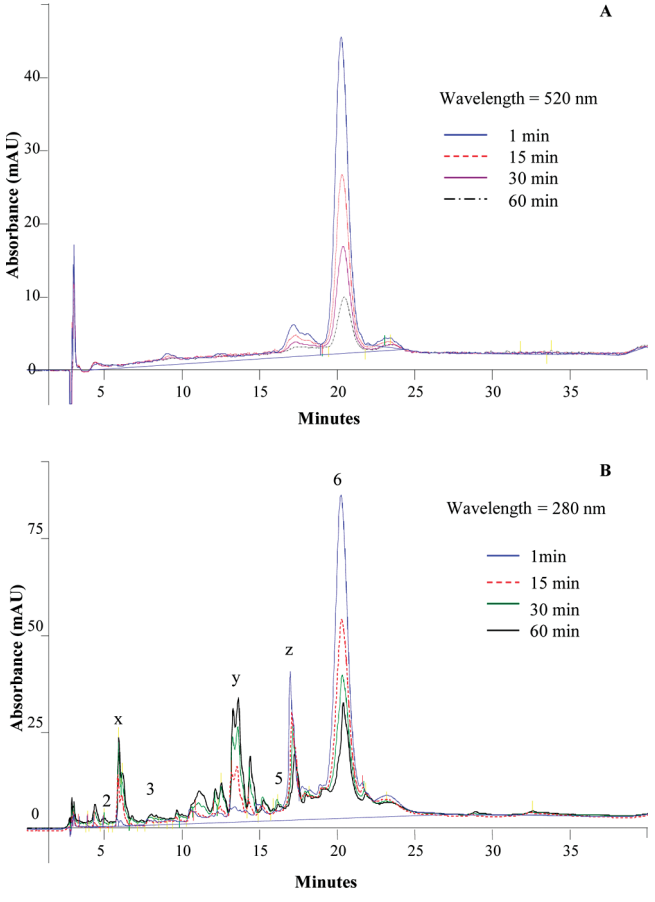


Figure 6. Chromatogram of purified anthocyanins from 'Purple Majesty' potato heated at 100 °C for 1−60 min using a reverse phase HPLC: (A) peaks detected at 520 nm; (B) peaks detected at 280 nm. Peaks 2, 3, 5, and 6 are protocatechuic, caffeic, *p*-coumaric, and ferulic acids, respectively. x, y, and z are unknown peaks.

their TAC values. The TAC of the degradation products from the anthocyanins ranged from 1243 ± 97 to 1860 ± 21 μg TE/g DW sample using the DPPH assay (Table 5) and from 1302 ± 82 to 1715 ± 21 μg TE/g DW sample using the ABTS assay (Table 6).

Table 3. Estimation of the Order of Anthocyanin Degradation by Examining Coefficients of Determination (r^2) from Plots of Zero-, Half-, First-, and Second-Order Reactions[a]

temperature (°C)	zero-order	half-order	first-order	second-order
100	0.9316	0.9644	0.9849	0.9792
110	0.8451	0.9207	0.9759	0.8428
120	0.7404	0.8958	0.9978	0.8632
130	0.7041	0.8670	0.9918	0.8428
140	0.4993	0.7040	0.9655	0.8511
150	0.4886	0.7194	0.9828	0.8428
mean	0.7015	0.8452	0.9831	0.8703

[a] Purified anthocyanins from 'Purple Majesty' potato in specially designed thermal kinetic test (TKT) cells were heat treated for 0−60 min in an oil bath ($n = 2$).

Thermally induced changes for the production of compounds from anthocyanin degradation did not follow a particular trend. The ratios of TAC and TA in the control unheated purified anthocyanins were 2.54 and 3.17 using DPPH and ABTS assays, respectively, and increased to maxima of 1316 (DPPH assay) and 1581 (ABTS assay) after heating at 150 °C (Table 7).

