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Probing Anthocyanin Profiles in Purple Sweet Potato Cell Line (*Ipomoea batatas* L. Cv. Ayamurasaki) by High-Performance Liquid Chromatography and Electrospray Ionization Tandem Mass Spectrometry

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A purple line cell line (PL) generated from the storage root of purple-fleshed sweet potato (*Ipomoea batatas* L.) cv. Ayamurasaki produces a complex mixture of anthocyanins, and seven major anthocyanins have been isolated and identified to date. All these anthocyanins are exclusively cyanidin or peonidin 3-sophoroside-5-glucosides and their acylated derivatives. High-performance liquid chromatography (HPLC) coupled to photodiode array (PDA) detection and electrospray ionization tandem mass spectrometry (ESI-MS/MS) on a triple quadrupole instrument was employed to further investigate the anthocyanin composition of the PL extract. Precursor-ion analysis, product-ion analysis, and selected reaction monitoring (SRM) MS/MS experiments were conducted sequentially to screen and characterize anthocyanins in the aqueous extract of the PL cell line. Precursor-ion analysis specifically detected the molecular cations of each category of anthocyanins by scanning the precursors of anthocyanidins (cyanidin, peonidin, and pelargonidin). The detected molecular cation of each anthocyanin was fragmented using product-ion analysis by collisionally activated dissociation (CAD). MS/MS using SRM detection was conducted to further confirm the fragmentation observed during product-ion analysis. In comparison to the commonly used product-ion analysis technique, the combined use of precursor-ion analysis, product-ion analysis, and SRM is particularly useful for positive identification of anthocyanins in complex matrixes and provides important information to confirm the proposed structures. Twenty-six anthocyanins were detected and characterized in the aqueous extract of the PL cell line. Several anthocyanins, including two pelargonidin derivatives, were tentatively identified for the first time in these cells.

KEYWORDS: Anthocyanins; sweet potato (*Ipomoea batatas* L.); cell line; tandem mass spectrometry

INTRODUCTION

Anthocyanins represent a group of water-soluble phenolic compounds responsible for the blue, red, and purple colors of many fruits and vegetables. More than 600 types of anthocyanins have been isolated from nature to date and cyanidin, delphinidin, malvidin, peonidin, pelargonidin, and petunidin are the most prevalent anthocyanins found in fruits and vegetables (*1*). Anthocyanins are present in almost all higher plants and have been reported to exhibit several physiological functions, such as antioxidative (*2*, *3*), antimutagenic (*4*, *5*), antidiabetic (*6*, *7*), and anticarcinogenic activities (*8*, *9*). As a result, anthocyanins have been regarded as potentially functional food colorants (*10*−*12*).

Previous studies demonstrated that acylated anthocyanins exhibited higher thermostability (*13*) and enhanced antioxidative (*14*, *15*) and antimutagenic activities (*4*, *5*). The storage roots of a purple-fleshed sweet potato (*Ipomoea batatas* L.) cultivar "Ayamurasaki" accumulate mono- and diacylated anthocyanins at high levels (*16*, *17*). A cell line (PL; purple line) derived from the Ayamurasaki storage root was found to contain a complex mixture of anthocyanins (*18*), exhibiting potent antimutagenic activity against Trp-P-1 and inhibition of the proliferation of HL-60 cells (*19*). When the cell line is cultured under controlled conditions to enhance pigment production, accumulation of cell line specific anthocyanins has been observed (*18*, *20*). The major cell line specific anthocyanins have been identified (*21*, *22*) using high-performance liquid chromatography (HPLC) and nuclear magnetic resonance spectroscopy (NMR). Other anthocyanins have not been identified because of their presence in the crude cell line extract at very low levels. Chemopreventive qualities of anthocyanins are

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Table 1. Mass Spectrometric Data of Anthocyanidinanins in PL Cell Line

