

Anthocyanins in Purple Sweet Potato (*Ipomoea batatas* L.) Varieties

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ABSTRACT

Anthocyanins from purple sweet potatoes can serve as natural colorants due to their high heat and light stability and are commonly used in juices, alcoholic beverages, jams, confectioneries, bread, snacks and noodles. There are several commercially available varieties of purple sweet potatoes, which can vary in storage root size, shape, and color. During the last decade sweet potato cultivars with purple flesh were mainly grown in Japan and new varieties with high contents of anthocyanins have been developed due to the low anthocyanin accumulation in indigenous purple-fleshed sweet potatoes. Among them, important cultivars are the 'Yamagawamurasaki' and 'Ayamurasaki' varieties. Chromatographic analyses show a very complex anthocyanin composition. Ten major pigments with non-, mono- or diacylated structures of 3-*O*-(2-*O*- β -D-glucoopyranosyl- β -D-glucoopyranoside)-5-*O*- β -D-glucosides of cyanidin and peonidin were characterized by ESI-MSⁿ and NMR analyses. A comparison of different Japanese purple sweet potato cultivars shows a remarkable variation of anthocyanin profile. According to this, they can be categorized into two groups (blue and red dominant) based on the shade of color and the peonidin/cyanidin ratio. By means of the four Japanese cultivars 'Chiran murasaki', 'Tanegashima murasaki', 'Naka murasaki' and 'Purple Sweet' the differences in anthocyanin composition will be discussed in detail.

Keywords: acylated anthocyanins, anthocyanin composition, anthocyanin content, *Ipomoea batatas* L., peonidin/cyanidin ratio, purple sweet potato

CONTENTS

INTRODUCTION.....	19
STRUCTURE OF ANTHOCYANINS.....	20
ANTHOCYANIN COMPOSITION.....	21
ANTHOCYANIN CONTENT.....	22
CONCLUSIONS.....	23
REFERENCES.....	23

INTRODUCTION

Anthocyanins (anthos = flower, kyanos = blue) are the most abundant flavonoid constituents of red fruits and vegetables, and are often used as water-soluble natural pigments (Pazmino-Duran *et al.* 2001). A detailed overview about the structure and occurrence of anthocyanins is given by Mazza and Miniati (1993). With respect to molecular structure, some anthocyanins are more stable than others. Generally, increased hydroxylation decreases stability, whereas methylation increases it (Brouillard 1982). Recent researches have shown that anthocyanins with acylated substituents are more stable during processing and storage (Giusti and Wrolstad 2003; Cevallos-Casals and Cisneros-Zevallos 2004).

Acylated anthocyanins from purple sweet potato (PSP) can serve as natural colorants due to their high heat and light stability (Tsukui *et al.* 2002; Cevallos-Casals and Cisneros-Zevallos 2004). 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 like FD&C red 40 (Bovell-Benjamin 2007). Investigation of *Ipomoea batatas* L. anthocyanin composition was already carried out by Otake *et al.* (1992), Goda *et al.* (1997) and continued more recently by Terahara

et al. (2004) and Steed and Truong (2008). In all studies, the predominance of acylated anthocyanins in pigmented sweet potato varieties was reported. There are several commercially available varieties of purple sweet potato, which can vary in storage root size, shape, flavor, texture and color (Philpott *et al.* 2004). During the last decade sweet potato cultivars with purple flesh were mainly grown in Japan, Korea or New Zealand (Steed and Truong 2008) and new varieties with high contents of anthocyanins have been developed due to the low anthocyanin accumulation in indigenous purple-fleshed sweet potatoes (Mano *et al.* 2007). Among them, important cultivars are the 'Yamagawamurasaki' and 'Ayamurasaki' varieties which are cultivated in Japan. **Table 1** summarizes the different PSP varieties previously found in literature. The analysis of the anthocyanins extracted from 19 Japanese clones by Yoshinaga *et al.* (1999) and 16 Japanese cultivars by Oki *et al.* (2003) by spectrophotometry and high-performance liquid chromatography (HPLC) revealed considerable differences in the color value and anthocyanin composition among the sweet potato clones. Based on the peonidin/cyanidin (peo/cy) ratio, the sweet potato clones were classified into two groups: cyanidin types (peo/cy < 1.0) with a greater degree of blueness (blue dominant group) and peonidin types (peo/cy > 1.0) with a greater degree of redness (red dominant group).