DISCUSSION

Under the HPLC conditions applied to detect phenolic standards, it was observed that every standard has shoulders at 280 nm (Figure 2). This may be due to the HPLC condition applied in this study or the injection volume of sample (20 μL). Reduction in the injection volume or changing the flow rate of elution solvents might improve the separation and form a sharp peak of the standard. Protocatechuic, chlorogenic, caffeic, and *p*-coumaric acids (peaks 2, 3, 4, and 5 in Figure 3) in the crude anthocyanin extract were not observed in the purified anthocyanins when detected at 280 nm. During purification of anthocyanins, washing of the Sep-Pak C18 column by ethyl acetate removed the colorless nonanthocyanin phenolics (peaks 2, 3, 4, and 5 in Figure 3). Prior to the removal of colorless phenolics, salts and sugars were also removed from the crude extract by acidified water. The HPLC chromatogram of the purified anthocyanins showed one large peak eluting at the same time as ferulic acid, and smaller peaks were

detected for other anthocyanins at 520 nm. However, the final anthocyanin-rich extract may still contain some substances other than salts, sugars, and colorless phenolics that could not be detected at 280 or 520 nm. Purified anthocyanins from elderberry, strawberry, and black carrot have shown similar chromatograms when detected at 280 and 520 nm.[11,13] From the HPLC and MALDI information, the presence of petunidin glucosides acylated with a phenolic acid in the purified anthocyanins was indicated. Stushnoff et al.[8] reported the presence of petunidin-3-rutinoside-5-glucoside acylated with coumaric and ferulic acid in addition to other types of anthocyanins in 'Purple Majesty' potato.

Degradation of anthocyanins in the extract is associated with reduction in its color. Similar observations of reduction in color with the decrease in the amount of anthocyanins were previously reported for purple potatoes dried by drum drying with a drum temperature of 135−138 °C.[32] In this study, anthocyanins in the extracts were reduced to negligible level after 15 min of heating at 140 °C and after 10 min of heating at 150 °C, whereas Yue and Xu[16] observed undetectable levels of anthocyanins in bilberry extracts after 10 min of heating at 150 °C. Reduction to negligible content of anthocyanins in the heated samples could be due to chalcone formation from the anthocyanins in the process of thermal exposure or loss of glycosyl moieties.[33] The breakdown of the anthocyanin compounds to other colorless small molecular compounds caused the sample to lose its original color.[15] Over the range of 100−150 °C, the degradation kinetics followed a first-order reaction, which is in agreement with previous results.[15,17,34−36] The reaction rate constants and half-lives over the range of 100−150 °C confirmed the influence of temperature on the anthocyanins and agreed with a previous paper by Yue et al.[16] on dry heating of bilberry extract. However, the k value for the purified anthocyanins in the present study was 10-fold higher than the reported values for crude anthocyanins in a black currant juice model at 100 °C[17] and purple-fleshed potato at 98 °C.[15] This shows that purified anthocyanins degrade at a faster rate compared to anthocyanins in the crude or unpurified extracts directly from the food material, which could be due to inter- and intramolecular copigmentation reactions in the color pigments and cofactors such as colorless nonanthocyanin phenolic compounds in the extracts.[9,10] The values of half-lives over the temperature of 100−150 °C at pH 5.95 ± 0.05 were 2.4−26.5 min (Table 4) and are comparable with those of bilberry extracts during dry heating at 80−125 °C (i.e., 8−25 min).[16] However, they were significantly lower than reported half-life values of 2.18 h at 100 °C, in a model black currant juice,[17] and 1.6, 5.6, 2, and 4.9 h at 98 °C for purple-flesh potato, red-flesh potato, grape, and purple carrot, respectively.[15] Sadilova et al.[11,13] reported that the half-lives of purified anthocyanins at 95 °C were 1.95, 1.96, and 2.81 h at pH 3.5 and 3.2, 1.9, and 4.1 h at pH 1.0 for strawberry, elderberry, and black carrot, respectively. This indicates that purified anthocyanins from 'Purple Majesty' potato are less heat stable than those of strawberry, elderberry, and black carrot. One