			MS/MS			
			precursor of			
peak label[a]	anthocyanins	MW	cyanidin *m/z* 287	peonidin *m/z* 301	pelargonidin *m/z* 271	fragment ions *m/z*
1	cyanidin 3-sophoroside-5-glucoside	773	×			611, 449, 287
2[b]	cyanidin 3,5-diglucoside	611	×			449,287
3	pelargonidin 3-sophoroside-5-glucoside	757			×	595, 433, 271
4	peonidin 3-sophoroside-5-glucoside	787		×		625, 463, 301
5	cyanidin 3-*p*-hydroxybenzoylsophoroside-5-glucoside	893	×			731, 449, 287
6	cyanidin 3-(6″-caffeoylsophoroside)-5-glucoside	935	×			773, 449, 287
7	peonidin 3-*p*-hydroxybenzoylsophoroside-5-glucoside	907		×		745, 463, 301
8	peonidin 3-caffeoylsophoroside-5-glucoside	949		×		787, 463, 301
9	cyanidin 3-(6″-*p*-coumarylsophoroside)-5-glucoside	919	×			757, 449, 287
10	cyanidin 3-(6″-feruloylsophoroside)-5-glucoside	949	×			787, 449, 287
11	peonidin 3-(6″-*p*-coumarylsophoroside)-5-glucoside	933		×		771, 463, 301
12	peonidin 3-(6″-feruloylsophoroside)-5-glucoside	963		×		801, 663, 301
13	pelargonidin 3-feruloylsophoroside-5-glucoside	933			×	741, 433, 271
14	unidentified	947	×			757, 477, 287
15	unidentified	977	×			787, 477, 287
16	cyanidin 3-(6″, 6‴-dicaffeoylsophoroside)-5-glucoside	1097	×			935, 449, 287
17	cyanidin 3-caffeoylsophoroside-5-glucoside	935	×			773, 449, 287
18	cyanidin 3-(6″-caffeoyl-6‴-feruloylsophoroside)-5-glucoside	1111	×			949, 449, 287
19	cyanidin 3-caffeoyl-*p*-coumarylsophoroside-5-glucoside	1081	×			919, 449, 287
20	peonidin 3-caffeoyl-*p*-hydroxybenzoyl-sophoroside-5-glucoside	1069		×		907, 463, 301
21	peonidin 3-caffeoylsophoroside-5-glucoside	949		×		787, 463, 301
22	peonidin 3-feruloyl-*p*-caffeoylsophoroside-5-glucoside	1127		×		965, 463, 301
23	cyanidin 3-feruloyl-*p*-coumarylsophoroside-5-glucoside	1095	×			933, 449, 287
24	cyanidin 3-(6″, 6‴-dicoumarylsophoroside)-5-glucoside	1065	×			903, 449, 287
25	peonidin 3-feruloyl-*p*-coumarylsophoroside-5-glucoside	1109		×	×	947, 463, 301
26	peonidin 3-(6″, 6‴-dicoumarylsophoroside)-5-glucoside	1079		×	×	917, 463, 301

[a] See Figure 2. [b] Peaks labeled in bold are detected for the first time in the PL cell line.

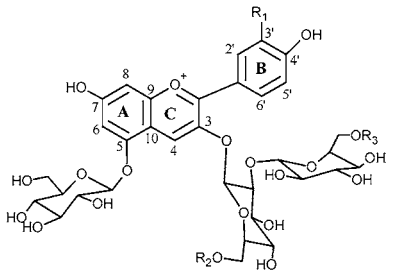


Figure 1. Structure of anthocyanins isolated from purple-fleshed sweet potato (*Ipomoea batatas* L.) storage root and PL cell line. Cyanidin: R_1 = OH; peonidin: R_1 = OCH_3; R_2, R_3 = H, *p*-hydroxybenzoic acid, caffeic acid, *p*-coumaric acid, and ferulic acid.

governed by their molecular structures (*5*, *9*, *23*), and for potential application of cell line extracts as food colorants with nutraceutical properties, complete knowledge of the extracts' composition is required.

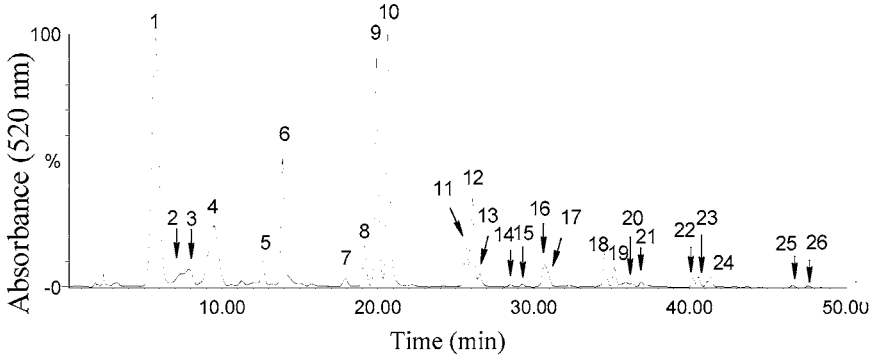
Electrospray ionization mass spectrometry (ESI-MS) and tandem spectrometry (MS/MS) have been used as a powerful technique for anthocyanin identification and characterization (*24*−*26*). In comparison to the most commonly used MS/MS product-ion analysis technique, combined use of precursor-ion analysis, product-ion analysis, and selected reaction monitoring (SRM) tandem mass spectrometry (MS/MS) techniques is particularly useful for screening anthocyanins in complex biological samples. In the current study, this technique was applied to probe anthocyanin profiles in the PL cell line extract that has been reported to contain a complex mixture of anthocyanins.

