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Antioxidant activities, phenolic and β-carotene contents of sweet potato genotypes with varying flesh colours

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Abstract

Antioxidant activities (μmol Trolox equivalent (TE)/g fresh weight) of 19 sweet potato genotypes with distinctive flesh colour (white, cream, yellow, orange and purple) were measured by oxygen radical absorbance capacity (ORAC), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2′-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS). Total phenolics were measured using the Folin–Ciocalteau method, total anthocyanins by the pH-differential method, and β-carotene by HPLC. The total antioxidant activity (hydrophilic + lipophilic ORAC) was highest (27.2 μmol TE/g fresh weight (fw)) for NC415 (purple-fleshed) and lowest (2.72 μmol TE/g fw) for Xushu 18 (white-fleshed). The hydrophilic-ORAC values were significantly correlated with the DPPH ($R^2 = 0.859$) and ABTS ($R^2 = 0.761$) values. However, the lipophilic-ORAC values were poorly correlated with the β-carotene contents ($R^2 = 0.480$). The total phenolic contents (0.011–0.949 mg chlorogenic acid equivalent/g fw) were highly correlated with the hydrophilic-ORAC ($R^2 = 0.937$) and DPPH ($R^2 = 0.820$) values. Therefore, the total phenolic content can serve as a useful indicator for the antioxidant activities of sweet potatoes.

Keywords: Sweet potatoes; Phenols; Carotene; Antioxidant activity; ORAC; ABTS; DPPH

1. Introduction

Fruits and vegetables are a rich source of phytochemicals, such as carotenoids, flavonoids and other phenolic compounds. Studies have indicated that these phytochemicals, especially polyphenols, have high free-radical scavenging activity, which helps to reduce the risk of chronic diseases, such as cardiovascular disease, cancer, and age-related neuronal degeneration (Ames, Shigena, & Hagen, 1993). The free radicals are generated in the human body through aerobic respiration and exist in different forms, including superoxide, hydroxyl, hydroperoxyl, peroxyl and alkoxyl radicals. Generally, natural antioxidant enzymes in healthy individuals remove these free radicals (Rimbach, Fuchs, & Packer, 2005). However, dietary antioxidants are helpful in assisting the body to neutralise free radicals. Therefore, it is important to consume a diet high in antioxidants, such as fruits and vegetables, to reduce the harmful effects of oxidative stress.

Sweet potatoes (*Ipomoea batatas* L.) are rich in dietary fibre, minerals, vitamins, and antioxidants, such as phenolic acids, anthocyanins, tocopherol and β-carotene (Woolfe, 1993). Besides acting as antioxidants, carotenoids and phenolic compounds also provide sweet potatoes with their distinctive flesh colours (cream, deep yellow, orange and purple). The phenolic contents of the sweet potatoes cultivated in the US ranged from 0.14 to 0.51 mg of chlorogenic acid equivalent/g fresh weight of hand-peeled storage roots, with chlorogenic acid and isochlorogenic acids

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as the main components (Walter, Purcell, & McCollum, 1979). Purple-fleshed sweet potatoes developed in Japan contained about 0.4–0.6 mg anthocyanins/g fw (Furuta, Suda, Nishiba, & Yamakawa, 1998). In recent years, several reports have indicated that the phytochemicals in sweet potatoes displayed antioxidative or radical-scavenging activity and exerted several health-promoting functions in humans (Konczak-Islam, Yoshimoto, Hou, Terahara, & Yamakawa, 2003; Rabah, Hou, Komine, & Fujii, 2004; Suda et al., 2003). A red-fleshed sweet potato cultivar grown in the Andean region has been reported to have higher antioxidant activity and phenolic content than a cultivar of blueberry, a fruit with high levels of antioxidants (Cevallos-Casals & Cisneros-Zevallos, 2003). On the other hand, Cao, Sofic, and Prior (1996) included an unknown sweet potato cultivar in their study and reported that its antioxidant activity was lower than those of leafy vegetables and broccoli. However, it was in the range of other vegetables, such as carrot, squash and potato. With the flesh colour varying from white, yellow to orange and even purple among the sweet potato cultivars, the antioxidant capacity of sweet potato roots may vary over a wide range.

Several methods are available to evaluate antioxidant activities of natural compounds in foods or biological systems. Two methods commonly used in antioxidant activity assays are the DPPH and ABTS procedures, which use 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) as free radical generators, respectively. The mechanisms of both methods are similar, in that the absorption spectra of the stable, free radical changes when the molecule is reduced by an antioxidant or a free radical species. ABTS is soluble in both aqueous and organic solvents, and it reacts relatively rapidly compared to DPPH, which normally takes several hours for the reaction to be completed. Colour interference of the DPPH assay with samples that contain anthocyanins leads to under-estimation of antioxidant activity. However this problem does not occur with the ABTS assay, especially when the absorbance is measured at 734 nm (Arnao, 2000). Nevertheless, the DPPH procedure has been used by several investigators to assay the antioxidant activity of sweet potatoes (Cevallos-Casals & Cisneros-Zevallos, 2003; Oki et al., 2002; Rabah et al., 2004).

Oxygen radical absorbance capacity (ORAC) is another method used to measure antioxidant capacity *in vitro*. This method is based on the inhibition of the peroxyl-radical-induced oxidation initiated by thermal decomposition of azo-compounds, such as 2,2′-azobis (2-amidino propane) dihydrochloride (AAPH). The antioxidants react with the peroxyl radicals and delay the degradation of fluorescein, a fluorescent probe. The ORAC method uses biologically relevant free radicals, integrates both time and degree of antioxidant activity into one data value, and it is readily adaptable to a high-throughput assay system. The advantage of the ORAC method is its ability to assay both hydrophilic and lipophilic antioxidants, which results in better measurements of total antioxidant activity (Prior et al., 2003). Randomly methylated β-cyclodextrin (RMBC) was used to increase the water solubility of lipophilic antioxidants, which consist mostly of vitamin E-related compounds and carotenoids (Huang, Ou, Hampsch-Woodill, Flanagan, & Deemer, 2002). The disadvantage is that this method usually requires the use of a specialised instrument. Wu et al. (2004a) analysed the antioxidant activity of a sweet potato cultivar, based on the hydrophilic and lipophilic ORAC procedures. They found the total antioxidant activity to be 9.02 μmol Trolox equivalents (TE)/g fresh weight (fw).

Several studies have reported correlations among the antioxidant activities measured by different methods, as well as the correlations between those methods and phytochemical concentrations in various food commodities (Awika, Rooney, Wu, Prior, & Cisneros-Zevallos, 2003). However, this type of information is very limited for sweet potatoes. Moreover, the common methods for assaying antioxidant capacity are based on different reaction mechanisms (Prior, Wu, & Schaich, 2005). It is necessary to compare the antioxidant values of sweet potato samples generated from these methods, in order to establish a suitable method for antioxidant capacity assay, which can be readily adapted in sweet potato breeding efforts and food processing research programmes. The information on the antioxidant activity of sweetpotatoes would also be helpful in increasing the awareness of the consumers regarding the level of beneficial phytochemicals present in this nutritious vegetable.