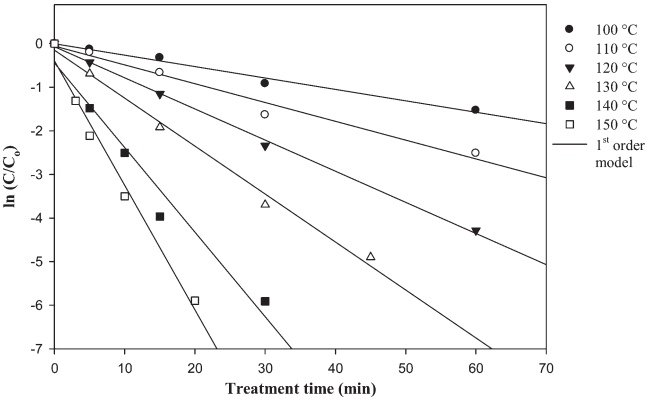


Figure 7. First-order plot for the degradation of purified anthocyanins from 'Purple Majesty' potato during heating over the temperature range of 100−150 °C for 0−60 min. Purified anthocyanins were heat treated in an oil bath. Data are the mean of duplicate samples.

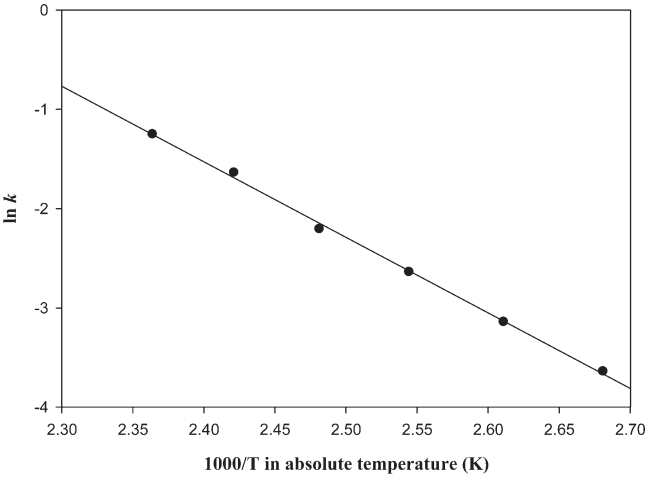


Figure 8. Plot of ln(k) versus ($1/T$) for degradation of purified anthocyanins from 'Purple Majesty' potato during heating over the temperature range of 100−150 °C for 0−60 min.

Table 4. Parameters for First-Order Kinetics and Transition State Equations for Degradation of Anthocyanins from 'Purple Majesty' Potato after Heat Treatment over the Temperature Range of 100−150 °C for 0−60 min[a]

temperature (°C)	k (min^{-1}) × 10^3	r^2	$t_{1/2}$ (min)	D value (min)	z value (°C)	E_a (kJ/mol)	ΔH (kJ/mol)	ΔS (J/mol·K)
100	26.2	0.9849	26.456	87.885	47.9	72.89	59.97	− 116.46
110	43.2	0.9759	16.045	53.301				
120	71.4	0.9978	9.708	32.249				
130	110	0.9918	6.301	20.933				
140	194.1	0.9655	3.571	11.863				
150	285.5	0.9828	2.428	8.065				

[a] Purified anthocyanins in specially designed thermal kinetic test (TKT) cells were heat treated in an oil bath (n = 2). k, reaction rate constant; r^2, coefficient of determination; $t_{1/2}$, half-time; D value, thermal reduction time, that is, heating time required to reduce anthocyanin concentration by 90%; z value, temperature change necessary to alter the thermal death time by one log cycle; E_a, energy of activation; ΔH, enthalpy of activation; ΔS, entropy of activation.

Table 5. Total Antioxidant Capacity of Purified Anthocyanins from 'Purple Majesty' Potato upon Heating at 100−150 °C Measured by DPPH Radical Scavenging Assay[a]

	total antioxidant capacity (μg trolox equiv/g dry weight sample)					
heating time (min)	100 °C	110 °C	120 °C	130 °C	140 °C	150 °C
control	1237 ± 14e	1237 ± 14b	1237 ± 14b	1237 ± 14c	1237 ± 14c	1237 ± 14b
0	1352 ± 21d	1404 ± 83a	1336 ± 7a	1639 ± 56a	1500 ± 34a	1326 ± 34a
3						1364 ± 89a
5	1511 ± 21b	1355 ± 41b	1341 ± 28a	1525 ± 7b	1500 ± 7a	1355 ± 21a
10					1476 ± 41a	1277 ± 117a
15	1860 ± 21a	1574 ± 117a	1356 ± 49a	1719 ± 28a	1340 ± 27b	
20						1316 ± 21a
30	1431 ± 7c	1277 ± 124b	1252 ± 14b	1525 ± 63b	1359 ± 14b	
45				1510 ± 98b		
60	1431 ± 21c	1389 ± 255a	1243 ± 97a			