MATERIALS AND METHODS

Plant Material. Callus culture of the PL cell line, previously established from the sweet potato (*Ipomoea batatas* L.) storage root cv. Ayamurasaki (*27*), was used for this study. Suspended cell cultures were initiated by transferring about 1.0 g fresh weight of callus to 50 mL liquid medium in 250-mL Erlenmeyer flasks. Basal Murashige and Skoog (MS) medium supplemented with 1.0 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) and 3% sucrose was used to multiply the cultures. Subcultures were done at 7-day intervals. The cultures were incubated on a rotary shaker (130 rpm) at 25 °C in the dark. Subsequently, they were moved into a high-anthocyanin-producing medium, which was a modified MS medium containing 9.4 mM KNO_3 and 5% sucrose, without NH_4NO_3 and growth regulators (*18*, *20*). Medium pH was adjusted to 5.8 before autoclaving. Five-hundred milligrams of cell aggregates was placed in each 250-mL Erlenmeyer flask containing 50 mL medium. The cultures were harvested after 14 days, and the aggregates were removed from the culture media, rinsed with distilled water, and separated from the liquid by vacuum filtration. Fresh aggregates were instantly frozen using liquid nitrogen and were freeze-dried. The freeze-dried tissues were stored at −20 °C until analyzed.

Extraction of Anthocyanins. Approximately 40 mg of lyophilized tissues was extracted two times with 10 mL deionized water containing 5% formic acid and was vortexed for 2 min. The suspensions were centrifuged at 4000 rpm for 10 min, and the supernatants were combined and filtered through a 0.20 μm nylon filter.

LC/MS/MS Analysis of Anthocyanins. HPLC analysis was performed using a Waters 2695 gradient HPLC separation module equipped with an autoinjector and a 996 photodiode array (PDA) UV/vis absorbance detector (Milford, MA). Separation was carried out on a 150 × 3.0 mm i.d., 3 μ Atlantis dC_{18} column (Waters Corporation, Milford, MA) at 35 °C. The solvent system consisted of a step gradient from 95% A to 90% A in 20 min, to 78% A in 30 min, and then returned to 95% A in 5 min (the total run was 55 min). Solvent A was formic acid:water (5/95, v/v) and solvent B was acetonitrile:formic acid (95/5, v/v). UV/vis spectra of anthocyanins were recorded from 200 to 600 nm using the in-line PDA detector. Mass spectrometry was conducted on a triple quadrupole ion-tunnel mass spectrometer (Quattro Ultima, Micromass Limited, Manchester, U.K.) equipped with a Z-spray ESI source. Calibration of the mass spectrometer was performed using sodium iodide and cesium iodide. Instrument control and data analysis



Peak number	UV/Vis spectra			
	Vis-Max (nm)	$A_{UV\text{-}max}/A_{Vis\text{-}max}$ (×100)	$A_{acyl\text{-}max}/A_{Vis\text{-}max}$ (×100)	$A_{440}/A_{Vis\text{-}max}$ (×100)
1	513	63		18
2	514	108		18
3	500	64		25
4	515	65		18
5	520	66	16	17
6	521	74	52	18
7	517	74	27	16
8	521	87	55	18
9	521	82	69	17
10	520	74	55	19
11	520	79	65	17
12	520	70	49	18
13	505	81	62	21
14	522	144	125	18
15	526	88	66	18
16	532	102	89	19
17	521	82	52	18
18	526	100	89	18
19	526	108	106	18
20	522	92	98	19
21	522	88	55	17
22	529	96	93	16
23	529	106	106	18
24	528	114	117	17
25	532	106	104	15
26	526	115	115	15

Figure 2. Representative HPLC chromatogram and UV/vis spectra of anthocyanins detected in the extract of PL cell line generated from the storage root of sweet potato (*Ipomoea batatas* L.) cv. Ayamurasaki.

