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Comparisons of *in vitro* antioxidant activities of storage proteins in tuber of two *Dioscorea* species

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Abstract. Dioscorin was purified by DE-52 ion exchange chromatography from two yam species, *Dioscorea alata* L. cv. Tainong 1 (TN1) and *Dioscorea batatas* Decne (imported from Japan, JP). By different *in vitro* antioxidant tests, including DPPH radical and hydroxyl radical scavenging activity assay, a reducing power test, an anti-lipid peroxidation test, DNA damage protection, and inhibition of dihydrorhodamine 123 oxidation by peroxynitrite, it was shown that dioscorins from the two species exhibited different scavenging activities against DPPH and hydroxyl radicals, even after heating 100˚C for 5 min. Dioscorins from TN1 were hydrolyzed by pepsin for different durations and the peptic hydrolysates exhibited DPPH radical scavenging activities. Peptic hydrolysates separated by Sephadex G-50 (F) gel filtration were tested for anti-DPPH radical activity. Results showed that fractions of smaller molecular weight still have antioxidant activities.

Keywords: Antioxidant; Dioscorin; Peptic hydrolysates; Storage protein; Yam.

INTRODUCTION

Yam (*Dioscorea* species) is a member of the monocotyledonous family Dioscoreaceae and is a staple food in West Africa, Southeast Asia, and the Caribbean (Akoruda, 1984). Yam is recognized as an herbal plant since the tuber dried slices have freqently been used as Chinese herbal medicines. The tuber storage proteins of yam, dioscorin, exhibited carbonic anhydrase, trypsin inhibitor activities (Hou et al., 1999a; Hou et al., 2000), and both dehydroascorbate reductase and monodehydroascorbate reductase activities (Hou et al., 1999b). Chang et al. (2004) reported that Chinese yam (*D. alata* cv. Tainong No. 2) feeding had antioxidant effects in hyperhomocysteinemia rats. However, the component in the freeze-dried powder of Chinese yam was responsible for this activity was not clear. We reported previously that the storage proteins (dioscorin) from Japanese yam (*D. batatas*) exhibited scavenging activities against DPPH and hydroxyl radicals (Hou et al., 2001). We also reported that the crude and purified mucilages from Japanese yam exhibited antioxidant activities (Lee et al., 2003). We discovered this by testing anti-DPPH and anti-hydroxyl radicals, reducing powers, and anti-lipid peroxidation activities (Hou et al., 2002).

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Liao et al. (2004) used near infrared Fourier transform Raman spectroscopy to analyze the secondary structure of dioscorin from three yam species [*D. Japonica* (Japanese yam), *D. alata* L., and *D. alata* L. var. *purpurea*]. Although the dioscorin from three species has a similar molecular mass, the amino acid compositions and the secondary structure of dioscorin from *D. alata* L., and *D. alata* L. var. *purpurea* were apparently different from those of *D. Japonica* (Japanese yam). Dioscorin from *D. Japonica* had lower contents of Cys, Ile, Lys, and total essential amino acids than did the other two yam species. The dioscorin from *D. alata* L., *D. alata* L. var. *purpurea*, and *D. Japonica* exhibited structures mostly of the α-helix, antiparallel β-sheet, mixed α-helix, and antiparallel β-sheet types, respectively. These differences among yam species might result in different biological activities. In this work we used dioscorin from two different yam species, Chinese yam (*D. alata* cv. Tainong No. 1, TN1) and Japanese yam (*D. batatas* Decne, imported from Japan, JP), and compared their antioxidant activity, using DPPH radical and hydroxyl radical scavenging activity assay, reducing power test, anti-lipid peroxidation test, DNA damage protection, and inhibition of dihydrorhodamine 123 oxidation by peroxynitrite. The results showed that dioscorins from two species exhibited different scavenging activities even with heating 100ºC for 5 min against DPPH and hydroxyl radicals. The peptic hydrolysates of TN1 dioscorin were separated

by Sephadex G-50(F) column and were then analyzed for anti-DPPH activity. It was found that the smaller molecular weight fractions still had antioxidant activities.