[a] Purified anthocyanins in specially designed thermal kinetic test (TKT) cells were heat treated in an oil bath. Time = 0 min was considered as the come-up time, that is, time to reach ±0.5 °C of the samples at center of the cells (mean ± SD, $n = 2$). Significant differences within the values in the same column are indicated by different letters ($p < 0.05$, Tukey's pairwise comparison test).

Table 6. Total Antioxidant Capacity of Purified Anthocyanins from 'Purple Majesty' Potato over the Heating Range of 100−150 °C Measured by ABTS Radical Scavenging Assay[a]

	total antioxidant capacity (μg trolox equiv/g dry weight sample)					
heating time (min)	100 °C	110 °C	120 °C	130 °C	140 °C	150 °C
control	1546 ± 5c	1546 ± 5a	1546 ± 5a	1546 ± 5b	1546 ± 5a	1546 ± 5c
0	1653 ± 22b	1491 ± 32a	1538 ± 5ab	1663 ± 22a	1539 ± 32a	1715 ± 21a
3						1622 ± 55b
5	1710 ± 19a	1433 ± 34b	1501 ± 21b	1573 ± 39a	1421 ± 14b	1619 ± 64b
10					1400 ± 20b	1617 ± 12b
15	1687 ± 38a	1433 ± 3b	1488 ± 8b	1576 ± 28a	1386 ± 6c	
20						1581 ± 40b
30	1649 ± 22b	1370 ± 119b	1482 ± 11b	1537 ± 45ab	1365 ± 23c	
45				1460 ± 64c		
60	1559 ± 41c	1302 ± 82b	1469 ± 13c			

[a] Purified anthocyanins in specially designed thermal kinetic test (TKT) cells were heat treated in an oil bath. Time = 0 min was considered as the come-up time, that is, the time to reach ±0.5 °C of the samples at center of the cells (mean ± SD, $n = 2$; come-up time = 0 min). Significant differences within the values in the same column are indicated by different letters ($p < 0.05$, Tukey's pairwise comparison test).

possible explanation is the difference in the chemical structure of anthocyanin,[37] intramolecular stacking of acylated anthocyanins,[38] types of acylation of anthocyanins,[11] and types of sugar moieties.[39] For example, cyanidin-3-galactoside-xyloside-glucoside-sinapic acid, cyanidin-3-galactoside-xyloside-glucoside-ferulic acid, and cyanidin-3-galactoside-xyloside-glucoside-coumaric acid in black carrot have half-lives of 2.94, 3.43, and 3.1 h, respectively, at pH 3.5 and 2.57, 2.39, and 2.16 h, respectively, at pH 1.0 when heated at 95 °C.[11,13] The same investigators also reported that different anthocyanins such as pelargonidin-3-glucoside in strawberry and cyanindin-3-glucoside in elderberry have half-lives of 2.12 and 1.82 at pH 3.5 and 2.12 and 1.95 at pH 1.0 when heated at 95 °C. In 'Purple Majesty' potato, anthocyanins such as petunidin/malvidin/delphidin/peonidin-3-rutinoside-5-glucoside are acylated with coumaric or ferulic acids,[8] which might have provided the anthocyanins with some heat stability. Matsufuji et al.[40] reported that anthocyanins in red radish extract, acylated with *p*-coumaric acid or ferulic acid, had more stability than anthocyanins acylated with caffeic acids. The *z* value of 'Purple Majesty' potato anthocyanin extracts was higher than the reported values for all blue and CO94165-3P/P potato varieties,[15] but comparable to that of roselle anthocyanin extract.[41]

