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Characterization and Quantitation of Anthocyanins in Purple-Fleshed Sweet Potatoes Cultivated in Korea by HPLC-DAD and HPLC-ESI-QTOF-MS/MS

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ABSTRACT: The characterization and quantitative analysis of anthocyanins in four purple-fleshed sweet potato varieties (Borami, Mokpo 62, Shinzami, and Zami) cultivated in Korea were carried out by HPLC/diode array detector (DAD), HPLC-TOF/MS, and HPLC-MS/MS analyses. For the identification of anthocyanins, molecular formulas were first calculated by using the exact mass data of the molecular ions ($[M]^+$). The patterns of isotope ions of M^+ were also monitored to confirm the assignment of the molecular formulas. HPLC-MS^2 analysis was further conducted for elucidating their molecular structures. Twenty-seven different anthocyanins were tentatively identified in the sweet potatoes. Six of them are the first reported in sweet potatoes roots. The quantity and profiles of anthocyanins in sweet potatoes varied greatly with variety. Borami was found, for the first time, to be a rare sweet potato variety with an exceptionally high quantity of pelargonidin-based anthocyanins.

KEYWORDS: *anthocyanin, high-performance liquid chromatography, time-of-flight mass spectrometry*

INTRODUCTION

Anthocyanins are a class of flavonoids responsible for the attractive red, blue, and purple colors in a range of fruits and vegetables. In nature, thousands of anthocyanins have been identified, whose structures vary with the number and variety of sugars attached to their basic anthocyanidin molecules, the position of this attachment, and the nature and number of aliphatic or phenolic acids attached to the sugar moiety in the molecules. Anthocyanins and anthocyanin-rich extracts could provide, reportedly, a range of health benefits such as protection from DNA damage,[1,2] anti-inflamatory activity,[3] anticancer activity,[4,5] antioxidative activity,[6−8] antidiabetes activity,[9,10] and prevention of cardiovascular and neuro-degenerative diseases.[11,12] The bioavailability and physiological and functional properties of the anthocyanins differ with their sources and molecular structures.[13−16]

Purple-fleshed sweet potatoes contain large quantities of anthocyanins.[17] Anthocyanin-rich sweet potato cultivars are mainly grown in Korea, Japan, and New Zealand.[18] Sweet potato anthocyanins possess high stability[19] and various health benefits such as antioxidative activity,[18,20,21] antimutagenicity,[22,23] antidiabetic activity,[24,25] and hepatoprotective activity.[26] To date, a total 25 anthocyanins have been identified in sweet potato (*Ipomoea batatas* L.) storage roots and cell lines.[18,27−30] The main anthocyanins in purple-fleshed sweet potatoes are 3,5-diglucoside derivatives of cyanidin or peonidin with acylated *p*-hydroxybenzoic acid, ferulic acid, or caffeic acid.[17] These acylated pigments constitute more than 93% of the total anthocyanin content in sweet potatoes.[17,31,36] Only small quantities of pelargonidin-based anthocyanins in purple-fleshed sweet potatoes have been reported previously.

In Korea, new purple-fleshed sweet potato varieties with high contents of anthocyanins have been developed during the last few decades and widely cultivated. Among them, important cultivars are Borami, Mokpo 62, Shinzami, and Zami varieties. The chemical composition of anthocyanins in the Shinzami variety has been reported after LC-DAD-ESI-MS analysis.[17] The authors reported 15 anthocyanins in the variety, which were composed of mono- or diacylated forms of *p*-hydroxybenzoic acid, caffeic acid, and/or ferulic acid with the basic structure of cyanidin 3-sophoroside-5-glucoside or peonidin 3-sophoroside-5-glucoside by single quadrupole mass spectrometry followed by a high-performance liquid chromatography.[17] However, the structure identification and quantitative analysis of anthocyanins in the other important purple-fleshed sweet potato varieties (Borami, Mokpo 62, and Zami) have never been previously carried out. Therefore, an urgent need still exists to analyze individual anthocyanins in these purple-fleshed sweet potatoes widely cultivated in Korea for the evaluation of their nutritional and health-promoting properties and commercial application values.

Thus, the objectives of this study were to characterize the anthocyanins in the four different purple-fleshed sweet potato varieties (Borami, Mokpo 62, Shinzami, and Zami) cultivated in Korea by HPLC-TOF/MS, HPLC/MS/MS, and UV/vis spectroscopy and to quantitate the individual and total anthocyanins in these purple-fleshed sweet potatoes by HPLC/DAD analysis.

MATERIALS AND METHODS

Chemicals and Materials. The HPLC-grade acetonitrile and water were obtained from Fisher Scientific (Pittsburgh, PA, USA). Formic acid was purchased from Sigma (St. Louis, MO, USA). Cyanidin 3-*O*-glycoside was obtained from Extrasynthese (Z.I Lyon

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Nord, French). Four varieties of purple-fleshed sweet potato (Borami, Mokpo 62, Shinzami, and Zami) were obtained from Iksan-si Agricultural Technology Service Center located in Iksan, Korea.

Sample Extraction. On the day of harvest, sweet potatoes were first sliced into thin pieces (ca. 4.0 mm thickness), then freeze-dried to minimize changes in the anthocyanins. The freeze-dried samples were ground to make a fine powder. Solvent extraction was carried out by the common extraction method for anthocyanins from the plant sources with a solvent of 0.2% concentrated HCl in methanol. Five-hundred milligrams of the dried purple sweet potato powders was weighed, in duplicate, in a 15 mL capacity tube. Then, 5 mL of extracting solvent with 0.2% HCl in MeOH was added to the sample tube. The sample tubes were capped, mixed briefly, and then placed on a shaker (EYELA MMV-1000W, Tokyo, Japan) at a speed of 280 rpm for 2 h. Then, the samples were centrifuged at 2224.5*g* for 20 min at 5 °C with a refrigerated multipurpose centrifuge (Combi-514R, Hanil Science, Seoul, Korea). The supernatant extract was transferred into a 50 mL tube. The extraction process was repeated seven more times until no more red color was observed in the solvent. The extracts were then pooled into the 50 mL capacity volumetric flask and were brought to 50 mL with the extraction solvent.

Anthocyanin Purification. Ten milliliters of the sweet potato extract and 2 mL of H_2O were transferred into a 15 mL tube. Then, the solvent was evaporated to 1.5 mL under nitrogen flush. A Sep-Pak C18 cartridge (Waters, Milford, MA, USA) was flushed with 2 mL of MeOH, followed by the addition of 2 mL of H_2O for activation. After loading of the evaporated sample, the cartridge was washed with 4 mL of H_2O and eluted with 1 mL of 0.2% HCl in MeOH. The eluted anthocyanin fraction was concentrated to dryness using N_2 gas in an evaporator. Then, the anthocyanins were dissolved with 1 mL of a solvent mixture of H_2O and MeOH (1:1, v/v) prior to HPLC-TOF/MS and HPLC-MS^2 analysis.[17]

HPLC/DAD for Separation and Quantitation of Anthocyanins. The HPLC/DAD analyses were performed on a HPLC equipped with a 1200 Series diode array detector (Agilent Technologies Inc., Santa Clara, CA, USA). The column used was a 150 mm × 4.6 mm i.d., S-3 μm, YMC-Pack Pro C8 (YMC Co., Ltd. Shimogyo-ku, Kyoto, Japan). The temperature-programmable column oven was used to maintain the column temperature at 30 °C during the HPLC analysis. The injection volume of the prepared sample was 10 μL. To determine the contents of anthocyanins, the extracted samples without Sep-Pak C18 cartridge purification were injected into the HPLC to avoid the possible loss of anthocyanins during the purification procedures. Solvent A was formic acid/water (5:95), and solvent B was formic acid/acetonitrile (5:95). The solvent gradient was 0 to 10 min, 5 to 10% B; 10 to 30 min, 10 to 15% B; 30 to 40 min, 15 to 15% B; 40 to 50 min, 15 to 20% B; 50 to 60 min, 20 to 30% B; 60 to 65 min, 30% B; and 65 to 70 min, 30 to 5% B.[32] Quantitation of individual anthocyanin in sweet potatoes was obtained by HPLC-DAD at 520 nm using the standard calibration curve based on the molar concentration with authentic cyanidin 3-glucoside. The contents of anthocyanins were then expressed as mg cyanidin 3-glucoside equivalent/100 g dry weight sweet potato.

