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Preparative Isolation of Anthocyanins from Japanese Purple Sweet Potato (*Ipomoea batatas* L.) Varieties by High-Speed Countercurrent Chromatography

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Purple-fleshed sweet potatoes (*Ipomoea batatas* L.) contain a very complex anthocyanin profile due to the presence of several non-, mono-, and diacylated glucosides of cyanidin and peonidin. In this study, the anthocyanin composition of four Japanese purple sweet potato cultivars (Chiran Murasaki, Tanegashima Murasaki, Naka Murasaki, and Purple Sweet) were investigated by HPLC-DAD and ESI-MS^n analyses. The HPLC chromatograms of the different cultivars show a remarkable variation of the two major pigments, cyanidin-3-(6″-caffeoylsophoroside)-5-glucoside and peonidin-3-(6″-caffeoylsophoroside)-5-glucoside, respectively. According to this, they can be categorized into two groups on the basis of the peonidin/cyanidin ratio: the cultivars Chiran Murasaki and Purple Sweet showed a high content of peonidin derivatives (peonidin type), whereas the varieties Tanegashima Murasaki and Naka Murasaki were classified as cyanidin types. By means of high-speed countercurrent chromatography (HSCCC) the nonacylated 3-sophoroside-5-glucoside of cyanidin was isolated on a preparative scale. Furthermore, it was possible to isolate the monoacylated cyanidin-3-(6″-caffeoylsophoroside)-5-glucoside as well as three diacylated major pigments, cyanidin-3-(6″,6‴-dicaffeoylsophoroside)-5-glucoside, cyanidin-3-(6″-caffeoyl-6‴-*p*-hydroxy-benzoylsophoroside)-5-glucoside, and peonidin-3-(6″-caffeoyl-6‴-*p*-hydroxybenzoyl-sophoroside)-5-glucoside. The purity and identity of the so-obtained pigments were confirmed by NMR measurements.

KEYWORDS: Purple sweet potato; HSCCC; Chiran Murasaki; Tanegashima Murasaki; Naka Murasaki; Purple Sweet

INTRODUCTION

The sweet potato (*Ipomoea batatas* L.) is considered to originate from the tropical Americas, most probably Central America. Today, the storage roots are grown worldwide in tropical and subtropical regions. Sweet potatoes are a major food crop in developing countries with a total world production of approximately 110 million tons per annum (*1*). It ranks as the world's seventh most important staple food after wheat, rice, maize, potato, barley, and cassava (*2*).

Purple-fleshed sweet potatoes (PSP) exhibit an intense purple color in the skins and flesh of the storage root due to the accumulation of mono- and diacylated anthocyanins (*3*, *4*). These cultivars were mainly grown in Japan, Korea, or New Zealand (*5*), and new varieties were developed in breeding programs for use as natural food colorants. Today, these varieties are gaining popularity as a dietary source rich in anthocyanins. A prominent example is the Japanese cultivar Ayamurasaki, which is the second generation of Yamagawamurasaki. Anthocyanin pigments in the purple sweet potato cultivar Yamagawamurasaki have been analyzed, isolated, and characterized by NMR analyses by several investigators (*6*, *7*). These anthocyanins belong either to the cyanidin or peonidin type and are linked with sophorose and glucose. Moreover, acylation with ferulic, caffeic, and *p*-hydroxybenzoic acid is typical. **Figure 1** shows the structures of major anthocyanins found in purple sweet potatoes.

Acylated anthocyanins from purple sweet potato can serve as natural colorants due to their high heat and light stability (*8*, *9*). They are commonly used in juices, alcoholic beverages, jams, confectioneries, bread, snacks, and noodles. The high content of anthocyanins combined with the high color stability affords a healthier alternative to synthetic colorants such as FD&C red 40 (*8*, *10*). The consumption of purple sweet potato tubers has always been associated with health-protecting effects, which have mainly been ascribed to their strong antioxidant properties. Extracts from purple sweet potato are reported to possess antimutagenic activities (*11*) and antihypertensive (*12*) as well as antihyperglycemic (*13*) and hepatoprotective effects (*14*). There are several commercially available varieties of purple sweet potato, which can vary in storage root size, shape, flavor, texture, and color (*15*). A comparison of 19 Japanese clones by Yoshinaga et al. (*16*) and 16 Japanese

