Intracellular Reactive Oxygen Species (ROS) Removal and Cytoprotection Effects of Sweet Potatoes of Various Flesh Colors and Their Polyphenols, Including Anthocyanin

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ABSTRACT: The flesh color of sweet potatoes varies based on the antioxidant pigments in the cultivar. In this study, the antioxidant characteristics of various flesh color sweet potato cultivars (Jinyulmi, Juhwangmi, Pungwonmi, and Sinjami) were investigated. The polyphenol contents were highest in the purple-fleshed cultivar, Sinjami (39, 68, and 71 µg gallic acid equivalent/g in distilled water, fermented ethanol, and ethanol extracts, respectively). The Sinjami cultivar contained 29 mg/100 g of anthocyanin, which is the major component resulting in increased concentrations of polyphenols. Using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), 2,2-diphenyl-1-picrylhydrazyl, and ferric reducing ability of plasma assays, Sinjami showed greater antioxidant activity than the other cultivars. Additionally, the Sinjami extracts could recover cellular reactive oxygen species levels in *tert*-butyl hydroperoxide-stimulated HepG2 cells to a normal level. In conclusion, anthocyanin-enriched Sinjami has strong antioxidant activities and could improve health by suppressing oxidative damage.

Keywords: sweet potato, antioxidant activity, phytochemical

INTRODUCTION

Sweet potato (*Ipomoea batatas* L.) is mainly consumed in Latin America, South America, China, and Korea. Asia is the largest area where sweet potato is cultivated, and China produces 65% of the sweet potato in the world (Bovell-Benjamin, 2007). Nutritionally, sweet potato contains carbohydrates, lipids, and proteins in proportions of 20.0, 0.1, and 1.6%, respectively, and contains bioactive compounds including dietary fiber, minerals (calcium, iron, and potassium), vitamins (B₆, C, E, and folate), and polyphenols (Mohanraj and Sivasankar, 2014). Natural pigments such as β-carotene and anthocyanin are the major bioactive compounds in sweet potatoes that determine the flesh color (Teow et al., 2007). The major pigment in purple-fleshed sweet potato is anthocyanin, which has antimutagenic and antioxidative activity, anti-inflammatory properties, and hepatocyte protective effects. The anthocyanin contents of purple-fleshed sweet potatoes vary depending on the cultivar and can be about 530 µg/g fresh weight (fw) (Teow et al., 2007).

Oxidative stress is a major risk factor for chronic and degenerative diseases. The importance of antioxidant consumption is emphasized since antioxidants improve health by eliminating free radicals and reactive oxygen species (ROS) in the body. The antioxidant activity of anthocyanin operates by the hydroxyl group of catechol donating a hydrogen atom to a free radical, resulting in a stable semiquinone. Antioxidant efficacy depends on the location and number of hydroxyl groups, determined by the substitution pattern of the anthocyanin (Castañeda-Ovando et al., 2009). More than 500 anthocyanins have been reported, and six (pelargonidin, peonidin, cyanidin, malvidin, petunidin, and delphinidin) are most commonly found in plants (Clifford, 2000). Purple-fleshed sweet potatoes contain specific glycosides, such as peonidin-3-caffeoyl-*p*-hydroxybenzoyl-sophoroside-5-glucoside and cyanidin-3-caffeoyl-*p*-hydroxybenzoyl-sophoroside-5-glucoside, which must also be studied to further elucidate the antioxidant activities (Kim et al., 2012).

In this study, extracts were prepared from four different cultivars of sweet potato using water, fermented eth-

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anol, and ethanol as solvents. The chemical composition, radical scavenging ability, intracellular ROS scavenging activity, and cytoprotective effects of the extracts were examined, and correlations between the sweet potatoes' chemical compositions and biological effects were calculated. The data from this study will be useful for the future production of sweet potato.

MATERIALS AND METHODS

Sample preparation

Four cultivars of sweet potato (Jinyulmi, JY; Juhwangmi, JH; Pungwonmi, PW; Sinjami, SJ) were grown at the National Institute of Crop Science, Rural Development Administration, Muan, Jeonnam, Korea. The sweet potatoes were harvested in 2017 and stored at $13 \pm 1^\circ\text{C}$ (humidity $90 \pm 2\%$) for 3 months. Samples (100 g) were extracted using distilled water (DW), fermented ethanol, or ethanol for 48 h at room temperature. Extracts were filtered through Whatman No. 4 paper (GE Healthcare, Chicago, IL, USA), and then solvents were evaporated using nitrogen gas or a freeze dryer. The extracts were re-dissolved in 70% ethanol for the cell-free assay or dimethyl sulfoxide (DMSO) for the cell-based assay.