The objectives of this study were (1) to determine the antioxidant activity of sweet potatoes using ORAC, DPPH and ABTS assays and to assess the correlations between these methods, and (2) to assess the relationship between the antioxidant activities of the hydrophilic and lipophilic extracts with the concentrations of phenolic compounds, and β-carotene contents of sweet potatoes.

2. Materials and methods

2.1. Chemicals

Trolox (2,5,7,8-tetramethylchroman-2-carboxylic acid), Folin–Ciocalteau reagent, chlorogenic acid, β-carotene, 2,2-diphenyl-1-picryl hydrazyl (DPPH), 2,2′-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), fluorescein, and potassium persulfate were obtained from Sigma–Aldrich (St. Louis, MO). 2,2′-Azobis (2-amidino propane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA (Richmond, VA). Randomly methylated β-cyclodextrin (RMCD) was purchased from Cyclodextrin Technologies Development Inc. (High Spring, FL). All other reagents were of analytical and HPLC grades.

2.2. Storage root samples

Nineteen sweet potato clones with varying flesh colours (white, yellow, orange, purple) were analysed. Four of the

clones, Beauregard, Hernandez, Covington (NC98-608), and Xushu 18, are grown commercially. The other 15 breeding lines were obtained from the Sweetpotato Breeding Program at NC State University. These breeding lines were identified as collection code (NC415) or based on the family and the selection number (e.g., 11-5, 12-17, 13-18) of the f1 generation in a 5×5 NC Design II mating design. The harvested storage roots were cured and stored at 13–16 °C and 80–90% relative humidity for 5–6 months, before samples were taken for analysis. Commercial sweet potatoes were readily available, and therefore, two replications were taken from two different lots of each cultivar. For the breeding lines, two samples were taken from a limited harvest of each clone.

2.3. Preparation of raw storage roots for analysis

The storage roots were thoroughly washed with tap water, manually peeled and ground into small pieces with a food processor. The ground samples were then freeze-dried in a Stoppering Trap Freeze Dryer 77560 (Labconco Corporation, Kansas City, MO) for several days at −45 to −30 °C. Dry matter content of a sample was calculated by the weight difference before and after freeze drying. Sample preparation, extraction and analysis were carried out in a laboratory with UV-filtered light, to minimise light degradation of components.

2.4. Extraction of lipophilic and hydrophilic fractions

Freeze-dried samples were ground into fine powder with a Cyclotec mill (Rose Scientific, Ontario, Canada). Five grams of freeze-dried powder were vortexed for 2 min in 25 ml hexane, and the mixture was filtered using a Büchner funnel. The hexane extraction was repeated twice, and the combined lipophilic extracts were evaporated to dryness at 50 °C using a vacuum evaporator. The residue after hexane extraction was then extracted two times with 25 ml of acidified methanol (7% acetic acid in 80% methanol) to obtain the hydrophilic fraction. The final volume of the hydrophilic fraction was made to 50 ml with acidified methanol.

2.5. Hydrophilic and lipophilic ORAC assay

For the lipophilic antioxidant assay, the dried hexane extract was re-dissolved in 50 mL of 50% acetone/50% water containing 7% randomly methylated β-cyclodextrin solution, (RMCD; Huang et al., 2002). Any further dilution was made with the 7% RMCD solution. The 7% RMCD solution was also used as a blank and to dissolve the Trolox standards for the lipophilic assay. For the hydrophilic assay, phosphate buffer (0.075 M, pH 7.4) was used. The phosphate buffer was also used as a blank and solvent for the Trolox standards in the hydrophilic assay.

The ORAC procedure was carried out following the procedure established by Prior et al. (2003). The fluorescence intensity measurement was performed using a Safire monochromator-based microplate reader equipped with Magellan V4-W reader software (Tecan USA, Research Triangle Park, NC) with the sample loaded on a Costar, polystyrene, flat-bottom 96-well plate (Corning, Acton, MA). The concentrations of reagents prepared were identical to those of Wu et al. (2004a), except that the samples were diluted 100-fold. The diluted samples, 20 μl, were mixed with 120 μl fluorescein solution in a clear, 96-well microplate and incubated at 37 °C for 15 min. Then, 20 μl of AAPH was rapidly added to each well using a multi-channel pipette. Immediately following the addition of AAPH, the plate was agitated for 5 s prior to the first reading and for 2 s before each subsequent reading. Readings were done at 1 min intervals for 80 min. Excitation and emission filter wavelengths were set at 484 nm and 520 nm, respectively. Data were expressed as μmol Trolox equivalents (TE) per gram of sweet potato on a fresh weight (fw) or dry weight (dw) basis.

The ORAC values were calculated by using a regression equation ($Y = a + bX$, linear; or $Y = a + bX + cX^2$, quadratic) between concentration (Y) (μM) and the net area under the fluorescence decay curve (X). Linear regression was used in the range of 6.25–50 μM Trolox. The area under curve (AUC) was calculated as follows:

$$\mathrm{AUC} = \left(0.5 + \frac{f_5}{f_4} + \frac{f_6}{f_4} + \frac{f_7}{f_4} \cdots + \frac{f_i}{f_4}\right) \times \mathrm{CT}$$

where f_4 is the initial fluorescence reading at cycle 4, f_i is the fluorescence reading at cycle i, and CT is the cycle time in minutes. The net area under the curve was obtained by subtracting the blank value from that of a sample or standard.

2.6. Assay of DPPH radical scavenging activity

Aliquots of the hydrophilic fractions were diluted (1:10) with ethanol, and the assay was performed following the procedure described by Brand-Williams, Cuvelier, and Berset (1995), with minor modifications. The diluted sample, 0.1 ml, was pipetted into 3.9 ml of DPPH$^{\cdot}$ solution to initiate the reaction. The absorbance was read every minute at 515 nm for 180 min using the Safire microplate reader equipped with a Magellan V4-W reader software (Tecan USA). Under these conditions, the decrease in absorbance reached a plateau within the 3 h sampling period, which indicated that the reaction had reached completion. Therefore, a reaction time of 3 h was used for all the DPPH$^{\cdot}$ assays. Ethanol (95%) was used as a blank. Trolox (0, 100, 200, 300, 400 and 500 μM) was used as a standard. Analysis was done in triplicate for each sample and each concentration of standard. The antioxidant activity was reported in μmoles of Trolox equivalents per gram sample (μmol TE/g fw or dw).