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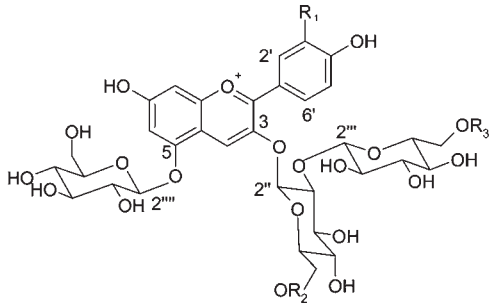

compd[a]	R_1	R_2	R_3
1	OH	H	H
2	OCH_3	H	H
9	OH	caf	caf
10	OH	caf	*p*hb
11	OH	caf	H
12	OH	caf	fer
13	OCH_3	caf	caf
14a	OCH_3	caf	H
14b	OCH_3	caf	*p*hb
15	OCH_3	caf	fer

Figure 1. Structures of major anthocyanins found in purple sweet potatoes. [a]For compound labeling cf. **Table 1**. Abbreviations: caf, caffeoyl; phb, *p*-hydroxybenzoyl; fer, feruloyl.

cultivars by Oki et al. (*17*) showed a remarkable variation in the anthocyanin profile. According to their composition, the Japanese cultivars can be categorized into two groups on the basis of the shade of color and the peonidin/cyanidin (pn/cy) ratio: cyanidin types (pn/cy < 1.0) with a greater degree of blueness (blue dominant group) and peonidin types (pn/cy >1.0) with a greater degree of redness (red dominant group) (*9*, *16*). New varieties of purple-fleshed sweet potatoes contain high amounts of anthocyanins (between 0.4 and 0.6 mg of anthocyanins/g of fresh weight) (*14*, *18*).

The objective of this study was the preparative isolation of anthocyanins from Japanese purple sweet potato cultivars Chiran Murasaki, Tanegashima Murasaki, Naka Murasaki, and Purple Sweet, by application of high-speed countercurrent chromatography (HSCCC). HSCCC is an automated and easy to handle liquid−liquid chromatographic technique that has been used for the preparative isolation of numerous natural products (*19*). The identity and purity of the isolated compounds were confirmed by high-performance liquid chromatography with photo diode array detection (HPLC-PDA) and HPLC−multiple mass spectrometry (HPLC-ESI-MS^n) as well as ionization multiple mass spectrometry (ESI-MS^n). To our knowledge, this is the first application of HSCCC for the preparative separation of pure PSP pigments on a large scale.

MATERIALS AND METHODS

Plant Material. The purple sweet potato varieties Chiran Murasaki (CM), Tanegashima Murasaki (TM), Naka Murasaki (N), and Purple Sweet (PS) were obtained from a local market (Chiran no Sato) in Kagoshima (Japan) in October 2009.

Extraction of Anthocyanins and Cleanup by Precipitation. Purple sweet potato slices (approximately 1 kg including peel) were blanched at 100 °C for 3 min with 1 L of demineralized water. The same volume of a solution of water/hydrochloric acid (19:1, v/v) was added. The suspension was cooled at 0 °C for 3 h and then stored at room temperature for 8 h without stirring. To remove solid material, the suspension was filtered prior to application onto an Amberlite XAD-7 column. The column was rinsed with water, and the anthocyanins were eluted with a mixture of methanol/acetic acid (19:1, v/v). The eluate was concentrated in vacuo, dissolved in water, and freeze-dried. Due to the remaining high content of polymeric compounds (e.g., starch), a further cleanup step was carried out according to the method described by Hillebrand et al. (*20*). Approximately 4 g of the XAD-7 extract was redissolved in 90 mL of a mixture of methanol/water (1:1, v/v). After the addition of 800 mL of a mixture of *tert*-butyl methyl ether/methanol (7:2, v/v), the precipitate was removed by filtration. The combined clear filtrates were evaporated in vacuo, and the aqueous phase was lyophilized. Depending on the cultivar, between 1.5 and 2.4 g of purified XAD-7 extracts was obtained from 1 kg of fresh material.