The activation energy (Table 4) for the degradation of purified anthocyanins was 72.89 kJ/mol, which is in agreement with reported values of 72.49 kJ/mol for purple-flesh potato over the temperature range of 25−98 °C.[15] It is also in agreement with the activation energy of blood orange juice and concentrate, 73.2−89.5 kJ/mol over the temperature range of 5−90 °C;[36] sour cherry concentrate, 73.06 kJ/mol over the temperature range of −18 to 80 °C;[35] and blueberry extract, 60−80 kJ/mol over the temperature range of 80−150 °C.[16] The enthalpy of activation in the degradation of purified anthocyanins from 'Purple Majesty' potato was 59.97 kJ/mol. This value was higher than those reported for blackberry of 34 kJ/mol and roselle extracts (except Thai variety) of 44−48 kJ/mol, but less than that reported for blood orange of 63 kJ/mol, over the temperature range of 30−100 °C.[41] The former data indicate that the degradation rate of purified anthocyanins from 'Purple Majesty'

Table 7. Progression of Degradation Products from Purified Anthocyanin of 'Purple Majesty' Potato Heated over the Range of 100−150 °C and Measured by the Ratio of Total Antioxidant Capacity (TAC) and Total Anthocyanins (TA)[a]

	ratio of total antioxidant capacity versus total anthocyanins											
	100 °C		110 °C		120 °C		130 °C		140 °C		150 °C	
heating time (min)	DPPH	ABTS	DPPH	ABTS	DPPH	ABTS	DPPH	ABTS	DPPH	ABTS	DPPH	ABTS
control	2.54	3.17	2.54	3.17	2.54	3.17	2.54	3.17	2.54	3.17	2.54	3.17
0	2.89	3.54	3.34	3.55	3.06	3.53	4.08	4.14	4.08	4.18	3.64	4.71
3											13.92	16.55
5	3.69	4.17	3.94	4.17	4.71	5.27	7.55	7.79	17.86	16.92	30.79	36.80
10									49.19	46.67	116.10	147
15	5.49	4.98	7.29	6.63	9.82	10.78	29.13	26.71	191.44	198.57		
20											1315.84	1581
30	7.68	8.85	15.57	16.71	29.82	35.29	152.51	153.70	1359.46	1365		
45							503.40	486.67				
60	14.14	15.40	40.85	38.29	207.09	244.83						

[a] DPPH values, TAC measured by DPPH assay; ABTS values, TAC measure by ABTS assay.

potato was less affected by temperature, even over the higher temperature range of 100−150 °C, than those from blood orange juice. Enthalpy of activation measures the energy barrier, which must be overcome by reacting molecules, and is related to the strength of bonds that are broken and made in the formation of the transition state from the reactants. The more negative entropy of activation indicates a smaller number of species in the transition state.[42] The estimated entropy of activation in the purified anthocyanins from 'Purple Majesty' potato (−116.46 J/mol·K) was less negative than those of blood orange juice (−149 J/mol·K), blackberry juice (−233 J/mol·K), and roselle extracts (−165 to −205 J/mol·K).[41]

After thermal treatment of the purified extracts from 'Purple Majesty' potato, degradation of native anthocyanins occurred. The MALDI data show spectra of pigments in the samples heat treated at 100 °C for 30 and 60 min and the formation of new compounds at different heating times (Figure 5B,C). Some possible explanations related to the observed new compounds include chalcone formation, deglycosylation, and formation of products such as coumarin derivatives,[43] benzoic derivatives,[44] and trihydroxybenzaldehyde.[11] The formation of five different types of anthocyanidins from the heated bilberry extract after cleavage of conjugated sugars from anthocyanins has been reported.[16] A significant increase in the TAC from degradation products of the heated purified anthocyanin preparation, compared to unheated purified anthocyanins, is emphasized by the progressive increasing ratio of TAC to TA in the samples over time (Table 7). It is also emphasized by the formations of new compounds, as observed by increasing peak areas detected at 280 nm in chromatogram, from the degradation of anthocyanins when heated at 100 °C for 0−60 min (Figure 6). The ratio of TAC to TA using the DPPH assay varied from 2.54 to 1359.46, whereas using ABTS assay the ratio was 3.17−1581 over the temperature range of 100−150 °C at 0−60 min. This showed that the anthocyanin degradation products exhibited higher antioxidant activity than the unheated anthocyanin samples, which agrees with previous results.[11,16,40] It is possible that the application of heat may have induced the cleavage of the acylated anthocyanins into their corresponding acyl-glucosides, then into intermediate chalcones, and finally into colorless phenolics (such as phenolic acids and aldehydes) that contributed to an increase the TAC in the samples. Sadilova et al.[11] reported ratios of TAC (measured by ABTS) to TA of 3.41, 10.08, and 8.85 in the anthocyanin degradation products after heating for 4 h at 95 °C in strawberry, black carrot, and elderberry anthocyanin isolates, respectively. The researchers added that these results were due to the formation of compounds such as protocatechuic acid, phloroglucinaldehyde, and 4-hydroxybenzoic acid. Sreeram et al.[44] reported that the antioxidant activity (measured by inhibition of lipid peroxidation) of degradation products of cyanidin glycosides, that is, protocatechuic acid at a concentration of 50 μM, from tart cherries was comparable to those of commercial antioxidants such as butylated hydroxytoulene (BHT), butylated hydroxyanisole (BHA), *tert*-butylhydroquinone (TBHQ), and vitamin C when tested at 10 μM concentration.

