Anthocyanins from Skins and Fleshes of Potato Varieties

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Potatoes come in many various colors of tuber peels and/or fleshes. Anthocyanin pigment is responsible for red and purple colors of colored potato varieties. We report anthocyanin determination of a red potato cultivar *Kintoki-imo* and other colored cultivars grown in Hokkaido, Japan, using DAD-HPLC and ESI-TOF/MS. ¹³C-NMR and ¹H-NMR analyses showed that the major *Kintoki-imo* anthocyanin were pelargonidin 3-(6-*O*-(4-*O*-(*E*)-*p*-coumaroyl- α -L-rhamnopyranosyl)- β -D-glucopyranoside)-5- β -D-glucopyranoside (pelanin), and pelargonidin 3-(6-*O*-(4-*O*-(*E*)-feruloyl- α -L-rhamnopyranosyl)- β -D-glucopyranoside)-5- β -D-glucopyranoside. The anthocyanin content per 100 g flesh tuber was 2-816 mg. The anthocyanin composition patterns of potato cultivars were classified into five types: type A contained a lot of pelanin and Pg 3-Fr.Rut-5-Glc; type B was similar to type A, except that it included purple pigments such as peonanin and Pn 3-Rut-5-Glc; type C was also close to type A, except that the peonanin content was high; type D had pelanin as the main pigment, but no peonanin; and type E had petanin as the main pigment, but no pelanin.

Keywords: anthocyanin, potato varieties, HPLC, ESI-TOF/MS

Introduction

Potatoes (i.e., *Solanum tuberosum* ssp. *tuberosum*, *S.tuberosum* ssp. *Andigena* and *S.phureja*) are among the most important staple foods in the world. As potatoes are rich in starch, vitamins C and B₆, they are widely used worldwide for green goods, processed foods, starch, or seed materials (Hayashi *et al.*, 1997). Potatoes come in many colors in the tuber peels and/or fleshes. Anthocyanin pigment is responsible for the red and purple colors of potato varieties (Harbone, 1960; Howard, 1970; Andersen, 1991; Ishii *et al.*, 1996b; Lewis, 1998; Naito *et al.*, 1998; Fossen, 2000). Anthocyanin-containing red-fleshed potato *Inca Red* (red potato) and purple-fleshed potato *Inca Purple* (purple potato) have been bred from hybrid seedlings between cultivars of *Solanum*

tuberosum ssp. *tuberosum* and *S. tuberosum* ssp. *andigena* (Hayashi *et al.*, 1997; Naito *et al.*, 1998; Mori *et al.*, 2000). Recently, potato anthocyanins have been recognized for their contributions to health, as they have been shown to have strong antioxidative activity (Ishii *et al.*, 1996b), anti-influenza virus activity (Hayashi *et al.*, 2003) and anti-stomach cancer activity (Hayashi *et al.*, 2006).

In this study, we analyzed anthocyanin determination of a red potato cultivar (*Kintoki-imo*), as well as other colored cultivars grown in National Agricultural Research Center for the Hokkaido Region.

Materials and Methods

Materials A total of 24 anthocyanin-containing potato cultivars were cultivated in the fields of the National Agricultural Research Center for Hokkaido Region (Memuro, Kasai-Gun, Hokkaido).

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Isolation of Kintoki-imo anthocyanin The *Kintoki-imo* tubers (30 kg) were sliced and dipped in 5 L of 3% trifluoroacetic acid (TFA) overnight at 4°C and filtered through filter paper (No. 3, Toyo, Japan). The residue was immersed in 5 L of 3% TFA overnight and filtered. The combined pigment extract contained eight anthocyanins (1-8, numbered by elution order). UV-absorbing components were then analyzed by high performance liquid chromatography (HPLC). The pigment extract was adsorbed on an Amberlite XAD-7 column (450 mm × 30 mm i.d., Rohm and Haas Co., USA), washed with 5 L of water, eluted with 3 L of 0.3% TFA-EtOH, and evaporated under vacuum at 4°C. The residue was re-dissolved in a minimum amount of 0.1% TFA-EtOH and centrifuged. To the supernatant, an excess amount of diethyl ether was added. The red precipitate was centrifuged and dried *in vacuo* in a silica-gel desiccator overnight to give the crude pigment powder (8 g).

