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Full Length Research Paper

Studies on antioxidant capacity of anthocyanin extract from purple sweet potato (*Ipomoea batatas* L.)

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The radical scavenging effects by α,α-diphenyl-β-picrylhydrazyl (DPPH) and superoxide anions of anthocyanin extract from purple sweet potato were investigated. The antioxidation experiments showed that the reducing power of the anthocyanin extract reduced 0.572 at 0.5 mg/ml, while those of L-ascorbic acid (L-AA) and butylated hydroxytoluene (BHT) reduced 0.460 and 0.121, respectively. They also displayed potent antioxidant effects against the DPPH radical and superoxide anions radical, showing the IC_{50} values of 6.94 and 3.68 µg/ml, respectively. Moreover, this anthocyanin extract also could significantly inhibit the formation of lipid peroxidation compound. Sixteen kinds of anthocyanins in purple sweet potato were detected by high-performance liquid chromatography with diode-array detection (HPLC-DAD), and most of the anthocyanins were acylated.

Key words: Antioxidant activity, anthocyanins, purple sweet potato.

INTRODUCTION

Reactive oxygen species (ROS), including super oxide anion (O_2^-), hydroxyl radical (OH) and hydrogen peroxide (H_2O_2), exist in living organisms (Riley, 1994). ROS formed during normal metabolism naturally can damage biological structures such as proteins, lipids and DNA and induce a variety of human diseases (Elahi and Matata, 2006; Thrasivoulou et al., 2006). Accumulation of ROS in organisms is considered to be as a reason of food deterioration through inducing lipid peroxidation (Kinsella et al., 1993). Many synthesis antioxidants have been used to retard lipid peroxidation in foods and defend the human body against diseases, but have shown toxic and /or mutagenic effects (Mizutani et al., 1987). Therefore, the natural antioxidants from foods have attracted much attention and great effort has been made to search for safe and effective therapeutic agents for oxidative stress-related diseases. Several fruits and vegetables have been demonstrated to contain antioxidants and colorants to prevent lipid peroxidation in food and help the human body to reduce oxidative damages (Prior, 2003; Wang and Lin, 2000).

Purple sweet potato (*Ipomoea batatas* L.) contains high content of anthocyanin in the storage root, of which cyanidin and peonidin were major anthocyanidins (Goda et al., 1997; Odake et al., 1992; Teranara et al., 1999; Terahara and Konczak, 2004). Different purple sweet potato cultivars contain different anthocyanin compositions which possess many healthy benefits including antioxidative properties, antineoplastic properties, as well as anticancer properties. The anthocyanin-health properties are due to electron deficiency chemical structure, of which the peculiar chemical structure leads to the reactive nature towards with ROS (Galvano et al., 2004). Therefore, anthocyanins are utilized as high quality natural food antioxidant with potentially preventive function against life-style-related disease like antimutagnicity, anticarciongenic activity and antidiabetic action. Anthocyanins in fruits and vegetables have been extensively studied (Bao et al., 2005; Gulcin et al., 2005). Though only few studies have focused on *in vitro* antioxidant activity of anthocyanins in purple sweet

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Abbreviations: DPPH, α,α-Diphenyl-β-picrylhydrazyl; **L-AA,** L-ascorbic acid; **BHT,** butylated hydroxytoluene.

potato from China. The object of this paper was to study the antioxidant activity of anthocyanins from Chinese purple sweet potato, which have been determined by the four methods under study.

MATERIALS AND METHODS

Materials and chemicals

Purple sweet potato kindly supplied by National Sweet Potato Research Institute, in Xuzhou (Xuzhou City, Jiangsu Province, China) was washed in running tap water, cut into pieces of approximately 0.5 cm, dried in a heated air drier (50°C) (ZT-3, Jiangdu City, Jiangsu Province, China), and then pulverized by the disintegrator (FSD-100A, Taizhou city, Zhejiang Province, China) and sifted through a 100 mesh sieve. Samples were kept at 4°C.

α-Diphenyl-β-picrylhydrazyl (DPPH), L-ascorbic acid (L-AA), linoleic acid, butylated hydroxytoluene (BHT), standards of cyanidin and peonidin were purchased from Sigma Chemicals Co. AB-8 resin was obtained from Nankai University Chemicals Co. Ammonium thiocyanate and other reagents were of analytical grade.

Preparation of anthocyanins extracts

About 200 g (powder of purple sweet potato) were macerated with 800 ml ethanol for 24 h in the dark at 4°C. The crude extract obtained was filtered through filter paper and the remaining residues were washed with 400 ml of ethanol, and then collected the crude extract filtrate. The ethanol in the crude extract was evaporated by the rotary evaporator (0.1 MPa, 40°C). The extract solution was loaded on AB-8 resin columns (20 × 400 mm) (Lanxiao Company, China) and washed with nanopure water and eluted with 100% ethanol. The eluate was concentrated and evaporated, and then the solution was freeze-dried to obtain red powder. The red powder was dissolved with 250 ml nanopure water, which was done as the stock solution of anthocyanins. The stock solution was kept in 4°C for further analysis.