MATERIALS AND METHODS

Material

Peroxynitrite was obtained from Calbiochem-Novabiochem Co. (Darmstadt, Germany). Calf thymus DNA (activated, 25 A_{260} units) was purchased from Amersham Biosciences (Uppsala, Sweden). Hydrogen peroxide (33%) was from Wako Pure Chemical Industry (Osaka, Japan). Reduced glutathione, dihydrorhodamine 123 (DHR 123), 1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2-deoxy-ribose, and other chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Dioscorin extractions and purifications from TN1 and JP yam

Tubers of fresh yams, *D. alata* L. cv. Tainong No. 1 (TN1) and *D. batatas* Decne (JP), were purchased from Taipei Agricultural Products Marketing Co., Wanhua District, Taipei. After washing and peeling, the tubers were cut into strips for dioscorin extraction and purification. After extraction and centrifugation, dioscorins were purified from crude extracts successively by a DE-52 ion exchange column according to the methods of Hou et al. (2001). The concentrated dioscorin solution was dialyzed against deionized water overnight and lyophilized for further use.

DPPH radical scavenging activity of dioscorin

The DPPH radical scavenging activity of dioscorin with or without heating at 100ºC for 5 min and peptic hydrolysates from TN1 and JP yams were measured according to the method of Hou et al. (2001, 2002). Every 0.6 mL dioscorin solution (10, 20 and 40 nanomoles) was added to 0.05 mL of 1 M Tris-HCl buffer (pH 7.9) and then mixed with 0.6 mL of 50 μM DPPH in methanol for 20 min under light protection at room temperature. The absorbance at 517 nm was measured. Deionized water was used as a blank experiment. The scavenging activity of DPPH radicals (%) was calculated following the equation: $(A517_{blank} - A517_{sample}) \div A517_{blank} \times 100\%$.

Scavenging activity of dioscorin against metal ion-dependent hydroxyl radicals

The hydroxyl radical scavenging activity of dioscorin with or without heating at 100ºC for 5 min from TN1 and JP yams was determined by the deoxyribose method (Halliwell et al., 1987). Every 0.5 ml sample containing different amounts of dioscorin (0.357, 1.785, 3.57, 7.14, and 17.85 nanomole) were added to 1.0 ml solution of 20 mM potassium phosphate buffer (pH 7.4), 2.8 mM 2-deoxy-ribose, 104 μM EDTA, 100 μM $FeCl_3$, 100 μM ascorbate, and 1 mM hydrogen peroxide. The mixtures were incubated for 1 h at 37ºC. After incubation, an equal volume of 0.5% thiobarbituric acid in 10% trichloroacetic acid was added and the mixtures were boiled at 100ºC for 15 min. Deionized water was used as a blank experiment. The absorbance at 532 nm was measured. The scavenging activity of hydroxyl radicals (%) was calculated with the equation: $(A532_{blank} - A532_{sample}) \div A532_{blank} \times 100\%$.

The reducing power of dioscorin

The reducing power of different amounts (1.785, 3.57, 7.14, and 17.85 nmole) of dioscorin from TN1 and JP yams in a 1.25 mL sample solution was measured by ferric chloride-potassium ferricyanide methods (Yen and Chen, 1995) at a wavelength of 700 nm. Deionized water was used as a blank experiment, and the reduced glutathione (4.69, 9.37, 10.07, and 18.74 nmole) was used a positive control. Increase of absorbance of the reaction mixture at a wavelength of 700 nm (ΔA700 nm) indicates an increase of reducing power.

Anti-linoleic acid peroxidation of dioscorin

The antiperoxidation activity of 5 μg (0.018 nmole) dioscorin from TN1 and JP yams against 2.5 mL of 0.02 M linoleic acid emulsion at intervals after reactions at 37ºC was measured by the thiocyanate method (Pham et al., 2000). At intervals during incubation, a 0.1-mL aliquot of the reaction mixture was mixed with 4.7 mL of 75% ethanol, 0.1 mL of 30% ammonium thiocyanate, and 0.1 mL of 20 mM ferrous chloride in 3.5% hydrochloric acid. Precisely 3 min after the addition of ferrous chloride to the reaction mixture, the absorbance at a wavelength of 500 nm was determined. Deionized water was used in a control experiment. Increase of absorbance at a wavelength of 500 nm indicates a decrease of antioxidant activity against linoleic acid peroxidation.