The crude pigment powder (8 g) was dissolved in 80 mL of 5% HCOOH/50% EtOH and centrifuged. The supernatant was applied on a PVPP column (Polyclar VT, ISP Technologies, Inc., USA; 120 mm × 45 mm i.d.) and eluted with 5% HCOOH/50%EtOH.

The fractions were separated repeatedly by preparative HPLC, and each elution was evaporated to dryness *in vacuo*, dissolved in a minimum amount of TFA, and precipitated with excess diethyl ether. Red powders were obtained as TFA salts: **1**: 0.4 mg, **2**: 0.3 mg, **3**: 2 mg (0.03%, yield from crude pigment), **4**: 1 mg (0.01%), **5**: 76 mg (1%), **6**: 3 mg (0.04%), **7**: 43 mg (0.5%), and **8**: 5 mg (0.06%).

Analytical analysis of anthocyanins All reagents and solvents were of analytical grade and used without further purification.

HPLC analysis of *Kintoki-imo* anthocyanin was performed with a linear gradient elution from 25% to 55% solvent B (1.5% phosphoric acid, 20% acetic acid, and 25% acetonitrile in H₂O) in solvent A (1.5% phosphoric acid in H₂O) on an Inertsil ODS-3 column (250 mm × 4.6 mm i.d., GL Sciences, Inc., Japan) for 60 min at 30°C. The flow rate was 1.0 mL/min and the fractions were monitored at 520, 310 or 280 nm with the MD-1510 multi-wavelength diode array detection (DAD) system (Jasco Co., Ltd., Japan). Preparative HPLC of *Kintoki-imo* anthocyanins was performed with a 2:1 isocratic elution of solvent C (15% acetic acid in H₂O):solvent D (15% acetic acid and 30% acetonitrile in H₂O) on an Inertsil ODS column (GL Sciences, Inc.; 250 mm × 20 mm i.d.) at room temperature. The flow rate was 7.0 mL/min, and the fractions were monitored at 520 or 310 nm with an L-4200 UV-Vis detector and a D-2000 integrator (Hitachi Co., Ltd., Japan).

UV-Vis spectra were recorded on the DAD system

equipped with an analytical HPLC system for structural determination.

Electrospray ionization time-of-flight mass spectrometry (ESI-TOFMS) analysis was performed on a Mariner Workstation system (Applied Biosystems Co., Ltd., USA) with a positive mode in 50% aqueous acetonitrile containing 1% acetic acid. The high resolution mass spectra were measured using an electrospray ionization Fourier transform ion-cyclotron resonance mass spectrometry (ESI/FT-ICRMS) APEX II 70e system (Bruker Daltonics, Germany) with a positive mode in 50% aqueous MeOH containing 2% acetic acid. ¹³C-NMR at 200 MHz and ¹H-NMR at 800 MHz were run on an Avance 800 spectrometer (Bruker BioSpin, Germany) in DMSO- δ_6 :CF₃COOD (9:1) with tetramethylsilane as an internal standard at 30°C.

Anthocyanin composition The anthocyanin-containing potato cultivars were sliced to 1-mm thickness using a slicer and were extracted with 3% TFA aqueous solution overnight at 4°C.

The maximum absorbable wavelength in the visible region ($\lambda_{\text{vis.max}}$) of potato anthocyanins was measured by the DU-7500 spectrophotometer (Beckman Co., Ltd., USA). The anthocyanin samples used for analyses were filtered with 0.22- μ m filter (Millex-GV; Millipore Co., Ltd., USA).

HPLC analysis of anthocyanin compositions was performed with an isocratic elution of 0.1% TFA-acetonitrile (82.5:17.5, v/v) on an Inertsil ODS column (250 mm × 20 mm i.d.) at 45°C. The flow rate was 0.85 mL/min, and the fractions were monitored at 250 to 700 nm with a TM996 photodiode array detector (Waters Co., Ltd., USA).

Anthocyanin composition was determined from the HPLC peak area recorded at 525 nm (Hayashi *et al.*, 1996). Anthocyanin concentration (mg/100 g flesh weight of potato tuber) was calculated from the peak area compared the standard pigment of pelanin, petanin (Hayashi *et al.*, 1997) and *Kintoki-imo* anthocyanin.

Pigments were identified by comparing their retention time on HPLC with those of the standard pigments.