2.7. Assay of ABTS radical scavenging activity

ABTS radical-scavenging activity of the hydrophilic fractions was determined by a procedure reported by

Miller and Rice-Evans (1997). The $ABTS^{\cdot+}$ solution was prepared by mixing 8 mM of ABTS salt with 3 mM of potassium persulfate in 25 ml of distilled water. The solution was held at room temperature in the dark for 16 h before use. The $ABTS^{\cdot+}$ solution was diluted with 95% ethanol (approximately 600 μl ABTS to 40 ml 95% ethanol), in order to obtain an absorbance between 0.8 and 0.9 at 734 nm. Fresh $ABTS^{\cdot+}$ solution was prepared for each analysis. Antioxidant or standard solutions, 20 μl, were mixed with 1 ml of diluted $ABTS^{\cdot+}$ solution and incubated at 30 °C. The absorbance at 734 nm was read every minute for 30 min using the Safire microplate reader. Ethanol (95%) was used as a blank. Trolox with concentrations from 0 to 500 μM was used as a standard. The free-radical-scavenging activity was expressed as μmoles of Trolox per gram of sample (μmol TE/g fw or dw).

2.8. Total phenolic assay

Total phenolic content was measured using the modified Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventos, 1999). The hydrophilic extract (0.5 ml) was diluted with distilled water to 5.0 ml, to which 0.5 ml Folin–Ciocalteau reagent was added and allowed to react at room temperature for 3 min. One millilitre of 1 N sodium carbonate was added, and the mixture was incubated at room temperature for 1 h. The absorbance was measured at 725 nm using the Safire microplate reader with distilled water as a blank. Chlorogenic acid and gallic acid were used as standards. Total phenolic content was reported as milligrams of chlorogenic acid equivalents per gram fresh weight sample (mg CAE/g fw or dw), which can be converted into milligrams of gallic acid by multiplying by a factor of 0.445.

2.9. Total anthocyanin assay

Total anthocyanin contents of the hydrophilic extracts were measured by the pH-differential method described by Rodriguez-Saona et al. (2001). Samples were diluted with two different solutions: potassium chloride (0.025 M), pH 1.0; and sodium acetate (0.4 M), pH 4.5. The pH was adjusted with concentrated hydrochloric acid. Samples were diluted to give an absorbance at 530 nm of <1.2. Diluted samples were held for 15 min before measuring the absorbance. The absorbance was measured at 530 nm and 700 nm with distilled water as a blank. The absorbance difference between the pH 1.0 and pH 4.5 samples was calculated:

$$A = (A_{530\,\mathrm{nm}} - A_{700\,\mathrm{nm}})_{\mathrm{pH}\,1.0} - (A_{530\,\mathrm{nm}} - A_{700\,\mathrm{nm}})_{\mathrm{pH}\,4.5}$$

The monomeric anthocyanin pigment concentration was calculated using the following equation:

$$\text{Monomeric anthocyanin pigment (mg/l)} = (A \times \mathrm{MW} \times \mathrm{DF} \times 1000)/(\varepsilon \times l)$$

MW = 449.2 and $\varepsilon = 26{,}900$ are, respectively, molecular weight and molar absorptivity of cyanidin-3-glucoside, which was used as a standard; DF is the dilution factor; l is the path length. The total monomeric anthocyanins were reported on the basis of mg/g fw sweet potatoes.

2.10. β-Carotene analysis

Freeze-dried sweet potato powder (5 g) was mixed with approximately 2 g of calcium carbonate, 1 g of diatomaceous earth, and 25 ml of methanol. A hexane–acetone (1:1) mixture (50 ml) was added and stirred. The mixture was filtered under vacuum through a funnel with a fritted disk. The residue in the funnel was washed two more times with 25 ml of methanol and then by 50 ml of the hexane–acetone mixture. All of the extracts were combined in a 250 ml separating funnel and washed with water. A few drops of saturated sodium chloride solution were added to the funnel to facilitate phase separation. The aqueous phase was discarded and the upper layer was transferred to a 50 ml volumetric flask and made to volume with hexane (Chandler & Schwartz, 1998). Samples were stored in dark vials at −20 °C until analysis.

The carotene content was analysed by a ThermoQuest (San Jose, CA) HPLC system that included a P2000 binary pump, an AS 3000 autosampler, and an SCM 1000 degasser. Samples were placed in the sample tray cooled to 6 °C. Aluminum foil was placed over the sample tray to minimise light. Samples (20 μl) were injected onto a Sunfire C18 reverse phase column (4.6 × 100 mm, 3.5 μm particle size; Waters Associates, Milford, MA). Separation was performed at 35 °C with a mobile phase of methanol, acetonitrile and chloroform (42.5/42.5/15 v/v). The flow rate was maintained at 1.2 ml/min. Peaks were monitored at 450 nm with a UV 6000 LP diode array detector (ThermoQuest, San Jose, CA). Standard solutions of β-carotene with concentrations from 0.5 μg/ml to 10 μg/ml were used to obtain a standard curve. ThermoQuest Chromatography Data Acquisition Software (version 4.1) was used to collect and process the data.

2.11. Statistical analysis

Group differences were evaluated using *t*-tests, with $p < 0.05$ considered to be a statistically significant difference. Means were compared with Duncan's multiple range test with $\alpha = 0.05$ and Pearson correlations were performed using SAS Statistical Analysis System (v. 8.1; SAS Institute Inc., Cary, NC).

3. Results and discussion

3.1. Antioxidant capacity by ORAC, DPPH and ABTS assays

The antioxidant activities of 19 sweet potato clones used in this study are shown in Fig. 1. White and yellow fleshed

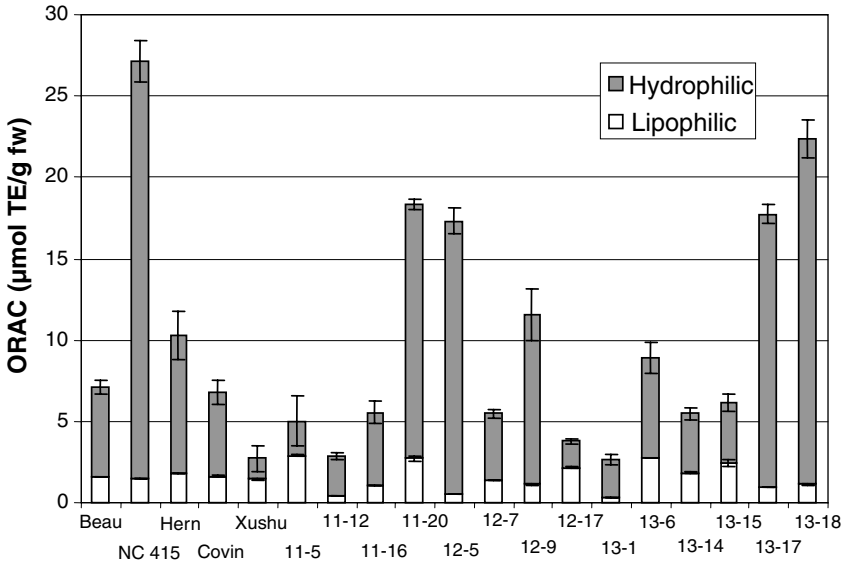

Fig. 1. Total antioxidant activity of sweet potato clones. (Bars indicate standard errors for commercial clones and standard deviation for breeding lines.)