Protecting hydroxyl radical-induced damages of calf thymus DNA by TN1 dioscorin

The hydroxyl radical was generated by Fenton reaction according to the method of Kohno et al. (1991). The 45 μL reaction mixture included TN1 dioscorin (20, 50, 100, and 200 μg), 15 μL of calf thymus DNA, 18 mM $FeSO_4$, and 60 mM hydroxygen peroxide at room temperature for 15 min or 30 min. Ten μL of 10 mM EDTA was added to stop the reaction. The only calf thymus DNA was used for the blank test, and the control test was without TN1 dioscorin additions. After agarose gel electrophoresis, the treated DNA solutions were stained with ethidium bromide and observed under UV light.

Protecting peroxynitrite-mediated DHR 123 oxidation by TN1 dioscorin

The protection against peroxynitrite-mediated DHR 123 oxidation was according to the methods of Kooy et al. (1994). The total 175 μl reaction mixture included different amounts of TN1 dioscorin (9, 12, and 16 μg), 10 μM DHR and 10 μl peroxynitrite in 50 mM phosphate

buffer (pH 7.4), containing 90 mM NaCl. After a 10-min reaction, the fluorescent intensity was measured at the excitation and emission wavelengths of 473 and 520 nm, respectively, and at excitation and emission slit widths of 2.5 nm and 3.0 nm, respectively. The control test was without dioscorin additions.

Determination of the DPPH scavenging activity of peptic hydrolysates of TN1 dioscorin

The 7 mg TN1 dioscorin was dissolved in 1 mL, 0.1 M KCl-HCl buffer (pH 2.0). The 0.1 mL, 14 mg pepsin was added for hydrolysis at 37ºC for 8, 12, 24 and 32 h. After hydrolysis, 0.5 mL of 0.5 M Tris-HCl buffer (pH 8.3) was added and heated at 100ºC for 5 min to stop hydrolysis. The pepsin was heated before dioscorin hydrolysis for zero hour reaction. Each dioscorin hydrolysate was used for determination of DPPH scavenging activity.

Chromatograms of peptic hydrolysates of TN1 dioscorin on Sephadex G-50 column and their DPPH scavenging activities

The peptic hydrolysates of TN1 dioscorin at 8, 12, 24 and 32 h were lyophilized and separated by Sephadex G-50(F) chromatography (1×75 cm). The column was eluted with 20 mM Tris-HCl buffer (pH 7.9). Flow rate was 30 mL/h, and each fraction contained 2 mL. Each fraction was determined at the absorbance of 210 nm for peptide contents and for DPPH scavenging activity (570 nm).

Statistical analysis

Student's t-test was used for comparisons between control and each experimental test. A difference was considered statistically significant when $P< 0.05$ (*) or $P< 0.01$ (**).

RESULTS AND DISCUSSION

Owing to the different amino acid compositions and the secondary structures of dioscorin from Chinese yam and Japanese yam (Liao et al., 2004), the antioxidant activity of the dioscorin from two different yam species, Chinese yam (*D. alata* cv. Tainong No. 1, TN1) and Japanese yam (*D. batatas* Decne, imported from Japan, JP), was compared. Our results show that dioscorin from Chinese yam (TN1 yam) had higher antioxidant or scavenging activities than did Japanese yam (JP yam) according to the DPPH radical and hydroxyl radical scavenging activity assay, reducing power test, anti-lipid peroxidation test.

Figure 1 shows the dioscorin scavenging activity against DPPH radical from TN1 and JP with or without heating at 100ºC for 5 min. When DPPH radical was scavenged, the color of the reaction mixture changed from purple to yellow with the decrease of absorbance at wavelength 517 nm. It was found that the dioscorin from TN1 and JP exhibited dose-dependent DPPH radical scavenging activity (Figure 1). Under the same 40 nmole of dioscorin (28 kDa), the scavenging activity of JP dioscorin decreased from 46% to 34% after heating at 100ºC for 5 min. However, an activation of scavenging activity (from 49% to 56%) was found in TN1 dioscorin. Dioscorin from *D. Japonica* was found to have a content of Cys roughly tenfold lower than the other two Taiwanese yam species (Liao et al., 2004). The Cys in patatin (Liu et al., 2003) and in sweet potato TIs (Hou et al., 2005) was reported to contribute to DPPH scavenging activities. It was proposed that the heating process could affect JP dioscorin stability and reduce the DPPH scavenging activity. However, it might also expose other inner Cys residues in TN1 dioscorin and elevate TN1 dioscorin scavenging activity.

