Involvement of Anthocyanins and other Phenolic Compounds in Radical-Scavenging Activity of Purple-Fleshed Sweet Potato Cultivars

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ABSTRACT: The 80% ethanol extracts of 5 purple-fleshed sweet potato cultivars were separated into 2 fractions, anthocyanins- and phenolic compounds-rich fractions, to clarify the contribution of these constituents to the radical-scavenging activity. The separation was accomplished with an ethyl acetate liquid/liquid extraction. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity in each fraction and the contributors varied according to the cultivars. The dominant DPPH radical-scavengers in "Ayamurasaki" and "Kyushu-132" were anthocyanins rather than phenolic compounds, while those in "Miyanou-36" and "Bise" were phenolic compounds, such as chlorogenic acid. Furthermore, the high-performance liquid chromatography analysis of anthocyanins showed that "Ayamurasaki" and "Kyushu-132" were rich in anthocyanins with peonidin aglycon, whereas "Miyanou-36," "Bise," and "Tanegashimamurasaki" contained cyanidin aglycon.

Keywords: sweet potato, *Ipomoea batatas*, radical-scavenging activity, anthocyanin, phenolic compound

Introduction

THE SWEET POTATO (*IPOMOEA BATATAS*) IS REGARDED AS A NUTRITIONALLY rich food. More than 142 million tons were produced globally in 2000, and it is the sixth most important food crop in the world according to data from the Food and Agriculture Organization. Yellow-fleshed sweet potatoes are most common in Japan. However, recently, new varieties of sweet potato cultivars with white, deep yellow, orange, and purple flesh have been released from National Agricultural Research Center for Kyushu Okinawa Region (KONARC), previously called the Kyushu National Agricultural Experiment Station (Yamakawa 1996). Their predominant pigments are flavones, β-carotene, and anthocyanins for deep yellow, orange, and purple sweet potatoes, respectively. Our focus is now on the purple-fleshed sweet potatoes with multiple physiological functions.

One of the purple-fleshed cultivars, "Ayamurasaki," has a high anthocyanin content. It was developed at our research center in 1995 (Yoshinaga 1995). At present, the paste and flour from the "Ayamurasaki" have been used in Japan for noodles, bread, jams, sweet potato chips, confectionery, juice, alcoholic drinks, and food dyes. The "Ayamurasaki" extract has been observed in vitro to be a potent antioxidant or radical scavenger (Furuta and others 1998), antimutagen (Yoshimoto and others 1999a), and angiotensin I-converting enzyme inhibitor (Suda and others 1999a). In a rat study, the purple-fleshed "Ayamurasaki" juice exhibited an ameliorative effect against carbon tetrachloride-induced liver injury (Suda and others 1997). In addition, restorative capacities were attributed to the juice when normal levels of serum γ-GTP, GOT, and GPT were achieved in human volunteers with impaired hepatic function; furthermore, blood pressures in volunteers with hypertension were reduced to normal levels (Suda and others 1998). Although these in vitro and in vivo studies suggested that some components in the purple-fleshed sweetpotato exhibited such physiological functions, it was unclear which compounds played a dominant role. Therefore, we are now attempting to clarify the components that are dominant relative to individual physiological functions. The present paper is a study of the contributors that exhibit radical-scavenging activity in the purple-fleshed sweet potato.

In a previous paper, we demonstrated that purple-fleshed sweet potato cultivars have a higher radical-scavenging or antioxidative activity than those with white, yellow, or orange flesh (Furuta and others 1998). Considering the components in purple-fleshed sweet potatoes, polyacylated anthocyanins (Odaka and others 1992: Goda and others 1997: Yoshimoto and others 1999b) were assumed to be the leading scavengers. On the other hand, a white mutant "Ayamurasaki" that did not contain anthocyanins exhibited a satisfactory amount of high tert-butylperoxyl (*t*-BuOO) radical-scavenging activity, though it was about one-half lower than that observed in the "Ayamurasaki" (Furuta and others 1998). In addition, the *t*-BuOO radical-scavenging activity from an 80% ethanol extract of the "Tanegashimamurasaki" cultivar was almost the same level as that from "Ayamurasaki," irrespective of the low anthocyanin content of the "Tanegashimamurasaki" relative to the "Ayamurasaki" (Furuta and others 1998). These observations led us to speculate that components other than anthocyanins could also participate as *t*-BuOO radical scavengers. Polyphenols (except anthocyanins) are highlighted as important candidates because the total *t*-BuOO radical-scavenging activity was elevated with an increase of polyphenol content in sweet potatoes (Furuta and others 1998).