were accomplished using Masslynx V3.5 software (Micromass Co. Ltd., U.K.). Approximately 1/10 of the HPLC eluate (0.6 mL/min) separated by a microsplitter valve (Upchurch Scientific, Oak Harbor, WA) was delivered to the ESI source. Cyanidin 3-sophoroside-5-glucoside, one of the major anthocyanins identified in the PL cell line (*21*), was obtained from Prof. Norihiko Terahara (Minami-Kyushu University, Japan) and was used to tune the quadrupole mass analyzer for each MS/MS experiment. During MS/MS precursor-ion analysis, the conditions of the mass spectrometer were optimized to produce the most abundant signals of both the daughter ion (*m*/*z* 287) and the precursor ion (*m*/*z* 773) (in approximately equal abundance). During product-ion analysis, the collision energy was set to attenuate the precursor ions ~50% and was optimized for each compound of interest. This collision energy was also used for the SRM experiments of the same compound. Typical settings of the quadrupole analyzer were as follows: capillary voltage, 3.0 kV; cone voltage, 35 V; radio frequency lens 1 voltage (RF-1), 50 V; desolvation gas temperature, 400 °C at a flow of 17 L/min; source temperature, 105 °C; collision gas (argon) pressure, 7 psi; collision energy, 25~35 eV.

RESULTS AND DISCUSSION

Previous research demonstrated that the PL suspension culture produced a complex mixture of anthocyanins, which differed from the anthocyanin mixture produced by the purple-fleshed Ayamurasaki storage root (cell line's donor tissue) (*27*). To date, many anthocyanins from the field-grown storage root (*15*−*17*) and seven major anthocyanins from the PL cell line (*18*, *21*, *22*) have been isolated and identified. All these anthocyanins are exclusively cyanidin or peonidin 3-sophoroside-5-glucosides (**Figure 1**) and their acylated derivatives. The anthocyanin mixture produced by field-grown storage root consists of mainly diacylated anthocyanins acylated with caffeic, ferulic, and hydroxybenzoic acids (*15*−*17*). In comparison, the cell line produced a mixture containing nonacylated, monoacylated, and diacylated anthocyanins acylated with *p*-coumaric and caffeic acids (*18*, *22*, *27*), exhibiting higher radical scavenging and antiproliferative activities (*19*).