Polyphenol contents

Total polyphenol content was determined by a previously described method, with some modification (Ainsworth and Gillespie, 2007). The sample or gallic acid (0.1%) standard was mixed with 1 mL sodium carbonate (2%, w/v) and 50 μL Folin-Ciocalteu reagent (50%, v/v). The mixtures were incubated for 30 min at room temperature prior to measurements. Samples were transferred into 96-well plates, and the absorbance was read at 750 nm (Varioskan LUX, Thermo Fisher Scientific, Inc., Waltham, MA, USA). The total polyphenolic compounds in the extracts were calculated as μg of gallic acid equivalent (GAE) per g of sample.

Total anthocyanin quantification

Total anthocyanin levels were spectrophotometrically determined, as described previously (Teow et al., 2007). Samples were added to potassium chloride (25 mM, pH 1.0) and sodium acetate (400 mM, pH 4.5) and incubated for 15 min. The absorbance was measured at 530 and 700 nm, and then the total amount was calculated using the following equation:

$$\text{Anthocyanin (mg/L)} = [(A_{530 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH } 1.0} - (A_{530 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH } 4.5}] \times 449.2 \times \text{DF} \times 1,000 / 26,900 \times l$$

where A is the absorbance at each spectrum, DF is the dilution factor, and l is the path length.

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The ABTS assay solution was prepared by mixing 7 mM ABTS with 2.45 mM potassium persulfate and incubating the solution in a dark room overnight (Re et al., 1999). The solution was diluted using methanol to obtain an absorbance between 1.4 and 1.5 at 735 nm. The samples and Trolox (Sigma-Aldrich Co., St. Louis, MO, USA) standards (50 μL) were then mixed with 1 mL of diluted ABTS working solution. Mixtures were incubated for 30 min at room temperature, and the radical scavenging activities were determined by reading the absorbance at 735 nm (Varioskan LUX, Thermo Fisher Scientific, Inc.). DPPH was dissolved in ethanol at a concentration of 0.2 mM. Samples and Trolox standards (50 μL) were mixed with 200 μL of DPPH solution and incubated for 30 min at room temperature. The mixtures were transferred to a 96-well plate and absorbances were measured at 520 nm (Varioskan LUX, Thermo Fisher Scientific, Inc.). The radical scavenging activities were calculated as mg of Trolox equivalent (TE) per g of sample.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed as previously described, with some modification (Benzie and Strain, 1996). In the FRAP assay, reductants in the sample reduce Fe(III) /tri-pyridyltriazine complexes, which are present in stoichiometric excess, to the blue ferrous form, which increases absorbance at 593 nm. Absorbance was measured after 0.5 s and once every 30 s thereafter through the monitoring period of 5 min. The readings at 4 min were used as the FRAP value ($\mu\text{M/g}$).

Cellular ROS determination

Human hepatoma HepG2 cells were grown in Dulbecco's modified Eagle's medium high glucose medium (Caisson, North Logan, UT, USA) with 10% fetal bovine serum (GenDEPOT, Barker, TX, USA) and 1% penicillin/streptomycin (Caisson). The cells were seeded on 96-well black plates at a density of 3×10^4 cells/well. Cells were stimulated with sweet potato extracts (100 $\mu\text{g/mL}$) or DMSO for vesicle control for 24 h. Growth medium was exchanged with medium containing 25 μM of a 2,7-dichlorofluorescein diacetate (Sigma-Aldrich Co.) fluorescent probe and cells were incubated for another 1 h at 37°C . *tert*-Butyl hydroperoxide (*t*-BHP, 1 mM, Sigma-Aldrich Co.) was then added to the wells, and fluorescence intensity was measured using a multi-plate reader (Varioskan LUX, Thermo Fisher Scientific, Inc.) at an excitation wavelength of 485 nm and emission wavelength of 530 nm for 2 h. The ROS levels were calculated by comparing the intensity from the DMSO-treated wells (Wang and Joseph, 1999).