HPLC-DAD-TOF/MS Analysis for Characterization. HPLC-TOF/MS analysis was performed on an UltiMate 3000 Series system HPLC (Dionex Technologies, Sunnyvale, CA, USA) equipped with a DAD (Dionex Technologies) and an ESI-QTOF/MS (electrospray ionization-quadrupole-time-of-flight mass spectrometer, micrOTOF-QII, Bruker Daltonik, Bremen, Germany) in series in the same chromatographic line.[33] The HPLC column and conditions for the mobile phase gradient were the same as those in HPLC/DAD analysis. The HPLC column oven temperature was maintained at 30 °C.[32] Mass spectra in the m/z range 100−1500 were obtained by electrospray ionization in positive-ion mode. The mass spectrometric conditions were optimized as follows: gas temperature 220 °C, drying gas flow rate 10.0 L min, nebulizer gas pressure 1.5 bar, and capillary and fragmentor potentials 4000 and 220 V, respectively. The mass axis was calibrated using the internal calibration solution (1 N lithium formate in a solvent mixture of 50% propanol in water).

HPLC-MS^2 Analysis for Characterization. The HPLC-MS^2 analysis was performed with the same instrument (micrOTOF-QII, Bruker Daltonik, Bremen, Germany) as described above. The mass spectrometric conditions for MS^2 were identical to those for TOF/MS analysis. For obtaining MS/MS fragments ions, argon was used as a collision gas. The collision energies were optimized for individual analytes by performing the repeated analysis. The optimal collision energies used were 10−25 eV in MS/MS analysis depending on the anthocyanins as shown in Table 1. The other mass spectomeric conditions were as follows: gas temperature 220 °C, drying gas flow rate 10.0 L min, nebulizer gas pressure 1.5 bar, and capillary and fragmentor potentials 4000 and 220 V, respectively. Mass spectra in the range m/z 100− 1500 were obtained by electrospray ionization in positive-ion mode.

RESULTS AND DISCUSSION

HPLC for Separation of Individual Anthocyanins. An HPLC reverse phase C18 column has been exclusively used for the separation of anthocyanins in sweet potatoes in the previous papers.[17−19,27−30] The two most commonly used HPLC mobile phases were formic acid in water/formic acid in acetonitrile and formic acid in water/formic acid in methanol for the separation of anthocyanins. However, we could not obtain the satisfactory separation of the anthocyanins in the sweet potatoes with a C18 column with the reported mobile phases. Thus, we decided to switch the C18 column to a C8 column. After several trials with the C8 column, we managed to achieve the satisfactory separation of anthocyanins in purple-fleshed sweet potatoes using the mobile phase system of formic acid in water/formic acid in acetonitrile. Since the C8 column has less hydrophobic properties than the C18 column, the less hydrophobic nature of the C8 column would be more appropriate for the separation of the anthocyanins, which have relatively polar properties, than the highly hydrophobic C18 column. Figure 1 shows the HPLC chromatograms of anthocyanins in the purple-fleshed sweet potato varieties obtained by C8 columns. The HPLC chromatograms showed a great difference in anthocyanin profiles among the sweet potato varieties (Figure 1). The results suggested the considerable variations in the compositions of anthocyanins among the sweet potato varieties.

Characterization of Anthocyanins. Twenty-seven different anthocyanins in the four different varieties (Borami, Mokpo 62, Shinzami, and Zami) were elucidated by a combination of high-resolution TOF/MS and MS/MS spectra and UV/vis scanning spectra. An LC-MS library of anthocyanins in sweet potatoes previously established[17] was modified and used as a reference for the efficient identification of anthocyanins in this study. Mass spectra in the range m/z 200−1500 were acquired with an LC-QTOF MS by use of electrospray ionization (ESI) in positive-ion mode. Mass spectrometric conditions such as capillary and fragmentor potential, nebulizer gas pressure, and drying gas flow rate were optimized to achieve maximum sensitivity for the components. The anthocyanins corresponding to the peaks (520 nm) in the HPLC-DAD chromatograms were first tentatively identified by the exact mass measurements of the molecular ions ($[M]^+$) obtained from the single TOF-MS analysis. In this study, excellent agreement was obtained between theoretical and actual experimental mass data of the peaks. The accuracy for confirmation of elemental compositions was less than ±5 ppm mass error, showing a high mass accuracy. The high resolution of TOF/MS allows the generation of the molecular formulas with the obtained molecular ion data. Besides exact mass measurements, the

Table 1. Identification of Anthocyanins in Purple Sweet Potato Using HPLC-ESI-MS, MS^2, and UV/Vis Scanning Spectra

