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Antioxidant Activities of Colored Sweet Potato Cultivars by Plant Parts

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Abstract Antioxidant activity of crude extracts from colored sweet potato cultivars by plant parts such as root, stem and leaf was evaluated. The highest TBARS values were obtained from root samples of sweet patato, and followed by stems and leaves, indicating that leaf sample showed the strongest antioxidant activity. Sweet potato cultivars with yellow flesh and leaf part exhibited strong antioxidant activities. Antioxidant activities of leaf and stem extracts were maintained for 21 days and were a little lower than that of BHT. The DPPH radical scavenging activity was the highest in "Sinhwangmi" leaf, and followed by "Jami" root. Chlorogenic acid was detected as the most abundant antioxidant substance among all fractions. These results suggest that the antioxidant activity of sweet potato differs depending on plant part and cultivar.

Keywords: antioxidant activity, phenolic compounds, TBA-reactive substance (TBARS), radical scavenging activity, 1,1-diphenyl-2-picrylhydrazyl(DPPH)

Introduction

Sweet potatoes are used as a livestock feed, and for starch and alcohol production. Sweet potato greens have been widely consumed as a fresh vegetable in the world (1, 2, 3). They are rich in polyphenols, vitamin B, iron, calcium, zinc, and protein, and are tolerant of many diseases and pests (4, 5, 6, 7, 8). A variety of health-related functions of sweet potatoes have now been identified, including the ability to inhibit lipid peroxide reactions and radical generation which cause aging and cancer. More formally, sweet potatoes possess antioxidant and radical scavenging activities. Such functions could be especially strong in colored cultivars which contain an anthocyanin or β-carotene giving it red or yellow flesh (9). Recently, antioxidants have attracted special attention because they can protect human body from oxidative stress which may cause many diseases including cancer and aging. In the past, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) as synthetic antioxidants had been widely used. However, many studies were carried out to screen more effective and safer antioxidant from natural sources. The objective of this study, therefore, was to compare antioxidant and radical scavenging activities among three colored sweet potato cultivars by plant parts and to quantify causative antioxidant substances such as coumarin, *trans*-cinnamic acid, *o*-coumaric acid, *p*-coumaric acid, caffeic acid, and chlorogenic acid through HPLC analysis. Sweet potato has various periderm colors such as white, yellow, and red. The pigmentation of such colors could be due to anthocyanins or β-carotene. This study was designed by the idea that antioxidant activity exhibit differently depending on periderm color and plant part.

Materials and Methods

Sampling and preparation of plant materials Three colored sweet potato cultivars, white sweet potato "Sinyulmi", yellow sweet potato "Sinhwangmi", and red sweet potato "Jami" were harvested at the Experimental Farm of Dongshin University. The plants were separately sampled into leaves, stems, and roots. The nine samples (3 cultivars × 3 plant parts) were immediately oven-dried at 60°C for 5 days (10), ground with a cutting mill to pass a 1 mm screen, and stored in a refrigerator at 2°C until used. The ground samples were extracted with 95% methanol at room temperature. The extract was then filtered through a Whatman No. 1 filter paper. The collected filtrate was evaporated to dryness under vacuum at 40°C using a rotary evaporator (N-1000V-W, Eyela, Japan). The yield from the original plant leaves was about 10% as the dried methanol extracts. The dried methanol extracts were used for the following measurement of antioxidant activity and radical scavenging activity.

Antioxidative activity of plant samples Antioxidant activity for the dried methanol extract was investigated by TBA method. The dry methanol extract of 0.1 g were homogenized with 10 g pork meat and then stored in a refrigerator at 2°C. At 168 hr after storage, the mixed samples were added with 25 mL of 20% trichloroacetic acid (TCA), homogenized at 14,000 rpm for 2 min, and diluted with distilled water to give final volume into 100 mL. The diluted solution was filtered with Whatman No.1 filter paper. The 5 mL-filtered solution was mixed with 5 mL TBA (5 mM) and transferred into test tube. The test tube was placed into dark room for 15 hr at 25°C. Then the solution was measured at the absorbance of 550 nm through UV-Vis spectrophotometer (Shimadzu UV-1650 PC, Japan). To evaluate long-term antioxidant activity of plant extracts in meat, TBA-reactive substance (TBARS) values of methanol extracts from the sample were measured at 7 days interval over 21 days, compared with a synthetic antioxidant, BHT 1%. TBARS value test was used to determine the degree of lipid oxidation according to the method of Witte (11). The values were calculated as follows;

TBA (MA mg/1000 g) = absorbance × 5.2.

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All measurements were replicated with 3 times.

Radical scavenging activity of methanol extracts In order to measure short-term antioxidant activity, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay was carried out according to the procedure designed by Blosi (12). Methanol extracts of each plant were added with a 1.5 x 10^{-4} M solution of DPPH in methanol, and the reaction mixture was shaken vigorously. The amount of DPPH remaining was determined at 520 nm, and the radical scavenging activity was obtained from the following equation;
Radical scavenging activity (%) = [(OD $_{control}$ - OD $_{sample}$) / OD $_{control}$] × 100.