Results and Discussion

As shown in Table 1, DAD-HPLC analysis confirmed eight anthocyanins (1-8) in *Kintoki-imo* tuber crude pigments. The anthocyanin content was in the order of **5** (23%) > **7** (22%) > **6** (12%), **8** (12%) > **3** (11%) > **4** (8%) > **1** (7%) > **2** (6%) based on the peak area% at 520 nm. The aglycon and acylation were elucidated by DAD UV-Vis spectral data of each anthocyanin. Aglycons **1**, **3**, **5** and **7** were estimated to be pelargonidin (Pg) since their maximum absorption wavelengths at visible region ($\lambda_{\text{vis.max}}$) were in the range of 499-507 nm. Similarly, aglycons **2**, **4**, **6** and **8** were estimated

Table 1. DAD-HPLC results of *Kintok-imo* anthocyanins.

Anthocyanin	tR ¹ (min)	PA ² (%)	$\lambda_{\text{vis.max}}$ (nm)	$\lambda_{\text{acyl.max}}$ (nm)	$\lambda_{\text{UV.max}}$ (nm)	R ³	Aglycon	n ⁴
1	12.1	7.3	499	327	275	0.20	Pg	0
2	14.4	5.7	515	323	279	0.30	Cy/Pn	0
3	43.0	10.5	507	327	283	0.60	Pg	1
4	44.5	8.2	523	327	283	0.74	Cy/Pn	1
5	51.8	22.5	507	315	287	0.79	Pg	1
6	53.3	12.3	523	311	283	0.74	Cy/Pn	1
7	55.5	21.6	507	327	287	0.65	Pg	1
8	57.0	11.9	523	327	283	0.70	Cy/Pn	1

¹t_R: retention time; ²PA: peak area% at 520 nm; ³R = $E_{\lambda_{\text{acyl.max}}}/E_{\lambda_{\text{vis.max}}}$: ratio of absorbances at $\lambda_{\text{acyl.max}}$ and at $\lambda_{\text{vis.max}}$;

⁴n: estimated number of binding acyl groups.

to be cyanidin (Cy) or peonidin (Pn) due to their $\lambda_{\text{vis.max}}$ at 515–523 nm (Harbone, 1967, 1968, 1973). Anthocyanins **3–8** were cinnamic acid-acylated due to their strong maximum absorption wavelengths at aromatic acyl region ($\lambda_{\text{acyl.max}}$) 310–335 nm, and the estimated binding acid numbers on each anthocyanin molecule were one mole because the ratios of absorbances at $\lambda_{\text{acyl.max}}$ and at $\lambda_{\text{vis.max}}$ ($R = E_{\lambda_{\text{acyl.max}}}/E_{\lambda_{\text{vis.max}}}$) were 0.6–0.7 (Harbone, 1967, 1968, 1973). Compounds **5** and **6** were estimated to contain p-coumaric acid (Pc) and **3**, **4**, **7** and **8** to contain caffeic (Cf) or ferulic (Fr) acid because $\lambda_{\text{acyl.max}}$ was 311–315 nm and 323–335 nm, respectively (Harbone, 1967, 1968, 1973). However, **1** and **2** were estimated to have no acylation by an aromatic acid due to the weak absorption at $\lambda_{\text{acyl.max}}$.

On ESI-TOF/MS, the isolated anthocyanins **3–8** all yielded clear mass numbers (m/z) of molecular ions (M^+) and the fragment ions such as de-monoglucosyl, monoglucosyl and aglycon ions. For example, **5** and **7** respectively gave m/z 887 ($C_{42}H_{47}O_{21}^+ = \text{Pg Pc.Rut-Glc}^+$) and 917 ($C_{43}H_{49}O_{22}^+ = \text{Pg Fr.Rut-Glc}^+$) as M^+ , 725 (Pg Pc.Rut^+) and 755 (Pg Fr.Rut^+) as the de-monoglucosyl ions, in addition to the common monoglucosyl ion 433 (Pg Glc^+), and aglycon ion 271 (Pg^+). High-resolution ESI/FT-ICRMS results of **5** (M^+ as m/z 887.2609 (err = +0.56 ppm) calcd for $C_{42}H_{47}O_{21}^+ = 887.2604$), and **7** (M^+ as m/z 917.2714 (err = +0.44 ppm) calcd for $C_{43}H_{49}O_{22}^+ = 917.2710$) supported the respective molecular formulas.