Cytotoxicity assay

The HepG2 cells were incubated with ethanolic extract of sweet potato (10 and 100 µg/mL) for 24 h. The medium was exchanged with medium containing *t*-BHP (1 mM, Sigma-Aldrich Co.) and incubated for a further 2 h. Cell viability was determined by adding 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (0.5 mg/mL). After 2 h, the medium was removed, and the formazan crystals were dissolved by adding DMSO. The intensity was measured at 570 nm using a spectrophotometer (Varioskan LUX, Thermo Fisher Scientific, Inc.) (Sylvester, 2011).

Statistical analysis

Data are expressed as mean±standard deviation (SD). The significance of differences among treatment means was determined by one-way analysis of variance (ANOVA) and Tukey tests using GraphPad Prism version 5 software (GraphPad Software, San Diego, CA, USA) with a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Flesh color and polyphenol contents of sweet potato according to cultivar

Representative images of each sweet potato cultivar and their total anthocyanin contents are shown in Fig. 1A. JY was bright yellow in color, JH and PW were orange, and SJ was dark purple. The anthocyanin was present in an amount of 29 mg/100 g in SJ, whereas the JY, JH, and PW cultivars contained trace amounts (0.07~0.30 mg/100 g). The total polyphenol concentration of each of the sweet potato cultivars was analyzed, and results are shown in Fig. 1B. The JY, JH, and PW cultivars contained polyphenols in amounts of 28~22 µg GAE/g irrespective of the solvents used for extraction, whereas the SJ cultivar contained 39, 68, and 71 µg GAE/g of polyphenols in the DW, fermented ethanol, and ethanol extracts, respectively. The polyphenol levels in the SJ extracts were 155, 309, and 311% of the levels found in the JY extracts prepared with DW, fermented ethanol, and ethanol, respectively.

Anthocyanins, a major group of polyphenols, are soluble in water and organic solvents and are extracted using alcohol in some industrial food processes (Castañeda-Ovando et al., 2009; Khoo et al., 2017). Anthocyanins are the major bioactive compounds in purple-fleshed sweet potatoes, with concentrations ranging from 3~53 mg/100 g fw (Teow et al., 2007). Anthocyanin content in ethanol extracts of SJ was also found to be within this range: 29 mg/100 g fw. Co-pigmentation of anthocyanins with other sweet potato compounds stabilizes the purple color (Castañeda-Ovando et al., 2009). However, isolated an-

A

Cultivar	JY	JH	PW	SJ
Flesh color
Anthocyanin (mg/100 g fw)	0.07±0.02 ^b	0.07±0.04 ^b	0.30±0.03 ^b	29.13±1.40 ^a

B

Fig. 1. Color and polyphenol contents of sweet potato cultivars and solvent extracts. Cut side of four sweet potatoes. (A) Representative images of Jinyulmi (JY), Juhwangmi (JH), Pungwonmi (PW), and Sinjami (SJ) are shown. (B) Total polyphenols were determined in the four sweet potato cultivars extracted with DW, fermented EtOH, and EtOH. The values are shown in µg GAE/g of extract. The data are mean±SD. Different letters (a-c) indicate significant differences at $P < 0.05$ as calculated using ANOVA. fw, fresh weight; DW, distilled water; EtOH, ethanol; GAE, gallic acid equivalent.

thocyanins are unstable and degrade easily (Giusti and Wrolstad, 2003). The stability of anthocyanins change based on pH, solvent, and temperature (Castañeda-Ovando et al., 2009; Khoo et al., 2017). Therefore, these findings suggest that methods to maximize and increase the stability of anthocyanin are needed to increase the functionality of purple-fleshed sweet potatoes.

Antioxidant activity of sweet potatoes according to cultivar

ABTS and DPPH radical scavenging activities of the four cultivars of sweet potato were derived and compared (Fig. 2A and 2B). The ABTS radical scavenging effects in the JY and JH cultivars were found to be 4~8 mg TE/g; these values did not differ significantly among the solvents used for extraction. The ABTS radical scavenging activity of the ethanolic extracts of PW was significantly higher than that of the DW extract (339% of the activity of the DW extract; $P < 0.05$). When comparing the DW extracts, the extract of the SJ cultivar showed the highest ABTS scavenging effect (13 mg TE/g, $P < 0.05$). The fermented ethanol and ethanolic extracts of SJ were most effective in scavenging ABTS radicals, with values of 54 and 57 mg TE/g, respectively. In the DPPH radical scavenging assay using DW extracts, the PW cultivar showed the lowest

Fig. 2. Antioxidant activities of sweet potato cultivars in cell-free assays. (A) 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and (B) 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. Antioxidant activities were compared between cultivars and extract solvents. The values are shown in mg Trolox equivalent (TE)/g of extract. (C) Ferric reducing antioxidant power (FRAP) assay. The values are shown in $\mu\text{M/g}$ of extract. The data are mean $\pm$ SD. Different letters (a-g) indicate significant differences at $P < 0.05$ as calculated using ANOVA. JY, Jinyulmi; JH, Juhwangmi; PW, Pungwonmi; SJ, Sinjami.

