DOI:10.1021/jf902799a

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Characterization of Anthocyanins and Anthocyanidins in Purple-Fleshed Sweetpotatoes by HPLC-DAD/ESI-MS/MS

Van-Den Truong,*,† Nigel Deighton,‡ Roger T. Thompson,† Roger F. McFeeters,† Lisa O. Dean,† Kenneth V. Pecota,§ and G. Craig Yencho§

†USDA-ARS, SAA, Food Science Research Unit, 322 Schaub Hall, Box 7624, North Carolina State University, Raleigh, North Carolina 27695-7624, ‡Genomic Sciences Laboratory, and §Department of Horticultural Science, North Carolina State University, Raleigh, North Carolina 27695

Purple-fleshed sweetpotatoes (PFSP) can be a healthy food choice for consumers and a potential source for natural food colorants. This study aimed to identify anthocyanins and anthocyanidins in PFSP, and to evaluate the effect of thermal processing on these polyphenolic compounds. Freeze-dried powder of raw and steamed samples of three PFSP varieties were extracted with acidified methanol using a Dionex ASE 200 accelerated solvent extractor. Seventeen anthocyanins were identified by HPLC-DAD/ESI-MS/MS for Stokes Purple and NC 415 varieties with five major compounds: cyanidin 3-caffeoylsophoroside-5-glucoside, peonidin 3-caffeoylsophoroside-5-glucoside, cyanidin 3-caffeoyl-*p*-hydroxybenzoylsophoroside-5-glucoside, peonidin 3-caffeoyl-*p*-hydroxybenzoyl-sophoroside-5-glucoside, and peonidin-caffeoyl-feruloylsophoroside-5-glucoside. Okinawa variety showed 12 pigments with 3 major peaks identified as cyanidin 3-caffeoylsophoroside-5-glucoside, cyanidin 3-(6″,6‴-dicaffeoylsophoroside)-5-glucoside and cyanidin 3-(6″-caffeoyl-6‴-feruloylsophoroside)-5-glucoside. Steam cooking had no significant effect on total anthocyanin content or the anthocyanin pigments. Cyanidin and peonidin, which were the major anthocyanidins in the acid hydrolyzed extracts, were well separated and quantified by HPLC with external standards. Cyanidin and peonidin, which contribute to the blue and red hues of PFSP, can be simply quantified by HPLC after acid hydrolysis of the anthocyanins.

KEYWORDS: Sweetpotatoes; *Ipomoea batatas*; cooking; puree; anthocyanins; polyphenolics

INTRODUCTION

Purple-fleshed sweetpotatoes (PFSP) have attractive reddish-purple color with high levels of anthocyanins, total phenolics and antioxidant activities (*1*−*3*). Recent research on nutraceutical properties of PFSP indicated that the extracted anthocyanins exhibited strong radical scavenging activity, antimutagenic activity, and significantly reduced high blood pressure and liver injury in rats (*4*−*7*). Other physiological functions of anthocyanins include anti-inflammatory activity, antimicrobial activity, ultraviolet light protection, and reduction in memory impairment effects (*8*, *9*). A study on healthy adult men with borderline hepatitis indicated that PFSP beverage intake (400 mg anthocyanins/day) may have a potential capacity for protection of the liver against oxidative stress (*8*).

In the past few years, sweetpotato cultivars with deep purple flesh were developed in many countries to meet a growing demand in the health food markets (*1*, *9*). Prominent examples are the Yamagawamurasaki and Ayamurasaki cultivars in Japan, which are utilized in a variety of processed commercial products including natural food colorants, juices, bread, noodles, jams, confectionary, and fermented beverages (*8*). In the United States, there is a growing interest in the sweetpotato industry in exploring the market opportunities for PFSP as a healthy food choice and potential source for natural food colorants. Breeding efforts to develop PFSP genotypes suitable for local growing conditions, postharvest handling practices and marketing systems have been carried out at North Carolina State University (NCSU). Processing technologies have been developed to convert PFSP into frozen and aseptic purees for various food applications (*1*, *10*). Therefore, characterization of anthocyanin pigments in PFSP is important for breeding programs and development of value-added products.

Anthocyanin pigments in sweetpotatoes have been analyzed using HPLC methods by several investigators (*11*, *12*). The chemical structures of six major anthocyanin compounds isolated from Yamagawamurasaki were elucidated using nuclear magnetic resonance (NMR) spectroscopy (*13*, *14*). These pigments are either cyanidin or peonidin linked with glucopyranoside and sophoroside which are acylated with caffeic acid, *p*-hydroxybenzoic acid and ferulic acid (**Figure 1**). With recent developments in HPLC coupled with mass spectrometry (MS), limited applications of this technique have been made in research on PFSP anthocyanins (*2*, *15*). Electronspray ionization mass spectrometry

*Corresponding author. Tel: (919) 513-7781. Fax: (919) 513-0180. E-mail: Den.Truong@ars.usda.gov.

 Published on Web 12/17/2009

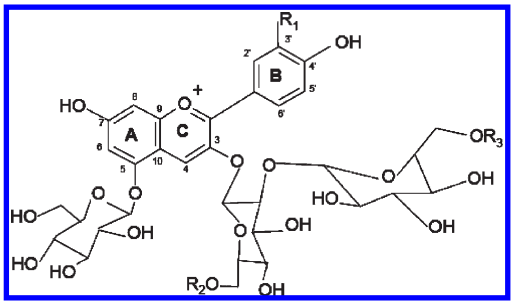

Figure 1. Structure of anthocyanins isolated from purple-fleshed sweetpotatoes: cyanidin, R_1 = OH; pelargonidin, R_1 = H; peonidin, R_1 = OCH_3; R_2, R_3 = H, caffeic acid, ferulic acid, *p*-hydroxybenzoic acid, and *p*-coumaric acid.

(ESI-MS) and tandem spectrometry (MS/MS) have emerged as powerful techniques for characterization of complex mixtures of anthocyanins in plant foods and biological materials (*16*). Using HPLC and ESI-MS/MS techniques in combination with precursor-ion analysis, product-ion analysis and selected reaction monitoring, Tian et al. (*17*) detected and characterized 26 anthocyanin pigments from a purple sweetpotato cell line with several anthocyanins and pelargonidin derivatives that were not reported in previous investigations. The objectives of this study were to profile the anthocyanins and anthocyanidins in PFSP using HPLC/ESI-MS/MS, and to evaluate the effect of steam cooking on anthocyanin pigments.

MATERIALS AND METHODS

Chemicals. Chlorogenic acid, Folin−Ciocalteau (FC) phenol reagent, and HPLC grade methanol were purchased from Sigma-Aldrich (St. Louis, MO). Standards of cyanidin, peonidin and pelargonidin were obtained from Chromadex (Santa Ana, CA). Water used for HPLC analysis was purified with a deionized water system (Eagle Water Systems of the Triangle, Inc., Durham, NC). All other chemicals were analytical grade (Fisher Scientific, Suwanee, GA).