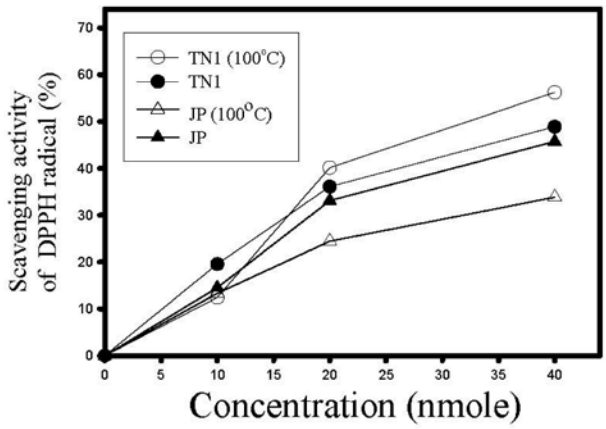

Figure 1. The scavenging activity against DPPH radical of dioscorin from TN1 and JP yams with or without heating at 100ºC for 5 min. Means of triplicates were measured. Deionized water was used as a blank experiment. The scavenging activity of DPPH radical (%) was calculated according to the following equation: $(A517_{blank} - A517_{sample}) \div A517_{blank} \times 100\%$.

Figure 2 shows the dioscorin scavenging activity against hydroxyl radical from TN1 and JP with or without heating at 100ºC for 5 min. The dioscorin from TN1 and JP was also found to exhibit dose-dependent hydroxyl radical scavenging activity, even after heating at 100ºC for 5 min (Figure 2). Under the same 17.85 nmole of dioscorin (28 kDa), the scavenging activity of TN1 (62%) was higher than that of JP (48%). The heating processing affected the hydroxyl radical scavenging activity in dioscorins from two yam species. The higher hydroxyl radical scavenging activity of the dioscorin from TN1 might be due partly to the different contents of Trp since dioscorin from *D. Japonica* had less Trp (intensity ratio of Raman spectra, $I_{878/759}$) than the other two Taiwanese yam species (Liao et al., 2004). The Trp in patatin (Liu et al., 2003) and in sweet potato TIs (Hou et al., 2005) was reported to contribute to hydroxyl radical scavenging activities. Water spinach constituents were also reported to have antioxidant activity (Huang et al., 2005). The heating process could affect dioscorin stability and reduced hydroxyl radical scavenging activity.

The reducing power of dioscorin from TN1 and JP is shown in Figure 3. The reduced glutathione was used as a positive control. TN1 dioscorin exhibited a dose-dependent reducing power activity within the applied concentrations (1.785, 3.57, 7.14, and 17.85 nmole) and had higher activities than glutathione under the same nmole concentration basis. The reducing power of TN1 dioscorin was about tenfold that of JP (Figure 3), which was comparable to the reported ratio of Cys content in each yam species (1.18±0.13 vs 0.13±0.07 mg/g protein, Liao et al., 2004).

The products of lipid peroxidation (such as malondialdehyde) could cause damage to proteins and DNA (Esterbauer et al., 1991). The anti-lipid peroxidation of 5 μg dioscorin from TN1 and JP is shown in Figure 4. Both the dioscorin from TN1 and JP could retard linoleic acid peroxidation during the intervals of 8 h at 37ºC compared to that of the control. Dioscorin from TN1 and JP were able to effect retardations of linoleic acid peroxidation that were about 15.60 and 8.3-fold of control, respectively, in the 12 h reaction.

Liao et al. (2004) reported the Cys content of dioscorin in Chinese yam was about tenfold that of Japanese yam. The secondary structures of dioscorin from *D. alata* L. (Chinese yam) and *D. Japonica* (Japanese yam) were mostly α-helix, a mixed α-helix type, and an antiparallel β-sheet type, respectively. From our present results, under the same weight basis, dioscorin from Chinese yam (TN1) had higher antioxidant or scavenging activities than Japanese yam (JP yam) based on DPPH radical and hydroxyl radical scavenging activity assay, a reducing power test, and an anti-lipid peroxidation test. These results might be attributable to the different amino acid compositions and protein conformations. Therefore, TN1 dioscorin was further studied in the following