High-Performance Liquid Chromatography (HPLC). HPLC analyses were performed on an MD-910 multiwavelength detector (wavelength range between 220 and 650 nm), equipped with a DG-980-50 3-line degasser and an LG-980-02 ternary gradient unit, a PU-980 Intelligent HPLC pump, an AS-950 Intelligent autosampler, and Borwin PDA chromatography software (Jasco, Gross-Umstadt, Germany). HPLC separation was carried out on a Luna RP-18 column (250 × 4.6 mm, 5 μm, Phenomenex, Aschaffenburg, Germany) at a flow rate of 0.5 mL/min, using an injection volume of 20 μL (in solvent system A). The binary gradient consisted of solvent system A (water/acetonitrile/formic acid 87:3:10, v/v/v) and solvent system B (water/acetonitrile/formic acid 40:50:10, v/v/v) and was as follows: 0 min, 6% B; 20 min, 20% B; 35 min, 40% B; 40 min, 60% B; 45 min, 90% B; and 55 min, 6% B, followed by a 5 min equilibration period.

High-Performance Liquid Chromatography−Electrospray Ionization Multiple Mass Spectrometry (HPLC-ESI-MS^n). Freeze-dried samples (XAD-7 extracts as well as CCC fractions containing a mixture of anthocyanins) were redissolved in a mixture of water/acetonitrile/formic acid (95:3:2, v/v/v) and were analyzed by HPLC-ESI-MS^n. HPLC analyses were performed using an Agilent system (Böblingen, Germany) equipped with a binary pump (1100 series) and an autosampler (1200 series). The same analytical conditions as described above were used. ESI-MS^n measurements were performed on a Bruker Esquire-LC multiple ion trap mass spectrometer (Bruker Daltonics, Bremen, Germany). Mass spectral analyses were recorded under the following operating conditions: positive ion mode; capillary, −2500 V; capillary exit offset, 70 V; end plate offset, −500 V; skimmer 1, 20 V; skimmer 2, 10 V; dry gas, N_2, 11 L/min; dry temperature, 325 °C; nebulizer, 60 psi; scan range, m/z 50−2200. Esquire NT 4.0 software (Bruker Daltonics) was used for analysis and data collection.

Electrospray Ionization Multiple Mass Spectrometry (ESI-MS^n). For ESI-MS^n experiments pure pigments were redissolved in a mixture of water/acetonitrile/formic acid (95:3:2, v/v/v) and were delivered directly via a syringe pump 74900 (Cole-Parmer, USA) into the ESI source (flow rate = 240 μL/h). Mass spectrometry parameters were the following: positive ion mode; capillary, −3500 V; capillary exit offset, 60 V; end plate offset, −500 V; skimmer 1, 30 V; skimmer 2, 10 V; dry gas, N_2, 4 L/min; dry temperature, 300 °C; nebulizer, 10 psi; scan range, m/z 50−1000.

Fractionation by High-Speed Countercurrent Chromatography (HSCCC). Separations were carried out with a high-speed model CCC-1000 (Triplecoil, diameter of tubing = 2.6 mm, total volume = 850 mL, revolution speed = 850 rpm) produced by Pharma-Tech Research Corp. (Baltimore, MD). A mixture of *tert*-butyl methyl ether/*n*-butanol/acetonitrile/water (1:3:1:5, v/v/v/v, acidified with 0.1% trifluoroacetic acid) was used as solvent system (less dense layer as stationary phase) with a flow rate of 4 mL/min delivered by a Biotronik HPLC-Pump-BT 3020 from Jasco (Gross-Umstadt, Germany). Approximately 500 mg of each XAD-7 extract (redissolved in 22 mL of solvent mixture) was injected for a single run. Fractions were collected with a Pharmacia LKB super Frac fraction collector (Bromma, Sweden). Anthocyanins were detected at 520 nm with a Knauer UV−vis detector (Berlin, Germany), and the resulting chromatograms were recorded by a plotter (BBC Goerz Metrawatt SE 120, Vienna, Austria).

