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Distribution of Antimutagenic Components in Colored Sweetpotatoes

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Abstract

Antimutagenicity of the water extracts prepared from the outer or inner portion of storage roots (hereafter referred to as 'outer and/or inner portion') of 4 varieties of sweetpotato differing in the flesh color was investigated using *Salmonella typhimurium* TA 98 to determine the distribution of the antimutagenic components. Analysis of anthocyanin pigments in both portions revealed a large distribution of anthocyanin pigments in the outer portion and showed that the content of cyanidin in the outer portion was higher than in the inner one. The strong antimutagenicity of Ayamurasaki outer portion was attributed chiefly to the high concentration of cyanidin. The extracts from the outer portions of Koganesengan, Sunny Red, and Joy White varieties showed an antimutagenic activity unlike the inner ones, suggesting that the antimutagenic component in the outer portions of these varieties was mainly associated with phenolics.

Discipline: Food
Additional key words: antimutagenicity, anthocyanin, phenolics

Introduction

Recently, new varieties of sweetpotato (*Ipomoea batatas*) differing in the flesh color have been released for new uses by the Kyushu National Agricultural Experiment Station. Several investigators have reported the presence of an antioxidative activity[2], reduction of liver injury induced by carbon tetrachloride in rats[7] and in man[8] which consumed purple-colored sweetpotato juice. Our recent study indicated that the anthocyanin pigments purified from purple-colored sweetpotato effectively decreased the reverse mutations induced by purified mutagens such as Trp-P-1, Trp-P-2, IQ, B[a]P, and 4-NQO, and also by dimethyl sulfoxide (DMSO) extracts of grilled beef[13]. The anthocyanin pigment from purple-colored sweetpotato is more stable than other anthocyanins from red cabbage, elderberry and purple corn[6]. At present, the flour from Ayamurasaki variety has been developed as a material for noodles, bread, a new type of alcohol drink, and a food colorant. However the distribution of functional components in sweetpotato is not clear. The functional components are not necessarily distributed uniformly in the sweetpotato storage roots. For instance, calcium[4] and phenolics[10] are abundant in the cortex of sweetpotato. Therefore it is important for effective application of sweetpotato to analyze the distribution of functional components such as nutritional or physiologically functional ones.

In this paper, we describe the distribution of the antimutagenic components of the storage roots of sweetpotato differing in the flesh color.

Materials and methods

1) Sweetpotato materials

Four varieties of sweetpotato differing in the flesh color, Koganesengan, Sunny Red, Joy White, and Ayamurasaki (Table 1) were cultivated in 1996 under the same conditions in an experimental field at Miyakonojo (Japan). Harvested roots were cut into 2 portions and one half was used as the whole root. In the remaining half, the peeled outer layer (about 0.5 cm thick) as the outer tissue containing the skin and the cortex was separated from the inner portion without outer layer as the inner tissue, respectively. The peeled outer layer comprised all of the cortical region, which included the periderm, laticiferous

Table 1. Effect of sweetpotato water extracts on the mutagenicity of Trp-P-1 against *Salmonella typhimurium* TA 98[a)]

Variety	Flesh color	Amount of extract (mg/plate)	Inhibition (%)[b)]
Ayamurasaki	Purple	1.0	41
		5.0	55
		10.0	62
Koganesengan	Yellow	1.0	0
		5.0	0
		10.0	5
Joy White	White	1.0	0
		5.0	0
		10.0	4
Sunny Red	Orange	1.0	5
		5.0	9
		10.0	8

a): Trp-P-1 was added at a dose of 0.075 μg/plate. Mutagenicity was tested with S-9 mix.
b): Each value represents mean ± SD of triplicate plates. In the values shown, the spontaneous mutation frequency was subtracted. The His^+ revertant value of the control was 625 ± 12.

ducts, and cambium. All the sections in each case were diced, lyophilized, and ground to flour. The flour samples were kept at −20°C until use.