The present study evaluated the thermal degradation of purified anthocyanins from 'Purple Majesty' potato after removal of salts, sugars, and colorless nonanthocyanin phenolics. The thermal degradation of purified anthocyanins followed first-order kinetics in an Arrhenius type relationship. The total antioxidant capacity of purified anthocyanins after heat treatment was greater than the TAC of the unheated samples, due to supplementary antioxidant activity resulting from the degradation products. Characterization of the degradation products from heat treatments based on their structure and functionality might be needed to understand the mechanism responsible for the measured antioxidant activity.

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■ REFERENCES

(1) Wagner, G. J. Cellular and subcellular localization in plant metabolism. In *Recent Advances in Phytochemistry*, Creasy, L. L., Hrazdina, G., Eds.; Plenum Press: New York, 1982; Vol. 16, pp 1−45.

(2) Downham, A.; Collins, P. Colouring our foods in the last and next millennium. *Int. J. Food Sci. Technol.* **2000**, *35* (1), 5–22.

(3) Stintzing, F. C.; Stintzing, A. S.; Carle, R.; Frei, B.; Wrolstad, R. E. Color and antioxidant properties of cyanidin-based anthocyanin pigments. *J. Agric. Food Chem.* **2002**, *50* (21), 6172–6181.

(4) Nayak, B.; Liu, R. H.; Berrios, J. J.; Tang, J.; Derito, C., Bioactivity of antioxidants in extruded products prepared from purple potato and dry pea flours. *J. Agric. Food Chem.* **2011**, *59* (15), 8233−8243.

(5) Han, K. H.; Hashimoto, N.; Shimada, K.; Sekikawa, M.; Noda, T.; Yamauchi, H.; Hashimoto, M.; Chiji, H.; Topping, D. L.; Fukushima, M. Hepatoprotective effects of purple potato extract against D-galactosamine-induced liver injury in rats. *Biosci., Biotechnol., Biochem.* **2006**, *70* (6), 1432–1437.

(6) Mishra, D. K.; Dolan, K. D.; Yang, L. Confidence intervals for modeling anthocyanin retention in grape pomace during nonisothermal heating. *J. Food Sci.* **2008**, *73* (1), E9–E15.

(7) Lachman, J.; Hamouz, K.; Orsak, M. Red and purple potatoes − a significant antioxidant source in human nutrition. *Chem. Listy* **2005**, *99* (7), 474–482.

(8) Stushnoff, C.; Holm, D.; Thompson, M. D.; Jiang, W.; Thompson, H. J.; Joyce, N. I.; Wilson, P. Antioxidant properties of cultivars and selections from the Colorado potato breeding program. *Am. J. Potato Res.* **2008**, *85* (4), 267–276.

(9) Malien-Aubert, C.; Dangles, O.; Amiot, M. J. Color stability of commercial anthocyanin-based extracts in relation to the phenolic composition. Protective effects by intra and intermolecular copigmentation. *J. Agric. Food Chem.* **2001**, *49* (1), 170–176.