To assess whether anthocyanins and other polyphenols contribute to radical-scavenging activity and the extent to which they do, we divided the extract of 80% ethanol obtained from various purple-fleshed sweet potato cultivars into fractions containing anthocyanins and other phenolic compounds and individually estimated their radical-scavenging activities. In addition, we determined the major components of each fraction by using high-performance liquid chromatography (HPLC) analysis.

Materials and Methods

Reagents

A Folin-Ciocalteu reagent, chlorogenic acid (ChA), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Wako Pure Chemicals Industries Ltd. (Osaka, Japan). Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was obtained from Aldrich Chemical Co. (Milwaukee, Wis., U.S.A.), and cyanidin 3-*O*- β-glucoside (C3G) from Funakoshi Co. Ltd. (Tokyo, Japan). Other reagents were of analytical grades and used without further purification.

Sweet potatoes

Three cultivars (Ayamurasaki, Kyushu-132, and Tanegashimamurasaki) were harvested at KONARC (Miyakonojyo Branch, Miyazaki, Japan) in 1998. "Bise" and "Miyanou-36" were supplied from Okinawa, Japan, in 1998. Five tubers of each cultivar were washed with tap water and cooled at 4 °C for 1 h. After peeling, the flesh was diced to about 0.5 cm, immediately frozen in liquid nitrogen, and then lyophilized. The lyophilizate was powdered in a mill and stored at –20 °C until use.

Colorimetric measurement of sweet potato powder

Color values (L*, a*, and b*) of sweet potato powder were measured with a colorimeter (Chroma Meters CR-200, geometry: diffuse illumination/0° viewing angle, Minolta Co., Ltd., Tokyo, Japan). Hue and chroma was calculated as arc tan (b*/a*) and $[a^{*2} + b^{*2}]^{1/2}$, respectively.

Preparation of sweet potato extract and its fractionation

Sweet potato powder (equivalent to 250 mg of flesh weight) was extracted twice with 2.5 mL of an 80% ethanol solution. The supernatant obtained by centrifugation (1200 × *g*, 10 min, 4 °C) was used as the sweet potato extract sample for measuring radical-scavenging activity. The extract was divided into fractions containing phenolic compounds and anthocyanins, according to the ethyl acetate liquid/liquid extraction method of Ghiselli and others (1998) (Figure 1). In brief, 40 μL of trifluoroacetic acid (TFA) and 1.6 mL of distilled water were added to 2.0 mL of sweet potato extract. After removing ethanol by evaporation, the solution was extracted 4 times with ethyl acetate (2.0 mL). In preliminary experiments, the recoveries of 2 major phenolic compounds (peak 1 and 2 as shown in Figure 3) from the solution by ethyl acetate extraction were 94.6% ± 3.9% (n = 3) and 99.8% ± 0.1% (n=3) for peak 1 and 2, respectively, when "Miyanou-36" was used as a cultivar with high phenolic contents. The ethyl acetate layer was collected, evaporated until dry in vacuo, and redissolved in 2.0 mL of an 80% ethanol solution; this fraction containing polyphenols except anthocyanins was labeled F-I. The aqueous residue subjected to ethyl acetate extraction was applied on an Amberlite XAD-7 column (10 mm × 40 mm) and eluted stepwise with 15 mL of distilled water and 15 mL of methanol containing 1% TFA. The methanol fraction was evaporated until dry under reduced pressure and redissolved in 2.0 mL of distilled water; this fraction containing anthocyanins was labeled F-II.

Assay for radical-scavenging activity. The radical-scavenging activity was examined by a modification of the method using DPPH described by Suda and others (1999b). DPPH dissolved in ethanol forms a stable free radical by ionization. The reaction mixture consists of 400 μL of 400 μM DPPH in ethanol, 400 μL of a 2-morpholinoethanesulphonic acid (MES) buffer (pH 6.0), x μL of a sample solution in 80% ethanol, x μL of 20% ethanol, and $(800 - 2x)$ μL of 50% ethanol. In the case of the sample of F-II redissolved in distilled water, the final concentration of ethanol in the assay was adjusted to 50% by changing the concentration of ethanol solution. The reaction was initiated by the addition of the sample solution. After the test tube stood for 20 min, the absorbance of the DPPH radical at 520 nm was measured (V-560 UV/VIS Spectrophotometer, JASCO Co., Ltd., Tokyo, Japan). The DPPH radical-scavenging activity was evaluated by the decrease of the absorbance at 520 nm and expressed as a Trolox equivalent per mL of the sample solution by using a calibration curve of Trolox.