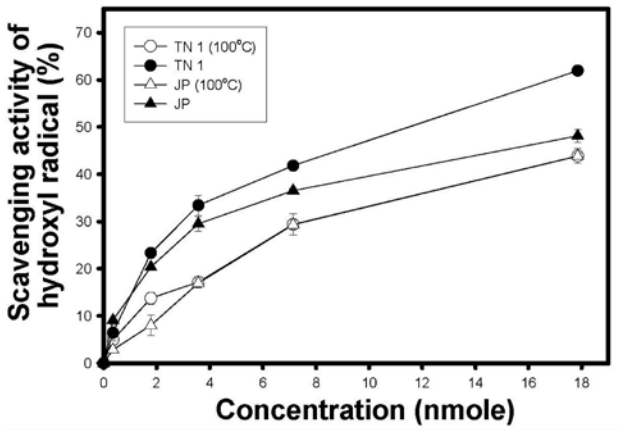

Figure 2. The scavenging activity against hydroxyl radical of dioscorin from TN1 and JP yam with or without heating at 100ºC for 5 min. Means of triplicates were measured. Deionized water was used as a blank experiment. The absorbance at 532 nm was measured. The scavenging activity of hydroxyl radicals (%) was calculated with the equation: $(A532_{blank} - A532_{sample}) \div A532_{blank} \times 100\%$.

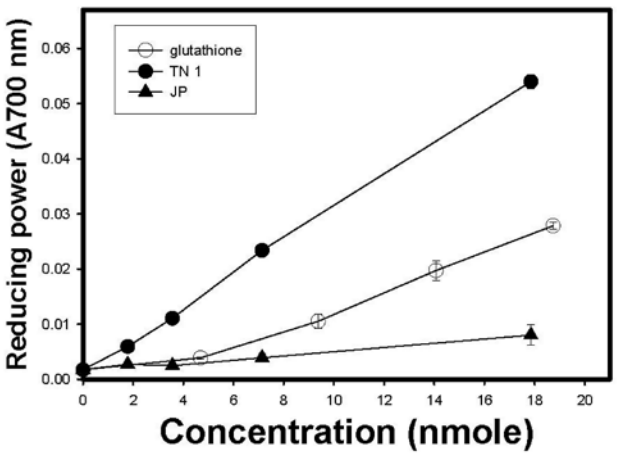

Figure 3. The reducing power of dioscorin from TN1 and JP yam (1.785, 3.57, 7.14, and 17.85 nmole). The glutathione (4.69, 9.37, 14.07, and 18.74 nmole) was used as a positive control. Means of triplicate were determined at the absorbance of 700 nm.

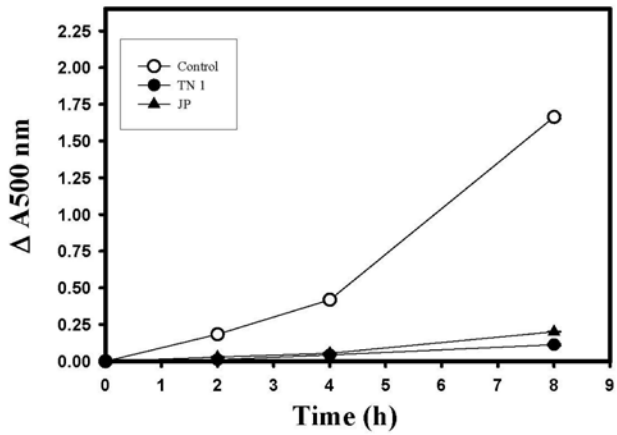

Figure 4. The effects of dioscorin from TN1 and JP yam on the anti-linoleic acid peroxidation. Each of 5 μg dioscorin was added to the emulsion and incubated at 37ºC for 0, 2, 4, and 8 h. At each time interval, 0.1 mL was picked and was determined by thiocyanide method (A500 nm).

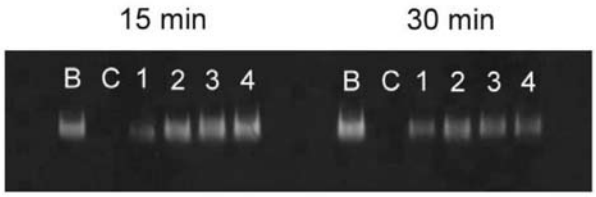

Figure 5. The effects of TN1 dioscorin on the Fe^{2+}-mediated DNA oxidation. Lane B was native calf thymus DNA; lane C was metal-mediated oxidized DNA; and lanes 1 to 4 were metal-mediated oxidized DNA with 20, 50, 100, and 200 μg TN1 dioscorin additions. The reaction was stopped after 15 min or 30 min by adding 10 mM EDTA. After electrophoresis, the gel was stained with ethidium bromide and observed under UV light.