clones (Xushu 18, 11-12, 13-10) had the lowest total antioxidant activities (hydrophilic + lipophilic) of 2.72–3.33 μmol (TE/g fw). Most of the orange-fleshed samples were in a middle range of 5.89–10.3 μmol TE/g fw, except the 11-20 clone, a dark orange flesh sample with 18.2 μmol TE/g fw. The total ORAC antioxidant activity of 9.02 μmol TE/g fw for a sweet potato cultivar reported by Wu et al. (2004a) is within this range. The dark purple-fleshed clones (NC415, 12-5, 12-9, 13-17, 13-18) were in the upper range of 14.7–29.2 μmol TE/g fw, with NC415 and 13-18 having highest ORAC values. The total ORAC values of the purple-fleshed sweet potatoes were comparable with those of fruits (apples, apricot, avocado, cherries, grapefruit, orange, pears) and vegetables (broccoli, cabbages, eggplants, lettuces; Wu et al., 2004a).

The results indicated that the antioxidant activity varied widely among the sweet potato clones. Purple sweet potatoes tended to be associated with high total antioxidant activity. Aside from sweet potatoes, other food commodities, such as sorghum, also show a similar relationship between colour intensity and antioxidant activity. Brown and black sorghums had the highest levels of freely extractable polyphenols. The total antioxidant activity of these sorghum clones was in the range of 271–878 μmol TE/g dw, as compared to less than 25 μmol TE/g dw for a white clone (Awika et al., 2003). Bao, Cai, Sun, Wang, and Corke (2005) reported a similar trend for Chinese bayberries in which black bayberry cultivars had much higher antioxidant activity than pink and yellowish varieties.

The constituents responsible for the hydrophilic antioxidant activity are primarily phenolic compounds and anthocyanins, whereas carotenoids and tocopherols are the main antioxidant constituents in lipophilic extracts. The hydrophilic and lipophilic antioxidant activities of the sweet potato clones ranged from 1.28 to 28.1 μmol TE/g fw and 0.32 to 2.87 μmol TE/g fw, respectively (Fig. 1). The differences between hydrophilic and lipophilic antioxidant activity were significant among the clones ($p < 0.05$). Clone 13-18 and NC415 (purple-fleshed) had the highest hydrophilic ORAC values of 28.1 and 25.7 μmol TE/g fw, while clone 11-5, 11-20 and 13-15 (orange-fleshed) had the highest lipophilic antioxidant activities (Fig. 1). On a dry weight basis, however, the hydrophilic ORAC value of NC415 was highest (95.7 μmol TE/g dw), followed by clone 13-18 (85.0 μmol TE/g dw). Among the orange-fleshed clones, 11-20 was highest in lipophilic antioxidant activity (11.0 μmol TE/g dw), followed by clone 13-15 (8.37 μmol TE/g dw) and 11-5 (8.27 μmol TE/g dw). Clone 13-6 was an exception; it had a light purple-flesh colour and lipophilic-ORAC values (2.70 μmol TE/g fw and 9.30 μmol TE/g dw) in the upper range of the orange-fleshed clones. The lipophilic ORAC values of the sweet potato clones were in a range of 0.85–2.87 μmol TE/g fw, which were higher than the lipophilic ORAC values of 0.59 and 0.24 μmol TE/g fw reported for other high carotene vegetables, such as carrots and tomatoes (Wu et al., 2004a). Avocado has the highest lipophilic antioxidant activity, 5.52 μmol TE/g fw among fruits and vegetables. The lipophilic antioxidant activity as a percentage of the total antioxidant activity ranged from 0.27% to 63.70% among the food commodities analysed by Wu et al. (2004b). The lipophilic ORAC values for the sweet potato clones in this study were in a range of 2.65–52.88% of the total antioxidant activity.

For DPPH radical-scavenging activity (Fig. 2), the rank order among the clones was similar to the ORAC results. The purple-fleshed clones (NC415, 12-5 and 13-18) had the highest antioxidant activities (>1.0 μmol TE/g fw), followed by the orange-fleshed clones, while the yellow- and white-fleshed clones (13-1, Xushu 18) had the lowest DPPH antioxidant activity. Statistical analysis indicated that Beauregard, Hernandez, Covington, and clone 12-9 were not significantly different ($p > 0.05$) from each other. In addition, clones 11-5, 11-16, 12-7, 13-14 and 13-15 were

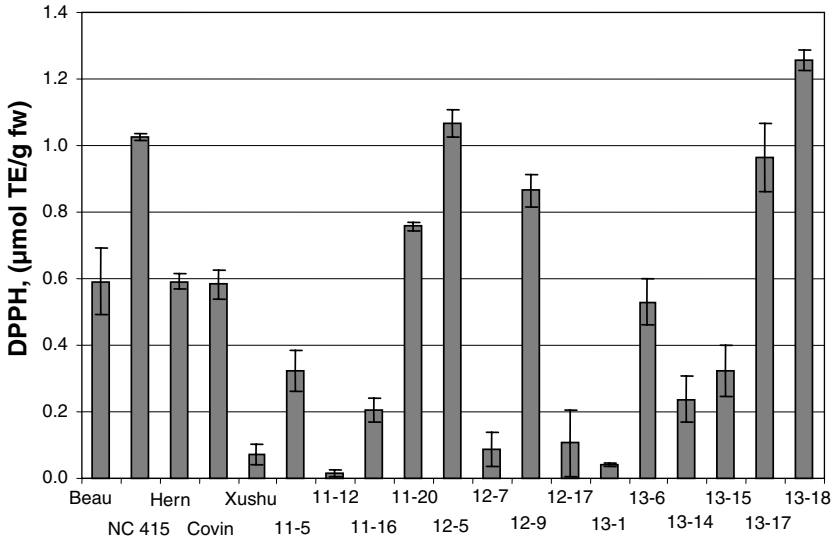

Fig. 2. DPPH antioxidant activity of sweet potato clones. (Bars indicate standard errors for commercial clones and standard deviation for breeding lines.)

not significantly different in DPPH values. The DPPH values of the clones studied were lower than the 8.6–49.0 μmol range of TE/g fw values for purple-fleshed sweet potato cultivars developed in Japan (Oki et al., 2003) and an Andean red-fleshed sweet potato (Cevallos-Casals & Cisneros-Zevallos, 2003). On a dry weight basis, the DPPH values obtained in this study were comparable with a range of 1.68–2.14 μmol TE/g dw for corn meal, whole wheat and buckwheat reported by Sensoy, Rosen, Ho, and Karwe (2006).