(10) Del Pozo-Insfran, D.; Brenes, C. H.; Talcottt, S. T. Phytochemical composition and pigment stability of acai (*Euterpe oleracea* Mart.). *J. Agric. Food Chem.* **2004**, *52* (6), 1539–1545.

(11) Sadilova, E.; Carle, R.; Stintzing, F. C. Thermal degradation of anthocyanins and its impact on color and in vitro antioxidant capacity. *Mol. Nutr. Food Res.* **2007**, *51* (12), 1461–1471.

(12) Dao, L. T.; Takeoka, G. R.; Edwards, R. H; Berrios, J. D. J. Improved method for the stabilization of anthocyanidins. *J. Agric. Food Chem.* **1998**, *46* (9), 3564–3569.

(13) Sadilova, E.; Stintzing, F. C.; Carle, R. Thermal degradation of acylated and nonacylated anthocyanins. *J. Food Sci.* **2006**, *71* (8), C504–C512.

(14) Kirca, A.; Ozkan, M.; Cemeroglu, B. Effects of temperature, solid content and pH on the stability of black carrot anthocyanins. *Food Chem.* **2007**, *101* (1), 212–218.

(15) Reyes, L. F.; Cisneros-Zevallos, L. Degradation kinetics and colour of anthocyanins in aqueous extracts of purple- and red-flesh potatoes (*Solanum tuberosum* L.). *Food Chem.* **2007**, *100* (3), 885–894.

(16) Yue, X.; Xu, Z. Changes of anthocyanins, anthocyanidins, and antioxidant activity in bilberry extract during dry heating. *J. Food Sci.* **2008**, *73* (6), C494–C499.

(17) Harbourne, N.; Jacquier, J. C.; Morgan, D. J.; Lyng, J. G. Determination of the degradation kinetics of anthocyanins in a model juice system using isothermal and non-isothermal methods. *Food Chem.* **2008**, *111* (1), 204–208.

(18) Nayak, B.; Berrios, J. J.; Powers, J. R.; Tang, J. Effect of extrusion on the antioxidant capacity and color attributes of expanded extrudates prepared from purple potato and yellow pea flour mixes. *J. Food Sci.* **2011**, *76* (6), C874−C883.

(19) AACC. *Approved Methods of the American Association of Cereal Chemists*, 10th ed.; American Association of Cereal Chemists: St. Paul, MN, 2000; Method 44-15A.

(20) Giusti, M. M.; Wrolstad, R. Characterization and measurement of anthocyanin by UV−visible spectroscopy In *Current Protocols in Food Analytical Chemistry*; Wrolstad, R. E., Ed.; Wiley: New York, 2001; Vol. F1.2, pp F1.2.1−F1.2.13.

(21) Reyes, L. F.; Cisneros-Zevallos, L. Wounding stress increases the phenolic content and antioxidant capacity of purple-flesh potatoes (*Solanum tuberosum* L.). *J. Agric. Food Chem.* **2003**, *51* (18), 5296–5300.

(22) Han, K. H.; Sekikawa, M.; Shimada, K.; Hashimoto, M.; Hashimoto, N.; Noda, T.; Tanaka, H.; Fukushima, M. Anthocyanin-rich purple potato flake extract has antioxidant capacity and improves antioxidant potential in rats. *Br. J. Nutr.* **2006**, *96* (6), 1125–1133.

(23) Jansen, G.; Flamme, W. Coloured potatoes (*Solanum tuberosum* L.) − anthocyanin content and tuber quality. *Genet. Resour. Crop Evol.* **2006**, *53* (7), 1321–1331.

(24) Lewis, C. E.; Walker, J. R. L.; Lancaster, J. E.; Sutton, K. H. Determination of anthocyanins, flavonoids and phenolic acids in potatoes. I: Coloured cultivars of *Solanum tuberosum* L. *J. Sci. Food Agric.* **1998**, *77* (1), 45–57.

(25) Brandwilliams, W.; Cuvelier, M. E.; Berset, C. Use of a free-radical method to evaluate antioxidant activity. *Food Sci. Technol.—Lebensm.-Wiss. Technol.* **1995**, *28* (1), 25–30.

(26) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26* (9−10), 1231–1237.

(27) Holdsworth, S. D. *Thermal Processing of Packaged Foods*; Blackie Academic and Professional: New York, 2000; pp 70−93.

