Functional Properties of Dioscorin, a Soluble Viscous Protein from Japanese Yam (*Dioscorea opposita* Thunb.) Tuber Mucilage *Tororo*

Takeshi Nagai* and Toshio Nagashima

Department of Food Science and Technology, Tokyo University of Agriculture, Hokkaido 0992493, Japan. Fax: +81-152-48-3850.
E-mail: t1nagai@bioindustry.nodai.ac.jp, nagatakenagatake@yahoo.co.jp

* Author for correspondence and reprint requests

Z. Naturforsch. **61c**, 792–798 (2006); received April 12/May 22, 2006

A soluble viscous protein was purified from yam (*Dioscorea opposita* Thunb.) tuber mucilage *tororo* by chromatographic steps, and its functional properties were estimated. The purified dioscorin having the molecular weight of about 200 kDa exhibited high scavenging activities against hydroxyl radicals (IC_{50} = 195.1 μg/ml) and superoxide anion radicals (IC_{50} = 92.7 μg/ml). Moreover, it showed extremely high angiotensin I-converting enzyme inhibitory activity (IC_{50} = 41.1 μg/ml). The results suggested that yam *D. opposita* tuber has a wide spectrum of strong antioxidative and antihypertensive activities and it could be utilized as a source of natural antioxidant.

Key words: Japanese Yam Tuber Mucilage, Viscous Protein, Functional Property

Introduction

Yam species is a member of the monocotyledonous family and belongs to the order Dioscoreaceae. Yams are cultivated all over the world and their number amounts to about six to seven hundred species (Satin, 1998). In China, yam tubers of *Dioscorea* spp. are widely consumed and are used as an ingredient of traditional herbal medicines. The Japanese have generally consumed yam tuber and yam as grated yam *"tororo"* is the main art of cooking in Japan. Although it is well-known that yam tuber mucilage *tororo* possesses distinctive properties such as viscosity, it is of great importance to characterize the viscous substance from it. However, there is few information about the functional properties of the viscous substance from yam tuber mucilage to date. The present study aims to purify the soluble viscous protein, dioscorin, from Japanese yam (*D. opposita* Thunb.) tuber mucilage and characterize the functional properties such as antioxidative activity, scavenging activities against active oxygen species, and angiotensin I-converting enzyme (ACE) inhibitory activity. It should be recognized as one of the health foods to prevent or improve lifestyle-related diseases such as cancer, diabetes, and hypertension by the effects of antioxidative activities, scavenging activities against active oxygen species, and antihypertensive activity.

Experimental

Samples

Fresh yam (*Dioscorea opposita* Thunb.) tubers were purchased from a local wholesale market (Abashiri, Hokkaido, Japan).

Preparation of soluble viscous protein from yam tuber mucilage tororo

After cleaning with water, yam tubers were peeled and immediately ground using a grater. After the extraction by gentle strring at 4 °C for 1 d, the viscous extracts were centrifuged at 50,000 × *g* for 1 h. The supernatants were brought to 40% solid ammonium sulfate saturation, and this solution was centrifuged at 28,500 × *g* for 30 min. The supernatants were pooled and applied to a Phenyl-Toyopearl 650M column (1.0 × 5.0 cm) previously equilibrated with 10 mM sodium phosphate buffer (pH 7.0) containing 1.8 M ammonium sulfate. The column was washed with the same buffer and the viscous protein was eluted with a linear gradient of 1.8–0 M ammonium sulfate in the same buffer (Fig. 1A). The pool of the viscous protein was dialyzed against 10 mM sodium phosphate buffer (pH 7.0) and this fraction was applied to a DEAE-Toyopearl 650M column (1.0 × 5.0 cm) equilibrated with the same buffer. The column was washed with the same buffer and the viscous pro-