Determination of total anthocyanin content of purple sweet potato extract

The anthocyanins were quantified following the spectrophotometric method proposed by Francis (1989). The concentration of anthocyanin was determined using the Lambert – Beer law. The factor 98.2 is the molar absorption value for the acid-ethanol solvent and it refers to the absorption of a mixture of cranberry anthocyanin in acid-ethanol, measured in a 1 cm cell at 535 nm, at a concentration of 1% (w/v).

The spectra recorded in a UV-2802 diode array spectrophotometer (UNIC, USA) were measured at 25°C and 530 nm, against the solvent. For that purpose 1 cm quartz cells were used. The anthocyanin content (mg/g) was calculated using the following equation:

Total anthocyanin (TA) = A_{530} × dilution factor/98.2.

Measurement of reducing power

The reducing powers of anthocyanins from purple sweet potato, L-AA and BHT were determined according to the method of Iqbal et al. (2006) with some modifications. Samples were mixed with 3.0 ml of 0.5 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide amd incubated at 50°C for 20 min. 10% acetic acid (2.5 ml) was added to the mixture to stop the reaction. The mixture was centrifuged at 2790 g for 10 min and the supernatant was mixed with distilled water and 0.1% $FeCl_3$ at a ratio of 5:5:1 (v/v/v). After the mixture reacting for 10 min, absorbance was measured at 700 nm. The reducing powers of the tested samples increased with the increasing absorbance values.

Measurement of antioxidant activity by inhibition of peroxidation of linoleic acid

The antioxidant activity of the anthocyanins from purple sweet potato on liposome acid were determined according to the ferric thiocyanate (FTC) method, as described in detail by Xu et al. (2005). Two microliters of anthocyanins, L-AA or BHT were added to 2 ml of 1.0% (w/v) linoleic acid in ethanol and then 4 ml of 0.05 M phosphate buffer (pH 7.0) and 2 ml of distilled water were mixed in a 10 ml vial with a screw cap and then incubated in a 40°C water bath in the dark. The above mixture (0.1 ml) was added to 9.7 ml of 75% (v/v) ethanol and 0.1 ml of 30% (w/v) ammonium thiocyanate. After precisely reacting for 5 min, 0.1 ml of 0.02 M ferrous chloride in 3.5% (v/v) hydrochloric acid were added to the above mixture, the absorbance of mixture was measured at 500 nm every 24 h for one week.

Measurement of antioxidant activity by DPPH radical-scavenging

DPPH radical-scavenging activity of anthocyanins, L-AA or BHT was determined using methods described by Chung et al. (2005) with some modifications. Two microliters of the anthocyanins, L-AA or BHT were mixed with 2.0 ml of 2×10^{-4} M DPPH in ethanol. The mixture was shaken vigorously and left in the dark at room temperature for 30 min. The absorbance of mixture was determined immediately at 517 nm. Initial and blank were measured without substrate and DPPH respectively. The ability to scavenge DPPH radical was calculated by the following equation: scavenging rate (%) = 100 $(A_i-A_s+A_b)$ $/A_i$, in which A_i, A_s, and A_b were the absorbance of initial, sample and blank solutions, respectively.

Measurement of antioxidant activity by superoxide radical-scavenging

Superoxide radical-scavenging rate was measured according to the method described by Giannopolites and Ries (1977), with some modifications. 195 mM methionine (0.2 ml), 0.1 ml 3 mM ethylenediaminetetraacetic acid (EDTA), the anthocyanins, L-AA or BHT, 0.2 ml 1.125 mM nitro blue tetrazolium, and 0.1 ml 60 μM riboflavin were sequentially added to 2.4 ml 0.05 M phosphate buffer (pH 7.8) in order. All solutions were prepared in 0.05 M phosphate buffer (pH 7.8). The mixtures were performed under 4000 lux fluorescent lamp for 10 min at 25°C. The mixture without sample was used as a control. The scavenging activity was calculated as follows: scavenging rate (%) $=100$ (A_c-A_s) $/A_c$, in which A_c and A_s were the absorbance of control and sample solutions, respectively.

Acid hydrolysis of purple sweet potato anthocyanins

One millilitre of concentrated purple sweet potato anthocyanins (PSPAs) solution was dissolved in 10 mL of hydrochloric acid (1.0 mol/L) in a screw-cap test tube. The solution was hydrolyzed at 98°C for 1 h, cooled in an ice bath (Luigia and Giuseppe, 2006). Samples were stored at 4°C.