2) Chemicals and bacteria

Trp-P-1 was obtained from Wako Pure Chemical Industries Ltd. Chlorogenic acid (ChA) was purchased from Sigma Chemical Co. S-9 Fraction prepared from rat liver treated with phenobarbital and 5, 6-benzoflavone and cofactors were purchased from Oriental Yeast Co., Ltd. Folin-Ciocalteau phenol reagent was purchased from nacalai tesque, Inc. (Kyoto, Japan). Other chemicals used were of special grade. Strain TA 98 of *Salmonella typhimurium* was supplied by the Institute for Fermentation, Osaka, Japan (IFO). The bacteria were cultured in nutrient broth for 16 h at 37°C before the mutagenicity assay was conducted.

3) Preparation of sweetpotato water extract

The extract was made from lyophilized flour (1 g), using 20 mL of ice-cold water for 1 h. The suspension was centrifuged at 18,000 × *g* for 20 min, and the resultant precipitate was re-extracted under the same conditions. The collected supernatant was lyophilized.

4) Assay of antimutagenicity

The mutagenicity assay was performed by a modification of the method of Yahagi et al.[11]. The antimutagenic activity was evaluated for TA 98 using the purified mutagen, Trp-P-1. These mutagens require metabolic activation to induce mutation in TA 98. S-9 Mix contained 50 μmol of sodium phosphate buffer (pH 7.4), 4 μmol of $MgCl_2$, 16.5 μmol of KCl, 2.5 μmol of glucose-6-phosphate, 2 μmol of NADH, 2 μmol of NADPH, and 50 μL of S-9 fraction in a total volume of 0.5 mL. For the inhibition test, 0.1 mL of each mutagen, 0.1 mL of sweetpotato water extract or DMSO-dissolved pigment solution, and 0.5 mL of S-9 mix or phosphate buffer were simultaneously incubated with 0.1 mL of bacterial suspension at 37°C for 20 min, and then poured on minimal glucose agar plates with 2 mL of soft agar.

5) Measurement of color value and HPLC analysis of anthocyanin pigments from extracts of Ayamurasaki inner or outer portion

The color value was measured by absorbance at 530 nm after the 0.5% sulfuric acid extract from each portion was diluted 4 times with McIlvaine's buffer (pH 3.0).

Individual anthocyanin pigments were analyzed using a HPLC (Model LC-9A, Shimadzu, Kyoto, Japan) equipped with a variable wavelength spectrum flow monitor. The samples for HPLC analysis of the anthocyanin pigments were prepared by extraction with 0.5% sulfuric acid. Separation of the pigments was carried out on a 4.6 mm × 10 cm Luna C18(2) column (Phenomenex) and detected at 530 nm. The column was conditioned with Solvent A (1.5% phosphoric acid), and the pigments were eluted using a linear solvent gradient of 0–100% Solvent B (1.5% phosphoric acid, 20% acetic acid, 25% acetonitrile) for 40 min at a flow rate of 1 mL/min.

6) Extraction and determination of phenolics

The lyophilized sweetpotato flour was vigorously mixed with 10 times its equivalent volume of 80% ethanol. The mixture was boiled for 5 min under a hood and centrifuged at 5,000 × *g* for 10 min. The residue was mixed with additional 80% ethanol and boiled for 10 min to re-extract the phenolics and centrifuged under the same conditions. The extracts were combined and made up to 10 mL. These extracts were used for the determination of total phenolics. Total phenolics were determined by the procedure described by Coseteng and Lee[1]. The alcohol extract was diluted to obtain an absorbance reading within the range of the standards (800-

40 μg ChA/mL). The absorbance in the microplate wells was measured at 600 nm with a dual wavelength flying spot scanning densitometer (Shimadzu, Kyoto), with a microplate system. The results were expressed as mg ChA/100 g flour.