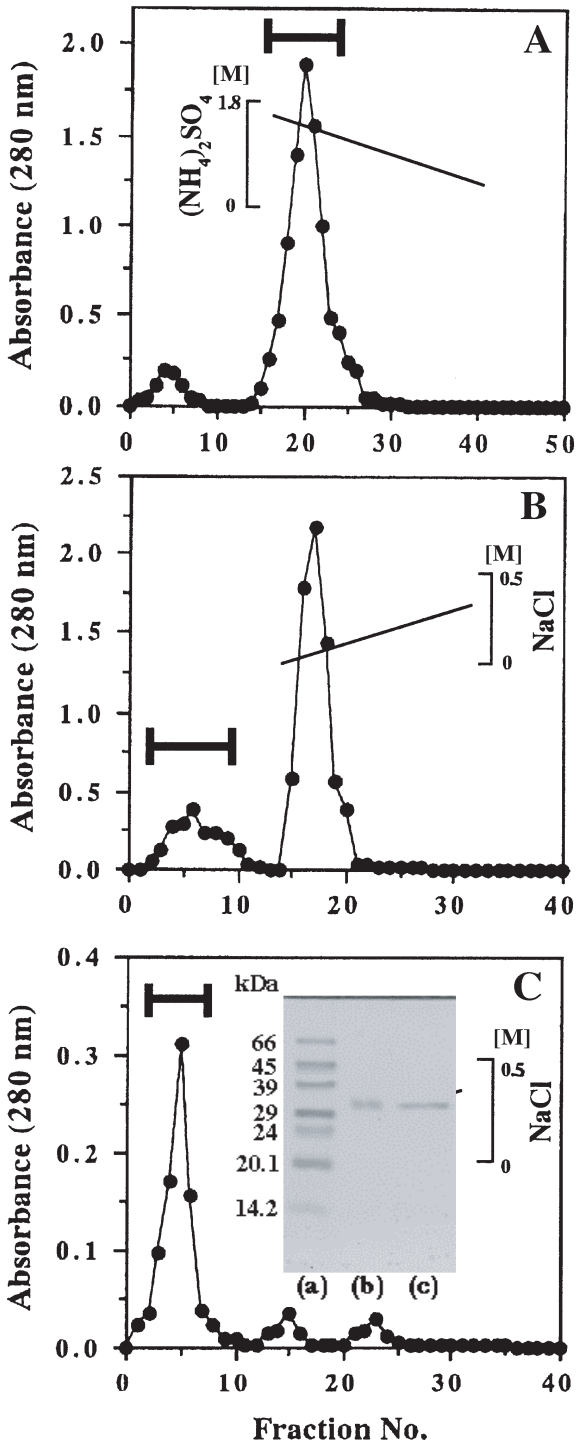

Fig. 1. Purification of the viscous protein from yam (*Dioscorea opposita* Thunb.) tuber mucilage *tororo*. (A) Phenyl-Toyopearl 650M column chromatography. The extracts (302.4 mg of protein) were put on a column (1.0 × 5.0 cm) equilibrated with 10 mM sodium phosphate buffer (pH 7.0) containing 1.8 M ammonium sulfate, and eluted with a linear gradient of 1.8–0 M ammonium sulfate in the same buffer at a flow rate of 1.0 ml/min. 2.5 ml fractions were collected. (B) DEAE-Toyopearl 650M column chromatography of the Phenyl-Toyopearl 650M fraction. The viscous solution (103.1 mg of protein) was applied on a column (1.0 × 5.0 cm) equilibrated with 10 mM sodium phoshate buffer (pH 7.0), and eluted with a linear gradient of 0–0.5 M NaCl in the same buffer at a flow rate of 1.0 ml/min. 2.0 ml fractions were collected. (C) CM-Toyopearl 650M column chromatography of the DEAE-Toyopearl 650M fraction. The eluate (17.8 mg of protein) was put on a column (1.0 × 3.0 cm) previously equilibrated with 10 mM sodium phosphate buffer (pH 6.0), and eluted with a linear gradient of 0–0.5 M NaCl in the same buffer at a flow rate of 1.0 ml/min. 2.0 ml fractions were collected. Insert: SDS-PAGE of molecular weight markers and purified viscous protein from yam (*D. opposita* Thunb.) tuber mucilage *tororo*. (a) Low molecular weight markers; (b) purified protein without reducing reagent; (c) purified protein with reducing reagent.

tein was eluated with a linear gradient of 0–0.5 M NaCl in the same buffer (Fig. 1B). After the sample was dialyzed against 10 mM sodium phosphate buffer (pH 6.0), the viscous solution was applied to a CM-Toyopearl 650 M column (1.0 × 3.0 cm) equilibrated with the same buffer. After washing with the same buffer, the viscous protein was eluated with a linear gradient of 0–0.5 M NaCl in the same buffer (Fig. 1C). The purified viscous protein was dialyzed against distilled water and used in the following tests.