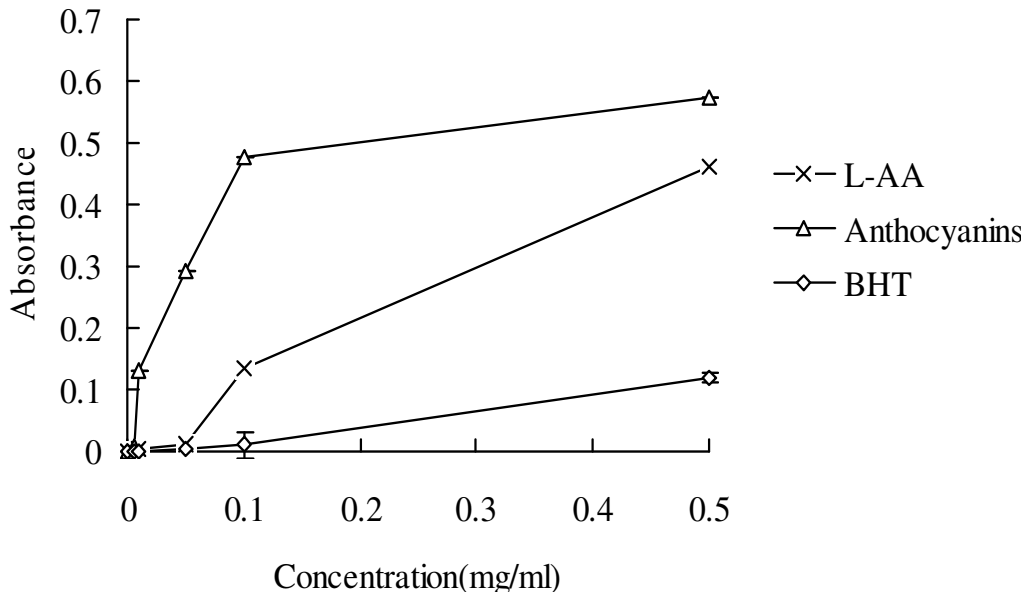

Figure 1. Reducing power of anthocyanins from purple sweet potato compared to BHT and L-AA. Each value represents means ± SD (n=6). L-AA, L-Ascorbic acid; BHT, butylated hydroxytoluene.

HPLC analysis of purple sweet potato anthocyanins

Purple sweet potato anthocyanins were separated by reverse-phase high performance liquid chromatography (HPLC) using Agilent 1100 (Palo Alto, USA) with a Prodigy C_{18} reversed-phase column (5 mm), 4.6 × 250mm i.d. (Agilent, USA), and were detected at 520 nm using diode-array absorbance detection (DAD). The text temperature was at 30℃, the flow rate was 1 mL/min, and the injection volume was 20 µl. The mobile phase A consisted of HPLC-grade acetonitrile, whereas mobile phase B was a mixture of 10% (v/v) acetic acid in distilled water. Separation of anthocyanins was carried out for 25 min. The elution profile was a linear gradient elution with 10 to 25% solvent A from 0 to 10 min, 25 to 30% from 15 to 20 min. Separation of anthocyanidins was carried out for 25 min. The elution profile was a linear gradient elution with 10 to 40% solvent A from 0 to 25 min.

Statistical analysis

All data were expressed as means ± standard deviation (SD). Analysis of variance was performed by ANOVA procedures. Multiple comparisons of means were done by least significant difference (LSD) test. And $P < 0.05$ was considered significant. All computations were made by employing the statistical software (SPSS, version 11.0).

RESULTS AND DISCUSSION

The content of anthocyanins in purple sweet potato

The content of anthocyanins from purple sweet potato was calculated to be 132 mg/100g (dry weight). Fan et al. (2008) reported that the highest anthocyanin content was 158 mg/100g (dry weight) of purple sweet potato which was reached at the best extraction conditions. Compared with other fruits, such as grape (25 to 260 mg/100g, fresh weight) (Arozarean et al., 2002), blackberry (67 to 230 mg/100g, fresh weight) (Wang and Xu, 2007) and Jaboticaba (4.4 to 16.3 mg/100g, fresh weight) (Montes et al, 2005), the content of anthocyanins was relatively high in purple sweet potato, which made this material a good source for anthocyanins.

Reducing power

Reducing power was measured using the potassium ferricyanide reduction method. In this assay, an antixidant donates electron to reduce the Fe^{3+} ferricyanide complex into its Fe^{2+} ferrous form (Hinneburg et al., 2006). Amount of Fe^{2+} compound can be monitored by detecting the absorbance of mixture at 700 nm. Therefore, the reducing power is believed to be strongly correlated with antioxidant activity, for it suggests the electron-donating capacity.

As shown in Figure 1, purple sweet potato anthocyanin, BHT and L-AA showed the reducing power as dose-dependent manner. In particular, anthocyanins exerted the strongest reducing power, indicating that anthocyanins had high electron-donating capacity. At a concentration of 0.5 mg/ml, the reducing power of anthocyanins, L-AA and BHT reached 0.572, 0.460 and 0.121, respectively. These results reveal that anthocyanins could donate electron easier and also had higher reducing power than BHT and L-AA at the same dosage. These results are in agreement with those reported by Duan et al. (2007) who found that the anthocyanins from litchi pericarp exhibited a higher reducing power than BHT and ascorbic acid, suggesting that the anthocyanins had strong electron-donating capacity.