Proton and Carbon Nuclear Magnetic Resonance Spectroscopy (NMR). ^1H and ^{13}C NMR measurements were performed on a Bruker AMX 600 spectrometer (Bruker Biospin, Rheinstetten, Germany) at 600.13 and 150.92 MHz, respectively. Pure pigments were dissolved in a mixture of methanol-d_4/TFA-d_1 (19:1, v/v), and data were processed by WIN-NMR software version 6.1.0.0.

RESULTS AND DISCUSSION

Variety Chiran Murasaki. The variety Chiran Murasaki is an indigenous sweet potato cultivar that is grown in Japan. The storage roots are large-sized and oval-shaped and exhibit a dark reddish-purpled skin and a deep purple flesh (*21*). The anthocyanin

Table 1. Mass Spectrometric Properties of Anthocyanins **1**−**15** Characterized from Different Japanese Purple Sweet Potato Cultivars

peak	compound	t_R (min)	$[M]^+$ (*m*/*z*)	fragments (*m*/*z*)
1	cy-3-soph-5-glc[a]	12.9	773	611, 449, 287
2	pn-3-soph-5-glc[b]	18.5	787	625, 463, 301
3	cy-3-*p*-hydroxybenzoylsoph-5-glc[b]	19.5	893	731, 449, 287
4	cy-3-(6‴caffeoylsoph)-5-glc[c]	20.1	935	773, 449, 287
5	pn-3-*p*-hydroxybenzoylsoph-5-glc[b]	25.0	907	745, 463, 301
6	unidentified	25.2	937	
7	cy-3-feruloylsoph-5-glc[b]	27.1	949	787, 449, 287
8	pn-3-feruloylsoph-5-glc[b]	32.0	963	801, 463, 301
9	cy-3-(6″,6‴-dicaffeoylsoph)-5-glc[c]	33.1	1097	935, 449, 287
10	cy-3-(6″-caffeoyl-6‴-*p*-hydroxybenzoylsoph)-5-glc[c]	33.6	1055	893, 449, 287
11	cy-3-(6″-caffeoylsoph)-5-glc[c]	34.0	935	773, 449, 287
12	cy-3-(6″-caffeoyl-6‴-feruloylsoph)-5-glc[c]	35.6	1111	949, 449, 287
13	pn-3-(6″,6‴-dicaffeoylsoph)-5-glc[c]	36.6	1111	949, 463, 301
14a	pn-3-(6″-caffeoylsoph)-5-glc[c]	37.2	949	787, 463, 301
14b	pn-3-(6″-caffeoyl-6‴-*p*-hydroxybenzoylsoph)-5-glc[c]		1069	907, 463, 301
15	pn-3-(6″-caffeoyl-6‴-feruloylsoph)-5-glc[c]	38.9	1125	963, 463, 301

[a] Structure elucidation based on ESI-MS^n analysis and NMR data. Abbreviations: cy = cyanidin; pn = peonidin; soph = sophoroside; glc = glucoside. [b] Structure identification based on ESI-MS^n analysis. [c] Structure identification according to literature data (*4*, *7*, *24*).