Quantification of phenolic compounds in sweet potato "sinhwangmi" leaves For fractionation, crude methanol extracts from leaves of yellow sweet potato "Sinhwangmi" mixed with distilled water and hexane to collect hexane extracts, were shaken for 2 hr. After hexane collection, the distilled water fractions were added with ethylacetate (EtOAc) to obtain EtOAc extracts in the same way. The same procedure was used in preparing butanol and water extraction. The fractions were taken to dryness on a rotary evaporator at 40-50. The four dried fractions were redissolved in HPLC grade MeOH to give 1,000 ppm for HPLC analysis. The standard phenol compounds used for HPLC analysis were coumarin, *trans*-cinnamic acid, *o*-coumaric acid, *p*-coumaric acid, caffeic acid, and chlorogenic acid (Aldrich Co., USA). All of chemicals were purchased as high purity standards and solvents were HPLC spectral grade. All solvents and distilled water were degassed before use. All solvent ratios were based on volume. Phenolic compounds were identified by retention times or standard materials, and amounts were calculated by comparing peak area with those of standards.

Results and Discussion

Antioxidant activity of plant samples The highest TBARS values were obtained from extracts of root samples and followed by stems and leaves, showing that the extracts from ground leaf sample possess the strongest antioxidant activity. Figure 1 shows also the antioxidant activities of the methanol extracts from three cultivars of sweet potato by plant part. The cultivar containing β-carotene, yellow sweet potato "Sinhwangmi" (13), showed a higher antioxidant activity than red one "Jami" or white one "Sinyulmi". This result indicates that sweet potato cultivars with yellow flesh and leaf part exhibit stronger antioxidant activity. The results are inconsistent with other finding that the cultivars of red or purple-fleshed sweet potato containing a high level of anthocyanins showed a higher antioxidant activity than the other cultivars (14). Further studies are required, in progress, to determine which compounds are responsible for the antioxidant activity.

TBARS values were higher in all samples at 7 days after storing than BHT as a synthetic antioxidant, and differed depending on plant part among plant part extracts. Particularly, TBARS values of methanol extracts of yellow sweet potato "Sinhwangmi" at 7 days after storing were

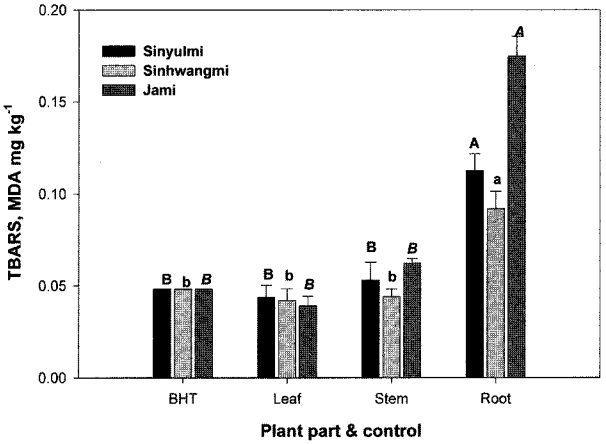

Fig. 1. TBARS values of methanol extracts from three sweet potato cultivars by plant part in meat. Each bar represents standard error of the mean. Means, within a cultivar, with same letter are not significantly different. Capital letters: cultivar 'Sinyulmi', Small letters: cultivar 'Sinhwangmi', and Italic capital letters: cultivar 'Jami'.

lower than those of "Jami" or "Sinyulmi", indicating "Sinhwangmi" cultivar had stronger antioxidant activity than "Jami" or "Sinyulmi" (Fig. 2). Antioxidant effects of methanol extracts was steadily kept over 3 weeks (Fig. 2). Antioxidative effect of leaf and stem extracts was similar to that of BHT. The result shows that methanol extracts of sweet potato would be a promising antioxidant as an alternative antioxidant.

In this study, we observed that the methanol extracts from sweet potatoes with yellow flesh exhibited stronger antioxidant activity. Therefore, the high amount of carotene in this crop makes it a healthier choice and an alternative for natural antioxidants, due to its high antioxidant activity.

Radical scavenging activity of methanol extracts The DPPH radical scavenging activities of the plant extracts are shown in Fig. 3. Extracts of "Sinhwangmi" leaf had the highest DPPH radical scavenging activity, and followed by "Jami" root. These results suggest that DPPH values were partly associated with TBARS values which is also related to the color or the plant parts of sweet potatoes. Thus, pigment such as anthocyanins and carotene in colored sweet potatoes could be largely involved in the high antioxidant activity. Antioxidant activity by DPPH method was different from the result by TBA method, however, the difference cannot be explained readily. Further investigations are also needed to determine the influence of variations according to evaluation methods of sample on antioxidant activity.