Based on the DAD-HPLC and ESI-TOF/MS results, in addition to previous reports relating to colored potatoes (Harbone, 1960; Howard, 1970; Andersen, 1991; Ishii *et al.*,

1996b; Lewis, 1998; Naito *et al.*, 1998; Fossen, 2000), **3–8** were estimated to be Pg 3-Cf.Rut-5-Glc, Pn 3-Cf.Rut-5-Glc, Pg 3-Pc.Rut-5-Glc (pelanin), Pn 3-Pc.Rut-5-Glc (peonanin), Pg 3-Fr.Rut-5-Glc, and Pn 3-Fr.Rut-5-Glc, respectively. Taken together, **1** and **2** were determined to be Pg 3-Rut-5-Glc and Pn 3-Rut-5-Glc, respectively.

To confirm the structures of the major anthocyanins **5** and **7**, ¹³C-NMR and ¹H-NMR analyses, including DQF-COSY, TOCSY, NOESY, HSQC and HMBC pulse techniques, were conducted, and the assignment data are summarized in Table 2. ¹H-NMR spectra of **5** and **7** showed the presence of the *p*-coumaroyl group (1,4-di-substituted benzene-type signal split) and feruloyl group (1,2,4-trisubstituted benzene-type signal split and a characteristic methoxy methyl signal at δ_H 3.83 and δ_C 55.8 ppm) bearing the (*E*)-olefinic α - and β -protons with large coupling constant signals ($J=16$ Hz), respectively. They were estimated to have the same sugar moieties, two D-glucopyranosyl (G_a and G_b) and one L-rhamnopyranosyl (Rh) moiety, based on the large J values (8.5–9.4 and 3.3–9.8 Hz, respectively) of the ring protons. G_a and G_b were oriented in the β -configuration as determined from the anomeric protons with large J values (7.6–7.8 Hz), and the Rh was in the α -configuration as the anomeric proton had a small J value (1.5–1.6 Hz). Since Rh was determined to be linked on the 6-hydroxyl of G_a (G_a -6OH) based on the glycosylation (lower field) shifts of δ_{G_b-6C} about $\Delta + 6$ ppm compared with one of δ_{G_b-6C} (Table 2), the interglycosidic linkage was α -Rh-(1→6)- G_a , indicating this sugar moiety was a rutinose. The connectivity was also supported by ob-

Table 2. ^{13}C (200 MHz)- and ^1H (800 MHz)-NMR chemical shifts of major *Kintoki-imo* anthocyanins 5 and 7 in $\text{DMSO-}d_6$ and CF_3COOD (9:1, v/v) with tetramethylsilane as an internal standard.