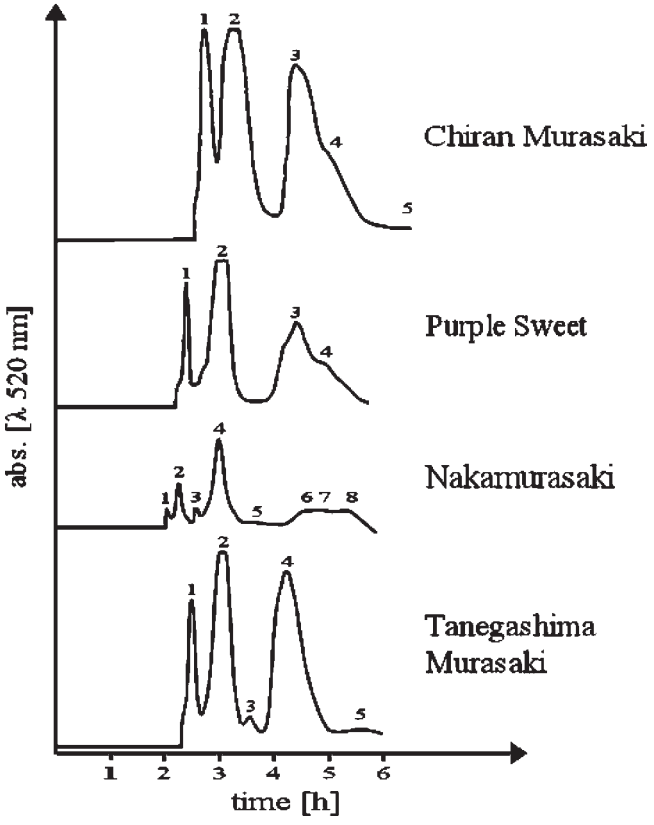

Figure 2. HSCCC separations of the purple sweet potato varieties Chiran Murasaki, Tanegashima Murasaki, Naka Murasaki, and Purple Sweet monitored at 520 nm.

composition of this variety was examined by HPLC-DAD and HPLC-ESI-MS^n analyses. Three major pigments (**2**, **14a**, and **15**) and eight minor anthocyanins (**1**, **5**, **7**, **8**, **11**−**13**, and **14b**) were detected at 520 nm (chromatogram not shown, cf. **Table 1**). By means of HSCCC five fractions as well as the coil residue were obtained. **Figure 2** shows the chromatogram of the HSCCC separation recorded at 520 nm. CCC fraction 1 (36 mg) contains a mixture of the two nonacylated 3-sophoroside-5-glucosides of cyanidin (**1**) and peonidin (**2**), respectively. The major pigment peonidin-3-(6″-caffeoylsophoroside)-5-glucoside (**14a**) could be obtained from fraction 2 in large yields (350 mg) in a purity of 63%. The minor anthocyanins peonidin-3-feruloylsophoroside-5-glucoside (**8**), cyanidin-3-(6″-caffeoylsophoroside)-5-glucoside (**11**), and cyanidin-3-(6″-caffeoyl-6‴-feruloylsophoroside)-5-glucoside (**12**) as well as the diacylated peonidin derivatives peonidin-3-(6″,6‴-dicaffeoylsophoroside)-5-glucoside (**13**) and peonidin-3-(6″-caffeoyl-6‴-feruloylsophoroside)-5-glucoside (**15**) coelute in CCC fractions 3 and 4 in amounts of 300 and 23 mg, respectively. Approximately 25 mg of peonidin-3-(6″-caffeoyl-6‴-*p*-hydroxybenzoylsophoroside)-5-glucoside (**14b**) could be isolated from fraction 5 in a purity of 90%. Mass spectrometric analysis of **14b** showed a molecular ion $[M]^+$ at *m*/*z* 1069 and fragments at *m*/*z* 907, 463, and 301. ^{1}H and ^{13}C NMR spectra were compared with literature data published by Terahara et al. (*4*). The monoacylated pigment cyanidin-3-feruloylsophoroside-5-glucoside (**7**) and the diacylated peonidin derivative peonidin-3-*p*-hydroxybenzoylsophoroside-5-glucoside (**5**) were characterized as minor compounds by HPLC-ESI-MS^n analysis in different CCC fractions. Mass spectrometric properties of the identified anthocyanins are presented in **Table 1**. In contrast to early results reported by Yoshinaga et al. (*16*), our investigations show a high content of peonidin derivatives. Due to this fact, the cultivar Chiran Murasaki could be classified as "peonidin-type"variety (**Table 2**).