			TOF/MS				MS/MS	DAD				
peak	t_R (min)	formula	theor mass (m/z)	exptl mass (m/z)	error (ppm)	collsion energy (eV)	fragment ions (m/z)	vis-max (nm)	A330/Avis-max	A440/Avis-max	identification[a]	found[c]
1	10.36	$C_{33}H_{41}O_{21}$	773.2135	773.2139	0.5	10	611.1637/449.1085/287.0554	514	0	19.44	Cy 3-soph-5-glc	B, M, S, Z
2	12.82	$C_{33}H_{41}O_{20}$	757.2186	757.2173	−1.7	10	595.1657/433.1111/271.0601	500	0	20.33	Pg 3-soph-5-glc	B
3	13.81	$C_{34}H_{43}O_{21}$	787.2291	787.2291	0.0	10	625.1753/463.1238/301.0703	512	0	12.41	Peo 3-soph-5-glc	B, M, S, Z
4	15.91	$C_{40}H_{45}O_{23}$	893.2346	893.2349	0.3	15	731.1835/449.1021/287.0569	520	93.33	13.33	Cy 3-*p*-hydroxybenzoylsoph-5-glc	B, M, S, Z
5	16.36	$C_{42}H_{47}O_{24}$	935.2452	935.2454	0.2	15	773.1872/449.1067/287.0560	520	48.84	18.60	Cy 3-(6‴-caffeoyl soph)-5-glc	B, S, Z
6	20.23	$C_{41}H_{47}O_{23}$	907.2503	907.2504	0.1	15	745.1922/463.1245/301.0692	510	83.33	13.33	Peo 3-*p*-hydroxybenzoylsoph-5-glc	B, M, S, Z
7	20.49	$C_{43}H_{49}O_{24}$	949.2608	949.2602	−0.6	15	787.2052/463.1219/301.0696	522	66.67	16.67	Peo 3-(6‴-caffeoyl soph)-5-glc	B, M, S, Z
8	20.67	$C_{42}H_{47}O_{23}$	919.2503	919.2493	−1.1	15	757.1951/433.1097/271.0595	506	63.00	22.50	Pg 3-(6‴-caffeoyl soph)-5-glc[b]	B
9	21.14	$C_{42}H_{47}O_{23}$	919.2503	919.2496	−0.8	15	757.1902/449.1028/287.0511	520			Cy 3-*p*-coumaryl soph-5-glc	B, Z
10	21.93	$C_{43}H_{49}O_{24}$	949.2608	949.2594	−1.5	15	787.2044/449.1057/287.0521	520	36.73	14.29	Cy 3-feruloyl soph-5-glc	B, M, S, Z
11	26.47	$C_{43}H_{49}O_{23}$	933.2659	933.2633	−2.8	15	771.2191/463.1224/301.0621	520			Peo 3-*p*-coumaryl soph-5-glc	B, S
12	27.07	$C_{44}H_{51}O_{24}$	963.2765	963.2760	−0.5	15	801.2216/463.1210/301.0718	518	63.98	15.31	Peo 3-feruloyl soph-5-glc	B, M, S, Z
13	27.69	$C_{43}H_{49}O_{23}$	933.2659	933.2653	−0.6	15	771.2131/433.1122/271.0603	506	63.93	22.13	Pg 3-feruloyl soph-5-glc	B
14	29.11	$C_{51}H_{53}O_{27}$	1097.2769	1097.2770	0.1	25	935.2267/449.1067/287.0548	528	106.92	18.27	Cy 3-dicaffeoyl soph-5-glc	B, S, Z
15	29.53	$C_{42}H_{47}O_{24}$	935.2452	935.2440	−1.3	15	773.1940/449.1057/287.0540	522	57.60	18.89	Cy 3-caffeoyl soph-5-glc	B, M, S, Z
16	29.73	$C_{49}H_{51}O_{26}$	1055.2663	1055.2657	−0.6	25	893.2171/449.1091/287.0544	526	37.65	17.65	Cy 3-caffeoyl-*p*-hydroxybenzoyl soph-5-glc	B, M, S, Z
17	33.26	$C_{52}H_{55}O_{27}$	1111.2925	1111.2922	−0.3	25	949.2361/449.1070/287.0547	530	103.33	19.17	Cy 3-caffeoyl-feruloylsoph-5-glc	B, M, S, Z
18	33.92	$C_{42}H_{47}O_{23}$	919.2503	919.2502	−0.1	15	757.1962/433.1114/271.0597	506	56.46	25.07	Pg 3-caffeoyl soph-5-glc[b]	B, M, Z
19	34.78	$C_{43}H_{49}O_{24}$	949.2608	949.2602	−0.6	15	787.2064/463.1245/301.0691	522	57.89	16.67	Peo 3-caffeoyl soph-5-glc	B, M, S, Z
20	35.04	$C_{52}H_{55}O_{27}$	1111.2925	1111.2927	0.2	25	949.2338/463.1238/301.0687	528	95.19	15.81	Peo 3-dicaffeoyl soph-5-glc	B, M, S, Z
21	35.19	$C_{51}H_{53}O_{26}$	1081.2820	1081.2819	−0.1	25	919.2217/433.1108/271.0601	510	106.50	27.35	Pg 3-dicaffeoyl soph-5-glc[b]	B, Z
22	36.26	$C_{50}H_{53}O_{26}$	1069.2820	1069.2811	−0.8	25	907.2269/463.1228/301.0690	525	55.65	16.30	Peo 3-caffeoyl-*p*-hydroxybenzoyl soph-5-glc	B, M, S, Z
23	36.45	$C_{49}H_{51}O_{25}$	1039.2714	1039.2713	−0.1	25	877.2200/433.1109/271.0588	508	51.75	29.82	Pg 3-caffeoyl-*p*-hydroxybenzoyl soph-5-glc[b]	B

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Table 1. continued

peak	t_R (min)	formula	TOF/MS theor mass (m/z)	TOF/MS exptl mass (m/z)	TOF/MS error (ppm)	MS/MS collision energy (eV)	MS/MS fragment ions (m/z)	DAD vis-max (nm)	DAD A330/Avis-max	DAD A440/Avis-max	identification[a]	found[c]
24	39.81	$C_{53}H_{57}O_{27}$	1125.3082	1125.3089	0.6	25	963.2522/463.1226/301.0687	528	90.86	14.29	Peo 3-caffeoyl-feruloylsoph-5-glc	B, M, S, Z
25	40.93	$C_{52}H_{55}O_{26}$	1095.2976	1095.2976	0.0	25	933.2549/463.1226/301.0679	526			Peo 3-caffeoyl-*p*-coumaryl soph-5-glc	B
26	41.49	$C_{52}H_{55}O_{26}$	1095.2976	1095.2977	0.1	25	933.2459/433.1105/271.0601	506	118.28	27.96	Pg 3-caffeoyl-feruloylsoph-5-glc[b]	B
27	42.6	$C_{51}H_{53}O_{25}$	1065.2870	1065.2863	−0.7	25	903.2390/433.1164/271.0591	506			Pg 3-caffeoyl-*p*-coumaryl soph-5-glc[b]	B, Z

[a]Cy = cyanidin, Peo = peonidin, Pg = pelargonidin, soph = sophorside, glc = glucoside. [b]Anthocyanins identified for the first time in sweet potatoes in this paper. [c]B = Borami, M = Mokpo, S = Shinzami, Z = Zami.

abundances of the isotope peaks of the molecular ions ($[M]^+$) were also monitored to confirm the identities of the compounds. Furthermore, LC-MS^2 analyses were carried out for structural identifications by using the data of the fragment patterns, in which the acylated phenolic acids such as *p*-hydroxybenzoic acid (m/z 120.0206), caffeic acid (m/z 162.0311), and ferulic acid (m/z 176.0468) were cleaved from their structures along with glucoside (m/z 162.0523) or sophoroside (m/z 324.1051).[17,34−36] UV/vis scanning spectra also provided valuable information supporting the characterization of individual anthocyanins. In general, diacylated anthocyanins exhibited a higher $A_{330}/A_{vis\text{-}max}$ (absorbance at 330 nm/absorbance at maximum visible wavelength) than monoacylated anthocyanins. Non-acylated anthocyanin did not exhibit distinct absorbance at 330 nm. Maximum visible wavelength (vis-max) and absorbance at 440 nm/absorbance at maximum visible wavelength ($A_{440}/A_{vis\text{-}max}$) also provided important information for the structures of the anthocyanins.[32] Thus, the UV/vis spectra were used as additional evidence for the structural identifications.