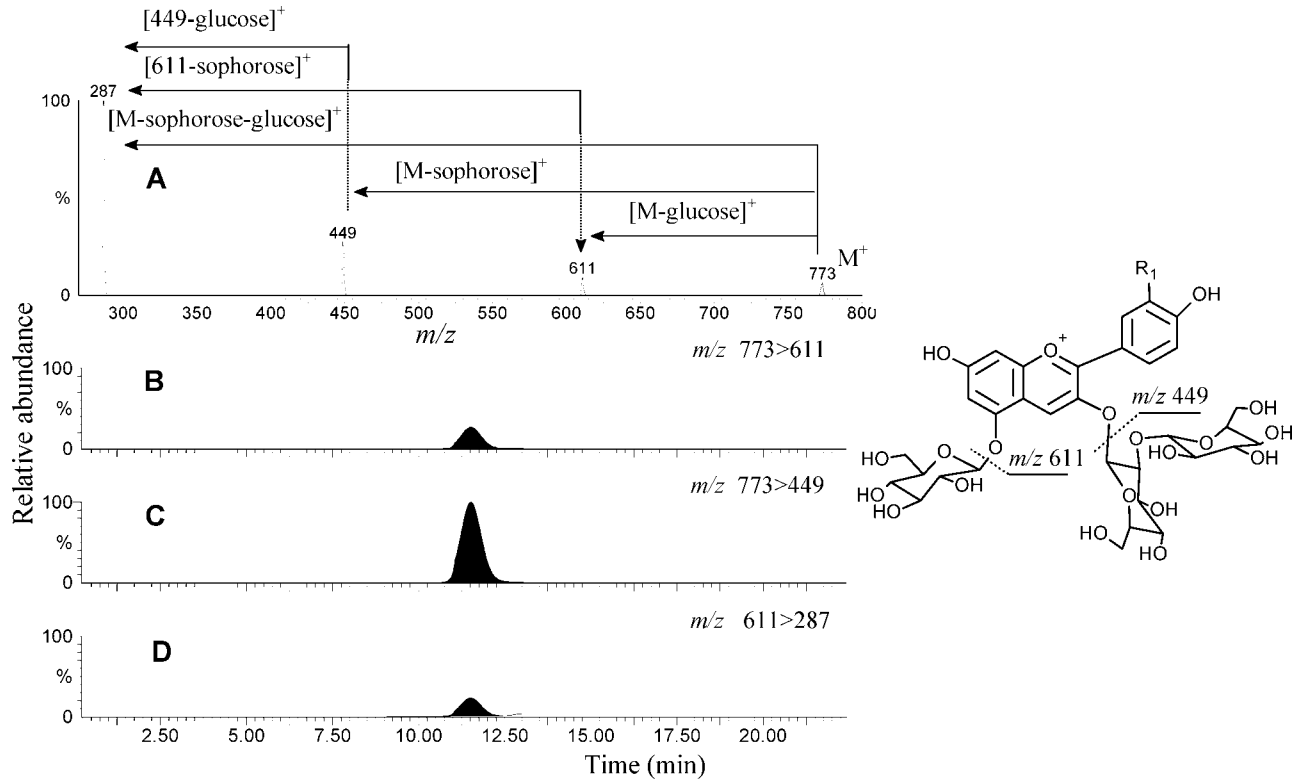


Figure 3. Tandem mass spectrometry (MS/MS) of cyanidin 3-sophoroside-5-glucoside using product-ion analysis and selected reaction monitoring (SRM). (**A**) MS/MS spectrum obtained using product-ion analysis; (**B**) SRM of *m*/*z* 773 > 611; (**C**) SRM of *m*/*z* 773 > 449; (**D**) SRM of *m*/*z* 611 > 287.

To identify the minor anthocyanins accumulated by the PL cell line, more sensitive and selective techniques are needed. In comparison to nuclear magnetic resonance spectroscopy which requires tremendous work to purify compounds of interest before characterization, HPLC coupled to a PDA detector and mass spectrometer possess a favorable advantage of direct analysis of anthocyanins in complex matrixes with high selectivity and sensitivity. For an unknown sample, an LC/MS/MS analysis by scanning the precursors of each anthocyanidin allows the specific detection of molecular cations of all anthocyanins present in the sample (unpublished results). By comparing to the ~600 types of known anthocyanins in our database, the overall profiles of anthocyanin composition in the sample can be obtained. However, for anthocyanins not characterized previously, other MS/MS experiments, such as product-ion analysis, common-neutral-loss analysis, and SRM, are helpful for tentative identification. For the PL cell line extract, six types of commonly found anthocyanins in fruits and vegetables were screened by scanning the precursors of each anthocyanidin (cyanidin, delphinidin, malvidin, peonidin, pelargonidin, and petunidin) during LC/MS/MS. Three types of anthocyanidins, cyanidin, peonidin, and pelargonidin, were detected in the PL cell line extract (**Table 1** and **Figure 2**). The UV/vis spectra of each compound recorded by the in-line PDA detector (**Figure 2**) provided important spectroscopic characteristics, including visible maximum and the ratios of A_{UV-max} to $A_{Vis-max}$, $A_{acyl-max}$ to $A_{Vis-max}$, and A_{440} to $A_{Vis-max}$, which can be used as important indexes for anthocyanin identification (*28*). However, because of the coelution of some anthocyanins, the assignment of each peak in the current study was primarily based on mass spectrometric data, as presented in **Table 1**.

ESI-MS/MS using product-ion analysis has been previously used as an important tool for anthocyanin identification (*26*, *29*, *30*). However, MS/MS using SRM detection is particularly useful for characterization of anthocyanidins glycosylated with more than two sugars or acylated substituents. For example, collisionally activated dissociation (CAD) of the molecular cation of standard cyanidin 3-sophoroside-5-glucoside produced fragment ions at *m*/*z* 611, 449, and 287 (**Figure 3A**). This fragmentation can be incorrectly explained as a cyanidin triglycoside, since the three fragments could be the result of the successive loss of one hexose (162 u) from the molecular cation. However, MS/MS using SRM can specifically detect the fragmentation that occurred during CAD, thus providing additional information to help identify the glycosidic linkage in the structure. The transitions of *m*/*z* 773 > 611 (**Figure 3B**), *m*/*z* 773 > 449 (**Figure 3C**), and *m*/*z* 611 > 287 (**Figure 3D**) observed during SRM detection indicated the fragmentation of the molecule by loss of glucose and sophorose substituents from the molecular cation and loss of sophorose substituent from the fragment *m*/*z* 611, respectively. The considerably higher abundance of *m*/*z* 773 > 449 than *m*/*z* 773 > 611 illustrates that the larger substituent (sophorose) is readily eliminated in comparison to the smaller substituent (glucose), which is consistent with the fragmentation of most organic compounds during mass spectrometry. However, only a trace *m*/*z* 611 > 449 (at least 1000-fold lower in comparison to the other transitions occurring during MS/MS) was observed (data not shown), indicating that the fragmentation of glycosidic bond within the sophorose is negligible. A similar finding was reported previously (*26*). Identical mass spectrometric fragmentation pattern, UV/vis spectra, and HPLC retention time of peak 1 (**Table 1** and **Figure 2**) as the standard cyanidin 3-sophoroside-5-glucoside confirmed its identity. Pelargonidin 3-sophoroside-5-glucoside (**Figure 2** and **Table 1**), which has not been reported in the PL cell line, exhibited a similar fragmentation pattern (**Figure 4**).