	5 (pelanin)		7	
	δ_{C} (ppm) *	δ_{H} (ppm)*	δ_{C} (ppm) *	δ_{H} (ppm) *
Pelargonidin				
2	163.3	—	162.9	—
3	145.0	—	144.8	—
4	133.6	8.96 s	133.2	8.91 s
5	155.7	—	155.2	—
6	104.7	7.05 d (1.9)	104.2	7.02 d (1.6)
7	168.3	—	167.8	—
8	96.6	7.20 d (1.9)	96.3	7.20 d (1.6)
9	155.9	—	155.5	—
10	112.3	—	111.9	—
1'	119.7	—	119.4	—
2'	135.5	8.67 d (9.5)	135.2	8.66 d (9.1)
3'	117.5	7.15 d (9.5)	117.1	7.12 d (9.1)
4'	165.9	—	165.4	—
5'	117.5	7.15 d (9.5)	117.1	7.12 d (9.1)
6'	135.5	8.67 d (9.5)	135.2	8.66 d (9.1)
Glucose-a (G_a)				
1	101.5	5.56 d (7.8)	101.3	5.49 d (7.8)
2	73.3	3.61 t (8.8)	72.9	3.56 t (8.5)
3	76.9	3.52 t (9.0)	76.5	3.46 t (9.0)
4	70.1	3.37 t (9.4)	69.5	3.34 t (9.3)
5	76.5	3.84 m	76.3	3.79 m
6a	66.4	3.66 dd (5.2, 11.8)	66.1	3.59 dd (5.6, 12.1)
6b	—	3.95 brd (10.6)	—	3.89 brd (10.6)
Glucose-b (G_b)				
1	104.7	5.18 d (7.6)	101.6	5.17 d (7.7)
2	73.8	3.55 t (8.8)	73.4	3.50 t (8.5)
3	76.4	3.48 t (9.0)	76.3	3.43 t (9.0)
4	69.8	3.43 t (9.3)	69.5	3.36 t (9.3)
5	78.0	3.57 m	77.6	3.53 m
6a	60.9	3.66 dd (5.2, 11.8)	60.5	3.64 dd (6.3, 9.8)
6b	—	3.81 brd (10.1)	—	3.76 brd (10.4)
Rhamnose (Rh)				
1	101.5	4.67 d (1.5)	100.7	4.63 d (1.6)
2	70.8	3.71 dd (1.5, 3.3)	70.4	3.67 dd (1.6, 3.3)
3	68.8	3.76 dd (3.3, 9.8)	68.4	3.72 dd (3.3, 9.8)
4	74.2	4.89 t (9.8)	73.8	4.87 t (9.8)
5	66.7	3.68 dd (6.4, 9.8)	66.3	3.64 dd (6.3, 9.8)
6 (CH_3)	17.8	0.98 d (6.4)	17.7	0.98 d (6.3)
Acid <i>p</i> -Coumaroyl				
1	125.8	—	125.9	—
2	130.8	7.54 d (8.7)	111.3	7.36 d (1.8)
3	116.3	6.85 d (8.6)	148.0	—
4	160.4	—	149.4	—
5	116.3	6.85 d (8.6)	115.6	6.83 d (8.2)
6	130.8	7.54 d (8.7)	123.3	7.14 dd (1.8, 8.2)
	114.8	6.35 d (15.9)	114.8	6.43 d (15.8)
	145.5	7.58 d (15.9)	145.4	7.54 d (15.8)
Feruloyl				
1	125.8	—	125.9	—
2	130.8	7.54 d (8.7)	111.3	7.36 d (1.8)
3	116.3	6.85 d (8.6)	148.0	—
4	160.4	—	149.4	—
5	116.3	6.85 d (8.6)	115.6	6.83 d (8.2)
6	130.8	7.54 d (8.7)	123.3	7.14 dd (1.8, 8.2)
	114.8	6.35 d (15.9)	114.8	6.43 d (15.8)
	145.5	7.58 d (15.9)	145.4	7.54 d (15.8)
Carbonyl				
OCH ₃	—	—	55.8	3.83 s

* Values in parentheses indicate coupling constants (J in Hz). δ_{C} and δ_{H} : ^{13}C -NMR and ^1H -NMR chemical shifts, respectively.

Fig. 1. Structures of major *Kintoki-imo* anthocyanins 5 and 7.

servation of the NOESY cross peak between Rh-1H and G_a-6Hs, and of the HMBC cross peak between Rh-1H and G_a-6C as shown in Figure 1. The deshielding shifts of Rh-4Hs (δ_{H} 4.8 ppm) and Rh-4C (δ_{C} 74 ppm) suggested that their acyl groups were attached to Rh-4OH. This was confirmed by the HMBC correlation peaks between Rh-4H and a acyl carbonyl carbon signals. Therefore, anthocyanins 5 and 7 were elucidated to have a 4-*O*-acyl- α -Rh-(1 $\rightarrow$ 6)-G_a structure, which was supported by the ¹³C-NMR results (Table 2). The connecting position of sugar residues in 5 and 7 were also confirmed by NOESY and HMBC. In the NOESY spectra, the correlations between Pg-4H and G_a-1H, and Pg-6H and G_b-1H indicated that G_a was β -glycosylated to Pg-3OH, and G_b was β -glycosylated to Pg-5OH, respectively, as shown in Figure 1. Taken together, the major *Kintoki-imo* anthocyanins 5 and 7 were unambiguously determined as pelargonidin 3-(6-*O*-(4-*O*-(*E*)-*p*-coumaroyl- α -L-rhamnopyranosyl)- β -D-glucopyranoside)-5- β -D-glucopyranoside (pelanin), and pelargonidin 3-(6-*O*-(4-*O*-(*E*)-feruloyl- α -L-rhamnopyranosyl)- β -D-glucopyranoside)-5- β -D-glucopyranoside, respectively (Figure 2). On co-HPLC analysis with standard pelanin, 5 was confirmed to be pelanin. These two major anthocyanins have been already found in other red-colored potato cultivars (Naito *et al.*, 1998).