Sweetpotato Varieties and Sample Preparation. Purple-fleshed sweetpotatoes, Stokes Purple and Okinawa varieties, were purchased from Saura Pride Sweetpotatoes (Walnut Cove, NC) and Pride of Sampson, Inc. (Clinton, NC), respectively. NC 415 clone was grown at the NCSU experimental fields of the Sweetpotato Breeding Program (Clinton, NC). The three varieties were cured at 30 °C, 85−90% relative humidity for 7 days, and stored at 13−16 °C, 80−90% relative humidity for about 4 months prior to sampling for analysis.

Duplicate samples were taken for each of the 3 varieties. At least ten roots from two batches were randomly taken for each sample, thoroughly washed with tap water and air-dried at room temperature. The roots were longitudinally cut and divided into four parts. Three parts of the roots were peeled with a manual vegetable peeler and used for raw and cooked samples. One part was ground at room temperature for 1 min into small particles using a heavy duty food processor, model RS1 2Y1 (Robo Coupe USA, Ridgeland, MS). The ground samples (100 g) were placed on a plastic tray (15 × 15 × 3.5 cm), covered with cheese cloth, and held at room temperature for 15 min before being put in a −80 °C freezer. These samples were designated as raw-ground (R-G) at room temperature. The second part was cut into 0.50 cm thick slices, double bagged in quart size Ziploc plastic bags, and immediately placed in the −80 °C freezer. The frozen slices in the bags were shattered with a hammer, mixed, sampled, and quickly placed in a plastic container covered with cheese cloth as described. These samples were coded as raw-sliced (R-S). The third part designated as cooked-flesh (C-F) was also cut into 0.50 cm thick slices, steamed cooked for 25 min in a vegetable steamer and placed in a closed container for cooling to room temperature prior to grinding into puree using the Robo Coupe food processor. The remaining portion was not peeled (whole), cut into 0.50 cm slices, steam cooked for 25 min, pureed, and designated as cooked-whole (C-W). The pureed samples were weighed (100 g), spread into plastic trays, covered, and frozen at −80 °C. All the frozen samples were freeze-dried for 4−6 days in the dark using a VirTis Genesis 25XL freeze-dryer (Gardiner, NY) operated at −35 °C to −40 °C. The freeze-dried samples were weighed and ground into powders using a Mr. Coffee precision coffee grinder (Sunbeam Boca Raton, FL), placed in sample vials and kept in −80 °C storage until analysis.

Pigment Extraction. Extraction of polyphenolic compounds from freeze-dried sweetpotato powders was performed using an accelerated solvent extractor, ASE 200 (Dionex Corporation, Sunnyvale, CA) equipped with a solvent controller. Three cellulose filters were placed in the bottom of a 22 mL stainless steel extraction cell and covered with 2 g of sea sand (Fisher Scientific, Pittsburgh, PA). Sweetpotato powder (0.25 g) was mixed with 26 g of sand, loaded into the cell, and then closed tightly. Extraction conditions were set as previously described by Steed and Truong (*1*): pressure, 1500 psi; temperature, 100 °C; extraction time, three 5 min cycles; flushing volume, 60% cell volume; and nitrogen purge time, 60 s. All powders were extracted with a solvent containing 7% acetic acid in 80% methanol that was purged with nitrogen gas prior to use to prevent oxidation of phenolic components during the extraction. Extracts were collected in amber glass vials, adjusted to 50 mL volume with solvent, and then transferred into several 10 mL serum tubes and kept at −80 °C until analysis.

Quantification of Total Phenolics and Anthocyanins. Total phenolics were quantified using a modified Folin−Ciocalteau (FC) method with chlorogenic acid as standard (*1*). Samples and standards (0.25 mL) were diluted in 4 mL of water to which 0.5 mL of the FC reagent was added and allowed to react at room temperature for 3 min. Sodium carbonate (1 N, 0.5 mL) was added and the reaction was carried out for 1 h. Sample absorbances were read at 725 nm using a Varian spectrophotometer, Cary WinUV model 300 (Palo Alto, CA). The baseline was established by reading a blank that contained 0.25 mL of water instead of sample, along with the same amount of water for dilution, FC reagent, and sodium carbonate solution. Total phenolic values were reported in milligrams chlorogenic acid equivalents per 100 g fresh weight (mg CAE/100 g fw).

Total monomeric anthocyanin (TMA) content was determined using the pH-differential method (*18*). Two dilutions were performed on each sample. The first used potassium chloride (0.025 M) at pH 1.0 and the second was with sodium acetate (0.4 M) at pH 4.5. Samples were diluted so that absorbance readings at 530 nm were less than 1.2. They were allowed to equilibrate for 15 min before absorbance at 530 and 700 nm were recorded using a spectrophotometer calibrated with distilled water as the blank. The difference in absorbance between the two pH values and wavelengths was used to calculate anthocyanin content as cyanidin-3-glucoside with molecular weight of 449.2 g/mol and molar absorptivity of 26,900 L/cm/mol (*18*). The TMA content was reported as milligrams anthocyanins per 100 g fresh weight or dry weight (mg anthocyanins/100 g fw or dw).

Acid Hydrolysis of Anthocyanins. A 200 μL aliquot of an anthocyanin extract was added to 300 μL of 6 N HCl in a screw-cap HPLC sampling vial. The solution was thoroughly mixed, and the pigments were hydrolyzed at 100 °C for times ranging from 30 min to 4 h. Sample was immediately cooled in an ice bath, brought to dryness using a SpeedVac, model SVC 100 (Savant Instruments, Inc., Farmingdale, NY), and redissolved in 200 μL of 1% formic acid acidified with 0.12 N HCl. Standards of cyanidin, peonidin and pelargonidin at 0.01 to 0.025 mg/mL were also hydrolyzed and redissolved in the same manner.

HPLC-DAD Analysis and LC/MS/MS identification. The anthocyanin extracts were analyzed using a Thermo Finnigan HPLC System equipped with a UV6000LP photodiode array detector, AS3000 autosampler, SCM1000 degasser, P2000 binary pump and ChromQuest software version 4.1 (Thermo Electron Corp., San Jose, CA). The column used was a 250 × 2 mm i.d., 4 μm, Synergi Polar-RP C18 (Phenomenex, Torrance, CA) equipped with a guard column of 7.5 × 4.6 mm i.d., guard column, Allsphere Phenyl 5 μm (Alltech, Deerfield, IL). The operating conditions were as follows: autosampler sample tray at 6 °C; column oven at 35 °C; injection volume, 10−20 μL; eluent flow rate, 0.23 mL/min. The elution solvents were A (1% v/v formic acid in water) and B (acetonitrile containing 1% formic acid) with the following gradient: 5% to 45% B from 0 to 50 min, 45% to 5% B from 50 to 55 min, and isocratic at 5% B from 55 to 60 min to equilibrate the column for the next injection. Spectral data from 200 to 600 nm were recorded, and the anthocyanin chromatograms were monitored at 530 nm. Identification of the anthocyanin