Variety Tanegashima Murasaki. The cultivar Tanegashima Murasaki is a traditional variety from Tanegashima (Japan). The storage roots are very sweet and, when steamed, quite tasty without being soggy. These features make it a popular crop for fresh consumption. The storage roots are medium-sized and oval-shaped with yellowish-white skin and blue-purple-marbled flesh (*21*). Chromatographic analyses (chromatogram not shown, cf. **Table 1**) show two major anthocyanins (**11** and **12**) and four minor pigments (**1**, **7**, **9**, and **14a**). The XAD-7 isolate of this variety was fractionated by HSCCC, and five fractions as well as the coil residue were obtained (**Figure 2**). Fraction 1 (105 mg) contains the nonacylated anthocyanin cyanidin-3-sophoroside-5-glucoside (**1**) in a purity of 90% exhibiting a pseudomolecular ion $[M]^+$ at *m*/*z* 773. The fragmentation pattern of this compound (*m*/*z* 611, 449, 287) indicated the presence of sophorose and glucose moieties. The NMR spectra of **1** were in accordance with previously published ^{1}H and ^{13}C NMR data for this nonacylated cyanidin derivative obtained from black rice (*22*). To our knowledge, this is the first report on the preparative isolation of cyanidin-3-sophoroside-5-glucoside from a purple sweet potato variety. Compound **1** has already been isolated from a sweet potato cell line by Tian et al. (*23*). **Table 3** summarizes the

Table 2. Major Anthocyanin Composition of Japanese Purple Sweet Potato Varieties Chiran Murasaki (CM), Tanegashima Murasaki (TM), Naka Murasaki (N), and Purple Sweet (PS)

	percentage of pigments[a]			percentage of major pigments[b]								
	Pn	Cy	Pn/Cy	**1**	**2**	**9**	**10**	**11**	**12**	**13**	**14a/b**	**15**
CM	80.2	19.8	4.05	3.9	12.2			9.4	6.5	5.7	44.8	17.5
TM	4.2	95.8	0.04	11.6		10.8		45.5	27.9		4.2	
N	9.3	90.7	0.10	14.6		10.2	13.1	40.0	12.8		9.3	
PS	81.9	18.1	4.52	7.9	33.8			4.5	5.7	10.2	29.1	8.8

[a] Cy, cyanidin derivatives (**1**, **9**, **10**, **11**, **12**); Pn, peonidin derivatives (**2**, **13**, **14a**, **14b**, **15**). Relative area (%). [b] For compound labeling cf. **Table 1**.

Table 3. ^{1}H and ^{13}C NMR Spectroscopic Data of Cyanidin-3-sophoroside-5-glucoside (**1**) in CD_3OD/TFA-d_1 (19:1, v/v)

position	^{1}H NMR[a] (ppm) ($J_{H,H}$ in Hz)	^{13}C NMR (ppm)
aglycone		
2		165.24
3		146.34
4	9.09 s	135.81
5		157.17
6	7.06 s	105.71
7		169.47
8	7.06 s_{br}	97.35
9		156.89
10		113.24
1′		121.17
2′	8.07 d (2.0)	118.72
3′		147.65
4′		156.55
5′	7.08 d (9.0)	117.76
6′	8.30 dd (9.0; 2.0)	129.02
3-glc		
1″	5.49 d (7.5)	102.40
2″	4.10 t (9.0)	81.73
3″	3.76 t (9.0)	78.09
4″	3.42−3.79 m	70.94
5″	3.42−3.79 m	78.68
6″-A	4.04 d_{br} (12.5)	62.4
6″-B	3.94 dd (12.0; 5.5)	62.4
2″-glc		
1‴	4.79 d (7.5)	104.61
2‴	3.26−3.33 m	75.79
3‴	3.26−3.33 m	77.69
4‴	3.27 t (9.0)	71.25
5‴	3.17−3.23 m	77.99
6‴	3.42−3.79 m	62.4
5-glc		
1⁗	5.18 d (8.0)	102.63
2⁗	3.66 t (9.0)	74.49
3⁗	3.55 t (9.0)	77.69
4⁗	3.42−3.79 m	71.10
5⁗	3.42−3.79 m	78.61
6⁗-A	4.04 d_{br} (12.5)	62.4
6⁗-B	3.94 dd (12.0; 5.5)	62.4

[a] Abbreviations: s, singulet; d, doublet; t, triplet; m, multiplet; br, broad.