DPPH radical scavenging activity, and SJ had the highest (1 and 9 mg TE/g in PW and SJ, respectively). The fermented ethanol and ethanolic extracts were not significantly different among the JY, JH, and PW cultivars (4~8 mg TE/g with the fermented ethanol extract and 5~8 mg TE/g with the ethanol extract). In contrast, SJ extracted with fermented ethanol showed a significant different effect on DPPH radical scavenging compared to other cultivars (40 mg TE/g in the fermented ethanol extract and 41 mg TE/g in the ethanol extract, $P < 0.05$). The FRAP assay measures the reducing power of antioxidants, which donate electrons to ferric ions (Fe^{3+}) to form blue ferrous ions (Fe^{2+}) (Gülçin, 2015). Of the DW extract, the PW cultivar had the lowest (27 $\mu\text{M/g}$) FRAP value and the SJ had the highest (129 $\mu\text{M/g}$) ($P < 0.05$, Fig. 2C). No significant differences were found between the fermented ethanol and ethanol extracts of JY, JH, and PW, whereas the SJ ethanol extracts showed a higher value (520 $\mu\text{M/g}$) than the fermented ethanol extract (425 $\mu\text{M/g}$, $P < 0.05$).

PW and JH are orange-fleshed cultivars (Fig. 1A) with β -carotene contents of 9.1 and 21.5 mg/100 g, respectively (Ahn et al., 2006; Lee et al., 2017). β -Carotene, a precursor of vitamin A, is a lipophilic molecule known to exhibit antioxidant activity through scavenging singlet molecular oxygen and peroxy radicals (Stahl and Sies, 2003). Therefore, the antioxidant effects shown in the organic solvent extracts were significantly increased in the ABTS, DPPH, and FRAP assays of PW and the FRAP assay of JH, compared to those of water extracts ($P < 0.05$). The fermented ethanol and ethanol extracts of SJ showed the highest antioxidant activities, which are related to the content of polyphenols, including anthocyanin. Teow et al. (2007) also showed that the antioxidant activities of 19 sweet potato cultivars increases by flesh color: yellow and orange, to purple. In a study of colored maize, DPPH and ABTS radical scavenging activities were found to be highly correlated with the anthocyanin content rather than with β -carotene content (Zilić et al., 2012). Of the

natural colorants present in sweet potatoes, anthocyanins have a higher radical scavenging activity than β -carotene.

Effects of sweet potato extracts on ROS scavenging and cell viability in *t*-BHP-stimulated HepG2 cells

HepG2 cells were treated with the sweet potato cultivar extracts. The hepatocytes were then stimulated with *t*-BHP to induce oxidative stress (Fig. 3A). After 1 h of *t*-BHP stimulation, cellular ROS levels increased by 295%. Cellular ROS levels were slightly reduced ($P > 0.05$) by the JY, JH, and PW extracts, with the exception of the DW extract of PW. The SJ extract induced a significantly greater reduction in cellular ROS than the *t*-BHP treatment group ($P < 0.05$). In addition, the fermented ethanol and ethanolic extracts of SJ caused the ROS level to recover by 125% compared with the vesicle control, although this finding was not statistically significant ($P > 0.05$). ROS levels after 2 h of *t*-BHP stimulation showed similar results. Cell viability was also affected in *t*-BHP-stimulated HepG2 cells (Fig. 3B), with *t*-BHP treatment significantly reducing cell viability by 59%. The ethanolic extract of JY, PW, and SJ sweet potatoes significantly recovered cell viability after stimulation with *t*-BHP in the 100 $\mu\text{g/mL}$ treatment group ($P < 0.05$). The SJ extract (100 $\mu\text{g/mL}$) showed the highest activity through increasing cell viability by 29% compared with the *t*-BHP treatment group.