Table 1 Different purple sweet potato varieties and literature data.

Varieties and clones	References
Stokes Purple ^a	Steed 2007; Steed and Truong 2008; Truong <i>et al.</i> 2010
clone NC415, 12-5 clone, 13-17 clone, 13-18 clone	Teow <i>et al.</i> 2007
Purple Okinawa	Truong <i>et al.</i> 2010
Yamagawa murasaki	Odake <i>et al.</i> 1994; Terahara <i>et al.</i> 1999; Kudoh and Matsuda 2000; Konczak-Islam <i>et al.</i> 2003
Aya murasaki	Yoshinaga <i>et al.</i> 1999; Oki <i>et al.</i> 2002; Matsui <i>et al.</i> 2002; Suda <i>et al.</i> 2002; Konczak-Islam <i>et al.</i> 2003; Oki <i>et al.</i> 2003; Suda <i>et al.</i> 2003; Harada <i>et al.</i> 2004; Konczak <i>et al.</i> 2004; Terahara <i>et al.</i> 2004; Kano <i>et al.</i> 2005; Kobayashi <i>et al.</i> 2005; Saigusa <i>et al.</i> 2005; Tian <i>et al.</i> 2005; Oki <i>et al.</i> 2006; Bovell-Benjamin 2007; Mano <i>et al.</i> 2007
Murasakimasari	Nagata <i>et al.</i> 2006; Mano <i>et al.</i> 2007
Kankei 55	Tsukui <i>et al.</i> 2002
Tanegashima murasaki	Yoshinaga <i>et al.</i> 1999; Tsukui <i>et al.</i> 2002; Oki <i>et al.</i> 2002, 2003
Chiran murasaki	Yoshinaga <i>et al.</i> 1999
Kyushu (3 different clones, and cultivars)	Yoshinaga <i>et al.</i> 1999; Oki <i>et al.</i> 2002, 2003
Miyanou-36	Oki <i>et al.</i> 2002
Bise	Oki <i>et al.</i> 2002, 2003
Purple Bom	Yoshinaga <i>et al.</i> 1999
Kuyukei (25 different clones and cultivars)	Yoshinaga <i>et al.</i> 1999; Oki <i>et al.</i> 2003
Purple Sweet Lord ^a	Tamiya <i>et al.</i> 2003
MSU (3 different clones)	Jusuf <i>et al.</i> 2006
n.n. ^b	Cevallos-Casals and Cisneros-Zevallos 2002, 2003, 2004
clone 97D	Philpott <i>et al.</i> 2004
n.n. ^b	Cho <i>et al.</i> 2003

^a new breeding cultivar, ^b unknown cultivar

In recent years the interest in anthocyanins has increased due to their possible health benefits (Giusti and Wrolstadt 2003). Anthocyanins are often associated with health preventive effects and reduced risks of e.g. aged-related macular degeneration (Jang *et al.* 2005), anticarcinogenic activity (Katsube *et al.* 2003), antioxidant capacity (Wang *et al.* 1997; Kähkönen and Heinonen 2003; Kähkönen *et al.* 2003; Kong *et al.* 2003), antiulcer activity (Cristoni and Magistretti 1987), and also reduced risks of cardiovascular disorders (Mazza 2007). The free-radical scavenging and antioxidant capacities of anthocyanins are the most significant and highly publicized of the *modus operandi* used by these pigments to intervene with human therapeutic targets, but, in fact, research clearly suggests that other mechanisms of action are also responsible for observed health benefits (Wang and Jiao 2000; Tsuda *et al.* 2002, 2003).

STRUCTURE OF ANTHOCYANINS

Due to the accumulation of anthocyanins, purple-fleshed sweet potatoes have intense purple color in the skins and flesh of the storage root (Philpott *et al.* 2004; Terahara *et al.* 2004). The structures of ten non-, mono- and diacylated major pigments were elucidated by means of high-performance liquid chromatography (HPLC), mass spectrometry (MS), and nuclear magnetic resonance spectroscopy (NMR). Due to the absence of commercially available standards, the identification of individual anthocyanins is mostly based on the data given by HPLC-DAD and HPLC-ESI-MSⁿ measurements. Especially ESI-MSⁿ analysis provides useful information about the molecular mass, the fragments, and the aglycon moiety. The major anthocyanins were characterized as pigments based on a peonidin and cyanidin core with *m/z* 301 and 287, respectively. Two non-acylated major pigments were found in purple sweet potatoes. One pigment was identified as cyanidin-3-sophoroside-5-glucoside (**1**) on the basis of the molecular ion [M]⁺ at *m/z* 773, which produces three fragment ions at *m/z* 611, 449, and 287 (loss of three glucosyl units), respectively. The second non-acylated anthocyanin had a molecular ion [M]⁺ at *m/z* 787 and three glucosyl moieties were cleaved resulting in two intermediate fragments at *m/z* 625 and 463 as well as a fragment of the aglycon with *m/z* 301. This indicated the presence of peonidin-3-sophoroside-5-glucoside (**2**). Most anthocyanins found in PSP cultivars are present as acylated forms. Among them, two monoacylated pigments linked with a caffeic acid residue, were characterized as 3-(6"-caffeoylsophoroside)-