Table 1 summarizes the peak identification of each peak in purple sweet potatoes. Some of them (peaks 5, 15, 7, 10, 19, and 22) are discussed here as typical examples to demonstrate identification of anthocyanins. Figure 2 shows the HPLC-TOF-MS spectra, isotope pattern of $[M]^+$ ion, HPLC-MS^2 spectra, and UV/vis scanning spectra of peaks 5 and 15. The TOF/MS data showed that peaks 5 and 15 had virtually the same molecular ion (m/z 935.2454 and 935.2440, respectively), which was calculated as a molecular formula of $C_{42}H_{47}O_{24}$. Furthermore, the experimental isotope information (exact masses and pattern of isotopes) of the $[M]^+$ ion was exactly matched with those of its theoretical isotope information (exact masses and pattern of isotopes) within the tolerable error ranges (Figure 2), confirming the correct assignment of its molecular formula. The MS^2 analysis showed that peaks 5 and 15 also had the same fragment ions. In the MS^2 analysis, the molecular ion of peaks 5 and 15 was fragmented to three product ions, 773.1940 ($[M - C_6H_{10}O_5]^+$), 449.1057 ($[M - C_9H_6O_3 - C_{12}H_{20}O_{10}]^+$), and 287.0540 ($[M - C_9H_6O_3 - C_{12}H_{20}O_{10} - C_6H_{10}O_5]^+$), corresponding to cyanidin caffeoyl-sophoroside, cyanidin glucoside, and cyanidin, respectively. Thus, both peaks 5 and 15 were assigned as cyanidin caffeoylsophoroside glucoside. These two anthocyanins seemed to be isomers with different acylation sites of their sophoroside moiety. The values of $A_{440}/A_{vis\text{-}max}$, $A_{330}/A_{vis\text{-}max}$, and maximum wavelength of peaks 5 and 15 were identical, showing their molecular similarity. The values of $A_{440}/A_{vis\text{-}max}$ and $A_{330}/A_{vis\text{-}max}$ showed that peaks 5 and 15 were acylated anthocyanin (Figure 2, Table 1).[35] Furthermore, the maximum wavelength of the peak was in good agreement with the previously reported value for cyanidin caffeoyl sophoroside glucoside.[35] The UV/vis spectroscopic data were supporting evidence for their structural identifications. Previous studies using NMR analysis also identified peaks 5 and 15 both as cyanidin 3-caffeoyl sophoroside-5-glucoside, but acylation occurred at a different position of the sophoroside moiety.[28,29] Peak 5 has been identified as cyanidin 3-(6‴-caffeoyl sophoroside)-5-glucoside,[29] and peak 15 has been identified as cyanidin 3-(6″-caffeoyl sophoroside)-5-glucoside.[28] These two anthocyanins (peaks 5 and 15) also have been reported in the purple sweet potato cell line.[35] It has been reported that the anthocyanin with an acylation site at the 6‴ position was eluted earlier than the anthyocyanin with acylation at the 6″ position.[28,29,35] Thus,

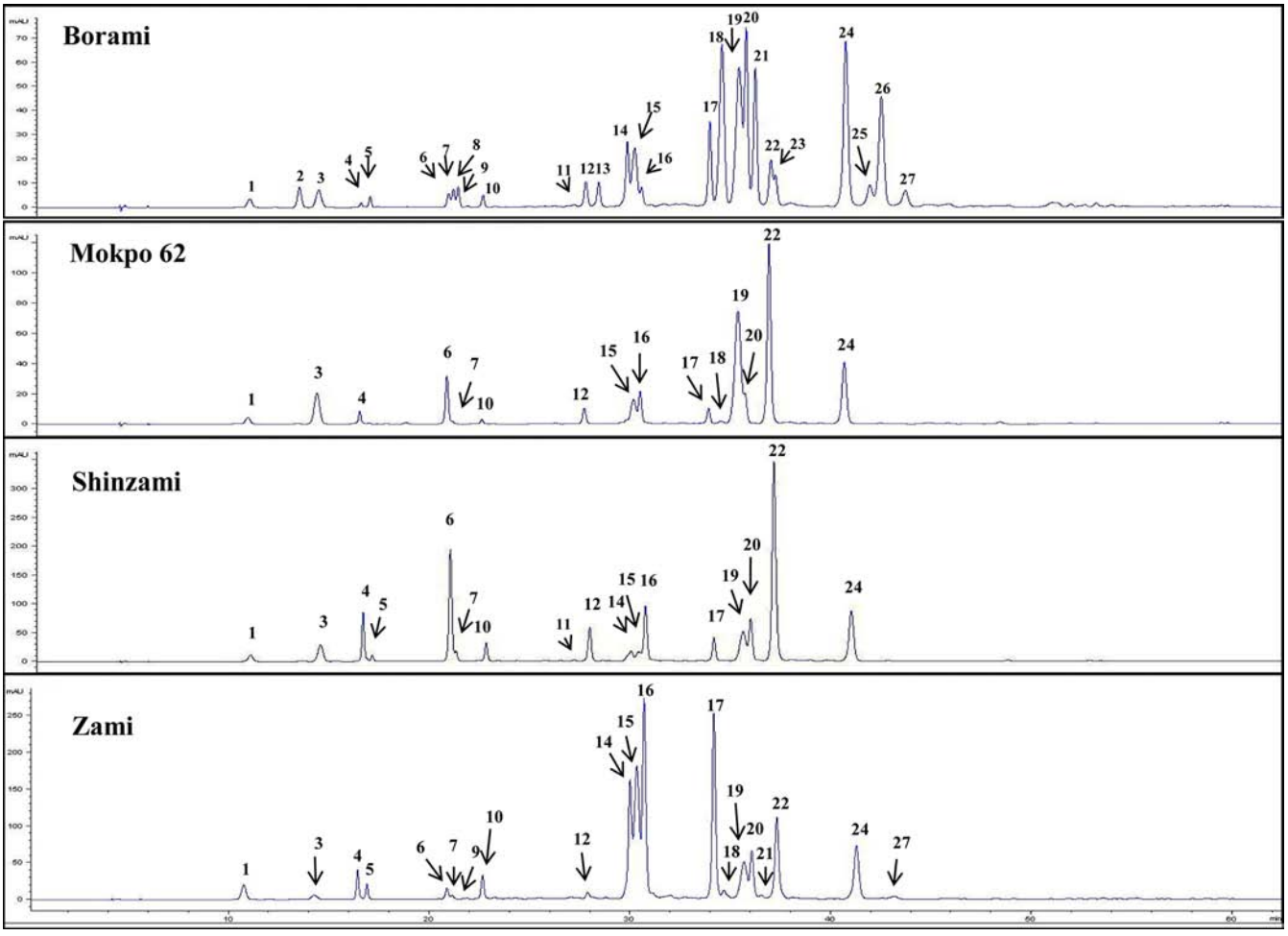

Figure 1. HPLC-DAD chromatogram of anthocyanins in purple-fleshed sweet potatoes (Borami, Mokpo 62, Shinzami, and Zami) cultivated in Korea.

peaks 5 and 15 were tentatively assigned as cyanidin 3-(6‴-caffeoyl sophoroside)-5-glucoside and cyanidin 3-(6″-caffeoyl sophoroside)-5-glucoside, respectively. In this research, we also found similar differences in acylation positions in the anthocyanins of peaks 7 and 19 and peaks 8 and 18.