Table 1. Total Phenolics and Anthocyanin Content of Raw and Steam Cooked PFSP[a]

treatment	dry matter (%)	total phenolics (mg CAE/100 g fw)	total anthocyanins (mg/100 g fw)
		Stokes Purple	
raw-ground (R-G)	29.2 bcd ± 0.3	323.8 cd ± 29.9	46.3c de ± 3.2
raw-sliced (R-S)	29.5 ab ± 0.0	593.5 ab ± 26.2	96.79 a ± 5.3
cooked flesh (C-F)	28.2 de ± 0.7	576.0 ab ± 43.1	78.3 abc ± 3.7
cooked whole (C-W)	28.8 cd ± 0.3	591.9 ab± 33.2	80.0 ab ± 2.0
		NC 415	
raw-ground (R-G)	29.4 bcd ± 0.5	332.2 cd ± 14.1	33.7 def ± 1.3
raw-sliced (R-S)	29.6 bc ± 0.3	533.4 abc ± 30.7	52.5 bcd ± 1.8
cooked flesh (C-F)	28.4 cde ± 1.1	690.9 a ± 46.3	69.0 abc ± 0.6
cooked whole (C-W)	27.3 e ± 1.2	713.0 a ± 23.0	69.6 abc ± 2.3
		Okinawa	
raw-ground (R-G)	31.6 a ± 0.7	261.4 d ± 13.5	10.0 f ± 0.5
raw-sliced (R-S)	31.5 a ± 0.9	432.7 bcd ± 29.3	19.1 ef ± 1.0
cooked flesh (C-F)	30.0 b ± 0.5	458.3 bcd ± 12.3	21.1 def ± 0.7
cooked whole (C-W)	29.9 b ± 0.2	594.8 ab ± 12.8	21.0 def ± 0.9

[a] Values with different letters within column designate statistically significant differences, $p < 0.05$ by Duncan's multiple range tests.

compounds was based on the retention time with references to LC/MS/MS analysis, and the UV spectra available in the literature (*15*, *17*).

For acid hydrolyzed samples, HPLC analysis was performed on YMC C18, ODS-AM column, 5 μ particle size, 150 × 4.6 mm with a gradient elution solvent: 3% to 45% B from 0 to 20 min, and a 10 min post run at 3% B for column equilibration. The eluent flow rate was 1 mL/min; other operating conditions were the same as described above. Identification of anthocyanidin compounds were based on the retention time and LC/MS m/z values with reference to cyanidin, peonidin and pelargonidin standards. Quantification of all compounds in the HPLC chromatograms was calculated from peak areas with reference to respective standard, and expressed in mg/g fresh weight sample (fw). All the calibration curves for the reference compounds were linear with $R^2 \geq 0.999$. Chromquest Version 4.1 software was used for data collection and analysis.

LC-DAD/MS/MS analyses were performed on an LTQ ion-trap mass spectrometer with a Surveyor autosampler, diode array detector and HPLC (Thermo Electron, San Jose, CA) equipped with an electrospray ionization (ESI) source. Briefly, a 5 μL sample was injected onto a 250 × 2 mm Synergi Hydro RP column (Phenomenex, Torrance, CA) equilibrated in 95:5 A:B where A = 1% formic acid in water; B = 1% formic acid in acetonitrile, flow rate = 0.23 mL/min. Anthocyanins were eluted through application of a linear gradient to 75:25 A:B in 50 min. Data dependent MS/MS and MS^3 were performed on abundant precursor ions to elucidate chemical structures. Precursor ion scans were obtained in the range m/z 600−1400 and all data manipulation was within Xcalibur software, version 2.0.7.

Statistical Analysis. The experiment was conducted with two replicates in a randomized complete block design. Two samples were taken and analyzed per replicate. Group differences were evaluated using t tests with $p < 0.05$ considered to be a statistically significant difference. Means were compared with Duncan's multiple range test with $\alpha = 0.05$ and Pearson correlations were performed using SAS Statistical Analysis System, v. 9.1 (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Total Phenolics and Total Monomeric Anthocyanins. The phenolic contents of the PFSP samples ranged from 261.4 to 712.8 mg CAE/100 g fw (**Table 1**), which were much higher than the values reported for the orange-fleshed sweetpotatoes (57.1 to 101.4 mg/100 g fw) (*19*), yellow carrots (7.7 mg/100 g fw) and purple carrots (74.6 mg/100 g fw) (*20*).

The total monomeric anthocyanin (TMA) content varied significantly ($p < 0.05$) among the three PFSP varieties (**Table 1**). The Stokes Purple and NC415 with dark purple-fleshed color had high anthocyanin content (33.7 to 96.8 mg/100 g fw), which was about 3- to 5-fold greater than in the Okinawa variety (10.0 to 21.1 mg/100 g fw). These anthocyanin values were comparable to those reported for several purple-fleshed sweetpotato cultivars in Japan (*8*), and 2 to 40 mg/100 g fw in various genotypes of red-fleshed potatoes (*18*). The anthocyanin contents for PFSP in the study were lower than those reported for black currant and blueberries (322−476 mg/100 g fw), but they were comparable with other commodities such as grapes (27−120 mg/100 g fw), plum (19−124 mg/100 g fw), sweet cherries (122 mg/100 g fw), raspberries (92 mg/100 g fw), eggplant (86 mg/100 g fw), and red radishes (100 mg/100 g fw) (*21*). The results indicated that PFSP fall in the middle of the spectrum of the high anthocyanin fruits and vegetables. The TMA and phenolic content in fresh weight of the tested PFSP genotypes can be converted into dry weight basis for comparison with other commodities using the dry matter contents of 28 to 32% (**Table 1**) which are typical for PFSP (*1*). Therefore, on dry weight basis the tested PFSP genotypes had total phenolics of 828−1992 mg CAE/100 g dw and TMA content of 33−328 mg/100 g dw.

Sample preparation such as peeling, freezing or steaming of PFSP slices before freeze-drying had no effect ($p > 0.05$) on total anthocyanin and phenolic content (**Table 1**). However, disrupting the raw tissue at room temperature during sample preparation resulted in significant decrease ($p < 0.05$) in both total phenolic and TMA content, especially for Stokes Purple and NC 415 genotypes. This effect can be attributed to the degradation of polyphenolics at room temperature by an active enzyme system in the tissue including anthocyanases, polyphenol oxidase and peroxidase (*11*). Jang and others (*22*) isolated polyphenoloxidase (PPO) in purple-fleshed potatoes and found that the enzyme is most active at room temperature and degraded at temperatures > 70 °C. Therefore, freezing or steaming of raw slices is recommended to minimize anthocyanin degradation during sample preparation.