glucosides of cyanidin (**5**) and peonidin (**8a**) with molecular ions at *m/z* 935 and 949, respectively. In addition, the mass spectra of **5** and **8a** showed fragment ions at *m/z* 773, 449, 287, and at *m/z* 787, 463, 301, respectively, corresponding to the loss of a glucose and caffeoylsophorose residues, and the aglycon moieties. In case of the six diacylated pigments an acylation with caffeic, ferulic, or *p*-hydroxybenzoic acids was indicated. Mass spectrometric analyses of these compounds indicated cyanidin derivatives for anthocyanins **3**, **4**, and **6** and peonidin derivatives for the pigments **7**, **8b**, and **9**. The pigments **3** and **7** had the same fragmentation pattern as was shown by the elimination of a glucose and dicaffeoylsophoroside moiety, and were identified as cyanidin-3-(6"-6"-dicaffeoylsophoroside)-5-glucoside (**3**) with a fragmentation pattern of *m/z* 1097, 935, 449, and 287, and peonidin-3-(6"-6"-dicaffeoylsophoroside)-5-glucoside (**7**) with *m/z* 1111, 949, 463, and 301, respectively. Similarly, the analysis of the anthocyanins **4** and **8b** showed molecular ions [M]⁺ and fragment ions at *m/z* 1055, 893, 449, 287 for compound **4** and *m/z* 1069, 907, 463, and 301 for pigment **8b**, whereas losses of a glucose and a sophorose diacylated with caffeic and *p*-hydroxybenzoic acid were produced. Consequently, the structures were determined to be 3-(6"-caffeoyl-6"-*p*-hydroxybenzoylsophoroside)-5-glucosides of cyanidin (**4**) and peonidin (**8b**). Furthermore, the pigments **6** and **9** exhibited similar fragmentation behaviour due to the loss of a glucosyl residue (162 u) and a sophoroyl moiety acylated with caffeic and ferulic acid. One compound has been identified as cyanidin-3-(6"-caffeoyl-6"-feruloylsophoroside)-5-glucoside (**6**) on the basis of its ESI-MSⁿ spectrum (*m/z* = 1111, 949, 449, and 287). The other pigment yielded a molecular ion [M]⁺ at *m/z* 1125 which yielded fragments at *m/z* 963, 463, 301, and was characterized as the analogue peonidin derivative peonidin-3-(6"-caffeoyl-6"-feruloylsophoroside)-5-glucoside (**9**). The mass spectrometric properties and identity of the peonidin- and cyanidin-based major anthocyanins are summarized in **Table 2**, while the structures are shown in **Fig. 1**. On the basis of ¹H- and ¹³C-NMR spectrometric data, the molecular structure of the monoacylated anthocyanins **5** and **8a** were reported by Goda *et al.* (1997), while the structure elucidation of the six diacylated major pigments **3**, **4**, **6**, **7**, **8b**, and **9** was established by Terahara *et al.* (1999). Recently, the attention of several researchers has been focused on the structure elucidation of minor anthocyanins in different breeding cultivars. A few reports demonstrated the presence of mainly cyanidin- and peonidin-derived minor pigments detected by HPLC-ESI-MSⁿ analyses (Tian *et al.* 2005; Tru-