t-BHP is an organic hydroperoxide that increases intracellular ROS to cause oxidative damage to DNA and cytotoxicity (Lazzé et al., 2003). Acute *t*-BHP stimulation reduced cell viability by 40% compared to normal cells. However, viability in the groups treated with sweet potato ethanol extracts tended to recover. In addition, there was a reduction in cellular ROS that induced cytotoxicity of cells treated with sweet potato extracts, especially the SJ group, which significantly down-regulated ROS to levels found in normal cells. These effects resulted from the radical scavenging activities of the sweet potato extracts,

Fig. 3. Antioxidant activities of sweet potato cultivars in cell-based assays. (A) Reactive oxygen species (ROS) levels were determined in HepG2 cells treated with sweet potatoes after 1 and 2 h of *tert*-butyl hydroperoxide (*t*-BHP) stimulation. (B) Viability of HepG2 cells after stimulation of sweet potatoes for 24 h, and treatment with *t*-BHP for 2 h. The data are mean $\pm$ SD. Different letters indicate significant differences at $P < 0.05$ as calculated using ANOVA analysis. DMSO, dimethyl sulfoxide; JY, Jinyulmi; JH, Juhwangmi; PW, Pungwonmi; SJ, Sinjami.

which are dependent on polyphenols and anthocyanins, as shown in Fig. 2. Antioxidant activity of sweet potatoes are may affected by their polyphenol contents including anthocyanins. Previous studies (Jiao et al., 2003; Alía et al., 2006) showed polyphenols improves antioxidant activity in cell models. Specifically, anthocyanin may prevent DNA damage caused by *t*-BHP in rat hepatocytes, suggesting that SJ cultivars have a significant effect on ROS normalization in oxidatively damaged cells (Lazzé et al., 2003).

Correlation coefficients of sweet potato antioxidant factors

Correlations between antioxidant compounds and their effects were calculated (Table 1). Polyphenol, anthocyanin, ABTS and DPPH radical scavenging activities, and FRAP data were positively correlated, above 0.9 ($P < 0.05$). The cellular ROS levels were negatively correlated, at above -0.9 , with polyphenol, anthocyanin, ABTS and DPPH radical scavenge activities, and FRAP.

In epidemiological studies, the intake of fruits and vegetables has been shown to lower the incidence of age-related diseases due to the cytoprotective effects of phenolic compounds against oxidative damage (Dudonné et al., 2009). The polyphenol and anthocyanin components of

sweet potato were highly correlated with antioxidant capacities, as measured by DPPH, ABTS, and FRAP assays (Fig. 2). Additionally, these components are beneficial for cellular ROS regulation and protection against *t*-BHP, even after uptake into HepG2 cells. The bioavailability of polyphenols differs depending on their structure. For example, quercetin and its glycoside have bioavailability values of 0.3 ~ 1.4%, whereas anthocyanins show relatively high bioavailability, with values of 1.0 ~ 6.7% (Scalbert and Williamson, 2000).

In this study, the antioxidant components and activities of four sweet potato cultivars were compared. The polyphenols and anthocyanins were found to be the highest in SJ, especially in the fermented ethanol and ethanol extracts. The high content of phenolic compounds in SJ show strong antioxidant activity compared with that of the other cultivars, as assessed using the DPPH and ABTS radical scavenging assays and the FRAP assay. In addition, pretreatment of HepG2 cells with the SJ extract impacted ROS removal, which may protect the cells from oxidative damage. As a result, the SJ cultivar, which has a high content of polyphenols including anthocyanin, may prevent age-related diseases caused by oxidative damage. Therefore, purple-fleshed sweet potatoes possess antiox-

Table 1. Correlation coefficients of polyphenol, ABTS, DPPH, and FRAP values for the four sweet potato cultivars with cellular ROS and cell viability

	Anthocyanin	ABTS	DPPH	FRAP	ROS (1 h)	ROS (2 h)	MTT
Polyphenol	1.000***	0.956*	0.997***	1.000***	-0.966^*	-0.987^*	0.965*
Anthocyanin		0.956*	0.998***	0.999***	-0.966^*	-0.984^*	0.971*
ABTS			0.959*	0.959*	-0.848	-0.912	0.947
DPPH				0.996***	-0.960^*	-0.973^*	0.983*
FRAP					-0.963^*	-0.987^*	0.965*
ROS (1 h)						0.978*	-0.916
ROS (2 h)							-0.914

Significant at * $P < 0.05$ and *** $P < 0.001$.

ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; ROS, reactive oxygen species; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

idant activity that may contribute to the maintenance of human health.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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