Results

1) Effects of water extracts from whole storage roots on the mutagenicity of Trp-P-1

The antimutagenic effect of the water extracts from whole storage roots of 4 varieties differing in the flesh color was examined using Trp-P-1 at a dose of 0.075 μg/plate (Table 1). The extract was used at doses of 1, 5, and 10 mg/plate. The Ayamurasaki extract inhibited the Trp-P-1-induced mutation by 41 to 62% and showed a dose-dependent antimutagenicity. The extracts from Koganesengan, Joy White, and Sunny Red varieties showed inhibitory activities of 5, 4, and 8% at a dose of 10 mg/plate, respectively. The antimutagenic effect of the extracts from whole storage roots of Koganesengan, Joy White, and Sunny Red on Trp-P-1 was negligible compared with that from Ayamurasaki.

2) Antimutagenic activity of the extracts from the inner or outer portion of sweetpotatoes

Table 2 shows the antimutagenic activity of the extracts from the inner or outer portion of Ayamurasaki, Joy White, Koganesengan, and Sunny Red. The extract in the Ayamurasaki variety was used at doses of 1, 5, and 10 mg/plate. The extract of Ayamurasaki inner portion inhibited the Trp-P-1-induced mutation by 14 to 74%, while that of the outer one by 53 to 80%. The extracts from both portions of Ayamurasaki showed a dose-dependent antimutagenicity. The extract of the outer portion showed an antimutagenic activity about 4 times stronger than that of the inner one at a dose of 1 mg/plate. The extracts from the portions in 3 varieties other than Ayamurasaki were used only at a dose of 10 mg/plate since the extracts from the whole root of these varieties did not display the antimutagenic activity at doses below 5 mg/plate as shown in Table 1. The extracts from the outer portion of Joy White, Sunny Red, and Koganesengan inhibited the mutation by 24, 25, and 18%, unlike those of the inner one.

3) Color value and HPLC analysis of anthocyanins of extracts from Ayamurasaki inner or outer portion

The color value of the extracts from both portions of Ayamurasaki was measured at an absorbance of 530 nm (Table 3). The color value of the extract from the outer portion was 15.8 and that from the inner portion 10.9. Anthocyanin content in the outer portion was 1.4 times higher than that

Table 2. Effect of extracts from sweetpotato inner or outer portion on the mutagenicity of Trp-P-1 against *Salmonella typhimurium* TA 98[a)]

Variety	Portion	Amount of extract (mg/plate)	Inhibition (%)[b)]
Ayamurasaki	Inner	1.0	14
		5.0	69
		10.0	74
	Outer	1.0	53
		5.0	77
		10.0	80
Joy White	Inner	10.0	0
	Outer	10.0	24
Sunny Red	Inner	10.0	0
	Outer	10.0	25
Koganesengan	Inner	10.0	0
	Outer	10.0	18

a): Trp-P-1 was added at a dose of 0.075 μg/plate. Mutagenicity was tested with S-9 mix.

b): Each value represents mean ±SD of triplicate plates. In the values shown, the spontaneous mutation frequency was subtracted. The His$^+$ revertant value of the control was 609±16/plate.

Table 3. Color value and ratio of anthocyanin pigments in extracts from Ayamurasaki outer or inner portion

Portion	Color value	YGM composition ratio (percentage of area in HPLC)										
		<YGM1	YGM1		YGM2	YGM3	YGM4	YGM5		YGM6	YGM6>	Total
			YGM1a	YGM1b				YGM5a	YGM5b			
Outer	15.8	9.4	6.1		6.8	5.7	11.1	38.0		17.9	4.0	99.0
			3.0	3.1				15.0	23.0			
Inner	10.9	12.7	3.1		5.7	3.4	11.0	37.0		17.7	7.1	97.7
			2.1	1.0				—*	—*			

* The percentage was not calculated when the separation of both peaks was not distinct.