The DPPH activities of all the clones studied were significantly lower than their ABTS values (Figs. 2 and 3). As with the DPPH and ORAC assays, clone NC415 exhibited highest ABTS radical-scavenging activity and 13-1 was the lowest. Beauregard, Hernandez, Covington, clones 11-5, 12-7, 13-6 and 13-14 were not significantly different ($p > 0.05$). The clones 11-20, 12-5, 12-9, 13-17 and 13-18 were not significantly different ($p > 0.05$). Apparently, the antioxidant activities of these clones were not differentiated as well with the ABTS method as with the ORAC and DPPH methods. Awika et al. (2003) reported minimal differences between the ABTS and DPPH values of brown sorghum samples. However, for the black sorghum, the authors observed that all of the ABTS values were significantly higher than the DPPH values. Colour interference by anthocyanins ($\lambda_{max} = 470–580$) with DPPH chromogen ($\lambda_{max} = 515$ nm) and carotenoids ($\lambda_{max} = 400–500$) may result in a lower measured DPPH activity (Arnao, 2000).

3.2. Total phenolic, anthocyanin and β-carotene contents

The total phenolic content was highest (0.949 mg CAE/g fw) for 13-18 (purple-fleshed) and lowest (0.003 mg CAE/g fw) for Xushu 18 (white-fleshed; Table 1). The purple-

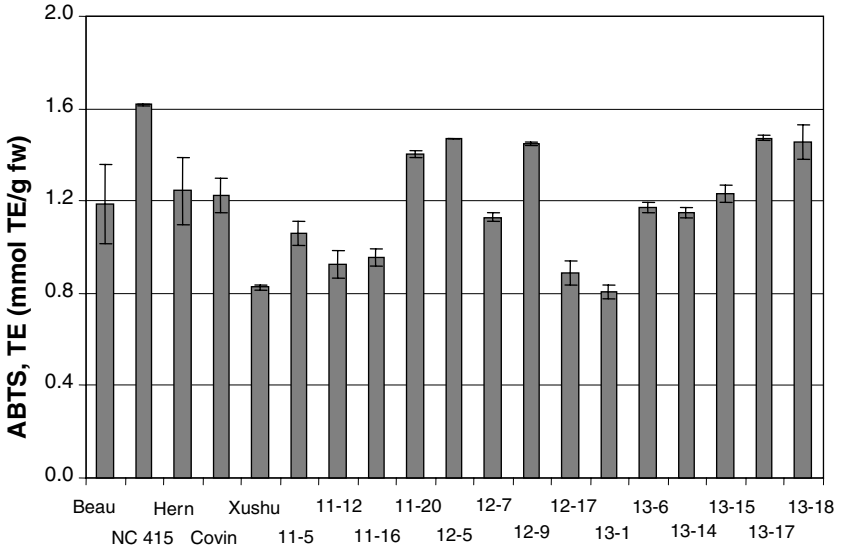

Fig. 3. ABTS antioxidant activity of sweet potato clones. (Bars indicate standard errors for commercial clones and standard deviation for breeding lines.)

fleshed clones had the highest total phenolic content, followed by orange, yellow and white-fleshed clones, respectively. An orange-fleshed clone, 11-20, which had high antioxidant activities (Figs. 1–3) also contained substantial amounts of phenolic compounds (0.472 mg CAE/g fw). The total phenolic contents of the sweet potato clones in this study were in the range of 0.14–0.51 mg CAE/g fw, and 2–18 mg CAE/g flour for various sweet potato cultivars reported by other investigators (Walter et al., 1979; Yoshimoto et al., 1999). The phenolic content of 9.45 mg/g fw (32.2 mg/g dw) of a red-fleshed sweet potato cultivar (Cevallos-Casals & Cisneros-Zevallos, 2003) was exceptionally high, and comparable with 21 mg CAE/g fw of berries, such as blackcurrants (Amakura, Umino, Tsuji, & Tonogai, 2000). The leaves of sweet potato genotypes are a rich source of phenolics, 14.2–171 mg CAE/g dw (Islam, Yoshimoto, Ishigure, Okuno, & Yamakawa, 2003). Genetic factors and growing conditions may play an important role in the formation of secondary metabolites, including phenolic acids (Howard, Clark, & Brownmiller, 2003; Islam et al., 2003).

There was a wide variation observed in the total anthocyanin content among the sweet potato breeding lines. The total anthocyanin content of the samples ranged from 0.017 to 0.531 mg/g sample fw (Table 1). Among all the samples, eight of the sweet potato clones with purple and orange flesh had detectable anthocyanins. The 11-20 clone with high total antioxidant activity (18.2 μM TE/g fw) did not have detectable anthocyanins. High anthocyanins with peonidin and cyanidin as the major components in purple and red-fleshed sweet potatoes have been reported by several investigators (Furuta et al., 1998; Harada, Kano, Takayanagi, Yamakawa, & Ishikawa, 2004; Oki et al., 2002; Suda et al., 2003; Yoshinaga, Yamakawa, & Nakatani, 1999). Cevallos-Casals and Cisneros-Zevallos (2003) reported that the anthocyanin content of a red-fleshed sweet potato cultivar was 1.82 mg anthocyanin/g fw. For a comparison, red-fleshed potatoes (*Solanum tuberosum*) were reported with anthocyanin contents ranging from 0.02 to 0.40 mg/g fw for 33 different genotypes (Rodriguez-Saona, Giusti, & Wrolstad, 1998). Anthocyanin contents of fruits and vegetables with red or purple colour ranged from 0.02 to 6 mg anthocyanins per gram fresh weight (Wrolstad, 2000).

For β-carotene, the orange-fleshed clones had the highest content among the samples analysed. Hernandez and clone 11-20, which are both dark orange-coloured, had the highest β-carotene content, with 167 and 226 μg/g fw, respectively (Table 1). Therefore, the high antioxidant activities of clone 11-20 can be attributed to its phenolic and β-carotene contents. Since anthocyanins were not detected in the clone, other phenolics appear to be responsible for elevated antioxidant activity. On the other hand, the white-fleshed sweet potato, Xushu 18, had the lowest β-carotene content, 0.18 μg/g fw. Interestingly, several purple-fleshed clones, such as 12-5, 13-6, and 13-17, contained substantial amounts of β-carotene (Table 1). Simonne, Kays, Koehler, and Eitenmiller (1993) observed a wide range of β-carotene, ranging from 1 to 190 μg/g dw, among various sweet potato breeding lines.