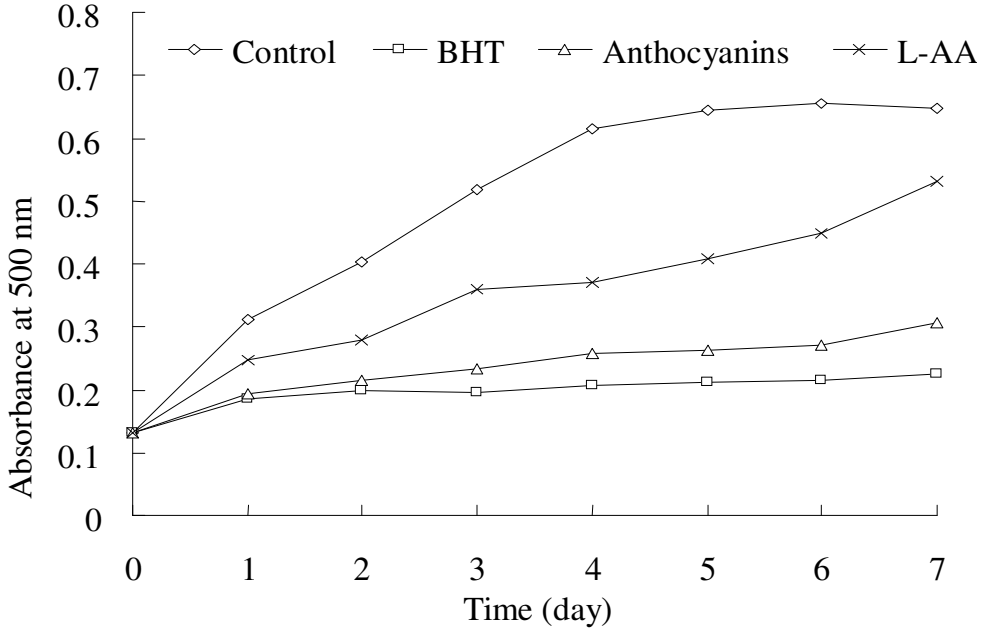

Figure 2. Antioxidant activities of anthocyanins from purple sweet potato compared to BHT and L-AA. Each value represents means ± SD (n=6). L-AA, L-Ascorbic acid; BHT, butylated hydroxytoluene.

Antioxidant activity in linoleic acid system

In present study, ferric thiocyanate method in linoleic acid emulsion was used to evaluate antioxidant activity of anthocyanins. The principle of FTC is that the Fe^{2+} ion was oxidized to Fe^{3+} ion by hydroperoxide, of which the red colour of ferric thiocyanate was produced by the direct addition of a ferric salt and Fe^{3+} ion. The content of hydroperoxide would be measured through colorimetry. The Fe^{3+} ion interacted with SCN^- and formed a resultant, which had a maximum absorbance at 500 nm. Thus, a high absorbance value suggests high peroxide formation during the emulsion incubation (Duan et al., 2007).

Figure 2 shows that the antioxidant activity of anthocyanins from purple sweet potato, BHT and L-AA exhibited peroxidation inhibition effect as a dose-dependent manner. In particular, the anthocyanins and BHT significantly retard peroxidation of linoleic acid and reduce formation of peroxide compounds. Although the absorbances of anthocyanins were slightly higher than those of BHT, a widely synthesized antioxidant, it still indicates that anthocyanins are natural antioxidants. Some authors (2007) reported that anthocyanis can significantly inhibit peroxidation of linoleic acid and reduce formation of hydroperoxide, thus implying that the anthocyains are powerful natural antioxidants.

DPPH radical-scavenging activity

The DPPH radical model has been widely used to valuate the antioxidant activity of fruit and vegetable extract. The method is based on the reaction that hydrogen-donating antioxidants reduce violet DPPH free radical to yellow DPHH-H, a non-radical form (Kumaran and karunakaran, 2005). The reduced amount of DPPH absorption at 517 nm indicated the radical-scavenging ability of anti-oxidants.

Figure 3 displays DPPH radical-scavenging activity of the anthocyanins from purple sweet potato. The results show that the radical-scavenging activities of antioxidants increased with the increment of concentrations. Terahara et al. (2004) investigated that the purple sweet potato anthocyanins were examined with respect to radical scavenging activity against the DPPH radical. Lachman et al. (2008) reported that the purple-fleshed potatoes have significantly higher antioxidant activity than yellow-fleshed cultivars. L-AA had the highest scavenging radical activity, followed by anthocyanins from purple sweet potato. Among them, the anthcyanins from purple sweet potato and L-AA exerted strong activities, showing 94.1 and 95.8% of DPPH radical scavenging activities at concentration of 50 µg/ml, respectively. On the hand, BHT showed relatively low DPPH radical scavenging activity (37.6%). The DPPH radical-scavenging effects of the anthocyanins, BHT and L-AA are shown in Table 1. The IC_{50} values of anthocyanins and L-AA were low, while BHT was higher.