Determination of phenolic content

The total phenolic content of F-I was determined by a modification of the Folin-Ciocalteu method already reported (Furuta and others 1998). Namely, 1.0 mL of 10% Folin-Ciocalteu solution was added to 1.0 mL of a sample solution. The concentration of ethanol in the sample solution was set at 40% by adding water and/or ethanol. After an interval of 3 min, 2.0 mL of a 10% sodium carbonate aqueous solution was added. The mixture was allowed to stand for 1 h at ambient temperature, and the absorbance was then measured at 750 nm. The phenolic content was expressed as a ChA equivalent.

Determination of anthocyanin content

Nine hundred μL of 1.1% TFA aqueous solution was added to 100 μL of F-II, and the absorbance at 530 nm was then measured. The anthocyanin content was calculated from a calibration curve for C3G.

HPLC of sweet potato extract

For analyzing phenolic compounds in F-I, an equivalent volume of a 0.4 M NaH_2PO_4-H_3PO_4 buffer (pH 3.9) containing 2% ascorbic acid and 0.01% EDTA-2Na was added to an aliquot of F-I. The mixture (20 μL) was put directly on a Wakosil-II AR column (4.6 mm × 250 mm, Wako Pure Chemicals Industries, Ltd., Osaka, Japan) at 35 °C. The elution system consisted of 2 solvents: (A) 2% acetic acid and (B) methanol. The following gradients were used: 7% to 50% (B) for 43 min at a flow rate of 0.75 mL/min. Phenolic compounds were monitored at 328 nm. The anthocyanin pigments in F-II were measured as follows: 20 μL of F-II diluted 5 times with a TFA solution (final concentration: 1.0%) was applied to a Wakosil-II AR column (4.6 mm × 250 mm) and eluted with a linear acetonitrile gradient (5% to 25%, 40 min) containing 1.5% phosphoric acid at a flow rate of 0.75 mL/min. The monitored absorbance was 530nm, and the column temperature was maintained at 35 °C. The identification of phenolic compounds and anthocyanins were performed by the comparison with the retention time and the UV-visible spectra obtained from JASCO MD-1580 photodiode array with Borwin as a data processing software (JASCO Co., Ltd., Tokyo, Japan). For the identification of anthocyanins, we used the anthocyanins isolated from "Ayamurasaki" as described previously (Terahara and others 1999).

Results and Discussion

Color value of sweet potato powder

The color of the sweet potato powder was measured with a colorimeter (Table 1). From the color values of L*, a*, and b*, hue, and chroma, the powder from "Ayamurasaki" and "Kyushu-132" was deep purple, and the powder from "Miyanou-36," "Bise," and "Tanegashimamurasaki" was light purple.

Table 1—Color values (L*, a*, and b*), hue, and chroma of sweet potato powders

	L*	a*	b*	Hue[a]	Chroma[b]
Ayamuasaki	44.0	21.6	–6.7	–0.30	22.6
Kyushu-132	45.5	21.5	–7.7	–0.34	22.9
Miyanou-36	57.2	14.8	–4.0	–0.27	15.4
Bise	59.2	13.1	–4.0	–0.30	13.7
Tanegashima-murasaki	57.5	17.4	–3.9	–0.22	17.8

Mean values of 5 tubers
[a]Hue was calculated as arc tan(b* / a*).
[b]Chroma was calculated as $[a^{*2} + b^{*2}]^{1/2}$

DPPH radical-scavenging activity of extract from purple-fleshed sweet potato and its subfraction