The peel and flesh color, 1% TFA extract color and anthocyanin concentration (mg/100 g) of potato cultivars are summarized in Table 3. Type I is composed of foreign

cultivar varieties, type II of Japanese bred varieties, type III of Japanese land races and type IV of newly bred colored varieties and their parental lines. *Jaga Kids Purple* and *Hanashibetu* have a purple peel, while the other varieties in types I and II have a red peel. Coloration of anthocyanins was slightly visible in flesh of *Benimaru*, *Jaga Kids Purple* and *Jaga Kid Red*, but most varieties did not contain an anthocyanin in their flesh; however, there was a little anthocyanin content per 100 g flesh tuber (2-36 mg). Among type II varieties, *Kintoki-imo* and *Nagasaki-murasaki* contained slight amounts of anthocyanin in their flesh; however, there was little anthocyanin content per the 100g flesh tuber (3-50 mg). Among type IV cultivars, the anthocyanin content of 553-4 was 15 mg, *Kitamurasaki* was 244 mg, *Northern Ruby* was 195 mg and *Shadow Queen* was 816 mg per 100 g flesh tuber.

The anthocyanin composition patterns of potato cultivars analyzed by HPLC are shown in Table 4. Compared with petanin, pelanin and *Kintoki-imo* anthocyanins P-a to P-h were determined to be Pg 3-Rut-5-Glc, Pn 3-Rut-5-Glc, Petunidin (Pt) 3-Cf.Rut-5-Glc (petanin), Pn 3-Cf.Rut-5-Glc, Pg 3-Pc.Rut-5-Glc (pelanin), Pn 3-Pc.Rut-5-Glc (peonanin), Pg 3-Fr.Rut-5-Glc, and Pn 3-Fr.Rut-5-Glc, respectively. The anthocyanin composition patterns were classified into five types (A-E) based on the HPLC analysis results. For the type A pattern, *Desiree*, *Red Pontiac* and *Red Warba* of Type I and *Aino-aka*, *Jaga Kids Red* and *Red Moon* of Type II

Table 3. Characteristics of anthocyanin in various varieties and breeding lines.

Variety	Color		Anthocyanin in flesh	Extract at 1% TFA		Anthocyanin content (mg/100 g flesh)
	Skin	Flesh		Color	$\lambda_{\text{vis,max}}$ (nm)	
Type I						
<i>Red Warba</i>	Red	Light yellow	–	Orange	508	4
<i>La Soda</i>	Red	White	–	Magenta	515	2
<i>Desiree</i>	Red	Light yellow	–	Orange	508	8
<i>Red Pontiac</i>	Red	White	–	Orange	503	3
Type II						
<i>Aino-aka</i>	Red	Light yellow	–	Orange	506	3
<i>Beni-akari</i>	Red	White	–	Magenta	516	3
<i>Benimaru</i>	Red	White	+/–	Orange	506	13
<i>Hanashibetsu</i>	Red-purple	White	–	Purple	514	15
<i>Red Moon</i>	Red	Light yellow	–	Orange	508	7
<i>Andes Red</i>	Red	Yellow	–	Orange	506	12
<i>Jaga Kids Purple</i>	Purple	Yellow	+/–	Purple	523	36
<i>Jaga Kids Red</i>	Red	Yellow	+/–	Orange	506	13
Type III						
<i>Nakatsugawa-imo</i>	Red	White	–	Magenta	514	12
<i>Kintoki-imo</i>	Dark red	White	+/–	Magenta	514	12
<i>Nagasaki-murasaki</i>	Purple	Light yellow	+/–	Purple	530	50
<i>Nagasaki-beni</i>	Red	White	–	Orange	508	3
<i>Nemuro-murasaki</i>	Purple	White	–	Purple	530	42
<i>Kushirosan</i>	Red	White		Orange	506	10
Type IV						
<i>553-4</i> ¹	Red	Yellow	+	Orange	506	15
<i>Inca Purple</i>	Purple	Purple	+	Purple	523	212
<i>Inca Red</i>	Red	Red	+	Orange	503	130
<i>Kitamurasaki</i>	Purple	Purple	+	Purple	525	244
<i>Shadow Queen</i>	Purple	Purple	+	Purple	523	816
<i>Northern Ruby</i>	Red	Red	+	Orange	503	195