Peaks 7, 10, and 19 showed virtually the same mass data (*m*/*z* 949.2602, 949.2594, and 949.2602, respectively) in the LC-TOF/MS analysis (Figure 3), suggesting that they had the same molecular formula of $C_{43}H_{49}O_{24}$. The experimental mass values obtained for these molecular ions were very close to the theoretical value (*m*/*z* 949.2608) of $C_{43}H_{49}O_{24}$ with an error of less than 1.5 ppm. The molecular formula was further confirmed by comparing the obtained ratios and abundance of the isotope ions for $[M]^+$ with those of theoretical values. The experimental mass data and abundance of isotope ions for the $[M]^+$ ion in samples were exactly matched with the theoretical values within a tolerable error range (Figure 3, Table 1), confirming the correct calculation of the molecular formula. The MS^2 analysis showed that peaks 7 and 19 had the same fragment ion pattern. However, peak 10 showed a completely different fragment ion pattern, indicating the difference in its structure from the others. The molecular ion of peaks 7 and 19 was fragmented to three product ions, 787.2064 ($[M - C_6H_{10}O_5]^+$), 463.1245 ($[M - C_9H_6O_3 - C_{12}H_{20}O_{10}]^+$), and 301.0691 ($[M - C_9H_6O_3 - C_{12}H_{20}O_{10} - C_6H_{10}O_5]^+$), corresponding to peonidin caffeoyl sophoroside, peonidin glucoside, and peonidin, respectively. Thus, peaks 7 and 19 both were assigned as peonidin 3-caffeoyl sophoroside-5-glucoside; however, the acylation may occur at different positions of the sophoroside moiety. The MS^2 spectrum showed that the molecular ion at *m*/*z* 949.2608 of peak 10 was fragmented to three product ions, 787.2044 ($[M - C_6H_{10}O_5]^+$), 449.1057 ($[M - C_{10}H_8O_3 - C_{12}H_{20}O_{10}]^+$), and 287.0521 ($[M - C_{10}H_8O_3 - C_{12}H_{20}O_{10} - C_6H_{10}O_5]^+$), corresponding to cyanidin feruloylsophoroside, cyanidin glucoside, and cyanidin, respectively. Thus, peak 10 could be assigned to cyandin feruloyl sophoroside glucoside. The UV/vis scanning spectra showed additional evidence for the structural identification for peaks 7, 10, and 19. Both peaks 7 and 19 showed the same maximum wavelength (vis-max) of 522 nm, indicating their structural similarity. Furthermore, the data of $A_{440}/A_{\text{vis-max}}$ and $A_{330}/A_{\text{vis-max}}$ for peaks 7 and 19 were very close to each other. However, the vis-max of peak 10 (520 nm) was different from those of peaks 7 and 19 (522 nm).

As shown in Table 1 and Figure 4, peak 21 had a molecular ion at *m*/*z* 1081.2819, which was calculated as a molecular formula of $C_{51}H_{53}O_{26}$. The experimental mass value (*m*/*z* 1081.2819) obtained for peak 21 was closely matched with the theoretical value (*m*/*z* 1081.2820) of $C_{51}H_{53}O_{26}$ with an error of −0.1 ppm. Furthermore, the experimental isotope information (exact masses and pattern of isotopes) of the $[M]^+$ ion was exactly matched with its theoretical isotope

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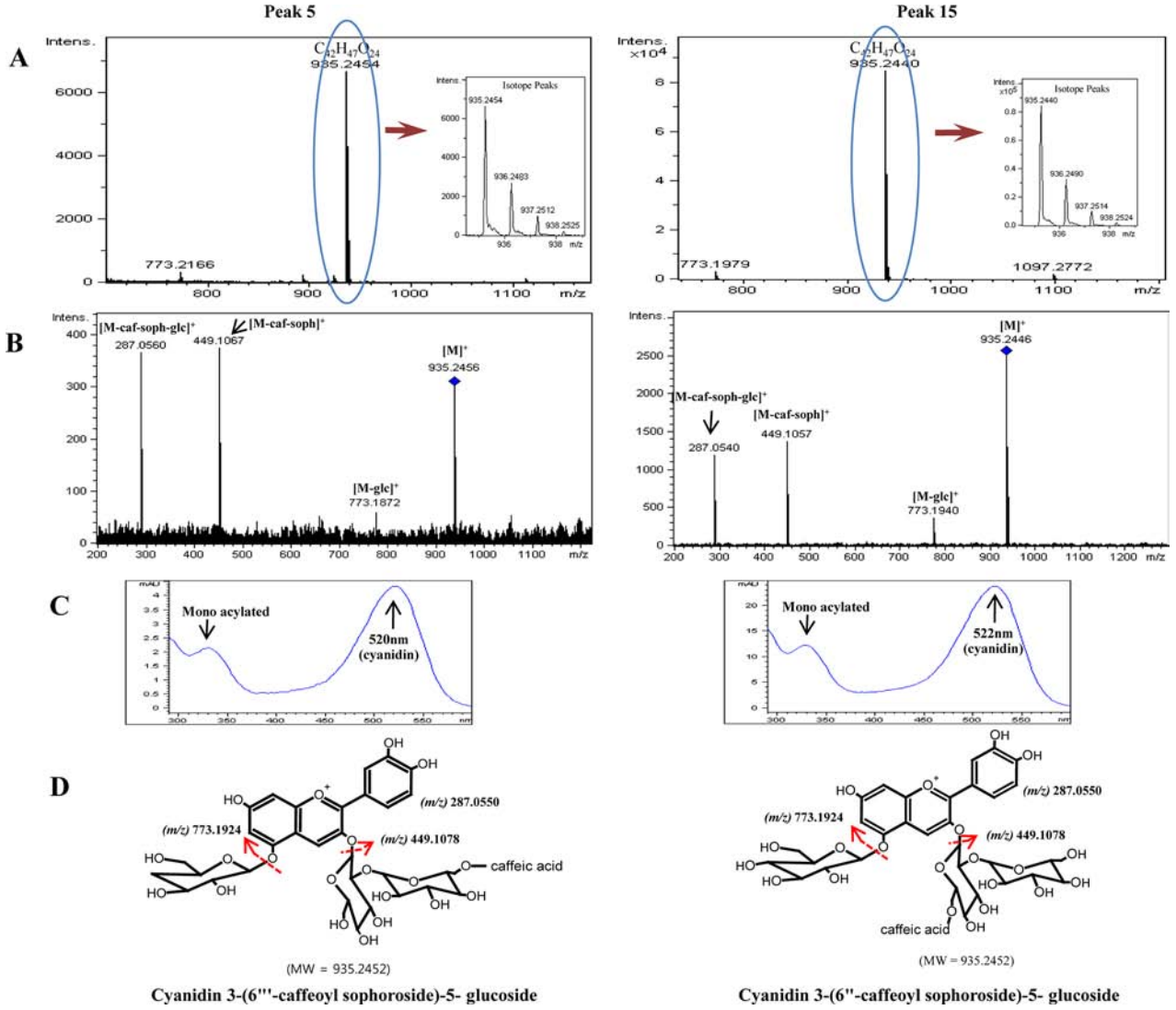

Figure 2. (A) HPLC-TOF/MS; (B) HPLC-MS/MS; (C) UV/vis scanning spectra; and (D) chemical structures of peaks 5 and 15.

information (exact masses and pattern of isotopes) within tolerable error ranges, confirming the correct assignment of its molecular formula ($C_{51}H_{53}O_{26}$). The MS^2 spectrum showed that the molecular ion at m/z 1081.2819 was fragmented to three product ions, 919.2217 ($[M - C_6H_{10}O_5]^+$), 433.1108 ($[M - C_9H_6O_3 - C_{12}H_{20}O_{10}]^+$), and 271.0601 ($[M - C_9H_6O_3 - C_{12}H_{20}O_{10} - C_6H_{10}O_5]^+$), corresponding to pelargonidin dicaffeoylsophoroside, pelargonidin glucoside, and pelargonidin, respectively. Therefore, peak 21 was assigned as pelargonidin 3-(dicaffeoyl sophoroside)-5-glucoside. UV/vis scanning data further confirmed the structural identification of peak 21. Peak 21 showed a visible maximum at 510 nm, which indicated the pelargonidin structure (Figure 4). The values of $A_{440}/A_{vis\text{-}max}$ of 27.4% and $A_{330}/A_{vis\text{-}max}$ of 106.5% indicated the diacylated structure with diglycoside at positions 3 and 5 of anthocyanidin (Figure 4).