The DPPH radical-scavenging activity from an 80% ethanol extract of the purple-fleshed sweet potato varied according to the cultivar. The cultivar with the highest DPPH radical-scavenging activity was "Miyanou-36," followed by "Bise," "Kyushu-132," "Ayamurasaki," and "Tanegashimamurasaki" (Figure 2A). To assess the involvement of anthocyanins and other polyphenols in radical-scavenging activity of an 80% ethanol extract, we fractionated the extract into 2 (aqueous and ethyl acetate) fractions, according to the scheme shown in Figure 1. In the ethyl acetate fraction (F-I), "Miyanou-36" and "Bise" showed higher radical-scavenging activities than the other cultivars (Figure 2B). The DPPH radical-scavenging activities in F-I from "Miyanou-36"and "Bise" corresponded to 78% and 71% of those in the 80% ethanol extract, respectively. In the aqueous fraction, F-II, the order of the DPPH radical-scavenging activity was "Ayamurasaki" > "Kyushu-132" > "Miyanou-36" > "Bise" > "Tanegashimamurasaki" (Figure 2C). The activities in F-II from "Ayamurasaki" and "Kyushu-132" ranked high at 87% and 76% of those in the 80% ethanol extract.

Phenolic content in F-I and anthocyanin content in F-II

Higher polyphenol contents were observed in F-I from "Miyanou-36" and "Bise" (Figure 2D). The phenolic content in F-1 showed the same tendency as the radical-scavenging activity in F-I (Figure 2B), suggesting that the radical-scavenging activity would be dependent on the content of phenolic compounds except the anthocyanins.

On the other hand, the anthocyanin content in F-II was the highest in "Ayamurasaki," followed by "Kyushu-132," "Miyanou-36," and "Bise," and the lowest in "Tanegashimamurasaki" shown in Figure 2E. This order was the same as the radical-scavenging activity in F-II (Figure 2C), indicating that the activity would be dependent on the content of anthocyanin.

Phenolic compounds in F-I

Figure 3 shows HPLC profiles of phenolic compounds in F-I prepared from "Miyanou-36" and "Bise." Two major peaks were observed in the retention time of 12.6 and 29.3 min. Two peaks were also observed in the other cultivars. However, the ratio of the peak area of peak 2 to that of Peak 1 varied according to the cultivars (Ayamurasaki: 1.20, Kyushu-132: 1.56, Miyanou-36: 0.69, Bise: 1.10, Tanegashimamurasaki: 1.34). When the monitoring absorbance was set at 280 nm, no other peaks than those monitored at 328 nm were observed (data not shown). Peak 1 (retention time = 12.6 min) was identified as ChA by comparison with the retention time and the photodiode array spectra of authentic ChA. In addition, the UV/VIS spectrum of the peak 2 was similar to that of the ChA and peak 1 (Figure 3).

Compositional Difference of Anthocyanins in F-II

Figure 4 shows the differences among cultivars by the HPLC profile of anthocyanins contained in F-II. In "Ayamurasaki," 7

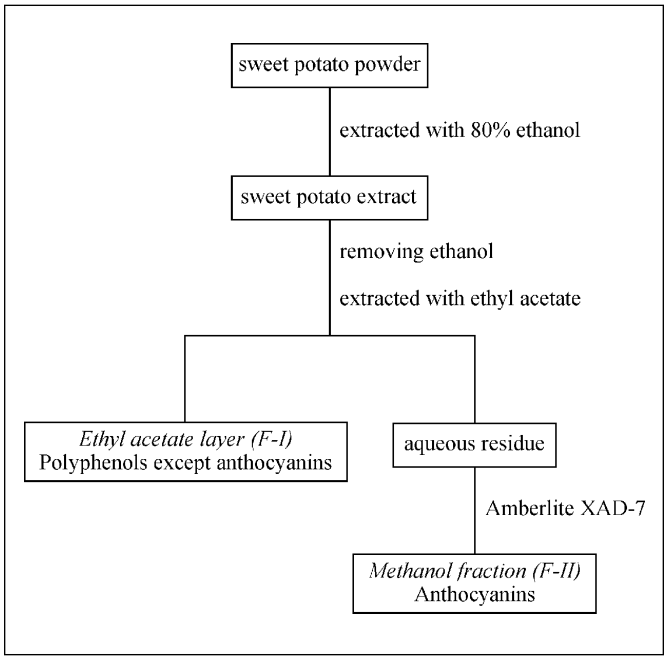

Figure 1—Scheme of the fractionation of polyphenols in purple-fleshed sweet potato