assignments of chemical shifts and coupling constants of pigment **1**. The major anthocyanin fraction 2 (300 mg) eluted after 3 h and contains the major pigment cyanidin-3-(6″-caffeoylsophoroside)-5-glucoside (**11**) in a purity of 85%. The structure of **11** was confirmed by ESI-MS^n and NMR measurements (*7*). The pseudomolecular ion ($[M]^+$ with m/z 935) yielded fragments of m/z 773, 449, and 287 due to the loss of sophorose and glucose as well as caffeic acid (**Table 1**). CCC fraction 4 (90 mg) contains mainly cyanidin-based compounds; among them the diacylated derivative cyanidin-3-(6″-caffeoyl-6‴-feruloylsophoroside)-5-glucoside (**12**) predominated. In addition, the acylated anthocyanins cyanidin-3-feruloylsophoroside-5-glucoside (**7**), cyanidin-3-(6″,6‴-dicaffeoylsophoroside)-5-glucoside (**9**), and peonidin-3-(6″-caffeoylsophoroside)-5-glucoside (**14a**) were found as minor pigments in different CCC fractions and could be characterized by HPLC-ESI-MS^n analyses. According to the data published by Yoshinaga et al. (*16*) the cultivar Tanegashima Murasaki could be characterized as "cyanidin-type" because of the high amount of cyanidin derivatives (**Table 2**).

Variety Naka Murasaki. The cultivar Naka Murasaki is a famous Japanese PSP variety. The storage roots are medium-sized, round-shaped, and dark reddish-purple-skinned. The flesh of this cultivar is white with a big purple vascular ring (*21*). The HPLC chromatogram of the XAD-7 extract shows four major (**1**, **10**, **11**, and **12**) and six minor (**2**, **3**, **4**, **7**, **9**, and **14a**) pigments (chromatogram not shown, cf. **Table 1**). By means of HSCCC, eight fractions were obtained (**Figure 2**). CCC fraction 2 afforded 25 mg of the nonacylated cyanidin derivative cyanidin-3-sophoroside-5-glucoside (**1**) in a purity of 85%. The major fraction 4 (125 mg) contains mainly cyanidin-3-(6″-caffeoylsophoroside)-5-glucoside (**11**) in a purity of 80%. Fraction 6 (15 mg) eluted after 4.5 h and contains the pigment **9** (purity = 90%). ESI-MS^n data of **9** showed a molecular ion at m/z 1097 and fragments at m/z 935, 449, and 287, respectively. This pigment was identified by ^{1}H and ^{13}C NMR analyses as cyanidin-3-(6″,6‴-dicaffeoylsophoroside)-5-glucoside (**9**) previously described by Terahara et al. (*4*). A mixture of different anthocyanins could be found in fraction 7, among them mainly the 3-(6″-caffeoyl-6‴-feruloylsophoroside)-5-glucoside of cyanidin (**12**). Approximately 25 mg of cyanidin-3-(6″-caffeoyl-6‴-*p*-hydroxybenzoylsophoroside)-5-glucoside (**10**) could be isolated from fraction 8 (85%). When analyzed by ESI-MS^n, pigment **10** showed a molecular ion at m/z 1055 with fragment ions at m/z 893, 449, and 287 (**Table 1**). The structure was elucidated by using ^{1}H and ^{13}C NMR spectroscopic data (*4*). Additional pigments (i.e., cyanidin-3-*p*-hydroxybenzoylsophoroside-5-glucoside (**3**) and peonidin-3-(6″-caffeoylsophoroside)-5- glucoside (**14a**)) were identified by HPLC-DAD and HPLC-ESI-MS^n analyses in different CCC fractions. In the Naka Murasaki variety, cyanidin derivatives are predominant (**Table 2**). On the basis of the calculated peonidin/cyanidin ratio (0.10) this cultivar belongs to the blue dominant group ("cyanidin-type").