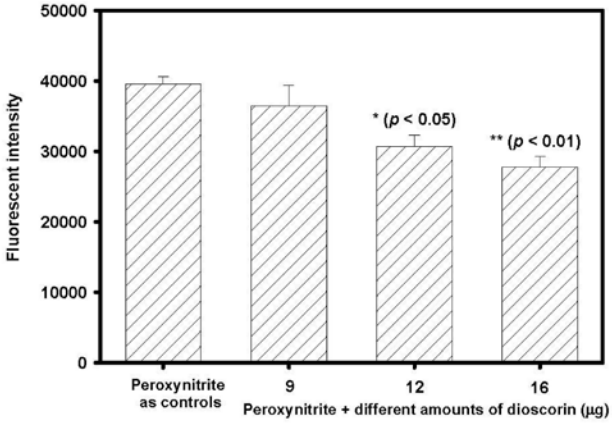

Figure 6. The TN1 dioscorin (9, 12, and 16 μg) protected peroxynitrite-mediated dihydrorhodamine 123 oxiadtion. The total 175 μl reaction mixture included different amounts of TN1 dioscorin, 10 μM DHR, and 10 μl peroxynitrite in 50 mM phosphate buffer (pH 7.4) containing 90 mM NaCl. After 10 min reaction, the fluorescent intensity was measured at the excitation and emission wavelengths of 473 and 520 nm, respectively, and excitation and emission slit widths of 2.5 nm and 3.0 nm, respectively. The control test was without dioscorins additions. A difference between the control and the experimental test was considered statistically significant when $p<0.05$ (*) or $p<0.01$ (**).

experiment of DNA damage protection, inhibition of dihydrorhodamine 123 oxidation by peroxynitrite, and DPPH scavenging activity of dioscorin peptic hydroxylates.

Free radicals can damage macromolecules in DNA, protein and the lipid cells in membranes (Halliwell, 1999). Figure 5 shows TN1 dioscorin protected against hydroxyl radical-induced calf thymus DNA damage in 15 or 30 min. The only calf thymus DNA was used for a blank test (lane B), and the control test (lane C) was without TN1 dioscorin additions. Compared to the blank test and control test, it was found that the added TN1 dioscorin above 50 μg (lane 2) could prevent hydroxyl radical-induced calf thymus DNA damage in both 15 min or 30 min reactions.

Peroxynitrite is formed from a nearly diffusion-limited reaction between nitric oxide and superoxide anion and as an initiator of potentially harmful oxidation reaction (Brannan et al., 2001). The results of Figure 6 demonstrate that the protective effect of peroxynitrite-mediated DHR oxidation of TN1 dioscorin was dose-dependent. Significant variation was observed among the peroxynitrite, peroxnitrite + 12 μg TN1 dioscorin ($p<0.05$), and peroxynitrite + 16 μg TN1 dioscorin ($p<0.01$).