Molecular weight determination

The molecular weight of the purified viscous protein was estimated by Toyopearl HW-55F (1.5 × 70 cm) gel filtration and polyacrylamide gel electrophoresis (Davis, 1964). Ferritin (450 kDa), catalase (240 kDa), aldolase (158 kDa), and albumin (68 kDa) were used as the standard markers for gel filtration and thyroglobulin (669 kDa), ferritin (440 kDa), catalase (232 kDa), lactate dehydrogenase (140 kDa), and albumin (66 kDa) were used as the standard markers for Native-PAGE. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method of Laemmli (1970) using a 12.5% gel. Myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase (97.4 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), bovine pancreas trypsinogen

(24 kDa), soybean trypsin inhibitor (20.1 kDa), and bovine milk α-lactoalbumin (14.2 kDa) were used as standards.

Protein concentration

Protein concentration was measured as by the method of Lowry *et al*. (1951) using bovine serum albumin as standard.

Glycoprotein staining

Glycoprotein was stained using periodic acid/Schiff staining.

Kinematic viscosity measurements

These were made on 5 ml sample solution in distilled water using a Cannon-Fenske type viscometer (SIBATA Scientific Technology Ltd., Tokyo, Japan) with a viscosity constant of 2.88×10^{-3} cSt/s. The kinematic viscosity of sample solution was obtained by measuring its viscosity at 25 °C The kinematic viscosity was calculated according to the following equation:

$$\nu = Ct = V/d, \quad (1)$$

where ν is the kinematic viscosity (in cSt), C the viscosity constant, t the flowing time (in s), V the viscosity (in cP: mPa·s), and d the density (in g/cm^3).

Antioxidative activity

The antioxidative activity was assayed as described by Nagai *et al*. (2005). 0.083 ml of sample solution and 0.208 ml of 0.2 M sodium phosphate buffer (pH 7.0) were mixed with 0.208 ml of 2.5% (w/v) linoleic acid in ethanol. The peroxidation was initiated by the addition of 20.8 μl of 0.1 M 2,2′-azobis(2-amidinopropane) dihydrochloride and carried out at 37 °C for 200 min in the dark. The degree of oxidation was measured for measuring peroxides by reading the absorbance at 500 nm after colouring with $FeCl_2$ and ammonium thiocyanate. Ascorbic acid (1 and 5 mM) and α-tocopherol (1 mM) were used as positive control. Distilled water was used as negative control.

Hydroxyl radical scavenging activity

2-Deoxyribose is oxidized by ·OH that was produced by the Fenton reaction and degraded to malonaldehyde. The reaction mixture contained 0.45 ml of 0.2 M sodium phosphate buffer (pH 7.0), 0.15 ml of 10 mM 2-deoxyribose, 0.15 ml of 10 mM $FeSO_4$ · ethylenediaminetetraacetic acid disodium salt (EDTA), 0.15 ml of 10 mM H_2O_2, 0.525 ml of H_2O, and 0.075 ml of sample solution in an Eppendorf tube. The reaction was started by the addition of H_2O_2. After incubation at 37 °C for 4 h, the reaction was stopped by adding 0.75 ml of 2.8% (w/v) trichloroacetic acid and 0.75 ml of 1.0% (w/v) TBA in 50 mM NaOH. The solution was boiled for 10 min, and then cooled in water. The absorbance of the solution was measured at 520 nm. Hydroxyl radical scavenging activity was evaluated as the inhibition rate of 2-deoxyribose oxidation by hydroxyl radicals (Chung *et al*., 1997). Ascorbic acid (1 and 5 mM) and α-tocopherol (1 mM) were used as positive control. Distilled water was used as negative control. Moreover, the IC_{50} value was defined as the concentration of inhibitor required to inhibit 50% of the hydroxyl radical scavenging activity.