Variety Purple Sweet. The variety Purple Sweet is a new Japanese breeding cultivar. The storage roots are fusiform and uniform. The skin is dark red-purple, and the flesh is purple. The taste is starchy, tasty, and sweet (*21*). The anthocyanin composition of this variety is very complex. Three major pigments (**2**, **13**, and **14a**) and nine minor anthocyanins (**1**, **5**−**8**, **11**, **12**, **14b**, and **15**) were detected at 520 nm (chromatogram not shown, cf. **Table 1**). The HSCCC separation of the XAD-7 lyophilisate yielded four fractions (**Figure 2**). Fraction 1 (50 mg) contains mainly the nonacylated 3-sophoroside-5-glucosides of cyanidin (**1**) and peonidin (**2**). The major anthocyanin fraction 2 (220 mg) eluted after 3 h and contains a mixture of various pigments, among them the major

anthocyanin of the cultivar Purple Sweet, the monoacylated peonidin-3-(6″-caffeoylsophoroside)-5-glucoside (**14a**). The pigments cyanidin-3-(6″-caffeoyl-6‴-feruloylsophoroside)-5-glucoside (**12**), peonidin-3-(6″,6‴-dicaffeoylsophoroside)-5-glucoside (**13**), and peonidin-3-(6″-caffeoyl-6‴-feruloylsophoroside)-5-glucoside (**15**) coelute in CCC fraction 3, whereas fraction 4 afforded 35 mg of the peonidin-3-(6″-caffeoyl-6‴-*p*-hydroxybenzoylsophoroside)-5-glucoside (**14b**) in a purity of 85%. Additional minor pigments (i.e., peonidin-3-*p*-hydroxybenzoylsophoroside-5-glucoside (**5**), cyanidin-3-feruloylsophoroside-5-glucoside (**7**), and cyanidin-3-(6″-caffeoylsophoroside)-5-glucoside (**11**)) could be identified by HPLC-ESI-MS^n analyses in different CCC fractions as minor compounds. According to its anthocyanin composition, this new Japanese sweet potato cultivar can be categorized as "peonidin-type". **Table 2** presents the percentage distribution of major anthocyanins and the calculated peonidin/cyanidin ratio of 4.52. This new Japanese cultivar shows an anthocyanin composition similar to that previously described by Truong et al. (*25*) for the new American variety Stokes Purple.

The anthocyanin composition of the four Japanese purple sweet potato cultivars showed remarkable differences. According to their amounts of peonidin and cyanidin derivatives they can be categorized into "peonidin-types" (Chiran Murasaki and Purple Sweet) and "cyanidin-types" (Tanegashima Murasaki and Naka Murasaki) corresponding to a significant variation of color shade ranging from reddish purple to bluish purple. A comparison of HPLC and HPLC-ESI-MS^n data showed that the varieties Chiran Murasaki and Purple Sweet present nearly the same anthocyanin composition as the new American cultivar Stokes Purple investigated by Truong et al. (*25*), and the storage roots of Tanegashima Murasaki and Naka Murasaki contain the same cyanidin-based major pigments as the old Japanese cultivar Ayamurasaki previously published by Tian et al. (*23*) and Konczak-Islam et al. (*26*).

Knowledge of the anthocyanin composition is very important for the successful utilization of purple sweet potatoes as natural colorants in foods and beverages. Our results could be used as the basis for further investigations of purified purple sweet potato pigments, for example, investigation of light and heat stability as well as color activity measurements (*27*, *28*). Furthermore, it would be desirable to isolate and characterize so far unknown minor pigments from different CCC fractions by preparative HPLC.

The analysis of the pigment pattern of different purple sweet potato cultivars shows impressively the complexity of anthocyanin composition. By means of HSCCC it was possible to isolate major pigments in high purity. Depending on the cultivar, between 15 and 300 mg of the cyanidin derivatives cyanidin-3-sophoroside-5-glucoside (**1**), cyanidin-3-(6″,6‴-dicaffeoylsophoroside-5)-glucoside (**9**), cyanidin-3-(6″-caffeoyl-6‴-*p*-hydroxybenzoylsophoroside-5)-glucoside (**10**), and cyanidin-3-(6″-caffeoylsophoroside-5)-glucoside (**11**) and the peonidin derivative peonidin-3-(6″-caffeoyl-6‴-*p*-hydroxybenzoylsophoroside-5)-glucoside (**14b**) were obtained in a single run. Our results evidenced, compared to HPLC, the advantage of HSCCC for the preparative isolation of anthocyanins: gentle operating conditions, fast separation, and isolation of compounds in high amounts with high purity, thus allowing them to be used for pharmacological and biological activity tests.

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