Anthocyanin Profiles. The HPLC-DAD chromatograms of anthocyanins extracted from the cooked flesh samples of the three tested varieties, recorded at 530 nm, are shown in **Figure 2**. Within the same genotype, the extracts from the raw and cooked-whole roots had similar anthocyanin profiles (data not shown) to that of the cooked flesh samples. However, there was a difference in the HPLC profiles (**Figure 2**) among the PFSP genotypes indicating a variation in anthocyanin composition. Such a variation has also been reported in PFSP cultivars grown in Japan (*5*). With the HPLC conditions used in this study, at least 10 peaks were separated from the anthocyanin extracts of the three genotypes analyzed (**Figure 2**). The chromatographic characteristics and percent peak areas of the separated compounds are shown in **Table 2**. Stokes Purple and NC 415 varieties had 13 and 11 anthocyanin compounds, respectively, with distinct major peaks of 5, 7, 11, 16, and 17 detected by the UV/vis detector. For the Okinawa variety, the HPLC profile showed 10 separated compounds with major peaks of 1, 4, 6, 7, 10, 11, and 12. The predominant compounds (13.2 to 39.1% peak area) in the pigment of Okinawa eluted at 34.70 (peak 7), 37.41 (peak 10) and 40.42 min (peak 12), which were distinctly different from 38.12 (peak 11) and 42.62 (peak 16) of Stokes Purple and NC 415 (**Table 2**). Yoshimoto et al. (*23*) reported at least 12 anthocyanins in the HPLC analysis of the extracts from the Ayamurasaki cultivar, and designated seven major compounds as YGM-1, -2, -3, -4, -5a, -5b and -6. Additional anthocyanin compounds designated as YGM0-a, -b, -c, -d, -e, -f, and -g were reported in

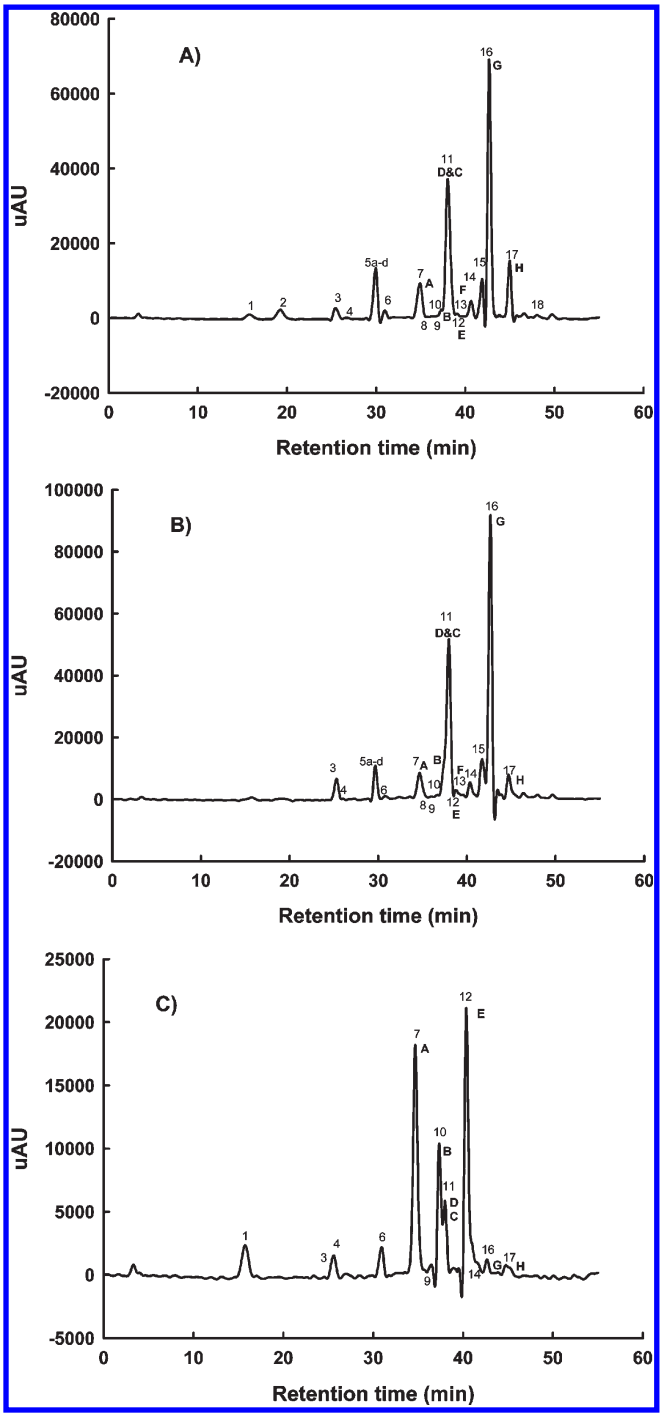

Figure 2. HPLC chromatograms of anthocyanin extracts of cooked-flesh samples: (**A**) Stokes Purple, (**B**) NC 415 and (**C**) Okinawa. Refer to **Table 3** for peaks A, B, C, D, E, F, G, H marked on the chromatograms.

sweetpotato leaves (*24*) and twenty six anthocyanin pigments were identified in purple sweetpotato cell cultures (*25*, *17*).

Anthocyanin compounds are not commercially available to serve as standards for identification, and UV−visible spectra do not allow a clear-cut identification of individual pigments (*26*). Therefore, identification of individual anthocyanin peaks was based on the data from HPLC-DAD/MS/MS, MS^3 in accordance with the information available in the literature especially on sweetpotato anthocyanins (*2*, *15*, *17*, *27*). It has been shown that the LC/MS/MS techniques are useful for anthocyanin characterization due to their highly sensitive ionization producing intact molecular ions and the corresponding anthocyanidin fragments.

Table 2. Anthocyanin Compounds of the PFSP Extracts Separated by HPLC

		genotype/treatment/peak area (%)[b]					
		S-Purple[c]		NC415		Okinawa	
peak label[a]	retention time	raw	puree[d]	raw	puree[d]	raw	puree[d]
1	15.85	1.48	1.62			8.37	8.65
2	19.27	2.52	3.62				
3	25.37	1.48	1.65	2.74	3.22		
4	25.80					2.90	2.71
5	29.77	5.00	6.57	4.28	4.14		
6	30.86	0.96	1.22	0.67	0.81	3.46	3.37
7	34.70	8.95	7.61	7.62	6.28	32.51	33.77
10	37.41	1.69	1.26	3.91	2.78	13.38	13.24
11	38.12	26.09	27.43	31.97	31.52	6.83	6.49
12	40.42	3.86	2.56	3.24	2.18	29.76	29.00
13	41.90	2.59	2.26	4.57	3.76		
14	41.98					1.37	1.58
15	42.21	2.93	2.82	2.07	2.32		
16	42.62	33.44	32.57	34.76	39.14	0.49	0.33
17	45.00	8.54	8.38	4.17	3.86	0.92	0.86

[a] Refer to **Figure 2**. [b] Mean values of two replicates. [c] Stokes Purple. [d] Cooked flesh.

These fragmentation patterns provide useful information for a tentative determination of the location of the glycosidation and acylated groups in an anthocyanin compound (*16*, *28*). **Table 3** summarizes the mass spectrometric data of all the peaks shown in the HPLC chromatograms (**Figure 2**), and the compounds that were coeluted in peaks 5 and 11. Peaks 8 and 9 were not clearly visible in the HPLC chromatograms, but there were signals in MS/MS fragment ions patterns. The identification was based on the m/z of molecular ion $[MH]^+$ and fragment ions associated with the elimination of known moieties attached to an aglycon of sweetpotato anthocyanins. For example, $[M - 162]^+$ and $[M - 2 \times 162]^+$ pointed to a loss of one molecule of glucose and sophorose. Fragment ions of acylated sophorose can be identified by m/z values at $[M - 2 \times 162 - 162]^+$, $[M - 2 \times 162 - 146]^+$, $[M - 2 \times 162 - 176]^+$, $[M - 2 \times 162 - 120]^+$ for caffeic, coumaric, ferulic and *p*-hydroxybenzoic acid moieties, respectively. These phenolic acids have been reported to be involved in the structures of sweetpotato anthocyanins using NMR techniques by previous investigators (*17*, *29*).