Superoxide anion radical scavenging activity

Superoxide anion radical scavenging activity was evaluated as described by Nagai *et al*. (2005). The reaction mixture contained 0.48 ml of 0.05 M sodium carbonate buffer (pH 10.5), 0.02 ml of 3 mM xanthine, 0.02 ml of 3 mM (EDTA), 0.02 ml of 0.15% bovine serum albumin, 0.02 ml of 0.75 mM NBT, and 0.02 ml of sample solution. After preincubation at 25 °C for 10 min, the reaction was started by addition of 6 mU XOD and carried out at 25 °C for 20 min. After 20 min the reaction was stopped by addition of 0.02 ml 6 mM CuCl. The absorbance of the reaction mixture was measured at 560 nm and the inhibition rate was calculated by measuring the amount of formazan that was reduced from NBT by superoxide. Ascorbic acid (1 and 5 mM) and α-tocopherol (1 mM) were used as positive control. Distilled water was used as negative control. Moreover, the IC_{50} value was defined as the concentration of inhibitor required to inhibit 50% of the superoxide anion radical scavenging activity.

DPPH radical scavenging activity

DPPH radical scavenging activity was evaluated by the method of Okada and Okada (1998) with a slight modification. The assay mixture contained 0.1 ml of 1.0 mM DPPH radical solution in ethanol, 0.8 ml of 99% ethanol, and 0.1 ml of sample solution. The solution was rapidly mixed and after

30 min the scavenging activity was measured spectrophotometrically by the decrease in absorbance at 517 nm. Ascorbic acid (0.1 and 1.0 mM) and α-tocopherol (1 mM) were used as positive control. Distilled water was used as negative control.

ACE inhibitory activity

The ACE inhibitory activity was measured as descrived by Nagai *et al.* (2005). 25 μl of sample solution and 75 μl of 0.1 M sodium borate (pH 8.3) containing 5.83 mM hippuryl-L-histidyl-L-leucine and 1.0 M NaCl were preincubated at 37 °C for 5 min, and then incubated with 25 μl of 0.1 M sodium borate buffer (pH 8.3) containing 1 mU ACE and 1.0 M NaCl at 37 °C for 60 min. The reaction was stopped by the addition of 125 μl of 1.0 M HCl. The resulting hippuric acid was extracted with 750 μl of ethyl acetate by mixing for 15 s. After centrifugation at 6,000 rpm for 3 min, 500 μl of the upper layer were transported into the tube and evaporated at 40 °C for 2 h. The hippuric acid was dissolved in 500 μl of distilled water, and the absorbance was measured at 228 nm using a PerkinElmer model Lambda 11 (PerkinElmer, Tokyo, Japan) UV/VIS spectrometer. The IC_{50} value was defined as the concentration of inhibitor required to inhibit 50% of the ACE inhibitory activity.

Results and Discussion

Preparation of soluble viscous protein from yam tuber mucilage tororo

The purification of the viscous protein is summarized in Table I. Finally, the specific kinematic viscosity of 0.18 cSt/mg protein and 3.6-fold purification with a recovery rate of 12.4% compared with the crude extract were attained. The protein content of the purified viscous protein was about 0.90 mg/ml.

Molecular weight determination of purified protein

The molecular weight of the purified viscous protein was about 200 kDa, estimated by Native-PAGE and Toyopearl HW-55F gel filtration (Fig. 2). According to SDS-PAGE, purified viscous protein appeared as a single protein band of molecular weight of about 32 kDa, either without or with 2-mercaptoethanol (2-ME), respectively (Fig. 1C). This suggests that this protein may be a