Superoxide anion-scavenging activity

Superoxide anion, which is a reductive form of molecular oxygen, is produced by a number of cellular reactions,

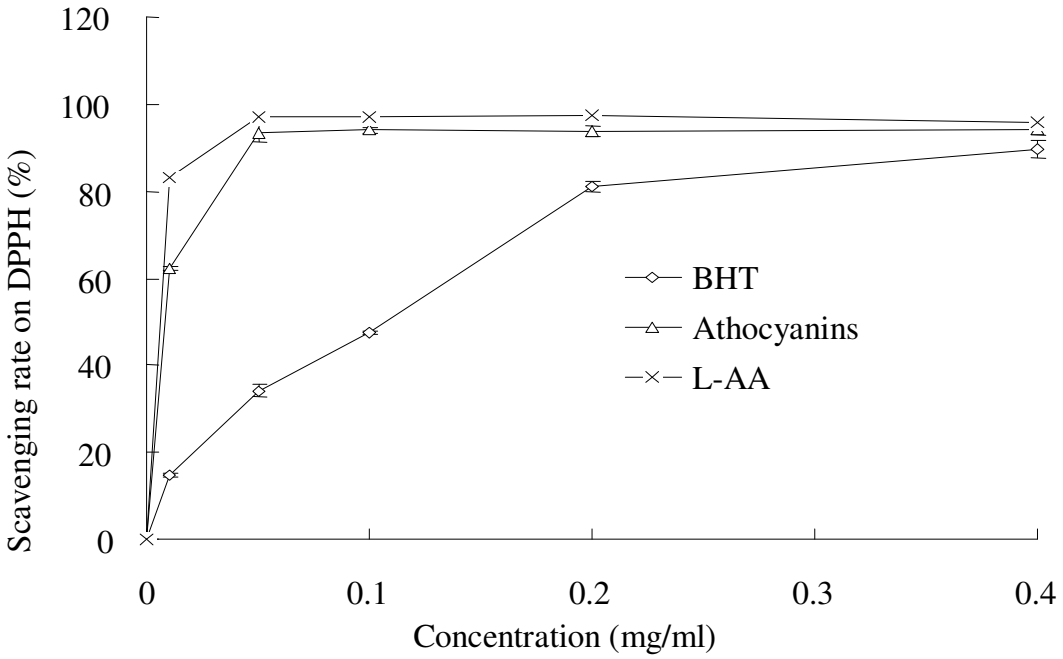

Figure 3. DPPH radical-scavenging activity of anthocyanins from purple sweet potato compared to BHT and L-AA. Each value represents means ± SD (n=6). DPPH, α,α-Diphenyl-β-picrylhydrazyl; L-AA, L-ascorbic acid; BHT, butylated hydroxytoluene.

Table 1. Half inhibition concentration (IC_{50}) of anthocyanins from purple sweet potato, BHT and L-AA scavenging DPPH, superoxide radical.

Antioxidant	DPPH radical-scavenging activity IC_{50} (μg/ml)	Superoxide onion radical-scavenging activity IC_{50} (μg/ml)
BHT	123.46 ± 0.14[a]	50.00 ± 0.14[a]
L-AA	6.10 ± 0.05[c]	10.01 ± 0.24[b]
Anthocyanins	6.94 ± 0.02[b]	3.68 ± 0.01[c]

Means in the same column followed by different letters are significantly different at P <0.05. HPLC characterization of purple sweet potato anthocyanins. L-AA, L-Ascorbic acid; BHT, butylated hydroxytoluene.

including a range of enzyme systems in autooxidation reaction and non-enzymatic electron transfers that reduce molecular oxygen (Gulcin et al., 2005). It was known that superoxide anion is very harmful because it could transform into more reactive oxygen species such as hydroxyl radical, which contributes to tissue damage and various diseases.

In this paper, illuminating a solution containing riboflavin was used to generate superoxide radical. Superoxide scavenging activities of the anthocyanins is shown in Figure 4. Anthocyanins, L-AA and BHT exhibited superoxide anoin-scavenging activity as dose-dependent manner. They exerted strong activities, showing 86.1, 65.8 and 50.7% of superoxide anoin-scavenging activities at concentration of 50 μg/ml, respectively. The IC_{50} value of anthocyanins was less than those of L-AA and BHT (Table 1). Thus, anthocyanins had higher superoxide anoin-scavenging activity than L-AA and BHT.