Peaks 1−3 were the early eluted compounds that were not identified in previous reports on anthocyanins in PFSP roots. Peak 1 had the molecular ion $[MH]^+$ at 773, and three mass fragment ions were detected by MS/MS analysis, one at m/z 611 due to the elimination of one molecule of glucose $[M - 162]^+$, and one at m/z 449 indicating the loss of sophorose, furnishing the cyanidin aglycon with m/z 287. Peak 1 was therefore tentatively identified as cyanidin 3-sophoroside-5-glucoside. For peak 2, the fragment ion patterns of m/z 625 $[M - 162]^+$, 463 $[M - 2 \times 162]^+$ and 301 $[peonidin]^+$ indicated that it was peonidin 3-sophorose-5-glucoside. Peak 3, their molecular ion and product ions of MS/MS results, m/z 893 $[MH]^+$, 731 $[M - 162]^+$ for the loss of glucose, 449 $[M - 2 \times 162 - 120]^+$ for the loss of a sophorose molecule and *p*-hydroxybenzoic acid 120 [*p*-hydroxybenzoic acid $- H_2O]^+$, and 287 $[cyanidin]^+$ pointed to a tentative identification as cyanidin 3-*p*-hydroxybenzoylsophoroside-5-glucoside. Peak 4 which appeared in the Okinawa chromatogram (**Figure 2**) is also a cyanidin compound (m/z 287); the fragment ions of m/z 773 $[M - 162]^+$, 449 $[M - 2 \times 162 - 162]^+$ indicated the elimination of glucose and sophorose and caffeoyl moiety 162 [caffeic acid $- H_2O]^+$, and 287 $[cyanidin]^+$. This compound was tentatively identified as cyaniding 3-(6″-cafeoylsophoroside)-5-glucoside which matched the structure of peak 1, a minor pigment, in the

Table 3. Mass Spectrometric Data and Identification of Anthocyanin Compounds in Purple-Fleshed Sweetpotatoes

		m/z				previously designated[b]		
peak label[a]	retention time (min)	MH^+	aglycon	other fragment ions	compound identity			
1	15.85	773	287	449, 611	cyanidin 3-sophoroside-5-glucoside	T1,	YGM-0a	
2	19.27	787	301	463, 625	peonidin 3-sophoroside-5-glucoside	T4,	YGM-0b	
3	25.37	893	287	449, 731	cyanidin 3-*p*-hydroxybenzoylsophoroside-5-glucoside	T5,	YGM-0c	
4	25.80	935	287	449, 773	cyanidin 3-(6″-caffeoylsophoroside)-5-glucoside	T6,	YGM-0d	
5a	29.77	937	301	463, 775	unknown			
5b	29.82	907	301	463, 745	peonidin 3-*p*-hydroxybenzoylsophoroside-5-glucoside	T7,	YGM-0e	
5c	29.82	877	271	433, 715	pelargonidin compound			
5d	30.00	937	301	463, 775	unknown			
6	30.86	949	287	449, 787	cyanidin 3-(6″-feruloylsophoroside)-5-glucoside	T10		
7	34.70	935	287	449, 773	cyanidin 3-caffeoylsophoroside-5-glucoside	T17,	YGM-2,	A
8	35.03	963	301	463, 801	peonidin 3-(6″-feruloylsophoroside)-5-glucoside	T12,	YGM-0 g	
9	36.40	1115	287	467, 629, 953	unknown			
10	37.41	1097	287	449, 629, 935	cyanidin 3-(6″,6‴-dicaffeoylsophoroside)-5-glucoside	T16,	YGM-1b,	B
11d	38.12	949	301	463, 787	peonidin 3-caffeoylsophoroside-5-glucoside	T21,	YGM-5b,	D
11c	38.17	1055	287	449, 893	cyanidin-3-caffeoyl-*p*-hydroxybenzoylsophoroside-5-glucoside		YGM-1a,	C
12	40.42	1111	287	449, 949	cyanidin 3-(6″-caffeoyl-6‴-feruloylsophoroside)-5-glucoside	T18,	YGM-3,	E
13	41.90	1111	301	463, 949	peonidin-dicaffeoylsophoroside-5-glucoside		YGM-4b,	F
14	41.98	1081	287	449, 919	cyanidin 3-caffeoyl-*p*-coumarylsophoroside-5-glucoside	T19		
15	42.21	1099	301	463, 937	unknown			
16	42.62	1069	301	463, 907	peonidin 3-caffeoyl-*p*-hydroxybenzoyl-sophoroside-5-glucoside	T20,	YGM-5a,	G
17	45.00	1125	301	463, 963	peonidin-caffeoyl-feruloylsophoroside-5-glucoside		YGM-6,	H
18	48.30	1083	301	463, 921	unknown			

[a] Refer to **Figure 2**. [b] References: ref *15* for compounds A−H; ref (*17*) for T1−T21; refs (*23*, *24*) for YGM-0e to YGM-6.

HPLC chromatogram of anthocyanin extract from PFSP roots of the Ayamurasaki cultivar elucidated by NMR spectroscopy (*27*). The anthocyanins identified for peaks 1, 2, 3, and 4 were reported as YGM-0a, YGM-0b, YGM-0c and YGM-0d in the HPLC chromatogram of sweetpotato cell line (*17*, *25*) and in sweetpotato leaves (*24*).

The mass spectrometric data revealed four anthocyanins in peak 5, and three of these compounds were unknown. Compounds 5a and 5d likely contained peonidin (*m/z* 301), and *m/z* of 271 in the fragment ions pattern of pigment 5c indicated a pelargonidin derivative. Pigment 5c had molecular ion and product ions of *m/z* 907 $[MH]^+$, 745 $[M - \text{glucose}]^+$, 463 $[M - \text{sophorose-}p\text{-hydroxybenzoic acid}]^+$, and 301 $[\text{peonidin}]^+$ pointed to a tentative identification as peonidin 3-*p*-hydroxybenzoylsophoroside-5-glucoside which matched with pigment 7 in sweetpotato cell line (*17*) and YGM-0e in sweetpotato leaves (*24*).