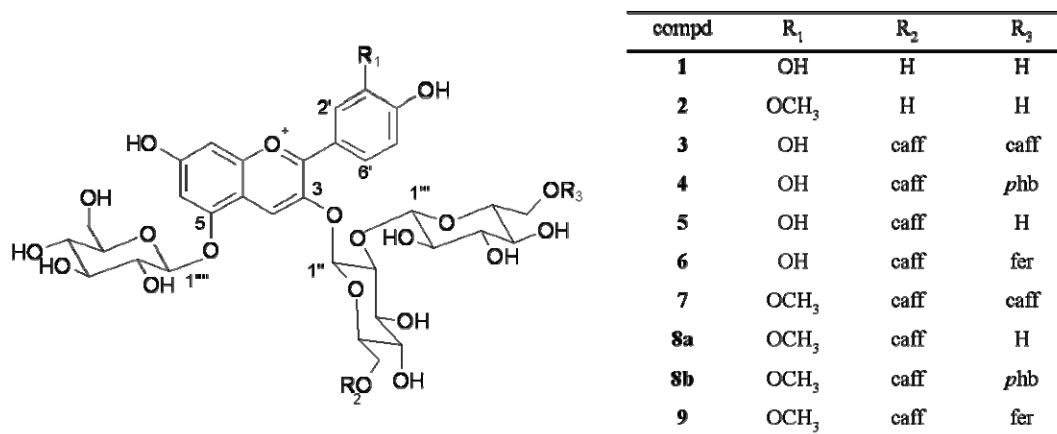


Fig. 1 Structures of major anthocyanins found in purple sweet potato storage roots. Abbreviations: caff = caffeoyl; fer = feruloyl; phb = *p*-hydroxybenzoyl.

Table 2 Mass spectrometric properties of major anthocyanins found in purple sweet potato varieties (5, 8a: Goda *et al.* 1997; 3, 4, 6, 7, 8b, 9: Terahara *et al.* 1999; 1, 2: Cuevas 2010 pers. comm.).

Peak no. ^a	Compound	t _R (min)	[M] ⁺ (m/z)	Fragments (m/z)
1	cy-3-soph-5-glc	12.9	773	611, 449, 287
2	peo-3-soph-5-glc	18.5	787	625, 463, 301
3	cy-3-(6'',6'''-dicaffeoylsoph)-5-glc	33.1	1097	935, 449, 287
4	cy-3-(6''-caffeoyl-6'''-p-hydroxybenzoylsoph)-5-glc	33.6	1055	893, 449, 287
5	cy-3-(6''-caffeoylsoph)-5-glc	34.0	935	773, 449, 287
6	cy-3-(6''-caffeoyl-6'''-feruloylsoph)-5-glc	35.6	1111	949, 449, 287
7	peo-3-(6'',6'''-dicaffeoylsoph)-5-glc	36.6	1111	949, 463, 301
8a	peo-3-(6''-caffeoylsoph)-5-glc	37.2	949	787, 463, 301
8b	peo-3-(6''-caffeoyl-6'''-p-hydroxybenzoylsoph)-5-glc	37.2	1069	907, 463, 301
9	peo-3-(6''-caffeoyl-6'''-feruloylsoph)-5-glc	38.9	1125	963, 463, 301

^a Peak labeling *cf.* Fig. 3

Abbreviations: cy = cyanidin; peo = peonidin; soph = sophoroside; glc = glucoside.

ong *et al.* 2010) as well as NMR measurements (Terahara *et al.* 2004).

ANTHOCYANIN COMPOSITION

HPLC-DAD analyses showed qualitative differences in the anthocyanin composition of different purple sweet potato varieties (e.g. 'Yamagawamurasaki', 'Ayamurasaki', 'Stokes Purple', 'Purple Okinawa') as previously described by several authors (Tsukui *et al.* 2002; Oki *et al.* 2003; Konczak-Islam *et al.* 2003; Suda *et al.* 2003; Tian *et al.* 2005; Truong *et al.* 2010). The non-acylated pigments eluate very early (t_R less than 20 min), while the monoacylated and diacylated major anthocyanins showed a retention time between 33 to 39 minutes under chromatographic conditions previously described by Hillebrand *et al.* (2009). Because of the coelution of some of the anthocyanins, the assignment of all pigments is based on HPLC analyses, coupled with mass spectrometric measurements (HPLC-ESI-MSⁿ). Anthocyanin composition was determined in 4 cultivars of purple sweet potato (*Ipomoea batatas* L.) grown in Japan (Fig. 2). The studied varieties included: three indigenous sweet potato cultivars ('Chiran murasaki', 'Tanegashima murasaki', 'Naka murasaki') and the variety 'Purple Sweet', a new breeding cultivar which was developed in breeding programs for use as natural food colorants. The predominant pigments of the varieties 'Chiran murasaki' and 'Purple Sweet' were identified as peonidin-3-(6''-caffeoylsophoroside)-5-glucoside (8a), whereas the storage roots of the 'Tanegashima murasaki' and 'Naka murasaki' cultivars were mainly composed by the analogue cyanidin derivative cyanidin-3-(6''-caffeoylsophoroside)-5-glucoside (5). HPLC chromatograms of the four breeding cultivars under investigation are shown in Fig. 3. The sweet potatoes with a high percentage content of cyanidin derivatives are blue dominant and belong to the so-called cyanidin-type (peo/cy ratio < 1), while the tubers with a high peonidin ratio (peo/cy