Figure 5 shows that 16 peaks appeared in the chromatograms of purple sweet potato antho-cyanins, which were detected at 520 nm. From the chromate-graphic and UV – visible spectral features, peaks 1, 2, 3 and 4 were accounting for 7.8, 16.3, 15.6 and 33.5%, respectively, of the total amount of all the anthocyanins, and they were eluted after 9.0, 10.8, 14.4 and 16.5 min, respectively. Suda et al. (2002) reported that 15 kind of anthocyanins in purple sweet potato from Japan. Eichhorn and Winterhalter (2005) found there were four anthocyanin peaks in the chromatograms of purple sweet potato from Germnay. An absorption peak appeared at 330 nm of the UV – Vis characteristic of the four major anthocyanins (Figure 6), which indicates that these anthocyanins are acylated anthocyanins. This result agreed with the research of Suda et al. (2003). They have

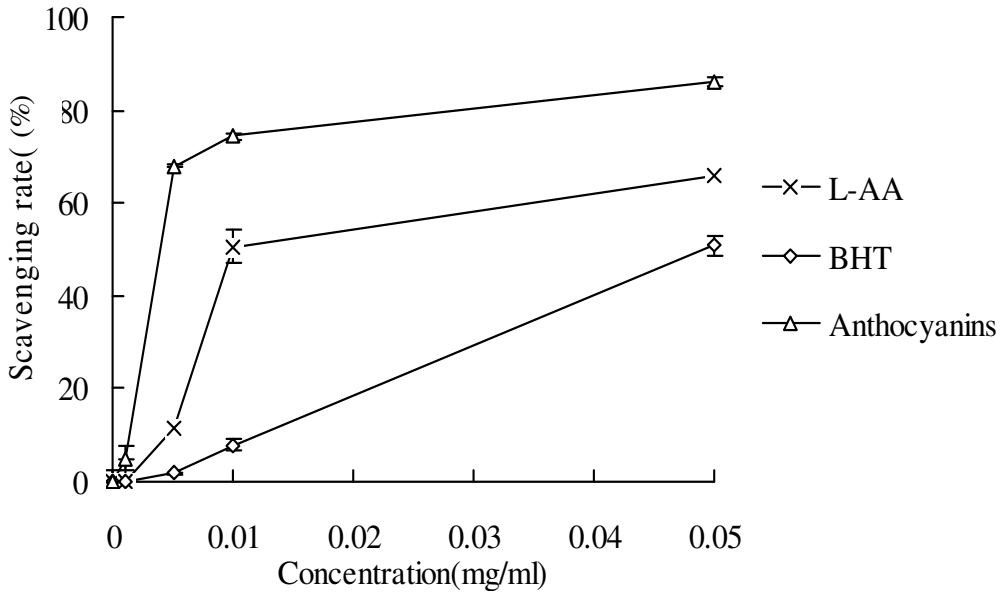

Figure 4. Superoxide radical-scavenging activity of anthocyanins from purple sweet potato compared to BHT. Each value represents means ± SD (n=6). BHT, Butylated hydroxytoluene.