Peak 6 had a molecular ion at *m/z* 949 $[MH]^+$, fragment ions at 787 from the loss of a glucose molecule, $[M - 162]^+$, at 449 $[M - 2 \times 162 - 176]^+$ from the elimination of sophorose and ferulic acid molecules and 287 $[\text{cyanidin}]^+$. The LC/MS/MS results pointed to a tentative identification of peak 6 as cyanidin 3-(6″-feruloylsophoroside)-5-glucoside which was reported as pigment 10 in sweetpotato cell line (*17*). Peak 7 was also a cyanidin compound (*m/z* 287) with moieties of glucose, *m/z* 773 $[M - 162]^+$ and caffeoylsophorose, 449 $[M - 2 \times 162 - 162]^+$. This pigment, cyanidin 3-(6″-caffeoylsophoroside)-5-glucoside, was identified and designated as YGM-2 in purple-fleshed roots of Yamagawa Murasaki and Ayamurasaki cultivars (*23*, *29*), sweetpotato leaves (*24*), and pigment 17 in the purple sweetpotato cell lines (*17*). Harada et al. (*15*) designated YGM-2 as pigment A in LC/MS analysis with molecular ion of *m/z* 935 which is in agreement with the present study. The concentration of pigment A or YGM-2 was about 6.8−7.3% of the total HPLC peak areas of the Ayamurasaki extract (*2*, *23*) which was comparable with a range of 5.9−7.9% in Stokes Purple and NC 415 samples. However, Okinawa had this pigment as the most abundant anthocyanin with 32.5−33.8% of the total peak areas (**Table 2**).

Peaks 8 and 9 were not seen in the chromatograms (**Figure 2**), but the signals were detected in the MS/MS analysis. The pigment in peak 8 was a peonidin-based anthocyanin (*m/z* 301) with attachments as those of the compound in peak 6 (**Table 3**) since these pigments had similar fragmentations. This minor compound (0.4−1.5%) was identified as pigment 12 in sweetpotato cell lines (*17*) and was not reported either in sweetpotato leaves or in the roots of PFSP. Peaks 9, 15, and 18 are among the five unidentified compounds in the HPLC chromatograms. Peak 10 appeared as a small shoulder in the chromatogram of Stokes Purple and NC415, but it was a major component (12.2%) in Okinawa cultivar (**Table 2**, **Figure 2**). The molecular ion *m/z* 1097 $[M - 162]^+$, and fragmentation ions of *m/z* 935 $[M - 162]^+$ and *m/z* 449 $[M - 2 \times 162 - 2 \times 162]^+$ due to a loss of a glucose and dicaffeoylsophoroside moieties, respectively, suggested a tentative identification of this pigment as cyanidin 3-(6″-6‴-dicaffeoylsophoroside)-5-glucoside. This pigment was identified and designated as YGM-1b or pigment B in purple-fleshed roots of Yamagawamurasaki and Ayamurasaki cultivars (*15*, *23*, *29*), sweetpotato leaves (*24*), and pigment 16 in the purple sweetpotato cell lines (*17*).

As shown in **Table 3**, two anthocyanins coeluted in peak 11. Pigment 11d was identified as peonidin 3-caffeoylsophoroside-5-glucoside based upon a molecular ion *m/z* at 949 $[M - H]^+$, and fragmentation ions of *m/z* 787 $[M - 162]^+$ and *m/z* 463 $[M - 2 \times 162 - 2 \times 162]^+$ corresponding to the elimination of a glucose and caffeoylsophoroside residues, and 301 $[\text{peonidin}]^+$. Pigment 11c was identified as cyanidin 3-caffeoyl-*p*-hydroxybenzoylsophoroside-5-glucoside with molecular ion *m/z* at 1055 $[M - H]^+$, fragment ions of *m/z* 287 $[\text{cyanidin}]^+$, *m/z* 893 $[M - 162]^+$ associated with the losses of a glucose and *m/z* 449 $[M - 2 \times 162 - 162 - 120]^+$, a caffeic acid (162), *p*-hydroxybenzoic acid (120) and sophorose (2 × 162). Peaks 11c and 11d were identified and named as YGM-1a and YGM-5b or C and D in PFSP (*15*, *23*, *29*). Peak 12 was also a cyanidin having a sophoroside acylated with caffeoyl and *p*-hydroxybenzoic acid. This pigment, cyanidin 3-(6″-caffeoyl-6‴-feruloylsophoroside)-5-glucoside or YGM-3, was a predominant anthocyanin in Okinawa

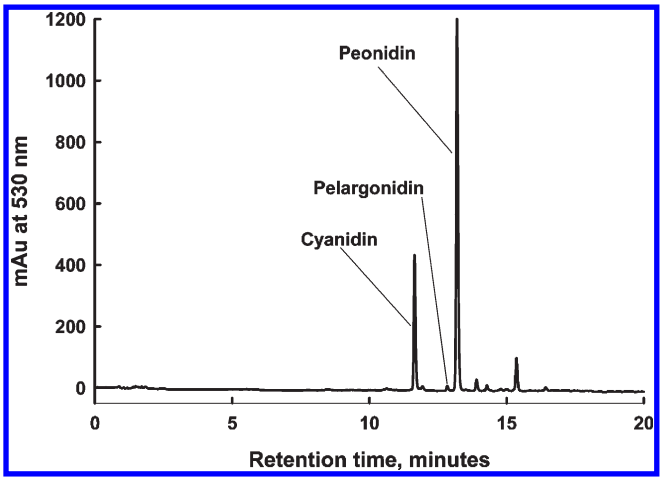

Figure 3. HPLC chromatogram of acid hydrolyzed anthocyanin extract from cooked-flesh sample of Stokes Purple variety.

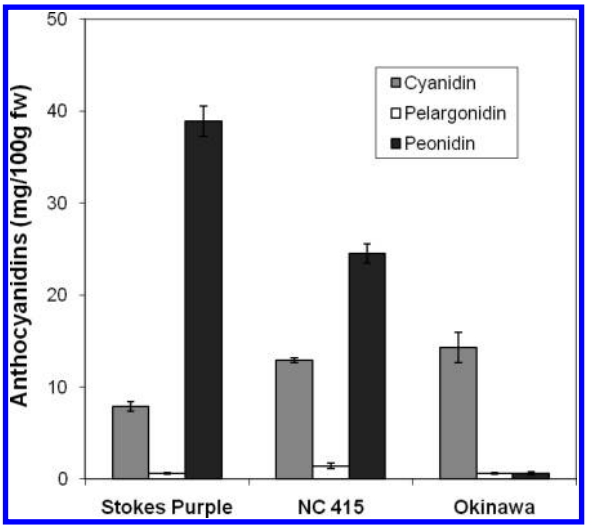

Figure 4. Anthocyanidin content in acid hydrolyzed extracts from cooked flesh of Stokes, NC415 and Okinawa sweetpotatoes.

(29%), and a minor pigment in Stokes Purple, NC 413 (**Table 2**) and Ayamurasaki (*2*). Peak 13 was identified as peonidin-dicaffeoylsophoroside-5-glucoside; a fragment ion of *m*/*z* 463 [M − 2 × 162- 162−162]$^+$ indicated the presence of sophorose and two caffeic acid substituents. This pigment was identified as YGM-4b, one of the four major pigments in leaves of several sweetpotato cultivars (*24*) and Ayamurasaki roots (*2*). Peak 14 was not observed in the chromatograms (**Figure 2**), but the MS/MS signal indicated the presence of this minor pigment in the NC 415 and Okinawa extracts.