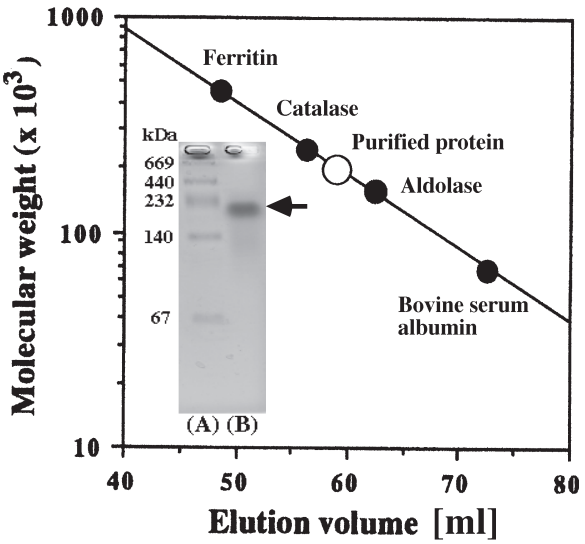

Fig. 2. Estimation of molecular weight of the purified viscous protein from yam (*D. opposita* Thunb.) tuber mucilage *tororo* by gel filtration on a Toyopearl HW-55F column (1.5 × 70 cm). The column was eluted with 10 mM sodium phosphate buffer (pH 6.0). The standard proteins used were as indicated. Insert: Nondenaturating polyacrylamide gel electrophoresis of the purified viscous protein from yam (*D. opposita* Thunb.) tuber mucilage *tororo*. (A) Molecular weight markers; (B) purified viscous protein.

Table I. Purification of the soluble viscous protein from yam tuber mucilage *tororo*.

Fraction	Volume [ml]	Total protein [mg]	Kinematic viscosity [cSt]	Specific kinematic viscosity [cSt/mg protein]	Yield[a] (%)	Purification[b] (fold)
Crude extract	160.0	302.4	15.3	0.05	100	1
Phenyl-Toyopearl 650M	21.0	103.1	3.1	0.03	20.3	0.6
DEAE-Toyopearl 650M	15.2	17.8	1.9	0.11	12.4	2.1
CM-Toyopearl 650M	11.6	10.4	1.9	0.18	12.4	3.6

[a] Yield against kinematic viscosity. [b] Purification against specific kinematic viscosity.

hexamer of identical subunits of molecular weight of about 32 kDa. Moreover, the purified viscous protein was glycoprotein which was stained using periodic acid/Schiff staining (data not shown). This suggests that this protein was dioscorin, the storage protein of yam (*D. opposita* Thunb.) tuber. It was reported that a major group of proteins accounted for about 85% of the total protein content of the tuber of *D. rotundata*, and the major protein comprised a number of isoforms with a molecular weight of about 31 kDa and was not glycosylated (Harvey and Boulter, 1983). The molecular weight of dioscorin from *D. opposita* tuber was similar to that of dioscorin from *D. rotundata* tuber, but the dioscorin from *D. opposita* was a glycoprotein different from that from *D. rotundata*. The dioscorin from *D. opposita* does not contain a disulfide bond having a single protein band of molecular weight of about 32 kDa, either without or with 2-ME. On the other hand, Harvey and Boulter (1983) reported that dioscorins usually contain a single disulfide bond, but Conlan *et al*. (1998) subsequently showed that the presence or absence of an intrachain disulfide bond could be used to discriminate between the major groups of components.

Functional properties of dioscorin from yam tuber mucilage

Antioxidative activity

The products of the lipid peroxidation (such as malondialdehyde) could cause damage to proteins and DNA. To evaluate the inhibition effects at the initiation stage of lipid peroxidation, the antioxidative activity was investigated *in vitro*. As a result, the antioxidative activity increased with increasing the content of sample solution (Table II).

Table II. Antioxidative activity of the soluble viscous protein from yam (*Dioscorea opposita* Thunb.) tuber mucilage *tororo*.

	Absorbance at 500 nm						
	Sample						
Time [min]	A	B	C	D	E	F	CN
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
50	0.18	0.16	0.09	0.02	0.02	0.01	0.38
100	0.60	0.34	0.19	0.14	0.03	0.03	0.72
200	0.74	0.43	0.35	0.47	0.09	0.03	1.41

A, 1% sample solution; B, 10% sample solution; C, 100% sample solution; D, 1 mM ascorbic acid; E, 5 mM ascorbic acid; F, 1 mM α-tocopherol; CN, control.

For 1% sample solution the activity was lower than that of 1 mM ascorbic acid. The activity for 10% sample solution was similar or slightly higher than that of 1 mM ascorbic acid. For 100% sample solution the activity was higher than that of 1 mM ascorbic acid, although it did not amount to those of 5 mM ascorbic acid and 1 mM α-tocopherol.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was investigated using the Fenton reaction system. For 1 and 10% sample solutions these activities were slightly higher than those of 1 and 5 mM ascorbic acid (Table III). Moreover, the sample solution for 100% showed extremely higher activity in comparison to 1 and 5 mM ascorbic acid, and the activity was much higher than that of 1 mM α-tocopherol. The activity tended to increase with an increasing degree of the content of the sample. The IC_{50} value was calculated as 195.1 μg/ml. The hydroxyl radicals are fearsomely reactive, and can be formed by transition of metal ion catalysis (iron, copper, as Fenton reaction). Our results suggest that this dioscorin may render the process of metal ion-dependent hydroxyl radical formation inactive or poorly active, thus, reduce the dangers of hydroxyl radicals toward the cells.