In a similar manner, we have isolated and identified 27 anthocyanins in Korean purple-fleshed sweet potatoes. To our knowledge, six of them are the first reported anthocyanins in sweet potatoes roots. In sweet potato varieties Borami, Mokpo 62, Shinzami, and Zami, 27, 15, 17, and 20 anthocyanins were identified, respectively. We found the varieties of sweet potatotes contained a range of anthocyanins. Note that Borami contained 27 anthocyanins. To date, a total of 25 anthocyanins have been identified in sweet potatoes (*Ipomoea batatas* L.) storage roots and cell lines.[18,27−30] Nine and eight anthocyanins mono- or diacylated with caffeic acid, *p*-hydroxybenzoic acid, and ferulic acid have been identified previously in Ayamurasaki[30,37−39] and Yamagawa murasaki,[40,41] respectively. It has been reported that there are 13, 10, and 11 different anthocyanins in purple-fleshed sweet potato varieties of Stoke purple sweet potato, Okinawa, and NC 415, respectively.[36] Chiran murasaki sweet potato has been also reported to have eight anthocyanins.[42−44] Recently, in Shinzami (Korean purple-fleshed sweet potato variety), 15 anthocyanins were isolated and identified.[17] Among the previously reported anthocyanins in Shinzami, peonidin-3-feruloyl *p*-hydrobenzoyl sophoroside 5-glucoside was not detected in our present research. In this report, however, two other anthocyanins (cyanidin-6‴-caffeoyl sophoroside-5-glucoside and peonidin caffeoyl sophoroside 5-glucoside) were tentatively newly identified in Shinzami. Previous research demonstrated that sweet potato cell line suspension culture contained a much more complex mixture of anthocyanins than its original storage roots.[35] In the present research, six pelargonidin-based anthocyanins (pelargonidin 3-(6‴-caffeoyl sophoroside)-5-glucoside, pelargonidin 3-dicaffeo-

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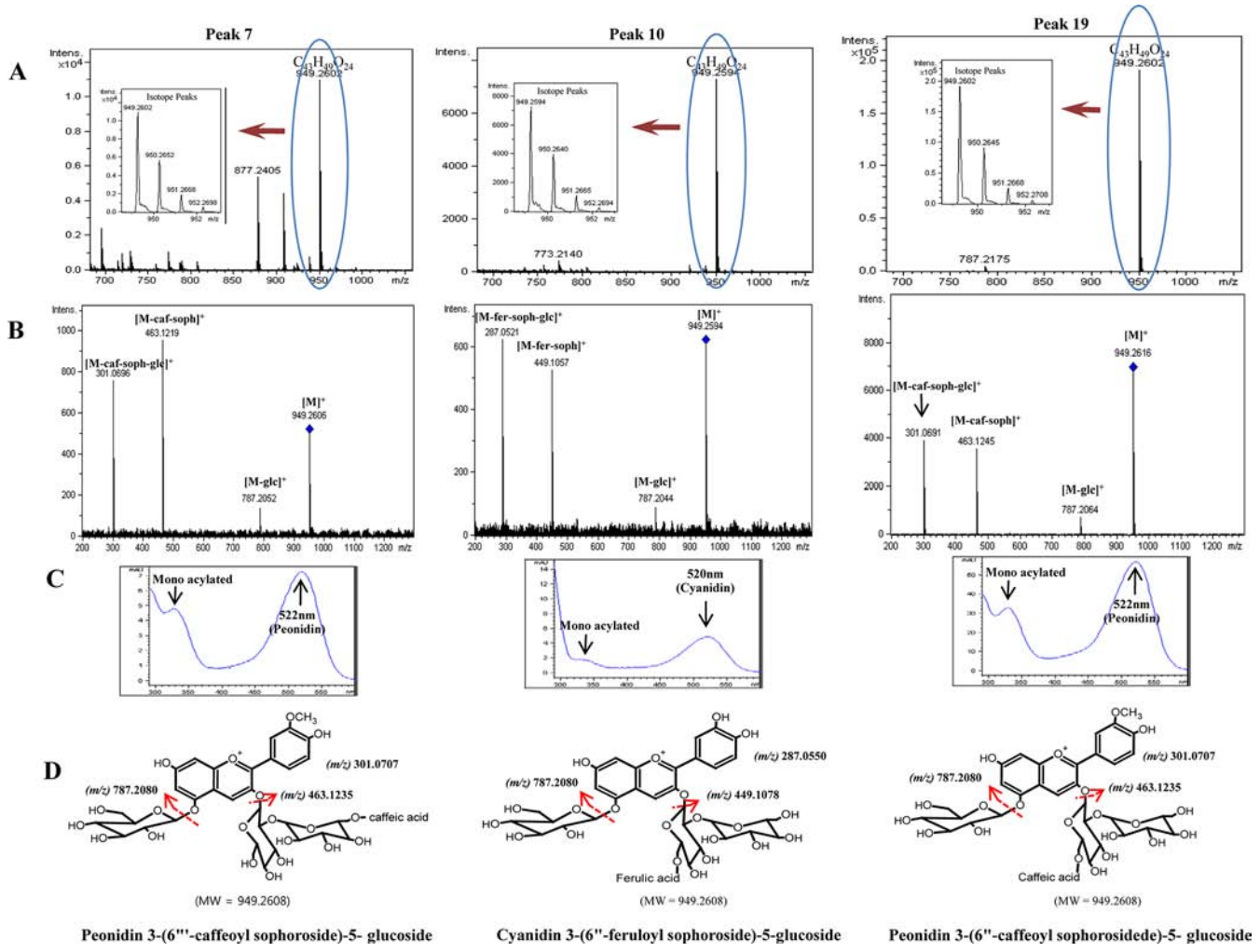

Figure 3. (A) HPLC-TOF/MS; (B) HPLC-MS/MS; (C) UV/vis scanning spectra; and (D) chemical structures of peaks 7, 10, and 19.

yl sophoroside-5-glucoside, pelargonidin 3-caffeoyl-*p*-hydroxybenzoyl sophoroside-5-glucoside, pelargonidin 3-caffeoyl-feruloyl sophoroside-5-glucoside, pelargonidin 3-caffeoyl-*p*-coumaryl sophoroside-5-glucoside, and pelargonidin 3-caffeoyl-*p*-coumaryl sophoroside-5-glucoside) were partially identified for the first time in purple-fleshed sweet potatoes. Two pelargonidin-based anthocyanins (peaks 2 and 13) identified here have also been reported in the previous studies.[17,34] However, the other six pelargonidin-based anthocyanins we found have never been identified previously in either storage roots or cell line suspension cultures. Pelagondin sophoroside glucosides acylated with caffeic acid, ferulic acid, and/or coumaroic acid have been reported previously to be present in red beets.[45,46]