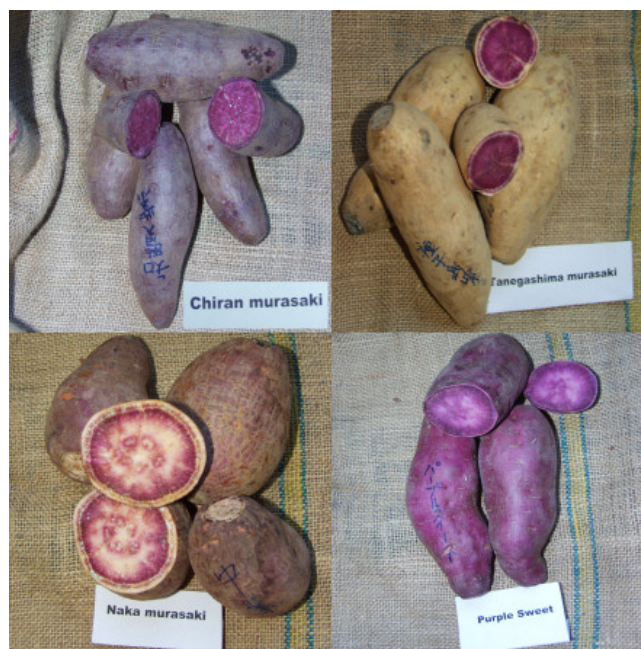


Fig. 2 The purple sweet potato varieties 'Chiran murasaki', 'Tanegashima murasaki', 'Naka murasaki', and 'Purple Sweet'.

ratio >1) contain mainly peonidin-based anthocyanins and are red dominant (peonidin-type). The percentage of peonidin and cyanidin of these four Japanese PSP cultivars were calculated from anthocyanin compositions of Table 3. The percentage peonidin content of the varieties 'Chiran murasaki' (CM), 'Tanegashima murasaki' (TM), 'Naka murasaki' (N), and 'Purple Sweet' (PS) were 80.2, 4.2, 9.3, and 81.9, respectively, while the percentage cyanidin con-