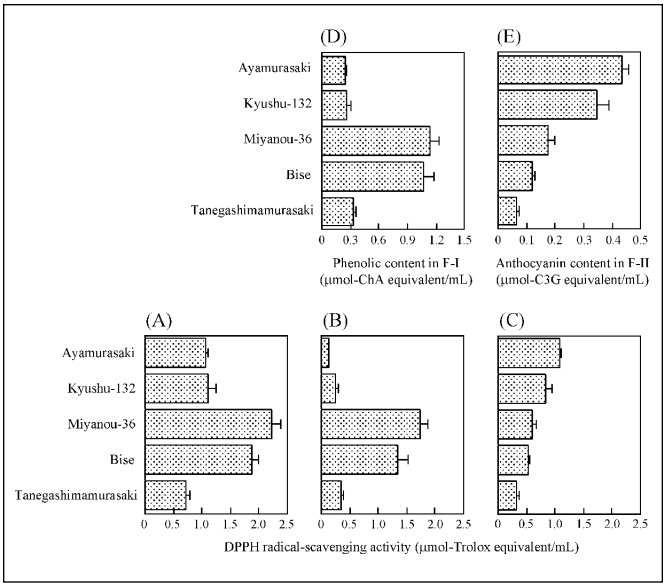

Figure 2—DPPH radical-scavenging activities of 80% ethanol extract from purple-fleshed sweet potato (A), F-I (B) and F-II (C), as well as phenolic content in F-I (D) and anthocyanin content in F-II (E). The DPPH radical-scavenging activity, phenolic content, and anthocyanin content were expressed as Trolox, ChA, and C3G equivalent per mL of a sample solution, respectively. Values are means ± SD of 3 replicate experiments for 5 tubers

major peaks of anthocyanins were observed as reported by Yoshimoto and others (1999b), who designated 7 anthocyanins as YGM-1, 2, 3, 4, 5a, 5b, and 6. In this study, these anthocyanins were identified by the comparison with the retention time and the photodiode array spectra of authentic anthocyanin samples isolated from "Ayamurasaki," and designated in a similar manner as Yoshimoto and others (1999b) reported. Table 2 summarized the ratio of their major anthocyanins (YGM-1 to YGM-6) in F-II obtained from 5 cultivars. "Ayamurasaki" and "Kyushu-132" were rich in anthocyanins from YGM-4 to YGM-6, while "Miyanou-36," "Bise," and "Tanegashimamurasaki" had anthocyanins from YGM-1 to YGM-3.

Discussion

In a previous paper (Furuta and others 1998), we reported that purple-fleshed sweet potato cultivars had higher antioxidant activity than those with white, yellow, and orange flesh by the assay based on inhibition of *t*-BuOO radical generation or linoleic acid auto-oxidation. Until this study was carried out, this finding led us to speculate that anthocyanins in purple-fleshed sweet potato might be the major components responsible for antioxidative activity. However, among sweet potatoes tested, a cultivar showing the highest radical-scavenging activity was "Miyanou-36," which estimated as a cultivar with low anthocyanin content judging from the flesh color of light purple (Table 1). This discrepancy could be explained by separating the 80% ethanol extract into anthocyanins and other phenolic-component fractions.

The present work clearly indicated that each radical-scavenging activity in F-I and F-II increased with the increase of phenolic content in F-I and anthocyanin content in F-II, respectively. Furthermore, the sum of radical-scavenging activities of F-I and F-II (Ayamurasaki: 1.23 μmol-Trolox equivalent/mL, Kyushu-132: 1.10, Miyanou-36: 2.34, Bise: 1.89, Tanegashimamurasaki: 0.71) was almost equal to the total activity in the 80% ethanol extract (Ayamurasaki: 1.24 μmol-Trolox equivalent/mL, Kyushu-132: 1.11, Miyanou-36: 2.22, Bise: 1.88, Tanegashimamurasaki: 0.66). Involvement of each component in the radical-scavenging activity of 80% ethanol extract varied according to the sweet potato cultivar. The major contributors were phenolic compounds ex-