Quantitation of Anthocyanins in Sweet Potatoes. Table 2 shows the contents of individual anthocyanins in sweet potatoes. The contents of anthocyanins were expressed as mg cyanidin-3-glucoside equivalent/100 g dry weight sweet potato. The total anthocyanin contents in the analyzed sweet potatoes varied with the variety, ranging from 383.2 mg/100 g to 1190.2 mg/100 g dry weight (DW). Zami showed the highest quantity of anthocynins (1190.2 mg/100 g DW), followed by Shinzami (964.3 mg/100 g DW), Borami (596.7 mg/100 g DW), and Mokpo 62 (383.2 mg/100 g DW) in decreasing order. The previously reported anthocyanin content in Shinzami cultivated in Korea was 1342 mg/100 g DW.[17] Our present data showed a similar quantity of anthocyanins in Shinzami to the previous reported value. As shown in Table 2, mono- and diacylated anthocyanins were major species, ranging from 93.36% to 98.18% of the total anthocyanins. The contents of non-acylated anthocyanins were found to be less than 6.4% of total anthocyanins in the sweet potato varieties. This result is in good agreement with the previous reports.[17,31,36] It has been reported that acylated anthocyanins were the main constituents among total anthocyanins in purple-fleshed sweet potatoes.[17,31,36] Borami contained the highest number of anthocyanins, followed by Zami, Shinzami, and Mokpo 62, in decreasing order. The numbers of anthocyanins found in Borami, Mokpo 62, Shinzami, and Zami were 27, 15, 17, and 20, respectively. In Borami, peonidin 3-caffeoyl-feruloylsophoroside-5-glucoside (peak 24) is the most abundant anthocyanin species, followed by peonidin 3-caffeoylsophoroside-5-glucoside (peak 19), pelargonidin 3-caffeoylsophoroside-5-glucoside (peak 18), peonidin 3-dicaffeoylsophoroside-5-glucoside (peak 20), pelargonidin 3-caffeoyl-feruloylsophoroside-5-glucoside (peak 26), and pelargonidin 3-dicaffeoylsophoroside-5-glucoside (peak 21), in decreasing order. These six major anthocyanins represented 66.8% of the total anthocyanins in Borami. It is interesting to note that Borami contained an exceptionally high quantity of pelargonidin-based anthocyanins (217.0 mg/100 g DW) (Table 2). It has been reported that the anthocyanins in sweet potatoes were almost exclusively

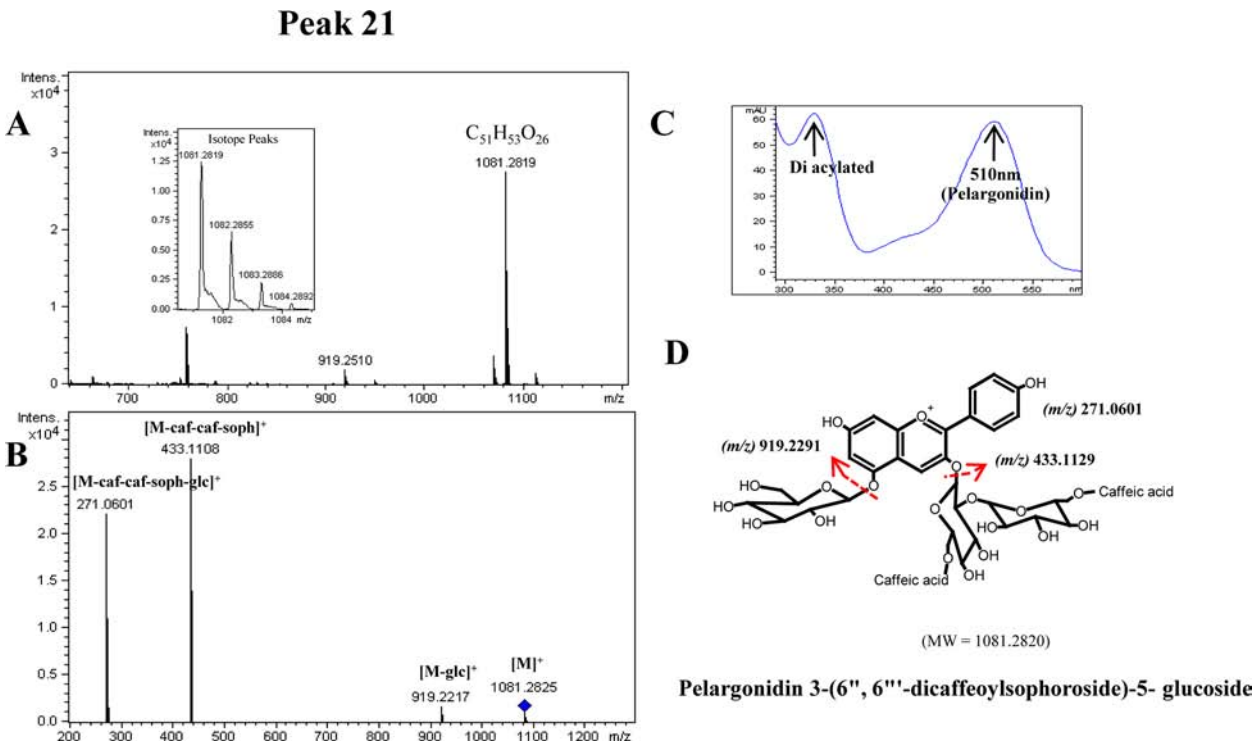

Figure 4. (A) HPLC-TOF/MS; (B) HPLC-MS/MS; (C) UV/vis scanning spectra; and (D) chemical structure of peak **21**.

cyanidin or peonidin 3-sophoroside 5-glucoside acylated with *p*-hydroxybenzoic acid, ferulic acid, or caffeic acid. Only a small amount of pelargonidin-based anthocyanins in all the purple-fleshed sweet potatoes has been reported previously. To our knowledge, this is the first reported purple-fleshed sweet potato variety containing an exceptionally high proportion of pelargonidin-based anthocyanin. The ratio of cyanidin, peonidin, and pelagondin was 1:2.9:2.2 (Table 2). The other sweet potato varieties analyzed here contained only low amounts of pelargonidin-based anthocyanins (Table 2). Mokpo 62 variety contained a high proportion of peonidin-based anthocyanin, which constituted 82.5% of the total anthocyanins. In the Mokpo 62 variety, peonidin 3-caffeoyl sophoroside-5-glucoside (peak 19), peonidin 3-caffeoyl-*p*-hydroxybenzoyl sophoroside-5-glucoside (peak 22), and peonidin 3-caffeoyl-feruloyl sophoroside-5-glucoside (peak 24) were the most predominant anthocyanins, representing 67.2% of the total anthocyanins. In Shinzami, peonidin 3-caffeoyl-*p*-hydroxybenzoyl sophoroside-5-glucoside (peak 22) was the most abundant anthocyanin, representing 32.1% of the total anthocyanins, followed by peonidin 3-*p*-hydroxybenzoyl sophoroside-5-glucoside (peak 6) and peonidin 3-caffeoyl-feruloyl sophoroside-5-glucoside (peak 24), in decreasing order. Our present results were consistent with the previously reported ones.[17] It has been reported that peonidin 3-caffeoyl-*p*-hydroxybenzoyl sophoroside-5-glucoside was the most abundant anthcyanin found in Shinzami, representing 42.2% of the total anthocyanins.[17] The Shinzami variety contains a high proportion of peonidin-based anthocyanins along with a minor constituent of cyanidin-based anthocyanin (Table 2). The peonidin-based anthocyanin constituted 76.8% of the total anthocyanins in the Shinzami variety. Pelargonidin-based anthocyanin was not found in Shinzami. In Zami variety, cyanidin 3-caffeoyl-*p*-hydroxybenzoyl sophoroside-5-glucoside (peak 16) was the most predominant anthcyanin, followed by cyanidin 3-caffeoyl-feruloyl sophoroside-5-glucoside (peak 17), cyanidin 3-caffeoyl sophoroside-5-glucoside (peak 15), cyanidin 3-dicaffeoyl sophoroside-5-glucoside (peak 14), peonidin 3-caffeoyl-*p*-hydroxybenzoyl sophoroside-5-glucoside (peak 22), peonidin 3-caffeoyl-feruloyl sophoroside-5-glucoside (peak 24), peonidin 3-caffeoyl sophoroside-5-glucoside (peak 19), and peonidin 3-dicaffeoyl sophoroside-5-glucoside (peak 20), in decreasing order. It is also interesting to note that Zami contained a higher quantity of cyanidin-based anthocyanins than peondin-based anthocyanins (Table 2). The proportions of cyanindin-, peonidin-, pelargonidin-based anthocyanins were 1:0.43:0.03.

