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Note

High *tert*-Butylperoxyl Radical Scavenging Activities of Sweet Potato Cultivars with Purple Flesh

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The *tert*-butylperoxyl radical (*t*-BuOO$^{\cdot}$) scavenging activities of ethanol extracts of 21 sweet potato cultivars with several flesh colors were examined using a *tert*-butyl hydroperoxide (*t*-BuOOH)/hemin/luminol system. Among them, sweet potato cultivars with purple flesh, which contained anthocyanins, had the highest *t*-BuOO$^{\cdot}$ scavenging activities. Those cultivars with purple flesh also had the highest antioxidative activities against lipid peroxidation induced by auto-oxidation of linoleic acid. Most of the sweet potato cultivars with white, white-yellow, yellow and orange flesh had low *t*-BuOO$^{\cdot}$ scavenging and antioxidative activities; however, some of them had higher activities. In all sweet potato cultivars tested, the *t*-BuOO$^{\cdot}$ scavenging activities became higher with an increase in the total phenolic content.

Keywords: *t*-BuOO$^{\cdot}$ scavenging activity, chemiluminescence intensity, antioxidative activity, lipid peroxidation, total phenolic content, anthocyanin, sweet potato

Recently, several colorful sweet potato cultivars with yellow, orange, or purple flesh have been released from our Kyushu National Agricultural Experiment Station (Yamakawa, 1996). The usual sweet potato cultivars generally contain ascorbic acid, α-tocopherol and flavonoids, and in addition, orange flesh cultivars are rich in β-carotene, while purple flesh cultivars are rich in anthocyanin; some orange ones contain more β-carotene than do carrots (Takahata *et al.*, 1993), and some purple ones, especially "Ayamurasaki," show an extremely high anthocyanin content (Yoshinaga, 1995). Our interest is now directed to the radical scavenging and antioxidative capacity of sweet potatoes. Numerous reports indicated that anthocyanins isolated from several plant materials, such as pea bean (Tsuda *et al.*, 1994), Muscat Bailey A grape (Tamura & Yamagami, 1994), wild grape (Igarashi *et al.*, 1989), Chouja-nasu (Igarashi *et al.*, 1993) and potato (Ishii *et al.*, 1996), exert antioxidative effects with high activities. Sweet potato cultivars with purple flesh, which contain anthocyanins, may possess the high radical scavenging and antioxidative capacity. However, less is known about their capacity.

In view of this background, the present investigation was carried out to clarify the high radical scavenging and antioxidative capacity of sweet potato cultivars with purple flesh.

Materials and Methods

Reagents Hemin, luminol, Folin-Ciocalteu reagent and trifluoroacetic acid (TFA) were obtained from Wako Pure Chemical Industries, Ltd. (Osaka). Diethylenetriamine-*N,N,N′,N″,N″*-pentaacetic acid (DTPA) was purchased from Dojindo Laboratories (Kumamoto), butylated hydroxytoluene (BHT) from Tokyo Kasei Kogyo Co., Ltd. (Tokyo), *tert*-butyl hydroperoxide (*t*-BuOOH) from Katayama Chemical Industries Co., Ltd. (Tokyo), chlorogenic acid from Sigma Chemical Co. (St. Louis, MO) and cyanidin-3-glucoside chloride from Extrasynthese (Genay, France). Other chemicals were of analytical grade.

Sweet potatoes Twenty-one cultivars of sweet potato (*Ipomoea batatas* POIR.) were grown at Kyushu National Agricultural Experiment Station (Miyakonojyo branch, Miyazaki) in 1995. Among them, one was a cultivar with white flesh (Joy white), five were cultivars with white-yellow flesh (Kyukei-116, Ayamurasaki white mutant, Koganesengan, Kyukei-170, Kyushu-125), four were cultivars with yellow flesh (Kokei-14, Kyushu-118, Beniotome, Kyushu-121), five were cultivars with orange flesh (Benihayato, Kyushu-114, J-red, Kyushu-122, Kyukei-173) and six were cultivars with purple flesh (Kyukei-165, Tanegashimamurasaki, Kyushu-119, Kyukei-174, Ayamurasaki, Kyukei-184). Four or five tubers were harvested from each cultivar, washed with tap water and cooled at 5°C for 10 min. After peeling, the inner portion was chopped into dice of $\approx$0.5 cm, immediately thrown into liquid nitrogen for instantaneous freezing and then lyophilized for 10–14 days. The lyophilized samples were powdered in a mill and stored at −20°C until used.