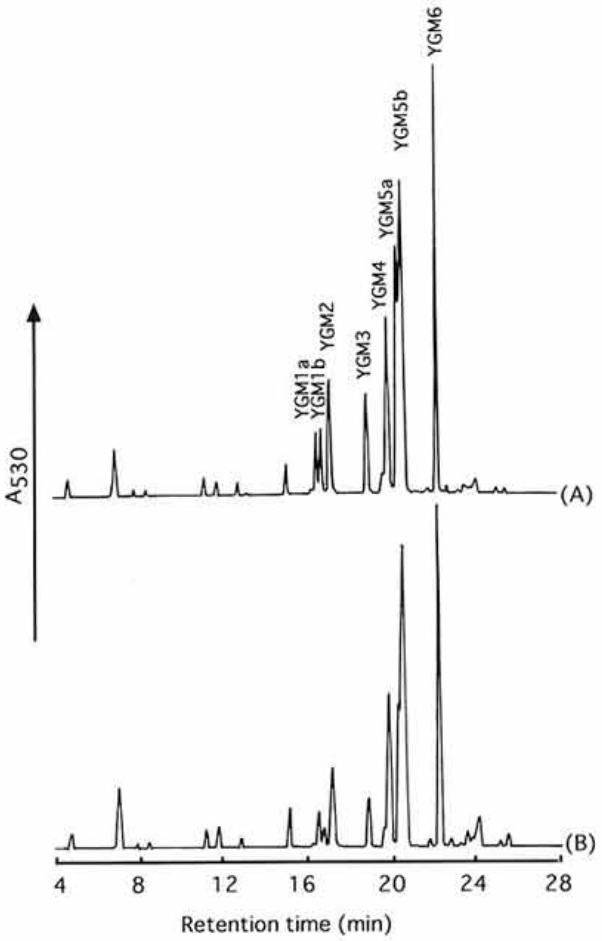

Fig. 1. HPLC pattern of anthocyanin pigments in extracts from Ayamurasaki outer or inner portion
(A): Outer portion, (B): Inner portion.

in the inner portion. These results indicate that anthocyanin pigments are abundant in the outer portion.

The difference in both color values was reflected in the HPLC pattern in that the peaks of the extract from the outer portion were generally higher than those of the inner one (Fig. 1). Sweetpotatoes with purple-colored flesh contain at least 12 anthocyanins[6] and their contents vary with the sweetpotato varieties[2]. The main anthocyanin pigments were respectively designated as YGM1 to YGM6. Ratios of each pigment in the extracts from both portions indicated in Fig. 1 are shown in Table 3. YGM5 in the extract from the outer portion was clearly separated into 2 components, YGM5a and YGM5b unlike the extract from the inner portion. The total content of YGM5 in the extracts from both portions was practically similar but the contents of HMG5a in the extracts from the outer portion were clearly higher than those in the inner portion. YGM1a, YGM1b, YGM2, YGM3, an YGM5a were more abundant in the outer portion, while the pigments with a ratio below that of YGM1 and above that of YGM6 showed a high concentration in the inner portion. Especially the contents of YGM1, YGM3, and YGM5a in the extract from the outer portion were about twice as high as those in the inner portion.

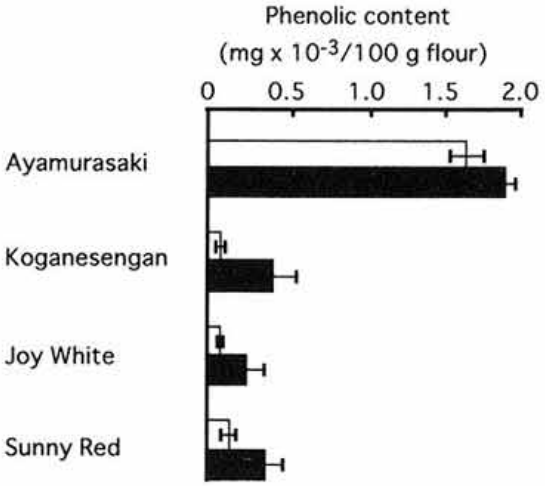

Fig. 2. Phenolic contents of extracts from the outer or inner portion from 4 varieties of sweetpotato
□: Inner portion, ■: Outer portion. Each value represents mean ±SD of 5 samples.