Quantification of phenolic compounds in sweet potato "sinhwangmi" leaves Phenolic substances present in the methanol extracts from "Sinhwangmi" leaf were analyzed by HPLC using standard compounds. The content was recorded as each or total phenol compounds in four fractions, and the related quantities referred to 100 g dry matter are showen in Table 1. The individual compounds identified were coumarin, *trans*-cinnamic acid, *o*-coumaric acid, *p*-coumaric acid, caffeic acid, and chlorogenic acid.

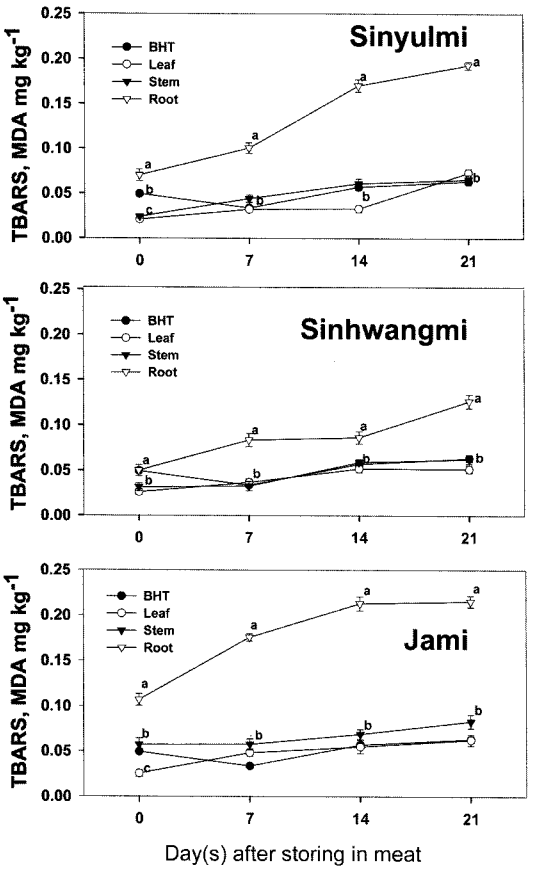

Fig. 2. Change in TBARS value of methanol extracts from three sweet potato cultivars by plant part in meat over 21 days. Each bar represents standard error of the mean. Means, within a storing day, with same letter are not significantly different.

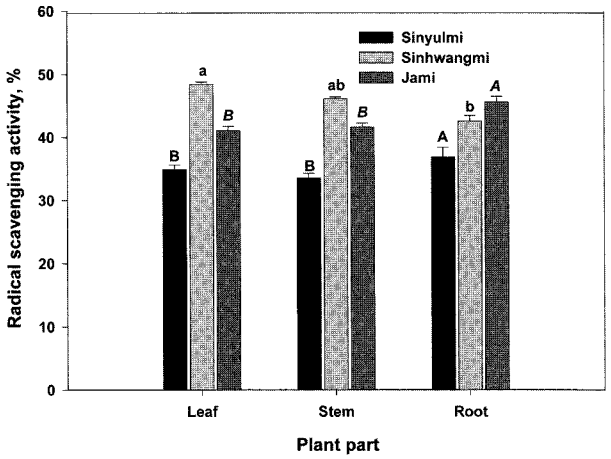

Fig. 3. DPPH radical scavenging activity of methanol extracts from three sweet potato cultivars by plant part. Each experiment was performed at least three times. Each bar represents standard error of the mean. Means, within a cultivar, with same letter are not significantly different. Capital letters: cultivar 'Sinyulmi', Small letters: cultivar 'Sinhwangmi', and Italic capital letters: cultivar 'Jami'.

Of these chlorogenic acid was detected in all fractions as the most abundant component (61.9 mg/100 g) and followed by caffeic acid (26.8 mg/100 g). The content of total phenolic compounds was the highest in the EtOAc fraction (55.3 mg/100 g), followed by water fraction (32.4 mg/100 g). These results also suggest that the antioxidant activity of sweet potato is highly correlated with the amount and type of phenolic compounds found in plant extracts. In accordance with others, who reported that high contents of total phenolic compounds can improve antioxidant activity (15, 16, 17), there was a linear correlation between phenol content and antioxidant activity. The crops contain a group of natural antioxidants that have not only a high antioxidant activity (18, 19), but also a good antioxidant quality (20). Therefore, the supplementation of natural antioxidants through a balanced diet containing enough crop and vegetables could be effective for protecting of human body against various oxidative stresses, and the use of natural phenols from sweet potato as food additives can be advantageous.

Table 1. Quantitative determination of HPLC analysis on some phenolic compounds present in leaves of sweet potato "Sinhwangmi"

Compound	Fractions				
	Hexane	EtOAc	BuOH	Water	Total
	(mg/100 g)				
Coumarin	0.354	0.747	0.187	0.361	1.648
trans-Cinnamic acid	0.091	0.102	0.024	0.058	0.274
o-Coumaric acid	0.116	0.246	0.062	0.119	0.542
p-Coumaric acid	-	1.499	-	0.571	2.070
Caffeic acid	0.077	16.031	1.165	9.507	26.780
Chlorogenic acid	0.721	36.717	2.667	21.774	61.879
Total	1.359	55.341	4.104	32.389	93.194

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