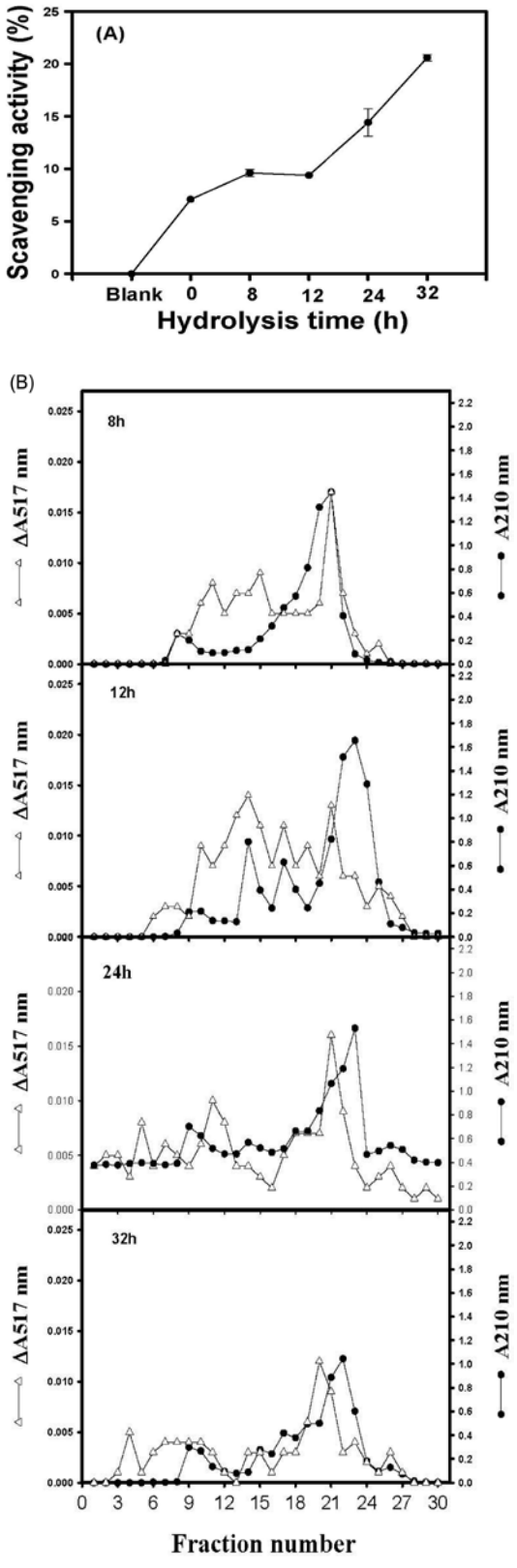

Figure 7. The DPPH scavenging activity (A) and chromatograms on Sephadex G-50(F) column (B) of peptic hydrolysates of TN1 dioscorin. TN1 dioscorin was hydrolyzed by pepsin at 37ºC for 8, 12, 24 and 32 h. After hydrolysis, 0.5 mL of 0.5 M Tris-HCl buffer (pH 8.3) was added and heated at 100ºC for 5 min to stop hydrolysis. The pepsin was heated before dioscorin hydrolysis for zero hour reaction. Each dioscorin hydrolysate was used for determinations of DPPH scavenging activity and was separated by Sephadex G-50(F).

Figure 7(A) shows the DPPH scavenging activity of TN1 dioscorin hydrolysates at different pepsin hydrolysis times. As the results of Figure 7 make clear, the scavenging activity against DPPH radical increased from 7.1% (0 h) to about 21% (32 h). Figure 7(B) shows the chromatograms of peptic dioscorin hydrolysates of 8, 12, 24 and 32 h on Sephadex G-50 chromatography. The smaller peptides were found to increase with increasing pepsin hydrolytic time and also exhibited DPPH radical scavenging activities. The purifications of antioxidant peptides will be investigated in the future.

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LITERATURE CITED

Akoruda, M.O. 1984. Genetic improvement of vegetable crops: yam (*Dioscorea* spp.). *In* M. Kasloo (ed.), Genetic improvement of vegetable crops. Pergamon Press, pp. 717-733.

Brannan, R.G., B.J. Connolly, and E.A. Decker. 2001. Peroxynitrite: a potential initiator of lipid oxidation in food. Trends Food Sci. & Technol. **12:** 164-173.

Chang, S.J., Y.C. Lee, S.Y. Liu, and T.W. Chang. 2004. Chinese yam (*Dioscorea alata* cv. Tainung No. 2) feeding exhibited antioxidant effects in hyperhomocysteinemia rats. J. Agric. Food Chem. **52:** 1720-1725.

Esterbauer, H., R.G. Schaur, and H. Zollner. 1991. Chemistry and biochemistry of 4-hydroxynonenal, malondialdehyde and related aldehyde. Free Rad. Biol. Med. **11:** 81-128.

Halliwell, B., J.M.C. Gutteridge, and O.I. Aruoma. 1987. The deoxyribose method: a simple test-tube assay for determination of rate constants for reactions of hydroxyl radicals. Anal. Biochem. **165:** 215-219.

Halliwell, B. 1999. Food-derived antioxidants. Evaluating their importance in food and in vivo. Food Sci. Agric. Chem. **1:** 67-109.

Hou, W.C., J.S. Liu, H.J. Chen, T.E. Chen, C.F. Chang, and Y.H. Lin. 1999a. Dioscorin, the major tuber storage protein of yam (*Dioscorea batatas* Decne), with carbonic anhydrase and trypsin inhibitor activities. J. Agric. Food Chem. **47:** 2168-2172.

Hou, W.C., H.J. Chen, and Y.H. Lin. 1999b. Dioscorin, the major tuber storage protein of yam (*Dioscorea batatas* Decne), with dehydroascorbate reductase and monodehydroascorbate reductase activities. Plant Sci. **149:** 151-156.