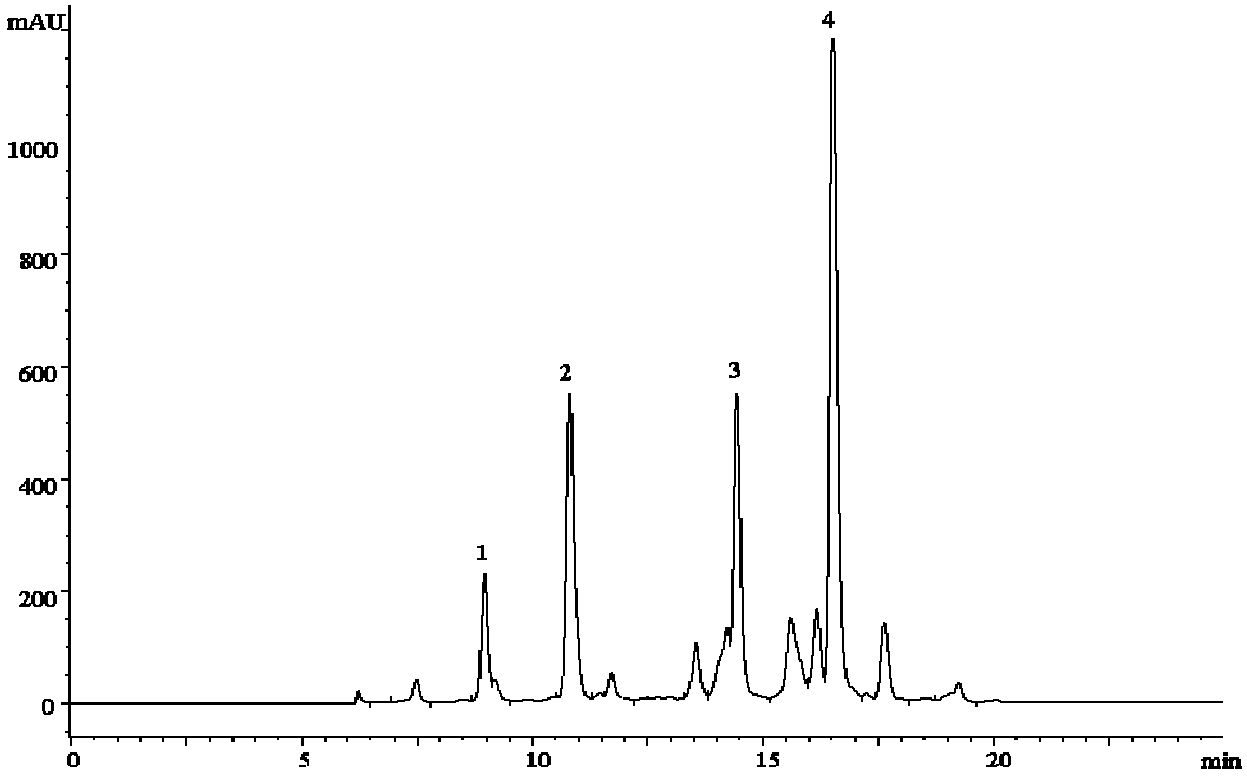

Figure 5. HPLC chromaographs of anthocyanins from purple sweet potato. HPLC, High-performance liquid chromatography.

indicated that the anthocyanins in purple sweet potato are mono- or diacylated forms. Calculation from the chromatograms showed that more than 84.7% of the purple sweet potato anthocyanins were acylated

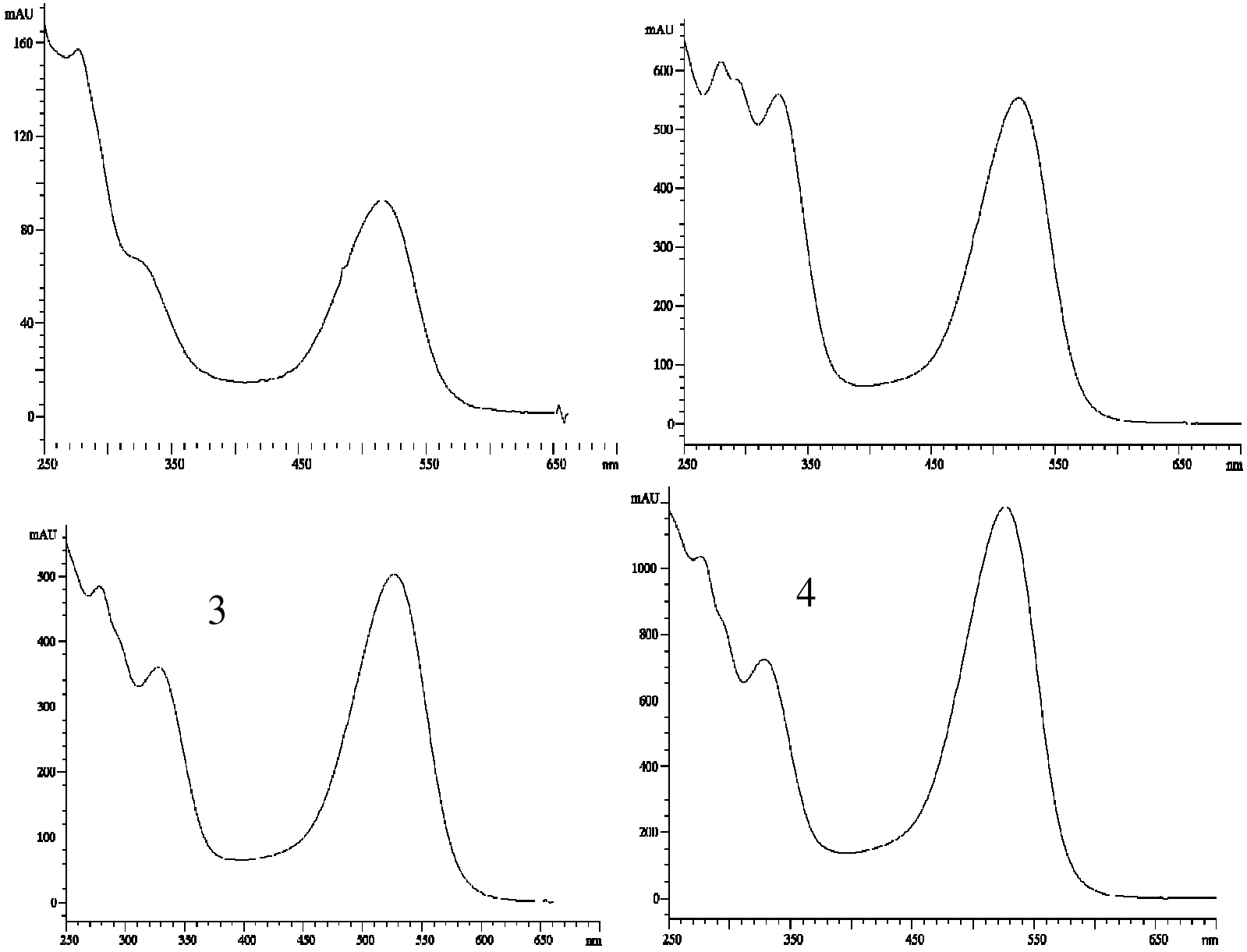

Figure 6. UV-Vis spectrum of major peaks in purple sweet potato anthocyanins recorded from 250 to 700 nm.