¹ *S. tuberosum* ssp. *andigena*

contained a lot of pelanin and Pg 3-Fr.Rut-5-Glc and a little of peonanin. The type B pattern was similar to that of type A, but with much more purple pigments such as peonanin and Pn 3-Rut-5-Glc; *Kintoki-imo* and *Nakatsugawa-imo* belonged to type B. The type C pattern was also similar to type A, but with high peonanin content; *Beni-akari*, *Hanashibetsu* and *La Soda* belonged to type C. As for the type D pattern, pelanin was the main pigment comprising more than 45%, but no peonanin was included; *553-4*, *Inca Red* and *Northern*

Ruby of the *Inca Red* breeding group, *Kushirosan*, *Andes Red* and *Benimaru* belong to type D. All purple skin colored potatoes had the type E pattern; type E included no pelanin, but petanin comprised 44% of the pigments. *Jaga Kids Red* and *Jaga Kids Purple* were protoclonal variants of type D. *Andes Red* (Okamura *et al.*, 1991), but were classified into types A and E, respectively. These results suggest that a minimal number of gene mutations can produce skin color variation in potatoes.

Table 4. Anthocyanin composition (%) characterized by HPLC in various varieties and breeding lines.

Peak	P-a	P-b	P-c	P-d	P-e	P-f	P-g	P-h	Others	Type
Red skin variety										
<i>Red Warba</i>	1	1			39	8	21	13	17	A
<i>Desiree</i>	12	3			37	6	21	4	17	A
<i>Red Pontiac</i>	1	1			27	4	31	7	29	A
<i>Aino-aka</i>	2	2			19	4	25	9	39	A
<i>Red Moon</i>	1	1			41	8	19	10	20	A
<i>Jaga Kids Red</i>	2	1			45	7	13	3	29	A
<i>Nagasaki-beni</i>	1	2			15	9	16	16	41	A
<i>Nakatugawa-imo</i>	8	10			24	15	21	15	7	B
<i>Kintoki-imo</i>	7	8			26	12	23	15	9	B
<i>Beni-akari</i>				5	8	24	12	29	22	C
<i>Hanashibetu</i>	3	11			8	14	9	17	38	C
<i>La Soda</i>	1	2			19	23	17	11	27	C
<i>Benimaru</i>					46		26	4	24	D
<i>Andes Red</i>					52		16	2	30	D
<i>Kushirosan</i>	6	2			45		21	4	22	D
<i>553-4</i> ¹	5	1			60		18	4	12	D
<i>Inca Red</i>	3				71		6		20	D
<i>Northern Ruby</i>	2				77		5		16	D
Purple skin variety										
<i>Jaga Kids Purple</i>		3	44			19		5	29	E
<i>Nagasaki-murasaki</i>		2	67	17		5		2	7	E
<i>Nemuro-murasaki</i>		3	50	21		9		7	10	E
<i>Inca Purple</i>		2	57	8		17		3	13	E
<i>Kitamurasaki</i>		1	63	7		13		2	14	E
<i>Shadow Queen</i>			72	3		14			11	E

¹⁾ *S. tuberosum* ssp. *andigena*

P-a: pelargonidin 3-rutinoside, 5-glucoside (Pg 3-Rut-5-G)

P-b: peonidin 3-rutinoside, 5-glucoside (Pn 3-Rut-5-G)

P-c: petunidin 3-p-coumaroylrutinoside, 5-glucoside (Petanin)

P-d: peonidin 3-caffeoylrutinoside, 5-glucoside (Pn 3-Cf.Rut-5-G)

P-e: pelargonidin 3-p-coumaroylrutinoside, 5-glucoside (Pelatin)

P-f: peonidin 3-p-coumaroylrutinoside, 5-glucoside (Peonatin)

P-g: pelargonidin 3-feruloylrutinoside, 5-glucoside (Pg 3-Fr.Rut-5-G)

P-h: peonidin 3-feruloylrutinoside, 5-glucoside (Pn 3-Fr.Rut-5-G)

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