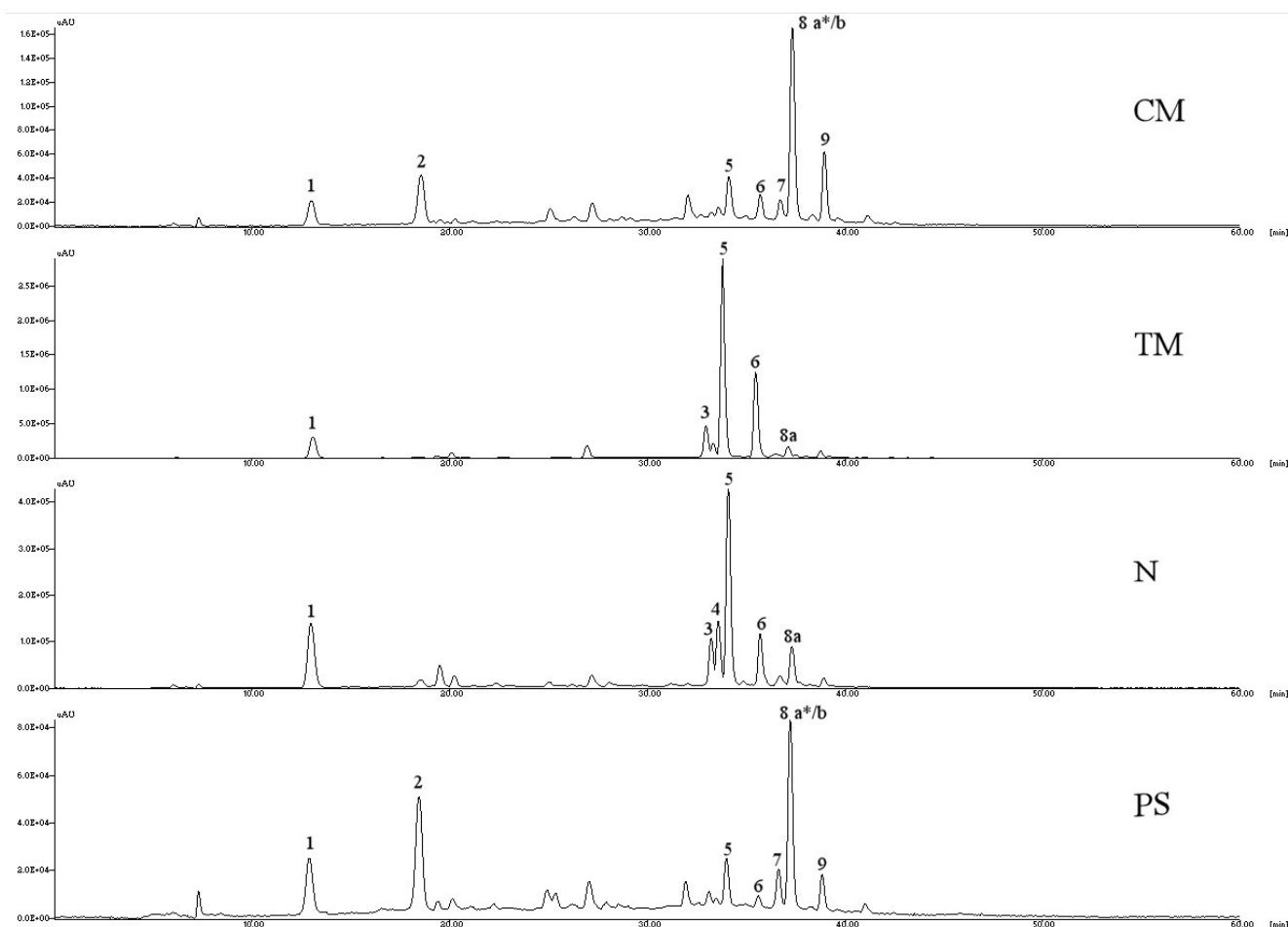


Fig. 3 HPLC chromatograms of the anthocyanin-enriched XAD-7 extracts of the PSP cultivars 'Chiran murasaki' (CM), 'Tanegashima murasaki' (TM), 'Naka murasaki' (N), and 'Purple Sweet' (PS) at 520 nm. For peak numbering see Fig. 1 and Table 2.

Table 3 Major anthocyanin composition of Japanese purple sweet potato varieties Chiran murasaki (CM), Tanegashima murasaki (TM), Naka murasaki (N), and Purple Sweet (PS) (Cuevas 2010 pers. comm.).

	percentage of pigments ^{a,b}			percentage of major pigments ^{c,d}								
	Peo	Cy	Peo/Cy	1	2	3	4	5	6	7	8a/b	9
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, 3, 4, 5, 6); Peo: peonidin derivatives (2, 7, 8a, 8b, 9)

^b relative area (%)

^c For peak labeling cf. Fig. 3

^d Minor pigments are not regarded

tent of these breeding cultivars were 19.8 (CM), 95.8 (TM), 90.7 (N), and 18.1 (PS), respectively (Cuevas 2010 pers. comm.). Ratio of peonidin and cyanidin content was 4.05 and 4.52 for the varieties 'Chiran murasaki' and 'Purple Sweet' (peonidin-types), and 0.04 and 0.10 for the cultivars 'Tanegashima murasaki' and 'Naka murasaki' (cyanidin-types).

ANTHOCYANIN CONTENT

Purple sweet potatoes attracted interest as a healthy food additive and a potential source of natural food colorants due to their high levels of anthocyanins. It was found by several studies that the anthocyanin content varies within the different PSP cultivar. The variety 'Ayamurasaki' contains anthocyanins of 59 mg of peonidin-3-caffeoylsophoroside-5-glucoside equivalents/100 g (Suda *et al.* 2002). Four American breeding clones were analyzed by Teow *et al.* (2007) and the total monomeric anthocyanin content of the samples ('NC 415', '12-5', '13-17', and '13-18') ranged from 24.6 to 45.1 mg/100 g fresh weight (fw) calculated as cyanidin-3-