3.3. Correlations

As shown in Figs. 4 and 5, the hydrophilic-ORAC values were highly correlated with the DPPH activities ($R^2 = 0.859$, $p < 0.0001$) and reasonably well correlated with the ABTS values ($R^2 = 0.761$, $p < 0.0001$). The correlation between the ABTS and DPPH values were also highly significant ($R^2 = 0.822$, $p < 0.0001$; Fig. 6). The results suggested that the three methods have similar predictive capacity for antioxidant activities of sweet potatoes. However, the correlations of ABTS values with ORAC and

Table 1
Total phenolic, anthocyanin and β-carotene contents of sweetpotato clones[a]

Sample	Flesh colour	Dry matter (%)	Phenols (mg CAE/g fw)	Total anthocyanins (mg/g fw)	β-Carotene (μg/g fw)
Beauregard (Beau)	Orange	28.4	0.211f	ND	92.3c
NC415	Purple	26.8	0.792b	0.430b	6.3h
Hernandez (Hern)	Orange	28.8	0.157g	ND	167b
Covington (Covin)	Orange	27.3	0.183f	0.038e	120g
Xushu 18 (Xushu)	White	31.1	0.003k	ND	0.2j
11-5	Orange	34.9	0.168gh	0.017f	77.1d
11-12	Yellow	28.4	0.011k	ND	1.5i
11-16	Light orange	31.8	0.118ij	ND	13.0g
11-20	Orange	24.7	0.472d	ND	226a
12-5	Purple	31.3	0.477c	0.246d	46.9e
12-7	Light orange	33.1	0.130hij	ND	29.8f
12-9	Light purple	32.6	0.248e	0.030f	22.3f
12-17	Light orange	33.5	0.108j	ND	11.8g
13-1	Yellow	32.6	0.033k	ND	2.3i
13-6	Light purple	29.7	0.257 e	0.069e	56.6d
13-14	Orange	29.5	0.130hi	ND	44.9e
13-15	Orange	29.1	0.140gh	ND	127b
13-17	Purple	27.6	0.571c	0.322c	31.3e
13-18	Purple	33.1	0.949a	0.531a	5.4hi

[a] a–h = Means within a column with the same superscript letter are not significantly different ($p = 0.05$). ND = non-detectable.

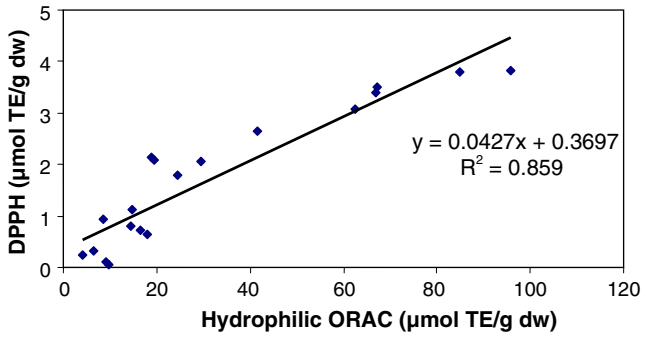

Fig. 4. Correlation between ORAC and DPPH activities.

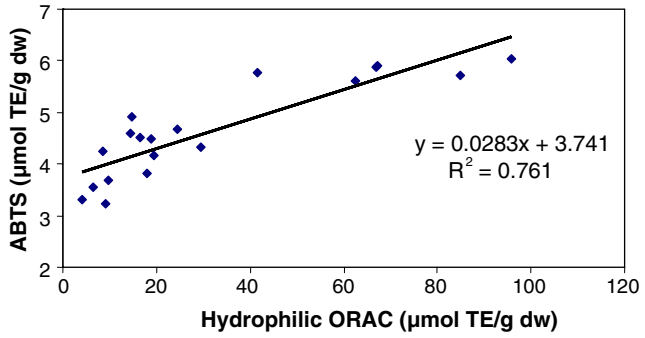

Fig. 5. Correlation between ORAC and ABTS activities.

DPPH activities for sweet potatoes were relatively lower than those found for other food commodities. Leong and Shui (2001) reported a high correlation ($R^2 = 0.90$) between ABTS and DPPH values for various fruit extracts. The R^2-values for correlations between ORAC and ABTS, and ORAC and DPPH were 0.99 and 0.98, respectively, for the antioxidant capacity of sorghum (Awika et al., 2003).

Both DPPH and ABTS procedures are relatively inexpensive and simple to perform. The ABTS method can be used over a wide pH range (Prior et al., 2005), whereas the DPPH method is limited to neutral and higher pH applications. Additionally, the ABTS assay is much faster than the DPPH assay and not affected by colour interference, as mentioned above. For sweet potato extracts, however, the DPPH method showed a better correlation with ORAC than ABTS (Figs. 4 and 5), and the colour interference would be minimal since at the pH of the DPPH assay medium (pH = 6.9), the coloured flavylium ion of anthocyanins is converted into a carbinol pseudo-base and chalcone which are colourless (Wrolstad, 2000).

The hydrophilic antioxidant activity data obtained from the ORAC procedure were highly correlated with the total phenolic contents ($R^2 = 0.937$, $p < 0.0001$; Fig. 7). The total phenolic content also correlated well with the DPPH values ($R^2 = 0.870$, $p < 0.0001$). However, the correlation between ABTS values and the total phenolic contents were not high ($R^2 = 0.6900$, $p < 0.0001$). The results indicated that about 87–97% of the variation of the ORAC and DPPH antioxidant activities could be explained by the phenolic components. The total phenolic content was also highly correlated with the total monomeric anthocyanin concentration in eight clones containing anthocyanins ($R^2 = 0.990$, figure not shown). Islam et al. (2003) reported a relatively lower, but significant correlation coefficient ($R^2 = 0.38$) between DPPH activities and the total phenolic content of sweet potato leaves. High correlation coefficients between the phenolic content and antioxidant activities have been reported for various food commodities such as sorghum ($R^2 = 0.971$), cactus pear ($R^2 = 0.970$–0.990; Rabah et al., 2004; Stintzing et al., 2005). Therefore, total phenolic components can be used as an indicator in assessing the antioxidant activity of fruits and vegetables, including sweet potatoes. Prior et al. (2005) recommended that the Folin–Ciocalteu method for total phenolic determination be standardised for comparison of the results between laboratories.

While the assay for total phenolic components correlated well with antioxidant activity assays on the hydrophilic extract of sweet potatoes, there was a low correlation ($R^2 = 0.48$, $p < 0.001$) between the lipophilic-ORAC values and β-carotene concentrations (Fig. 8). Poor correlations have also been found between the ORAC antioxidant capacity in broccoli and the contents of lutein ($R^2 = 0.32$, $p < 0.05$) and zeaxanthin ($R^2 = 0.65$, $p < 0.05$) in this vegetable (Kurilich, Jeffery, Juvik, Wallig, & Klein, 2002).