Peak 16 was the most abundant anthocyanin (32.6−39.1%) in Stokes Purple and NC415, but a minor component (0.3−0.5%) in Okinawa. It was designated as YGM-5a in Yamagawa Murasaki and Ayamurasaki at about 6.4% of the total peak area (*2*). This pigment was a peonidin, *m*/*z* 301 [peonidin]$^+$ and fragment ions of *m*/*z* 907 and 463 due to the losses of glucose and sophorose-caffeic-*p*-hydroxybenzoic moiety, respectively, pointing to its identification as peonidin 3-caffeoyl-*p*-hydroxybenzoyl-sophoroside-5-glucoside. Similarly, peak 17 had a molecular ion at *m*/*z* of 1125, and fragment ion of 463 [M − 2 × 162 − 162−176]$^+$ associated with a structure containing caffeoyl-feruloyl-sophoroside. This compound was among the six major anthocyanins (16%) designated as YGM-6 in Ayamurasaki cultivar (*2*).

Acid Hydrolysis of Anthocyanins. Acid hydrolysis detached the glycons from the flavylium ion of anthocyanin compounds and released the major anthocyanidins (aglycons) commonly known as cyanidin, delphinidin, malvidin, peonidin, pelargonidin and petunidin. A hydrolysis reaction time of 60 min was sufficient to convert all PFSP anthocyanins to anthocyanidins. The HPLC chromatograms were greatly simplified from 15 peaks to 3 well-separated peaks (**Figure 3**). Based on the retention time of the anthocyanidin standards that are commercially available, these peaks were identified as cyanidin, pelargonidin and peonidin. The corresponding *m*/*z* values of 287, 301, and 271 for these compounds from the LC/MS/MS data confirmed these identifications. Examination of mass spectral data for all other peaks in the chromatograms indicated that they were not anthocyanidin compounds such as malvidin, petunidin and delphinidin that have been commonly found in plants. As indicated in **Figure 3**, cyanidin and peonidin were the main anthocyanidins of the PFSP extract, and pelargonidin was a minor component. The concentrations of peonidin in Stokes Purple and NC 415 were 52.4 and 24.5 mg/100 g fresh weight which accounted for 81% and 63% of the total anthocyanidins, respectively. Cyanidin levels in this peonidin-dominant PFSP were 11.4 (18%) and 12.9 (33%) mg/100 g (**Figure 4**). For Okinawa, cyanidin was dominant with 14.3 mg/100 g equivalent to 92% of the total pigments, while peonidin and pelargonidin levels were as low as 0.6 mg/100 g (3.8%). The peonidin/cyanidin ratios were 4.58, 1.90, and 0.04 for the cooked samples of Stokes Purple, NC 415 and Okinawa, respectively. The raw PFSP samples had similar anthocyanidin contents and peonidin/cyanidin ratios (data not shown). These ratios were within the ranges of 1.86−19.89 for peonidin type and 0.02−0.90 for cyanidin type of PFSP reported by Yoshinaga et al. (*3*).

Pelargonidin has not been reported in the HPLC analysis of the anthocyanin extracts from the roots of PFSP. Our results on the acid hydrolysis of PFSP extracts showed the presence of pelargonidin in PFSP roots. Tian et al. (*17*) reported the detection of two pelargonidin-based anthocyanidins in a purple cell line derived from a PFSP cultivar, *Ipomoea batatas* L. cv. *'Ayamurasaki'*. Several pelargonidin-based compounds were reported in flowers of the subgenus Ipomoea, such as *I. nil* and *I. purpurea* (*30*, *31*). Further studies are required to confirm the presence of pelargonidin in PFSP roots.

The acid hydrolysis method allowed the quantification of anthocyanidins in PFSP with more accuracy since there was no coelution of the compounds as encountered in the HPLC analysis of the anthocyanin extracts (*32*). Acid hydrolysis of the standard compounds, cyanidin, pelargonidin and peonidin had recoveries of 98 ± 4% and coefficient of variation of 2.31−4.48%. The acid hydrolysis method would be useful in the breeding programs to select clones with various levels of cyanidin or peonidin for targeted reddish-purple flesh colors and antioxidant activities. Antioxidant activity of anthocyanins is related to the positions of the −OH group on the flavylium ion. It has been documented that the cyanidin with OH group on the B-ring of anthocyanins has higher antioxidant activity than peonidin, malvidin and pelargonidin (*33*). In a study on the antimutagenicity of deacylated anthocyanins isolated from PFSP, Yoshimoto et al. (*34*) reported that the activity of cyanidin was greater than that of peonidin. Therefore, the acid hydrolysis method would be useful for accurately evaluating PFSP for high cyanidin levels.

ACKNOWLEDGMENT

Paper no. FSR09-19 of the Journal Series of the Department of Food Science, NC State University, Raleigh, NC 27695-7624.

LITERATURE CITED

(1) Steed, L. E.; Truong, V. D. Anthocyanin content, antioxidant activity and selected physical properties of flowable purple-fleshed sweetpotato purees. *J. Food Sci.* **2008**, *73*, S215–S221.

(2) Oki, T.; Osame, M.; Masuda, M.; Kobayashi, M.; Furuta, S.; Nishiba, Y.; Kumagai, T.; Sato, T.; Suda, I. Simple and rapid spectrophotometric method for selecting purple-fleshed sweetpotato cultivars with a high radical-scavenging activity. *Breeding Sci.* **2003**, *52*, 101–107.

(3) Yoshinaga, M.; Yamakasa, O.; Nakatani, M. Genetic diversity of anthocyanin content and composition in purple-fleshed sweetpotato (*Ipomoea batatas* (L.) *Lam*). *Breeding Sci.* **1999**, *49*, 43–47.

(4) Kano, M.; Takayanagi, T.; Harada, K.; Makino, K.; Ishikawa, F. Antioxidant activity of anthocyanions from purple sweetpotato, *Ipomoea batatas* cultivar Ayamurasaki. *Biosci., Biotechnol., Biochem.* **2005**, *69*, 979–988.

(5) Oki, T.; Masuda, M.; Furuta, S.; Nishiba, Y.; Terahara, N.; Suda, I. Involvement of anthocyanins and other phenolic compounds in radical-scavenging activity of purple-fleshed sweetpotato cultivars. *J. Food Sci.* **2002**, *67*, 1752–1756.

(6) Suda, I.; Oki, T.; Masuda, M.; Kobayashi, M.; Nishiba, Y.; Furuta, S. Physilological functionality of purple-fleshed sweet potatoes containing anthocyanins and their utilization in foods. *Jpn. Agric. Res. Q.* **2003**, *37*, 167–173.

(7) Zhang, Z.-F.; Fan, S.-H.; Zheng, Y.-L.; Lu, J.; Wu, D.-M.; Shan, Q.; Hu, B. Purple sweetpotato color attenuates oxidative stress and inflammatory response induced by D-galactose in mouse liver. *Food Chem. Toxicol.* **2009**, *47*, 496–501.

(8) Suda, I.; Ishikawa, F.; Hatakeyama, M.; Miyawaki, M.; Kudo, T.; Hirano, K.; Ito, A.; Yamakawa, O.; Horiuchi, S. Intake of purple sweetpotato beverage affects on serum hepatic biomarker levels of healthy adult men with borderline hepatitis. *Eur. J. Clin. Nutr.* **2008**, *62*, 60–67.