(28) Hong, V.; Wrolstad, R. E. Use of HPLC separation photodiode array detection for characterization of anthocyanins. *J. Agric. Food Chem.* **1990**, *38* (3), 708–715.

(29) Wang, J.; Kalt, W.; Sporns, P. Comparison between HPLC and MALDI-TOF MS analysis of anthocyanins in highbush blueberries. *J. Agric. Food Chem.* **2000**, *48* (8), 3330–3335.

(30) Giusti, M. M.; Rodriguez-Saona, L. E.; Griffin, D.; Wrolstad, R. E. Electrospray and tandem mass spectroscopy as tools for anthocyanin characterization. *J. Agric. Food Chem.* **1999**, *47* (11), 4657–4664.

(31) Jones, C. M.; Mes, P.; Myers, J. R. Characterization and inheritance of the anthocyanin fruit (Aft) tomato. *J. Hered.* **2003**, *94* (6), 449–456.

(32) Nayak, B.; Berrios, J. J.; Powers, J. R.; Tang, J.; Ji, Y. Colored potatoes (*Solanum tuberosum* L.) dried for antioxidant-rich value-added foods. *J. Food Process. Preserv.* **2011**, doi: 10.1111/j.1745-4549.2010.00502.x.

(33) Adams, J. B. Thermal degradation of anthocyanins with particular reference to the 3-glycosides of cyanidin. I. In acidified aqueous solution at 100 °C. *J. Sci. Food Agric.* **1973**, *24* (7), 747–762.

(34) Wang, W. D.; Xu, S. Y. Degradation kinetics of anthocyanins in blackberry juice and concentrate. *J. Food Eng.* **2007**, *82* (3), 271–275.

(35) Cemeroglu, B.; Velioglu, S.; Isik, S. Degradation kinetics of anthocyanins in sour cherry juice and concentrate. *J. Food Sci.* **1994**, *59* (6), 1216–1218.

(36) Kirca, A.; Cemeroglu, B. Degradation kinetics of anthocyanins in blood orange juice and concentrate. *Food Chem.* **2003**, *81* (4), 583–587.

(37) Von Elbe, J. H.; Schwartz, S. J. Colorants. In *Food Chemistry*, 3rd ed.; Fennema, O. R., Ed.; Dekker: New York, 1996; pp 651−722.

(38) Dangles, O.; Saito, N.; Brouillard, R. Anthocyanin intramolecular copigment effect. *Phytochemistry* **1993**, *34* (1), 119–124.

(39) Attoe, E. L.; Von Elbe, J. H. Photochemial degradation of betanine and selected anthocyanins. *J. Food Sci.* **1981**, *46* (6), 1934–1937.

(40) Matsufuji, H.; Kido, H.; Misawa, H.; Yaguchi, J.; Otsuki, T.; Chino, M.; Takeda, M.; Yamagata, K. Stability to light, heat, and hydrogen peroxide at different pH values and DPPH radical scavenging activity of acylated anthocyanins from red radish extract. *J. Agric. Food Chem.* **2007**, *55* (9), 3692–3701.

(41) Cisse, M.; Vaillant, F.; Acosta, O.; Dhuique-Mayer, C.; Dornier, M. Thermal degradation kinetics of anthocyanins from blood orange, blackberry, and roselle using the Arrhenius, Eyring, and Ball models. *J. Agric. Food Chem.* **2009**, *57* (14), 6285–6291.

(42) Ariahu, C. C.; Ogunsua, A. O. Thermal degradation kinetics of thiamine in periwinkle based formulated low acidity foods. *Int. J. Food Sci. Technol.* **2000**, *35* (3), 315–321.

(43) Jackman, R. L.; Smith, J. L. Anthocyanins and betalains. In *Natural Food Colorants*, Hendry, G. A. F., Houghton, J. D., Eds.; Blackie and Son: London, U.K., 1992; pp 183−241.

(44) Seeram, N. P.; Bourquin, L. D.; Nair, M. G. Degradation products of cyanidin glycosides from tart cherries and their bioactivities. *J. Agric. Food Chem.* **2001**, *49* (10), 4924–4929.