In summary, the characterization and quantitative analysis of anthocyanins in purple-fleshed sweet potato cultivars (Borami, Mokpo 62, Shinzami, and Zami) cultivated in Korea were carried out by HPLC/diode array detector, TOF-MS, and MS^2 analyses. Twenty-seven different anthocyanins (three non-acylated and 19 acylated anthocyanins) were identified in the sweet potatoes. To our knowledge, six of them are the first reported anthocyanins in the sweet potatoes. The numbers of anthocyanins found in sweet potato varieties of Borami, Mokpo 62, Shinzami, and Zami were 27, 15, 17, and 20, respectively. The anthocyanin profiles and contents in sweet potatoes were greatly different among the cultivars. Zami contained the highest quantity of anthocyanins, followed by Shinzami, Borami, and Mokpo 62, in decreasing order. Here, we report for the first time a very rare sweet potato variety (Borami) containing an exceptionally high quantity of pelargonidin-based anthocyanins. Furthermore, this paper represents the first report on the systematic characterization and quantitative analysis of anthocyanins in various purple-fleshed sweet potatoes cultivated in Korea.

Table 2. Anthocyanin Contents and Compositions in Common Commercial Purple Sweet Potatoes As Determined by High-Performance Liquid Chromatography

peak	identification[a]	anthocyanin content in purple sweet potatoes (mg/100 g DW)			
		Borami	Mokpo	Shinzami	Zami
1	Cy 3-soph-5-glc	3.7 ± 0.2	4.6 ± 0.6	9.8 ± 0.2	15.1 ± 0.1
2	Pg 3-soph-5-glc	6.3 ± 0.0	–[b]	–	–
3	Peo 3-soph-5-glc	7.5 ± 0.0	20.8 ± 0.3	26.0 ± 0.2	6.6 ± 0.1
4	Cy 3-*p*-hydroxybenzoylsoph-5-glc	1.7 ± 0.0	5.0 ± 0.0	41.3 ± 0.6	19.7 ± 0.3
5	Cy 3-(6″′-caffeoyl soph)-5-glc	3.1 ± 0.0	–	6.3 ± 0.0	10.7 ± 0.3
6	Peo 3-*p*-hydroxybenzoylsoph-5-glc	4.4 ± 0.0	20.5 ± 0.3	115.3 ± 2.7	10.2 ± 0.2
7	Peo 3-(6″′-caffeoyl soph)-5-glc	5.3 ± 0.0	2.0 ± 0.0	10.5 ± 0.0	3.6 ± 0.0
8	Pg 3-(6″′-caffeoyl soph)-5-glc	5.5 ± 0.0	–	–	–
9	Cy 3-*p*-coumaryl soph-5-glc	1.2 ± 0.0	–	–	1.7 ± 0.0
10	Cy 3-feruloyl soph-5-glc	3.9 ± 0.0	2.8 ± 0.0	19.8 ± 0.4	19.8 ± 0.6
11	Peo 3-*p*-coumaryl soph-5-glc	2.4 ± 1.2	–	2.7 ± 0.1	–
12	Peo 3-feruloyl soph-5-glc	8.2 ± 0.0	8.1 ± 0.1	41.3 ± 0.5	6.7 ± 0.1
13	Pg 3-feruloyl soph-5-glc	7.9 ± 0.0	–	–	–
14	Cy 3-dicaffeoyl soph-5-glc	23.6 ± 0.1	–	22.7 ± 0.0	140.1 ± 0.9
15	Cy 3-caffeoyl soph-5-glc	25.7 ± 0.2	17.4 ± 0.3	15.0 ± 0.2	175.8 ± 6.8
16	Cy 3-caffeoyl-*p*-hydroxybenzoyl soph-5-glc	6.5 ± 0.0	17.8 ± 0.0	76.6 ± 1.1	225.4 ± 1.9
17	Cy 3-caffeoyl-feruloylsoph)-5-glc	28.8 ± 0.1	9.0 ± 0.1	32.0 ± 0.3	206.8 ± 4.1
18	Pg 3-caffeoyl soph-5-glc	67.0 ± 0.0	2.2 ± 0.0	–	10.9 ± 0.3
19	Peo 3-caffeoyl soph-5-glc	71.92 ± 0.2	100.2 ± 1.42	63.9 ± 0.5	64.3 ± 0.9
20	Peo 3-dicaffeoyl soph-5-glc	66.3 ± 0.1	15.4 ± 0.2	66.4 ± 0.4	59.7 ± 0.2
21	Pg 3-dicaffeoyl soph-5-glc	50.1 ± 0.1	–	–	5.0 ± 0.03
22	Peo 3-caffeoyl-*p*-hydroxybenzoyl soph-5-glc	18.6 ± 0.1	108.2 ± 1.3	309.6 ± 5.9	110.1 ± 0.4
23	Pg 3-caffeoyl-*p*-hydroxybenzoyl soph-5-glc	10.2 ± 0.2	–	–	–
24	Peo 3-caffeoyl-feruloylsoph-5-glc	83.8 ± 0.4	49.2 ± 0.2	105.1 ± 2. 6	92.7 ± 0.1
25	Peo 3-caffeoyl-*p*-coumaryl soph-5-glc	13.1 ± 0.0	–	–	–
26	Pg 3-caffeoyl-feruloylsoph-5-glc	59.4 ± 0.5	–	–	–
27	Pg 3-caffeoyl-*p*-coumaryl soph-5-glc	10.6 ± 0.1	–	–	5.3 ± 0.2
total anthocyanin		596.7 ± 3.9	383.2 ± 4.7	964.3 ± 15.7	1190.2 ± 17.4
non-acylated anthocyanin		17.5 ± 0.1	25.4 ± 0.8	35.8 ± 0.04	21.7 ± 0.1
monoacylated anthocyanin		208.2 ± 1.5	158.2 ± 2.1	316.1 ± 5.0	323.4 ± 9.2
diacylated anthocyanin		371.0 ± 1.3	199.6 ± 1.8	612.4 ± 8.0	845.1 ± 7.0
cyanidin-based anthocyanin		98.2 ± 0.6	56.6 ± 1.0	223.5 ± 0.1	815.1 ± 15.05
peonidin-based anthocyanin		281.5 ± 2.0	324.4 ± 3.8	740.8 ± 12.8	353.9 ± 0.8
pelargnidin-based anthocyanin		217.0 ± 0.2	2.2 ± 0.0	–	21.2 ± 0.5

[a]Cy = cyanidin, Peo = peonidin, Pg = pelargonidin, soph = sophorside, glc = glucoside. [b]Not detected.

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Notes

The authors declare no competing financial interest.

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