The high degree of correlation between the simple spectrophotometric assay for total phenolic compounds and antioxidant activity of the aqueous extracts, as determined

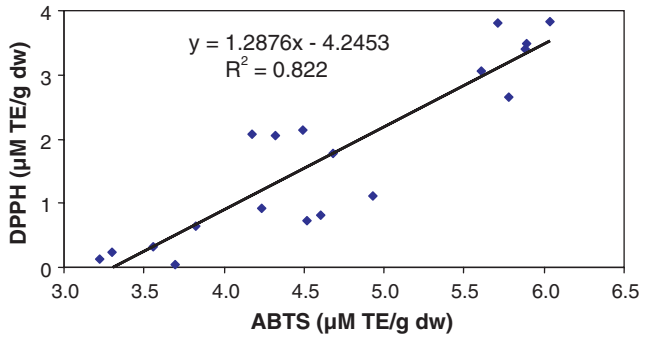

Fig. 6. Correlation between ABTS and DPPH activities.

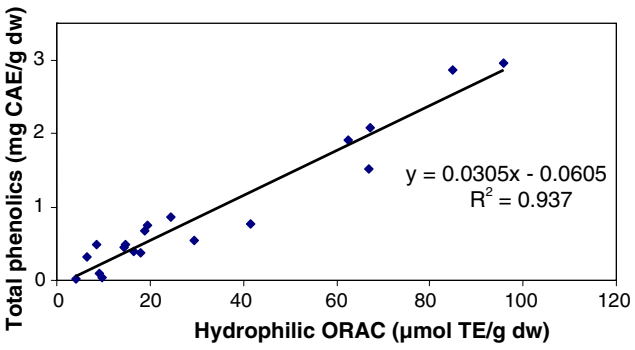

Fig. 7. Correlation between hydrophilic ORAC values and total phenolic contents.

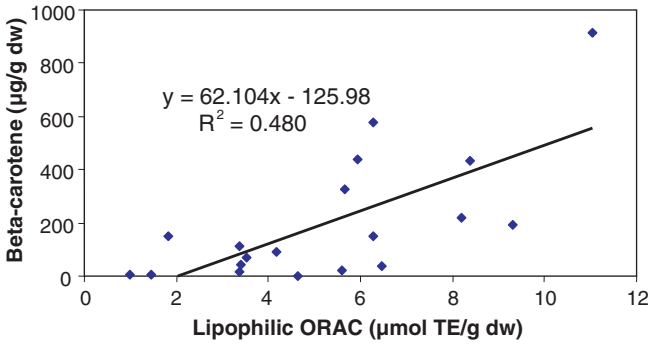

Fig. 8. Correlation between lipophilic ORAC values and β-carotene content.

by radical quenching assays, shows that the assay for total phenolics would be a useful technique for rapid evaluation of antioxidant activity in sweet potatoes. This would be important for breeding programmes where many samples must be evaluated or for investigating the effect of processing and storage treatments on the retention of antioxidant activity. The fact that there is a rather poor correlation between β-carotene content and the antioxidant activity in the lipophilic extracts of sweet potatoes may mean that other components, such as tocopherols (Woolfe, 1993), need to be investigated for their contribution to the lipophilic antioxidant activity. It is also possible that the peroxyl radicals used in the ORAC assay may not be effective in detecting the antioxidant activity of lipophilic antioxidants and that new assays need to be developed for assessing the singlet oxygen-scavenging capacity of carotenoids. For example, Aldini, Yeum, Russel, and Krinsky (2001) developed an antioxidant activity assay using a lipophilic radical generator, 2,2′-azobis(4-methoxy)-2,4-dimethylvaleronitrile, and a fluorescence probe. They reported that β-carotene was rapidly oxidised in this system, while the APPH radical had little effect on β-carotene oxidation.

4. Conclusions

Antioxidant activities varied widely among the sweet potato clones. The purple colour intensity of the sweet potatoes tended to be associated with high antioxidant activity. Purple-fleshed sweet potatoes would be a healthy food choice for consumers, as well as a potential source of natural food colourants.

There were good correlations among the hydrophilic antioxidant activities measured by ORAC, ABTS and DPPH, suggesting that these methods have similar predictive capacity for antioxidant activities of sweet potatoes. High correlations between the Folin–Ciocalteu, ORAC and DPPH methods indicated that the total phenolic contents can be used as indicator for hydrophilic antioxidant activities of sweet potatoes. The ORAC values of lipophilic extracts were poorly correlated with the β-carotene contents. Other lipophilic compounds such as tocopherols and their involvement in the antioxidant activities of sweet potatoes need to be analysed. Quantification of total phenolic components by a simple spectrophotometric method would be helpful in evaluating large number of sweet potato clones and processed products for antioxidant activities.

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References

Aldini, G., Yeum, K-J., Russel, R. M., & Krinsky, N. I. (2001). A method to measure the oxidizability of both the aqueous and lipid compartments of plasma. *Free Radical Biology and Medicine, 31*, 1043–1050.

Amakura, Y., Umino, U., Tsuji, S., & Tonogai, Y. (2000). Influence of jam processing on the radical scavenging activity and phenolic content in berries. *Journal of Agricultural and Food Chemistry, 48*, 6292–6297.

Ames, B. M., Shigena, M. K., & Hagen, T. M. (1993). Oxidants, antioxidant and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences, 90*, 7915–7922.

Arnao, M. B. (2000). Some methodological problems in the determination of antioxidant activity using chromogen radicals: A practical case. *Trends in Food Science and Technology, 11*, 419–421.

Awika, J. M., Rooney, L. W., Wu, X., Prior, R. L., & Cisneros-Zevallos, L. (2003). Screening methods to measure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products. *Journal of Agricultural and Food Chemistry, 51*, 6657–6662.

Bao, J., Cai, Y., Sun, M., Wang, G., & Corke, H. (2005). Anthocyanins, flavonols, and free radical scavenging activity of Chinese bayberry (*Myrica rubra*) extracts and their color properties and stability. *Journal of Agricultural and Food Chemistry, 53*, 2327–2332.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel- Wissenschaft und Technologie, 28*, 25–30.

Cao, G., Sofic, E., & Prior, R. L. (1996). Antioxidant activity of tea and common vegetables. *Journal of Agricultural and Food Chemistry, 44*, 3426–3431.

Cevallos-Casals, B. A., & Cisneros-Zevallos, L. A. (2003). Stoichiometric and kinetic studies of phenolic antioxidants from Andean purple corn and red-fleshed sweetpotato. *Journal of Agricultural and Food Chemistry, 51*, 3313–3319.

Chandler, L. A., & Schwartz, S. J. (1998). Isomerization and losses of *trans*-caretene in sweet potatoes as affected by processing treatments. *Journal of Agricultural and Food Chemistry, 36*, 129–133.