glucoside. Steed and Truong (2008) analyzed the anthocyanin content of 'Stokes Purple' variety cultivated in North Carolina. The level of total monomeric content of this new breeding cultivar varied from 57.5 mg/100 g fw for puree to 174.7 mg/100 g fw for raw potato peels. The anthocyanin content of the varieties 'Stokes Purple', 'NC 415', and 'Okinawa' was determined by Truong *et al.* (2010), whereas levels of pigments in the new breeding cultivars 'Stokes Purple' and 'NC 415' showed high levels of pigments (33.7 to 96.8 mg/100 g fw), which were about 3-5-fold higher than in the old variety 'Okinawa' (10.0 to 21.1 mg/100 g fw). These results show impressively that there is a high accumulation of anthocyanins in the peel of purple sweet potatoes. The monomeric anthocyanin content of four different Japanese breeding cultivars were evaluated by means of HPLC-DAD analyses and calculated as cyanidin-3-glucoside equivalents previously described by Hillebrand *et al.* (2010). The values ranged from 6.5 to 29.1 mg/100 g fw (Cuevas 2010 pers. comm.) and are comparable to that found in pigmented potatoes (*Solanum tuberosum* L.) previously described by Rodriguez-Saona *et al.* (1998). A good

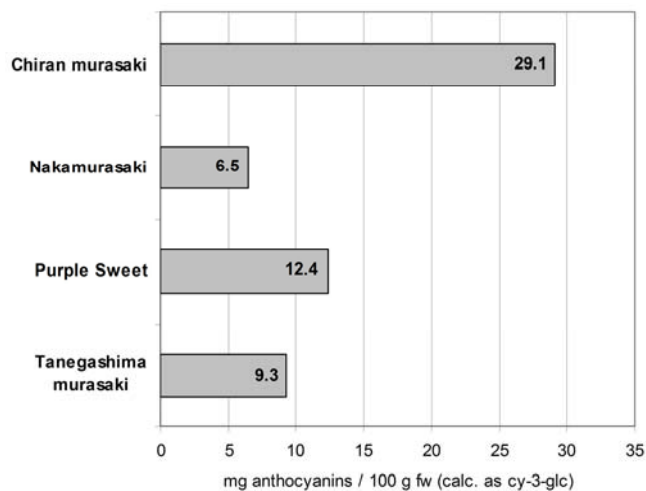


Fig. 4 Monomeric anthocyanin content of Japanese purple sweet potato cultivars. (Cuevas 2010 pers. comm.); fw = fresh weight; calc. = calculated.

correlation between the color intensity of the flesh and the anthocyanin content has been found, while the most intense color and anthocyanin accumulation was observed in the tuberous roots of 'Chiran murasaki'. Although information concerning the anthocyanin contents of different PSP breeding cultivars is very limited and the assays for determination of pigment levels are based on different methods (Rodríguez-Saona and Wrolstad 2001; Giusti and Wrolstad 2001; Hillebrand *et al.* 2010), the results indicated that the content of anthocyanins varied widely among the purple sweet potatoes. A better knowledge would be helpful in view of the consumer's awareness regarding the level of beneficial compounds as well as the increased application of PSP extracts and concentrates in foods and beverages as natural food colorant. **Fig. 4** summarizes the contents of anthocyanins found in four different Japanese purple sweet potato varieties under investigation.

CONCLUSIONS

The structures of ten non-, mono- and diacylated derivatives of 3-*O*-(2-*O*- β -D-glucopyranosyl- β -D-glucopyranoside)-5-*O*- β -D-glucosides of cyanidin and peonidin were elucidated by means of high-performance liquid chromatography (HPLC), mass spectrometry (MS), and nuclear magnetic resonance spectroscopy (NMR), and compared with literature data (Otake *et al.* 1992; Goda *et al.* 1997). The comparison of the four investigated Japanese purple sweet potato cultivars shows significant differences in their anthocyanin composition. Based on the peonidin/cyanidin ratio, they can be categorized into two groups (blue and red dominant): 'Chiran murasaki' and 'Purple Sweet' showed a high content of peonidin derivatives and could be classified as members of the red dominant group (peonidin-type) based on their color characteristics and their high peonidin/cyanidin ratio (>1); in counterpart, the cultivars 'Tanegashima murasaki' and 'Naka murasaki' showed a high content of cyanidin derivatives and could be classified as members of the blue dominant group (cyanidin-type, peonidin/cyanidin ratio < 1).

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