For monoacylated anthocyanins, the fragmentation between the aromatic acid (caffeic acid, coumaric acid, ferulic acid, and hydroxybenzoic acid) and sophoroside was barely visible.

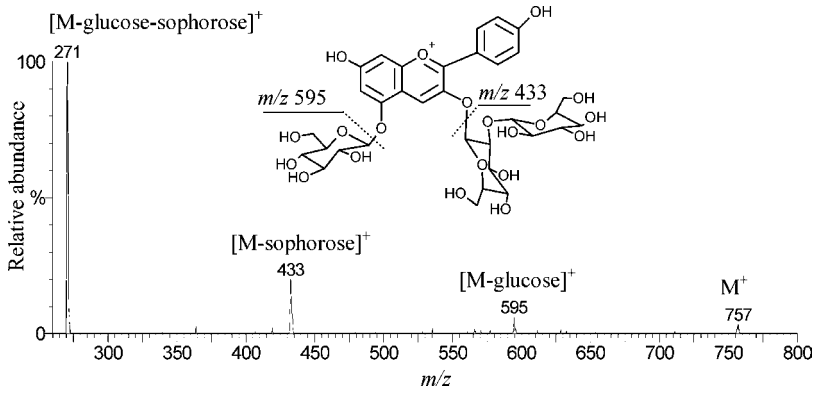


Figure 4. Tandem mass spectrometry (MS/MS) of pelargonidin 3-sophoroside-5-glucoside using product-ion analysis.