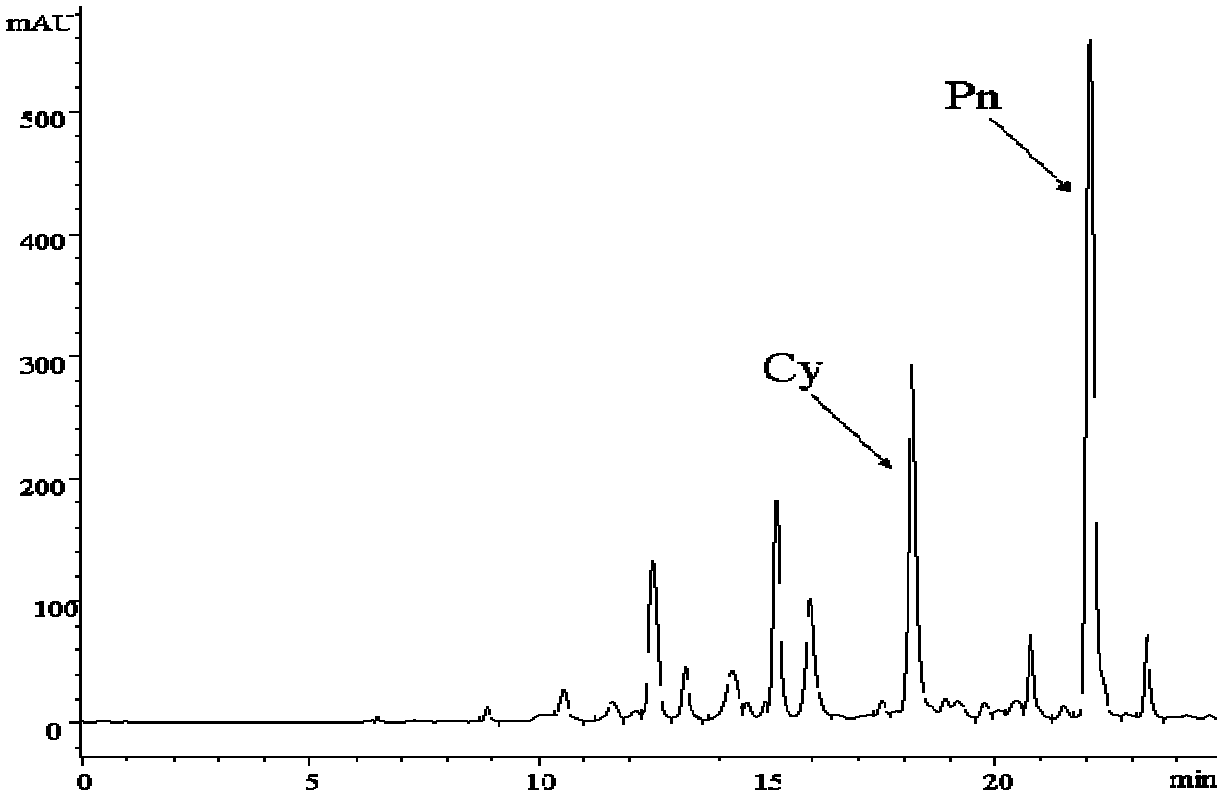

Figure 7. HPLC frofile of purple sweet potato anthocyanins after acid hydrolysis. Cy, Cyanidin; Pn, peonidin. HPLC, High-performance liquid chromatography.

anthocyanins. Fossen and Andersen (2000) also found that the acylated anthocyanis constitute more than 98% of the total anthocyani content in purple sweet potato. This suggests a high stability of the purple sweet potato anthocyanins and confirms the potential use of purple sweet potato anthocyanins as a source of natural colourants for the food industry (Fernando and Cisneros-Zevallos, 2007).

Figure 7 shows the chromatograms of purple sweet potato anthocyanins after acid hydrolysis, and peaks 1 and 2 were detected compared with the standard samples.They accounted for about 46.5% of the total anthocyanidins. Cyanidin accounted for about 16.7% of the anthocyanidins and was eluted after 18.2 min. Peonidin accounted for about 29.8% and was eluted after 22.1 min. These results are in agreement with those reported by Bridle and Timberlake (1997) and Terahara et al. (1999) who found that the anthocyanins in purple sweet potato are mono- or di-acylated forms of cyanidin and peonidin. Further studies would be needed to determine other anthocyanidins in purple sweet potato anthocyanins.

Conclusion

This study demonstrated that anthocyanins from purple sweet potato exerts DPPH radical and superoxide anion scavenging activities, resulting in a significant, dose-dependent scavenging radical. The anthocyanins in purple sweet potato are mono- or di-acylated forms of cyanidin and peonidin. However, the further study is needed to isolate and identify the monoanthocyanins and compare their antioxidant activities *in vitro* and *in vivo*.

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