Preparation of sweet potato ethanol extract Sweet potato ethanol extracts (equivalent to 100 mg of fresh weight/ml) were prepared according to the method described in our previous work (Furuta *et al.*, 1997). For analysis, each extract sample was diluted with ethanol.

Radical scavenging activity against t-BuOO$^{\cdot}$ generation The *t*-BuOO$^{\cdot}$ scavenging activity was evaluated with a chemiluminescence (CL) analyzer (Model CLD-100, Tohoku Electric Industrial Co., Ltd., Sendai, Japan). The procedure was based on reports of Maeda *et al.* (1992) and Akaike

et al. (1992) and modified as follows: components of the reaction mixture, such as *t*-BuOOH, hemin and luminol, were prepared just before use. The reaction mixture contained 0.8 ml of 75 mM sodium phosphate buffer (pH 7.0), 0.2 ml of extract sample diluted with ethanol, 0.2 ml of 10 mM DTPA in 50 mM phosphate buffer (pH 7.0), 0.2 ml of 1 mM *t*-BuOOH in ethanol, 0.4 ml of 5 μM hemin in 50 mM phosphate buffer (pH 7.0) and 0.2 ml of 10 μM luminol in 50 mM phosphate buffer (pH 7.0). All components, except for hemin, in a stainless steel plate (50 mm in diameter and 13 mm in height), were placed in a CL analyzer. After keeping the sample at 30°C for 30 s, the reaction was initiated by rapid injection of hemin solution through a microsyringe, and the CL intensity was measured for 30 s. The background count of the blank plate at 30°C was about 2400 counts/30 s. The net CL intensity was expressed after subtraction of the background count. The net CL intensity of the control containing no additives was about 500,000 counts/30 s, and this value represented 100% CL intensity. All data were expressed as means±SD (standard deviation) of 4 or 5 experiments. A low CL intensity indicated a high *t*-BuOO· scavenging activity. BHT was used as a standard sample.

Antioxidative activity against lipid peroxidation induced by auto-oxidation of linoleic acid Antioxidative activity was evaluated by a fluorometric assay which was developed by us for screening the antioxidative activity of agricultural products (Furuta *et al.*, 1997).

Total phenolic content The total phenolic content was determined by a modification of the Folin-Ciocalteu procedure of Sato *et al.* (1996). Each 80% ethanol extract (10 ml) was evaporated and redissolved in a total volume of 2.5 ml with distilled water (equivalent to 400 mg of fresh weight/ml). To 400 μl of sample extract, 2 ml of 10% Folin-Ciocalteu reagent was added, and the contents of the test tube were mixed. After an interval of 3 min, 2 ml of 10% sodium carbonate solution was added, and the mixture was allowed to stand for 1 h. The absorbance was measured at 765 nm with a spectrophotometer (Model DU-70, Beckman Instruments, Inc., Fullerton, CA). The total phenolic contents were expressed as the chlorogenic acid equivalent.

Anthocyanin content To 1 ml of sample extract (equivalent to 100 mg of fresh weight/ml), 0.4 ml of 10% TFA in ethanol and 2.6 ml of ethanol were added, and the absorbance of the solution (4 ml) was measured at 536 nm with a spectrophotometer. The anthocyanin contents were calculated from a calibration curve for cyanidin-3-glucoside chloride.

Results and Discussion

Sweet potato cultivars with purple flesh showed high radical scavenging activities against *t*-BuOO· generation as compared with those of other cultivars with white, white-yellow, yellow and orange flesh (Fig. 1). All the purple flesh cultivars also showed high antioxidative activities against linoleic acid auto-oxidation (Fig. 2). It has been known that purple flesh sweet potato contains at least six major anthocyanins, whose main aglycones are cyanidin and peonidin (Odake *et al.*, 1992; Muroi, 1993; Odake & Muroi, 1994); furthermore, anthocyanins isolated from vegetables (Tsuda *et al.*, 1994; Igarashi *et al.*, 1993) and fruits (Igarashi *et al.*, 1989), and commercial anthocyanins and their aglycones (Wang *et al.*, 1997) have high antioxidative and radical scavenging activities. These facts supported our idea that the anthocyanins present in sweet potato cultivars with purple flesh may have high radical scavenging and antioxidative capacity.