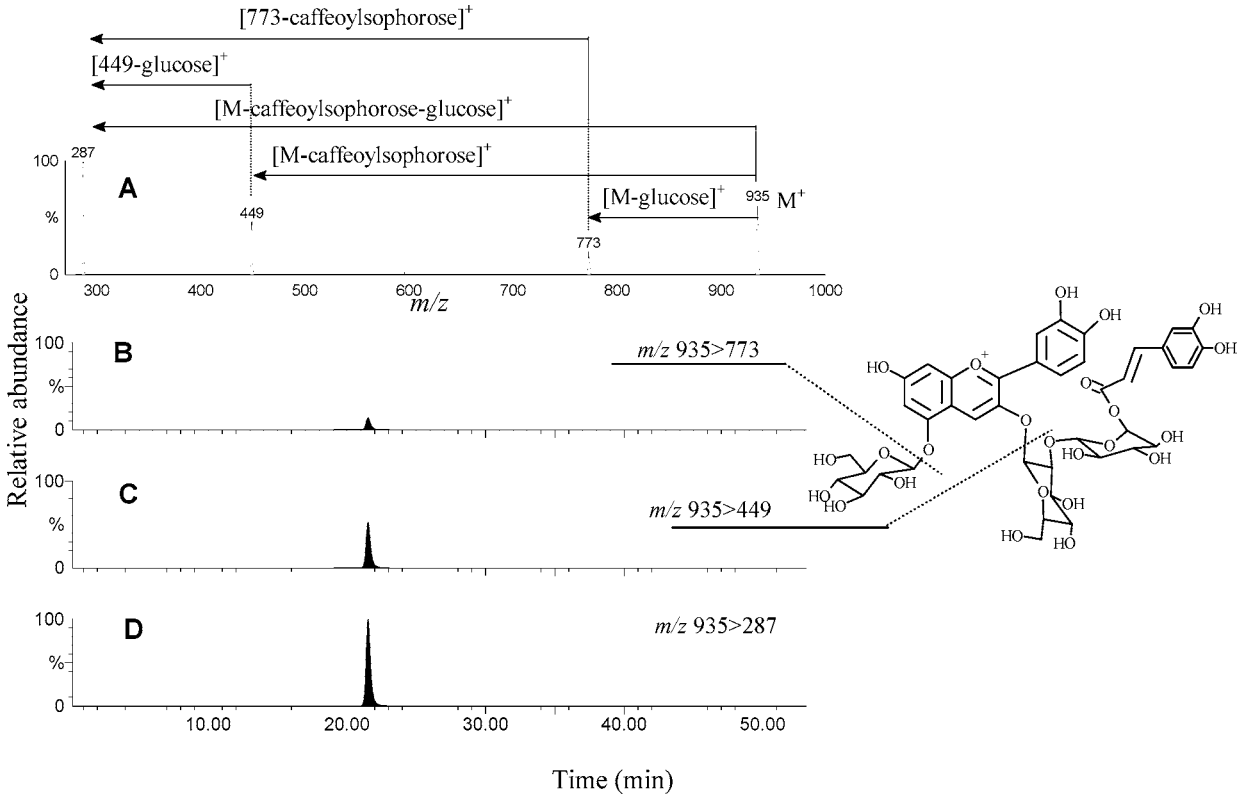


Figure 5. Tandem mass spectrometry (MS/MS) of cyanidin 3-caffeylsophoroside-5-glucoside using product-ion analysis and selected reaction monitoring (SRM). (**A**) MS/MS spectrum obtained using product-ion analysis; (**B**) SRM of *m*/*z* 935 > 773; (**C**) SRM of *m*/*z* 935 > 449; (**D**) SRM of *m*/*z* 935 > 287.

Figure 5 shows the LC/MS/MS chromatograms of cyanidin 3-caffeoylsophoroside-5-glucoside (**Figure 2**) which has been identified previously using NMR (*22*). The molecular cation (*m*/*z* 935) was fragmented into *m*/*z* 773, 449, and 287 during product-ion analysis (**Figure 5A**). However, we only detected *m*/*z* 935 > 773 (**Figure 5B**, $[M\text{-glucose}]^+$), *m*/*z* 935 > 449 (**Figure 5C**, $[M\text{-caffeoylsophorose}]^+$), *m*/*z* 935 > 287 (**Figure 5D**, $[M\text{-caffeoylsophorose-glucose}]^+$), as well as *m*/*z* 449 > 287 (loss of glucose) and *m*/*z* 773 > 287 (loss of caffeoylsophorose) (chromatograms not shown) using SRM detection. Only trace *m*/*z* 773 > 611 and *m*/*z* 773 > 449 signals were detected (data not shown), indicating that the caffeoylsophorose substituent was further fragmented with difficulty. As in the case of cyanidin 3-sophoroside-5-glucoside, the elimination of the larger substituent (*m*/*z* 935 > 449) produced a more abundant signal than the fragment resulting from loss of glucose (*m*/*z* 935 > 773) (**Figure 5C** and **5B**).

Diacylated anthocyanins exhibited similar fragmentation behavior to monoacylated anthocyanins. **Figure 6** shows the MS/MS spectrum of cyanidin 3-*di*-coumarylsophoroside-5-glucoside (**Table 1**) using product-ion analysis. This compound has also been isolated and identified by previous researchers (*22*). The fragmentations of the two glycosidic bonds (cyanidin-glucose, cyanidin-sophorose) dominated the spectrum, while the fragmentation between the sophorose and acylated coumaric acid was negligible.

Although mass spectrometry and tandem mass spectrometry provides important information for identification of anthocyanins, the glycosylated and acylated positions cannot be determined. For this determination, other techniques are needed. For example, peaks 6 and 17 as well as 8 and 21 (**Table 1**) exhibited identical mass spectrometric data (molecular weight, fragmentation, and UV/vis spectra), but these two pairs of compounds were eluted at different times (**Figure 2**). Previous studies using NMR identified peaks 6 and 17 both as cyanidin 3-caffeoylsophoroside-5-glucosides, however, the acylation occurred at different glucoses of the sophoroside moiety. Peak 6 has been identified as cyanidin 3-*O*-(2-*O*-(6-*O*-(*E*)-caffeoyl-*β*-D-gluco-