Hou, W.C., H.J. Chen, and Y.H. Lin. 2000. Dioscorins from different *Dioscorea* species all exhibit both carbonic anhydrase and trypsin inhibitor activities. Bot. Bull. Acad. Sin. **41:** 191-196.

Hou, W.C., M.H. Lee, H.J. Chen, W.L. Liang, C.H. Han, Y.W. Liu, and Y.H. Lin. 2001. Antioxidant activities of dioscorin, the storage protein of yam (*Dioscorea batatas* Decne) tuber. J. Agric. Food Chem. **49:** 4956-4960.

Hou, W.C., F.L. Hsu, and M.H. Lee. 2002. Yam (*Dioscorea batatas*) tuber mucilage exhibited antioxidant *in vitro*. Planta Med. **68:** 1072-1076.

Hou, W.C., C.H. Han, H.J. Chen, C.L. Wen, and Y.H. Lin. 2005. Storage proteins of two cultivars of sweet potato (*Ipomoea batatas* L.) and their protease hydrolysates exhibited antioxidant activity *in vitro*. Plant Sci. **168:** 449-456.

Huang, D.J., H.J. Chen, C.D. Lin, and Y.H. Lin. 2005. Antioxidant and antiproliferative activities of water spinach (*Ipomoea aquatica* Forsk) constituents. Bot. Bull. Acad. Sin. **46:** 99-106.

Kohno, M., M. Yamada, K. Mitsuta, Y. Mizuta, and T. Yoshikawa. 1991. Spin-trapping studies on the reaction of iron complexes with peroxides and the effects of water-soluble antioxidants. Bull. Chem. Soc. Jpn. **64:** 1447-1453.

Kooy, N.W., J.A. Royall, H. Ischiropoulos, and J.S. Beckman. 1994. Peroxynitrite-mediated oxidation of dihydrorhodamine 123. Free Rad. Biol. Med. **16:** 149-156.

Lee, M.H., Y.S. Lin, Y.H. Lin, F.L. Hsu, and W.C. Hou. 2003. The mucilage of yam (*Dioscorea batatas* Decne) tuber exhibited angiotensin converting enzyme inhibitory activities. Bot. Bull. Acad. Sin. **44:** 267-273.

Liao, Y.H., C.H. Wang, C.Y. Tseng, H.L. Chen, L.L. Lin, and W. Chen. 2004. Compositional and conformational analysis of yam proteins by near infrared Fourier transform Raman spectroscopy. J. Agric. Food Chem. **52:** 8190-8196.

Liu, Y.W., C.H. Han, M.H. Lee, F.L. Hsu, and W.C. Hou. 2003. Patatin, the tuber storage protein of potato (*Solanum tuberosum* L.), exhibits antioxidant activity *in vitro*. J. Agric. Food Chem. **51:** 4389-4393.

Pham, T.Q., F. Cormier, E. Farnworth, V.H. Tong, and M.V. Calsteren. 2000. Antioxidant properties of crocin from *Gardenia jasminoides* Ellis and study of reactions of crocin with linoleic acid and crocin with oxygen. J. Agric. Food Chem. **48:** 1455-1461.

Yen, G.C. and H.Y. Chen. 1995. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J. Agric. Food Chem. **43:** 27-32.

兩品種山藥塊莖儲藏性蛋白質體外抗氧化活性之研究

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兩品種山藥〈台農一號與日本山藥〉塊莖儲藏性蛋白質 dioscorin 經由 DE-52 離子交換層析法純化。經由一系列體外抗氧化實驗，包括清除 DPPH 與氫氧自由基，還原能力，抗脂質過氧化能力，保護去氧核糖核酸傷害能力，及抑制 peroxynitrite 氧化 dihydrorhodamine 123 能力。結果顯示，兩品種山藥 dioscorin 具有不同的抗氧化能力，即使 100℃ 加熱五分鐘也仍具有不同的清除 DPPH 與氫氧自由基的能力。以胃蛋白酶水解台農一號 dioscorin 不同時間之水解產物也具有清除 DPPH 自由基的能力。以 Sephadex G-50 (F) 膠濾層析進行分離並進行 DPPH 自由基清除實驗，結果顯示小分子水解產物也具有抗氧化能力。

關鍵詞：抗氧化；儲藏性蛋白質；胃蛋白酶水解物；山藥。