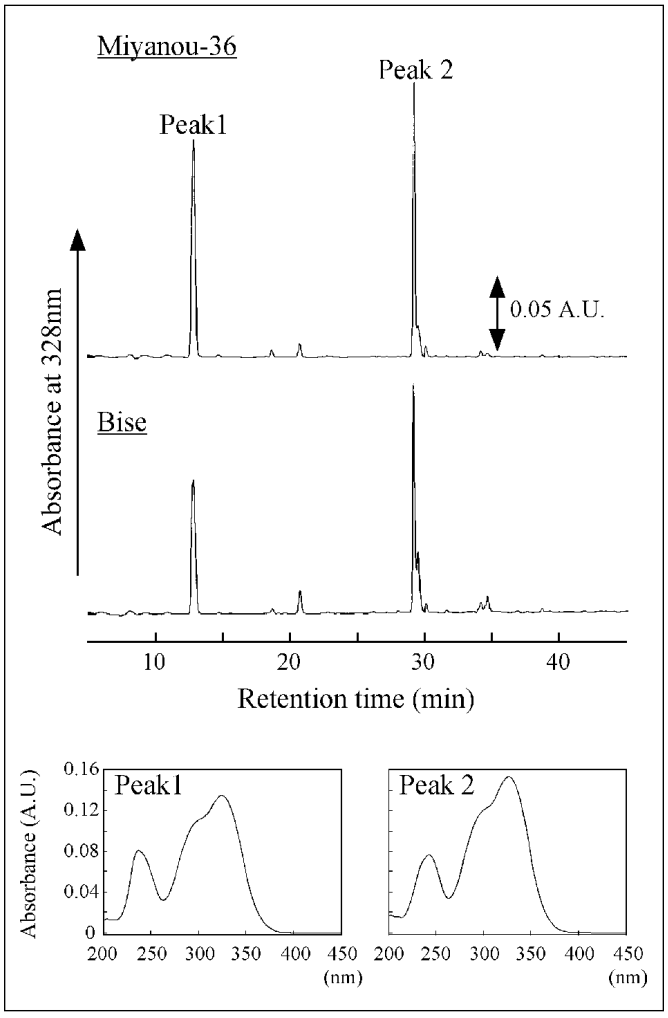

Figure 3—High-performance liquid chromatograms of F-I and UV/visible spectra of peak 1 and 2. Peak 1 was identified as ChA by the comparison with the retention time and UV/visible spectrum of an authentic sample of ChA. The spectra recorded by HPLC combined with a photodiode array detector. The spectrum of peak 2 was similar to that of peak 1 identified as ChA.

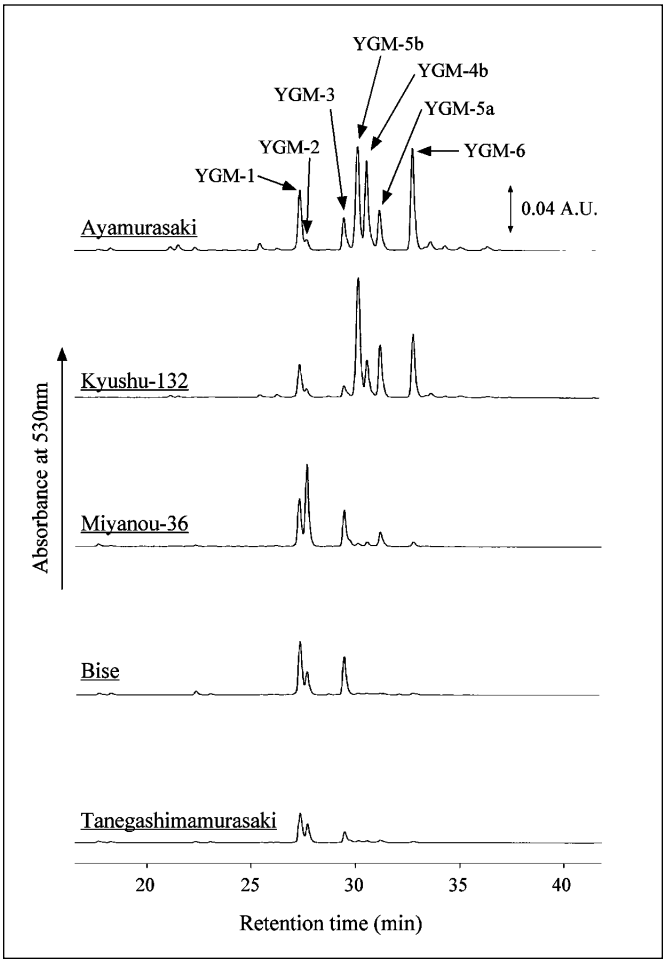

Figure 4—High-performace chromatograms of F-II, Seven major anthocyanins were identified by the comparison of the retention time and UV/visisble spectrum of authentic samples. The spectra recorded by HPLC combined with a photodiode array detector. The authentic samples were anthocyanins isolated from "Ayamurasaki" as previously described.