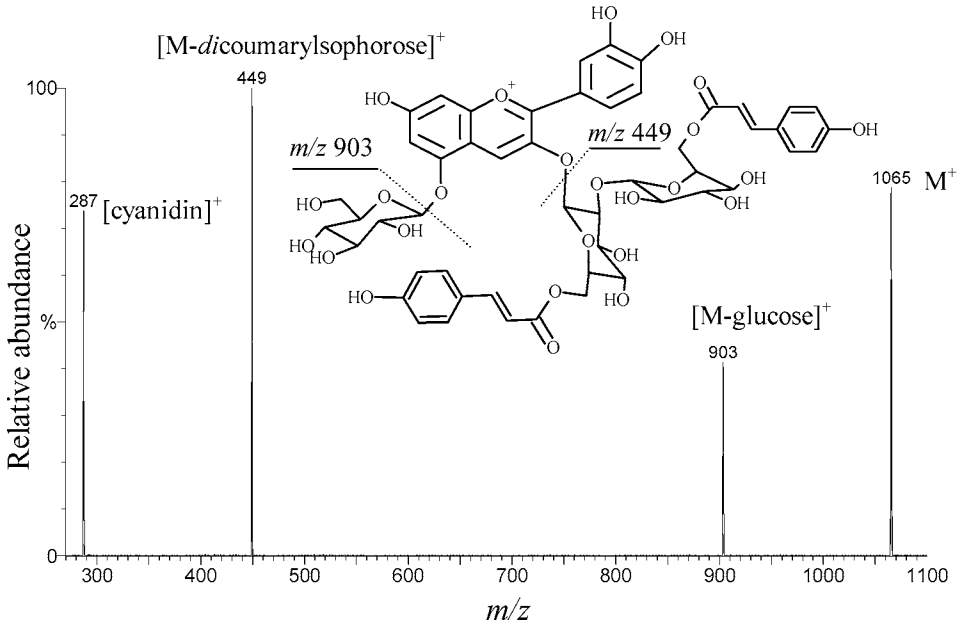


Figure 6. Tandem mass spectrometry (MS/MS) of cyanidin 3-*di*-coumarylsophoroside-5-glucoside using product-ion analysis.

pyranosyl)-β- D-glucopyranoside)-5-*O*-β-D-glucopyranoside (*22*) and peak 17 has been identified as cyanidin 3-*O*-(6-*O*-trans-caffeoyl-2-*O*-β-glucopyranosyl-β-glucopyranoside)-5-*O*-β-glucoside (*16*). Similar differences in acylation profile could be present for the two peonidin anthocyanins, peaks 8 and 21. In addition, UV/vis spectra also provide valuable supporting information which helps in the characterization of individual anthocyanins (**Figure 2**). In general, diacylated anthocyanins exhibited a higher A_{acylated} peak/$A_{\text{Vis-max}}$ value than monoacylated anthocyanins.

Furthermore, two unidentified minor cyanidin-based anthocyanins (peaks 14 and 15) were detected during LC/MS/MS analyses (**Figure 2**). Both anthocyanins exhibited similar fragmentation behavior during MS/MS precursor-ion analysis and product-ion analysis (**Table 1**). The fragmentations of *m*/*z* 947 > 477 and *m*/*z* 977 > 477 indicated the possible presence of coumarylsophorose and feruloylsophorose in the structures of anthocyanins 14 and 15, respectively. However, the loss of 190 u (*m*/*z* 947 > 757 and *m*/*z* 977 > 787) observed for both anthocyanins during MS/MS needs to be further characterized. In addition, different UV/vis spectra were also observed for the two compounds (**Figure 2**).

In the present study, we have detected 26 different anthocyanins in a crude mixture. Seven of them are reported for the first time (**Table 1**). Among them, two are pelargonidin-based anthocyanins: pelargonidin 3-sophoroside-5-glucoside and its acylated derivative pelargonidin 3-feruloylsophoroside-5-glucoside. Detection of pelargonidin-based anthocyanins in the storage root of purple-fleshed sweet potato and in the PL cell line has not yet been reported. Although not accumulated in the *Ipomoea batatas* storage root, the pelargonidin 3-sophoroside-5-glucoside and acylated derivatives were found in flowers of more primitive strains of the subgenus *Ipomoea*, such as *Pharabitis nil* (*Ipomoea nil*) (*31*) and *Ipomoea purpurea* (*32*), where they were considered to be the analogous pigments to cyanidin 3-sophoroside-5-glucoside and derivatives in other forms of *Ipomoea*. Detection of pelargonidin-based anthocyanins in the cell line extract may indicate that under in vitro conditions the activity of flavonoid 3′-hydroxylase is suppressed.

Peak 2 has been identified as cyanidin 3,5-diglucoside (**Table 1**). This anthocyanin was not previously found in *I. batatas*, where all reported anthocyanins are derivatives of cyanidin and peonidin 3-sophoroside-5-glucosides (*15*−*17*). Anthocyanins with less evolved molecular structure, pelargonidin 3-glucoside and 3,5-diglucoside, have been reported for maroon flowers of *Pharbitis nil* cultivars (*33*). Earlier we have reported suppression of methylation of cyanidin to peonidin in the cell line, which resulted in enhanced accumulation of cyanidin-based pigments in culture in comparison to the field-grown storage root (*34*). The above findings suggest that during the PL cell line tissue growth under in vitro conditions the activity of numerous enzymes of the anthocyanin-biosynthetic pathway is suppressed, while a tendency toward accumulation of pigments with a less evolved molecular structure is observed (*19*).

In conclusion, we have demonstrated a successful application of LC/MS/MS precursor-ion analysis, product-ion analysis, and SRM for characterization of a variety of anthocyanins in the crude extract of the PL cell line. Precursor-ion analysis and SRM are important supplementary techniques to commonly used MS/MS product-ion analysis, providing valuable information to confirm the proposed structures deduced from product-ion analysis.

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