The total phenolic contents of representative 14 sweet potato cultivars were determined and plotted against their *t*-BuOO· scavenging activities (Fig. 3). Table 1 shows the anthocyanin contents of those cultivars with purple flesh. Anthocyanin contents of cultivars with white, white-yellow, yellow and orange flesh were not detected. Purple flesh cultivars rich in phenolic components, including anthocyanins, formed a group with the highest *t*-BuOO· scavenging activity level. On the other hand, four cultivars, such as "Ayamurasaki white mutant," "Beniotome," "Benihayato," and "Kyushu-114," formed a secondary higher group rich in polyphenol besides anthocyanins. This result was consistent

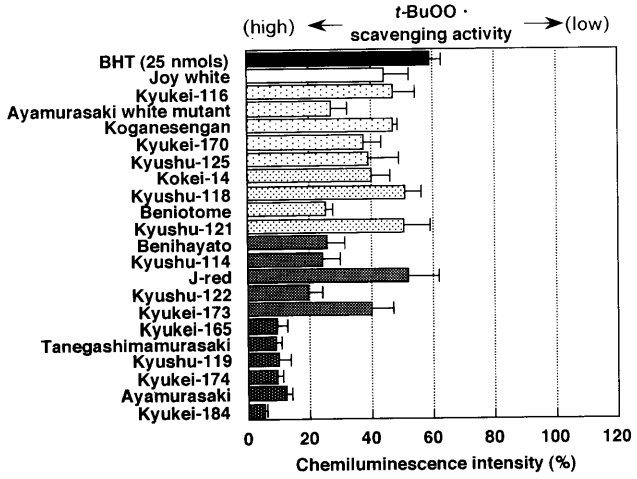

Fig. 1. Scavenging activities of sweet potato ethanol extracts against *t*-BuOO· generation. A sweet potato sample equivalent to 25 μg fresh weight was added to the assay. As a standard sample, 25 nmols of BHT was used.

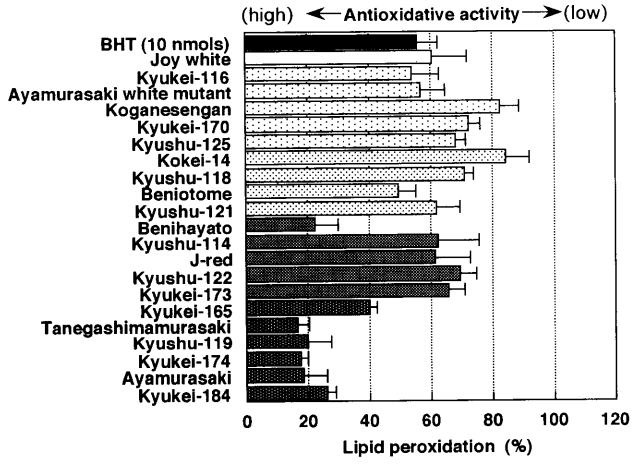

Fig. 2. Antioxidative activities of sweet potato ethanol extracts against lipid peroxidation. A sweet potato sample equivalent to 200 μg fresh weight was added to the assay. As a standard sample, 10 nmols of BHT was used.

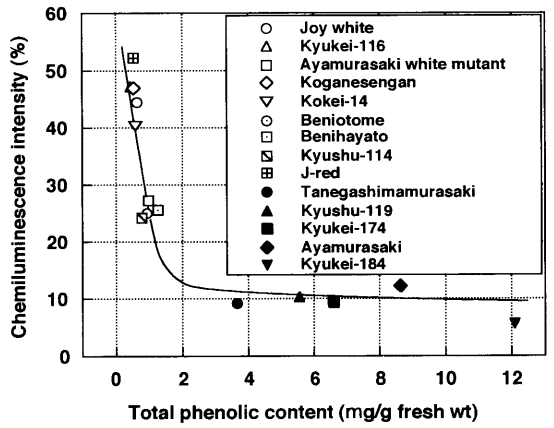

Fig. 3. Relationship between *t*-BuOO· scavenging activity and total phenolic content.

Table 1. Anthocyanin contents of sweet potato cultivars with purple flesh.

Cultivar	Anthocyanin content (mg/g fresh wt)
Tanegashimamurasaki	0.053
Kyushu-119	0.111
Kyukei-174	0.254
Ayamurasaki	0.405
Kyukei-184	0.536

with a report by Tsushida *et al.* (1994), in which the antioxidative activities of 43 kinds of vegetable extracts were correlated to their polyphenol contents. Thus, we consider that several phenolic components, for example, anthocyanin and other polyphenols in sweet potato, are among the contributors to the *t*-BuOO· scavenging activity.

In this study, we found that purple flesh cultivars are worthy of attention as natural colorants having high *t*-BuOO· and antioxidative capacity. We do not know at this time which components (e.g., anthocyanins, the other flavonoids, tocopherols) contribute to the total radical scavenging and antioxidative capacity of sweet potato cultivars or to what extent do so. Further studies are necessary to clarify the major contributor as radical scavenger.

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