4) Phenolic contents in the extracts from the inner or outer portion of 4 varieties of sweetpotato

Phenolics are known to inhibit the reverse mutation induced by various mutations. We determined the phenolic distribution of sweetpotato roots to analyze the relationship between the phenolic contents and the antimutagenicity. The contents of total phenolics in the extracts from both portions of the varieties tested were expressed as mg/100 g flour (Fig. 2). The phenolic contents in the extracts from the outer and inner portions in Ayamurasaki roots were 1,900 mg and 1,650 mg/100 g flour, respectively. In the extract from the inner portion of Koganesengan, Joy White, and Sunny Red the contents were about 100, 110, and 150 mg/100 g flour, while in the outer one 400, 240, and 360 mg/100 g flour. The high phenolic content of Ayamurasaki probably enhanced the reaction of anthocyanin pigments with Folin-Ciocalteau phenol reagent. Odake et al.[5] and Goda et al.[3] reported that anthocyanins of the purple-colored sweetpotato have a caffeyl or ferulyl group in their structural formula.

Discussion

The antimutagenic activity of the extracts from the inner or outer portion of the storage roots was investigated to analyze the distribution of the antimutagenic components in sweetpotatoes differing in the color of the flesh. In 4 varieties tested, only the extract of the whole root of Ayamurasaki inhibited effectively the Trp-P-1-induced mutation (Table 1). As anticipated, the extracts from both portions also decreased the reverse mutation, and the antimutagenic activity was stronger in the extract from the outer portion than in that from the inner portion (Table 2). The determination of the color value in both portions indicated that the content of anthocyanins in the outer portion was about 1.5 times higher than that in the inner portion (Table 3). These results appear to reflect the strong antimutagenicity detected in the outer portion of Ayamurasaki. However the antimutagenic activity of the extract from the outer portion was about 4 times stronger than that from the inner portion at a dose of 1 mg/plate. These results did not always reflect the difference in the anthocyanin contents in both portions.

HPLC analysis revealed the compositional difference of anthocyanin pigments in both portions. Namely, anthocyanin pigments corresponding to YGM1a, YGM1b, YGM2, YGM3, and YGM5a were more abundant in the outer portion. Anthocyanin pigments corresponding to YGM1 to YGM3 consist of cyanidin and those to YGM4 to YGM6 of peonidin[3,5,6]. Total percentage of YGM1 to YGM3 in the outer portion was 18.6%, while 12.2% in the inner portion. Furthermore, the total percentage of YGM4 to YGM6 in the outer portion was 66.9%, while 65.7% in the inner portion. The cyanidin content in the outer portion was 1.5 times higher than that in the inner portion. These values were in agreement with the increase of the color value in the outer portion, suggesting that cyanidin was abundant in the outer portion. Our previous data also indicated that cyanidin showed a relatively stronger antimutagenic activity than peonidin[13]. These results suggest that the high concentration of cyanidin in the outer portion partially contributes to the strong antimutagenicity.

The extracts of the outer portion of Joy White, Sunny Red, and Koganesengan without anthocyanin in the flesh also effectively inhibited the reverse mutation (Table 2). The phenolic contents in the extracts of the outer portion were several times higher than those in the inner portion (Fig. 2). Yamada and Tomita[12] reported that many kinds of extracts containing compounds analogous to caffeic acid or ChA effectively decreased the mutagenic activity of the mutagens as heterocyclic amines. We also confirmed that chlorogenic acid showed a dose-dependent antimutagenicity against Trp-P-1- or Trp-P-2-induced reverse mutation (data not shown). Walter and Schadel[10] indicated that phenolics including ChA are abundant in the outer tissues of the sweetpotato roots. Their results suggest that phenolics in the outer portion mainly suppress the reverse mutation.

In conclusion, the outer portion of Ayamurasaki contained a large amount of cyanidin reflecting the strong antimutagenicity of the outer portion. These results suggest that this new variety of sweetpotato with higher physiological functions should be selected. Furthermore our data showed that the functional components were more abundant in the outer portion of sweetpotatoes. Suzuki et al.[9] reported that the calcium content was high in the cortex of sweetpotatoes. These results suggest that the utilization of the whole root including the skin is important for maximizing the beneficial physiological functions of sweetpotato. Such an utilization may contribute to the effective use of biological resources, and result in the reduction of the negative environmental impact of wastes from sweetpotato processing.

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