Furuta, S., Suda, I., Nishiba, Y., & Yamakawa, O. (1998). High *tert*-butylperoxyl radical scavenging activities of sweetpotato cultivars with purple flesh. *Food Science and Technology International of Tokyo, 4*, 33–35.

Harada, K., Kano, M., Takayanagi, T., Yamakawa, O., & Ishikawa, F. (2004). Absorption of acylated anthocyanins in rats and humans after ingesting an extract of *Ipomoea batatas* purple sweet potato tuber. *Bioscience Biotechnology and Biochemistry, 68*, 1500–1507.

Howard, L. R., Clark, J. R., & Brownmiller, C. (2003). Antioxidant capacity and phenolic content in blueberries as affected by genotype and growing season. *Journal of the Science of Food and Agriculture, 83*, 1238–1247.

Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J., & Deemer, E. (2002). Development and validation of oxygen radical absorbance capacity assay for lipophilic antioxidants using randomly methylated beta-cyclodextrin as the solubility enhancer. *Journal of Agricultural and Food Chemistry, 50*, 1815–1821.

Islam, M. S., Yoshimoto, M., Ishigure, K., Okuno, S., & Yamakawa, O. (2003). Effect of artificial shading and temperature on radical scavenging activity and polyphenolic composition in sweetpotato (*Ipomoea batatas* L.) leaves. *Journal of the American Society of Horticultural Science, 128*, 182–187.

Konczak-Islam, I., Yoshimoto, Y., Hou, D., Terahara, N., & Yamakawa, O. (2003). Potential chemopreventive properties of anthocyanin-rich aqueous extracts from in vitro produced tissue of sweetpotato. *Journal of Agricultural and Food Chemistry, 51*, 5916–5922.

Kurilich, A. C., Jeffery, E. H., Juvik, J. A., Wallig, M. A., & Klein, B. P. (2002). Antioxidant capacity of different broccoli (*Brassica oleracea*) genotypes using the oxygen radical absorbance capacity (ORAC) assay. *Journal of Agricultural and Food Chemistry, 50*, 5053–5057.

Leong, L. P., & Shui, G. (2001). An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chemistry, 76*, 69–75.

Miller, N., & Rice-Evans, C. (1997). Factors influencing the antioxidant activity determined by the ABTS radical cation assay. *Free Radical Research, 26*, 195–199.

Oki, T., Masuda, M., Furuta, S., Nishiba, Y., Terahara, N., & Suda, I. (2002). Involvement of anthocyanins and other phenolic compounds in radical scavenging activity of purple-fleshed sweet potato cultivars. *Journal of Food Science, 67*, 1752–1756.

Oki, T., Osame, M., Masuda, M., Kobayashi, M., Furuta, S., Nishiba, Y., et al. (2003). Simple and rapid spectrophotometric method for selecting purple-fleshed sweetpotato cultivars with a high radical-scavenging activity. *Breeding Science, 53*, 101–107.

Prior, R. L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., et al. (2003). Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC)) of plasma and other biological and food samples. *Journal of Agricultural and Food Chemistry, 51*, 3273–3279.

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry, 53*, 4290–4302.

Rabah, I. O., Hou, D. X., Komine, S. I., & Fujii, M. (2004). Potential chemopreventive properties of extract from baked sweet potato (*Ipomoea batatas lam. Cv. Koganesengan*). *Journal of Agricultural and Food Chemistry, 23*, 7152–7157.

Rimbach, G., Fuchs, J., & Packer, L. (2005). Application of nutrigenomics tools to analyze the role of oxidants and antioxidants in gene expression. In G. Rimbach, J. Fuchs, & L. Packer (Eds.), *Nutrigenomics* (pp. 1–12). Boca Raton, FL: Taylor & Francis.

Rodriguez-Saona, L. E., Giusti, M. M., & Wrolstad, R. E. (1998). Anthocyanin pigment composition of red-fleshed potatoes. *Journal of Food Science, 63*, 458–465.

Rodriguez-Saona, L. E., & Wrolstad, R. E. (2001). Extraction, isolation, and purification of anthocyanins. In R. E. Wrolstad et al. (Eds.), *Current protocols in food analytical chemistry* (pp. 7–17). Indianapolis, IN: John Wiley.

Sensoy, I., Rosen, R. T., Ho, C-T., & Karwe, M. V. (2006). Effect of processing on buckwheat phenolics and antioxidant activity. *Food Chemistry, 99*(2), 388–393.

Simonne, A. H., Kays, S. J., Koehler, P. E., & Eitenmiller, R. R. (1993). Assessment of β-carotene content in sweetpotato breeding lines in relation to dietary requirements. *Journal of Food Composition and Analysis, 6*, 336–345.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenol and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods in Enzymology, 299*, 152–178.

Stintzing, F. C., Herbach, K. M., Mosshammer, M. R., Carle, R., Yi, W., Sellappan, S., et al. (2005). Color, betalain pattern, and antioxidant properties of cactus pear (*Opuntia spp.*) clones. *Journal of Agricultural and Food Chemistry, 53*, 422–451.

Suda, I., Oki, T., Masuda, M., Kobayashi, M., Nishiba, Y., & Furuta, S. (2003). Physiological functionality of purple-fleshed sweetpotatoes containing anthocyanins and their utilization in foods. *Japan Agricultural Research Quarterly, 37*, 167–173.

Walter, W. M., Jr., Purcell, A. E., & McCollum, G. K. (1979). Use of high-pressure liquid chromatography for analysis of sweetpotato phenolics. *Journal of Agricultural and Food Chemistry, 27*, 942–946.

Woolfe, J. (1993). *Sweet potato: An untapped food resource*. Cambridge: Cambridge University Press.

Wrolstad, R. E. (2000). Anthocyanins. In G. J. Lauro & F. J. Francis (Eds.), *Natural food colorants* (pp. 237–252). New York: Marcel Dekker.

Wu, X., Gu, L., Holden, J., Haytowitz, D. B., Gebhardt, S. E., Beecher, G., et al. (2004a). Lipophilic and hydrophilic antioxidant capacity of common foods in the US. *Journal of Agricultural and Food Chemistry, 52*, 4026–4037.

Wu, X., Gu, L., Holden, J., Haytowitz, D. B., Gebhardt, S. E., Beecher, G., et al. (2004b). Development of a database for total antioxidant capacity in foods: A preliminary study. *Journal of Food Composition and Analysis, 17*, 407–422.

Yoshinaga, M., Yamakawa, O., & Nakatani, M. (1999). Genotypic diversity of anthocyanins content and composition in purple-fleshed sweetpotato. *Breeding Science, 49*, 43–47.

Yoshimoto, M., Okuno, S., Yoshinaga, M., Yamakawa, O., Yamaguchi, M., & Yamada, J. (1999). Antimutagencity of sweetpotato (*Ipomoea batatas*) roots. *Bioscience Biotechnology and Biochemistry, 63*, 537–541.