(9) Wu, D.; Lu, J.; Zheng, Y.; Zhou, Z.; Shan, Q.; Ma, D. Purple sweetpotato color repairs D-galactose-induced spatial learning and memory impairment by regulating the expression of synaptic proteins. *Neurobiol. Learn. Mem.* **2008**, *90*, 19–27.

(10) Steed, L. E.; Truong, V. D.; Simunovic, J.; Sandeep, K. P.; Kumar, P.; Cartwright, G. D.; Swartzel, K. R. Continuous flow microwave-assisted processing and aseptic packaging of purple-fleshed sweetpotato purees. *J. Food Sci.* **2008**, *73*, E455–E462.

(11) Shi, Z.; Bassa, I. A.; Gabriel, S. L.; Francis, F. J. Anthocyanin pigments of sweetpotatoes—*Ipomea batatas*. *J. Food Sci.* **1992**, *57*, 755–770.

(12) Tsukui, A.; Kuwano, K.; Mitamura, T. Anthocyanin pigment isolated from purple root of sweetpotato. *Nihon Kaseigaku Zasshi* **1983**, *34*, 153−159. CA99:102276.

(13) Odake, K.; Terahara, N.; Saito, N.; Toki, K.; Honda, T. Chemical structures of two anthocyanins from purple sweet potato, *Ipomoea batatas*. *Phytochemistry* **1992**, *31*, 2127–2130.

(14) Goda, Y.; Shimizu, T.; Kato, Y.; Nakamura, M.; Maitani, T.; Yamada, T.; Terahar, N.; Yamaguchi, M. Two acylated anthocyanins from purple sweet potato. *Phytochemistry* **1997**, *44*, 183–186.

(15) Harada, K.; Kano, M.; Takayanagi, T.; Yamakawa, O.; Ishikawa, F. Absorption of acylated anthocyanins in rats and humans after ingesting an extract of *Ipomoea batatas* purple sweetpotato tuber. *Biosci., Biotechnol., Biochem.* **2004**, *68*, 1500–1507.

(16) 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*, 4657–4664.

(17) Tian, Q.; Konczak, I.; Schwartz, S. J. Probing anthocyanin profiles in purple sweetpotato cell line (*Ipomea batatas* L. cv. Ayamurasaki) by High-performance liquid chromatography and electrospray ionization tandem mass spectrometry. *J. Agric. Food Chem.* **2005**, *53*, 6503–6509.

(18) Rodriguez-Saona, L.; Giusti, M.; Wrolstad, R. Anthocyanin pigment composition of red-fleshed potatoes. *J. Food Sci.* **1998**, *63*, 457–465.

(19) Truong, V. D.; McFeeters, R. F.; Thompson, R. T.; Dean, L. O.; Shofran, B. Phenolic acid content and composition in commercial sweetpotato (*Ipomea batatas* L.) cultivars in the United States. *J. Food Sci.* **2007**, *72*, C343–C349.

(20) Alasalvar, C.; Grigor, G. M.; Zhang, D.; Quantick, P. C.; Shahidi, F. Comparison of volatiles, phenolics, sugars, antioxidant vitamins and sensory quality of different colored carrot varieties. *J. Agric. Food Chem.* **2001**, *49*, 1410–1416.

(21) Wu, X.; Beecher, G.; Holden, J.; Haytowitz, D.; Gebhardt, S.; Prior, R. Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. *J. Agric. Food Chem.* **2006**, *54* (11), 4069–4075.

(22) Jang, J.; Ma, Y.; Shin, J.; Song, K. Characterization of polyphenol-oxidase extracted from *Solanum tuberosum* Jasim. *Food Sci. Biotechnol.* **2005**, *14* (1), 117–122.

(23) Yoshimoto, M.; Okuno, S.; Kumagi, T.; Yoshinaga, M.; Yamakawa, O. Distribution of antimutagenic components in colored sweetpotato. *Jpn. Agric. Res. Q.* **1999**, *33*, 143–148.

(24) Islam, Md. S.; Yoshimoto, M.; Terahara, N.; Yamakawa, O. Anthocyanin composition in sweetpotato (*Ipomoea batatas* L.) leaves. *Biosci., Biotechnol., Biochem.* **2002**, *66* (11), 2483–2486.

(25) Konczak-Islam, I.; Okuno, S.; Yoshimoto, M.; Yamakawa, O. Composition of phenolics and anthocyanins in a sweetpotato cell suspension culture. *Biochem. Eng. J.* **2003**, *14*, 155–161.

(26) Longo, L.; Vasapollo, G.; Rescio, L. Identification of anthocyanins in *Rhamnus alaternus* L. berries. *J. Agric. Food Chem.* **2005**, *53*, 1723–1727.

(27) Terahara, N.; Konczak, I.; Ono, H.; Yoshimoto, M.; Yamakawa, O. Characterization of acylated anthocyanins in callus induced from storage root of purple-fleshed sweetpotato, *Ipomoea batatas* L. J. *Biomed. Biotechnol.* **2004**, *2004* (5), 279–286.

(28) Dugo, P.; Mondello, L.; Errante, G.; Zappia, G.; Dugo, G. Identification of anthocyanins in berries by narrow-bore high-performance liquid chromatography with electrospray ionization detection. *J. Agric. Food Chem.* **2001**, *49*, 3987–3992.

(29) Terahara, N.; Shimizu, T.; Kato, Y.; Nakamura, M.; Maitani, T.; Yamaguchi, M.; Goda, Y. Six diacylated anthocyanins from the storage root of purple sweetpotato. *Phytochemistry* **1999**, *54*, 919–922.

(30) Lu, T. S.; Saito, N.; Yoloi, M.; Shigihara, A.; Honda, T. Acylated pelargonidin glycosides in red-purple flowers of *Pharbitis nil*. *Phytochemistry* **1992**, *31*, 289–295.

(31) Saito, N.; Tatsuzawa, F.; Yokoi, M.; Kasahara, K.; Iida, S.; Shigihara, A.; Honda, T. Acylated pelargonidin glycosides in red-purple flowers of *Ipomoea purpurea*. *Phytochemistry* **1996**, *43*, 1365–1370.

(32) Zhang, Z.; Kou, X.; Fugal, K.; McLaughlin, J. Comparison of HPLC Methods for Determination of anthocyanins and anthocyanidins in bilberry extracts. *J. Agric. Food Chem.* **2004**, *52* (4), 688–691.

(33) Wang, H.; Cao, G.; Prior, R. Oxygen radical absorbing capacity of anthocyanins. *J. Agric. Food Chem.* **1997**, *45*, 304–309.

(34) Yoshimoto, M.; Okuno, S.; Yamaguchi, M.; Yamakawa, O. Antimutagenicity of deacylated anthocyanins in purple-fleshed sweetpotatoes. *Biosci., Biotechnol., Biochem.* **2001**, *65*, 1652–1655.

Received for review August 11, 2009. Revised manuscript received November 19, 2009. Accepted November 20, 2009.