Table 2—Area percentages of anthocyanins in F-II

	peak area (%) [a]							
	YGM-1	YGM-2	YGM-3	YGM-4b	YGM-5a	YGM-5b	YGM-6	Other
Ayamurasaki	1.9	2.0	5.7	17.7	9.0	20.4	21.3	13.0
Kyushu-132	9.6	2.4	3.2	10.3	15.4	32.5	17.5	9.0
Miyanou-36	24.3	35.5	15.1	2.6	8.8	2.1	2.9	8.7
Bise	34.8	16.2	35.1	0.9	1.1	1.1	2.0	8.8
Tanegashimamurasaki	42.9	20.4	12.5	2.0	2.9	2.6	2.7	14.0

[a]Mean values of 5 tubers

cept anthocyanins for "Miyanou-36" and "Bise," while they were anthocyanins for "Ayamurasaki" and "Kyushu-132." This evidence strongly supports the fact that both anthocyanins and polyphenols play an important role in the radical-scavenging activity of purple-fleshed sweet potatoes. The summation of both activities brings by their components expressed as total activity. Thus, attention should be given to the existence of anthocyanins and polyphenols when estimating the radical-scavenging activity of purple-fleshed sweet potatoes.

The most likely polyphenol contributors responsible for radical-scavenging activity are ChA and unknown substances (peak 2) confirmed in F-I by HPLC analysis (Figure 3). In addition, most of phenolic compounds in F-I would be cinnamic acids because the HPLC elution patterns at 328 nm were similar to those at 280 nm. Unknown substances (peak 2) appeared to be isochlorogenic acid (iso-ChA) due to the similarity of the UV/VIS spectrum to chlorogenic acid (Figure 3). Hayase and Kato (1984) reported that 6 phenolic compounds were observed on the HPLC chromatogram in the 70% methanol extract from sweet potato (Kintoki and Kokei-14). In these compounds, ChA and iso-ChAs were the most abundant components, comprising more than 80% of the total phenolic compounds. They also mentioned that ChA and iso-ChAs showed antioxidative activity in a linoleic acid-aqueous system. We also confirmed that ChA exhibited 1.6 times higher DPPH radical-scavenging activity than Trolox on a mole basis. Thus, ChA and iso-ChAs are suggested as possible major contributors to the radical-scavenging activity in F-I.

In F-II, which contains anthocyanins, the radical-scavenging activity increased with an increase of the anthocyanin content (Figure 2C, E), indicating that anthocyanins would be a major contributor in F-II. The present study further revealed that their anthocyanin compositions varied with the cultivars; "Ayamurasaki" and "Kyushu-132" were rich in YGM-4 to YGM-6, whereas "Miyanou-36," "Bise," and "Tanegashimamurasaki" were rich in YGM-1 to YGM-3 (Table 2). This finding on the differences in the cultivars agreed with the results reported by Tsukui and others (1999). It has also been reported that anthocyanins from YGM-1 to YGM-3 were comprised of cyanidin aglycon, while those from YGM-4 to YGM-6 were peonidin aglycon (Terahara and others 1999: Tsukui and others 1999). Therefore, anthocyanins with peonidin and cyanidin aglycon appeared to be major radical-scavenging compounds in F-II of the cultivars with higher anthocyanin contents (Ayamurasaki and Kyushu-132) and the other cultivars, respectively.

Conclusion

IT WAS FOUND THAT ANTHOCYANINS AND PHENOLIC COMPOUNDS were potent radical scavengers in purple-fleshed sweet potato cultivars. There are cultivar differences in the total radical-scavenging activity irrespective of the shade of purple, resulting from the sum total of activity by each component. Judging from the content and ratio of each component, the most active contributors to scavenging DPPH radicals were phenolic compounds such as ChA and iso-ChA in "Miyanou-36" and "Bise" and anthocyanins with peonidin aglycon in "Ayamurasaki" and "Kyushu-132."

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