Table III. Hydroxyl radical, superoxide anion radical, and DPPH radical scavenging activities of the soluble viscous protein from yam (*Dioscorea opposita* Thunb.) tuber mucilage *tororo*.

	Scavenging activity (%)		
Sample[a]	Hydroxyl radical	Superoxide anion radical	DPPH radical
A	8.2	3.0	5.4
B	25.6	53.9	10.7
C	89.4	92.1	18.6
D	13.2	14.7	5.6[b]
E	16.1	89.9	94.7[c]
F	67.3	52.6	94.7
CN	0.0	0.0	0.0

[a] See sample nomenclature in Table II.
[b] 0.1 mM ascorbic acid.
[c] 1.0 mM ascorbic acid.

Superoxide anion radical scavenging activity

Superoxide anion radical scavenging activity of dioscorin was measured using the xanthine/xanthine oxidase system (NBT method). For 1% sample solution the activity was very low, about 3% (Table III). On the other hand, the 10% sample

solution exhibited higher activity, about 54%, and this activity was similar to that of 1 mM α-tocopherol. For 100% sample solution the activity was extremely high, about 92%, and was higher than that of 5 mM ascorbic acid. The scavenging activities of these sample solutions increased with an increasing degree of the sample content. The IC_{50} value was calculated as 92.7 μg/ml.

DPPH radical scavenging activity

DPPH radicals were widely used in the model system to investigate the scavenging activities of several natural compounds. As the DPPH radical is scavenged by antioxidants through the donation of a hydrogen atom, it forms the reduced DPPH-H. The colour changes from purple to yellow, and its absorbance at 517 nm decreases. DPPH radical scavenging activity was investigated and the results were indicated as relative activity against a negative control. Most of these sample solutions hardly exhibited DPPH radical scavenging activities (data not shown).

ACE inhibitory activity

ACE inhibitory activity of dioscorin was measured and the result was indicated as IC_{50} value. As a result, the activity was extremely high and was about 41.1 μg/ml.

It is known that yam tuber contains relatively high contents of vitamins B_1 (0.08–0.15 mg/100 g edible portion) and C (4–17 mg/100 g edible portion), different micro and macro elements such as potassium (430–590 mg/100 g edible portion), iron (0.4–0.8 mg/100 g edible portion), zinc (0.3–0.7 mg/100 g edible portion), and enzymes such as amylase, urease, oxidase. Moreover, yam tuber is rich in dietary fibers as water-soluble (0.2–0.7 g/100 g edible portion) and -insoluble (0.8–1.8 g/100 g edible portion) ones. For this reason it is considered that yam tuber is a more attractive food as well as natural one. The Japanese have consumed yam tuber as yam rice boiled with barley, grated yam soup, fried food, food in sweetened vinegar, Japanese sweets, food made from fish paste, and a thickener for buckwheat flour. It is believed from old times that sticky foods such as okra, *Abelmoschus esculentus* (L.) Moench, and taro, *Colocasis esculenta* (L.) Schott, as well as yam are beneficial to the health.

In the present study we tried to isolate and partially characterize dioscorin, the soluble viscous protein in representative Japanese yam (*D. opposita* Thunb.) tuber mucilage *tororo*. The purified dioscorin possessed high antioxidative activity and scavenging activities against active oxygen species such as a superoxide anion radical and hydroxyl radical. Moreover, dioscorin exhibited highest angiotensin I-converting enzyme inhibitory activity. It is reported that the mucilage contained in yam (*Dioscorea alata* Linn.) tubers is a mamnan-protein complex (Tsai and Tai, 1984). Recently, it is also reported that antioxidative activity is exhibited in compounds from Taiwan yams *D. alata* tubers (Chen *et al*., 2004), a freeze-dried powder of Chinese yam *D. alata* cv. Tainung No. 2 (Chang *et al*., 2004), mucilage (Hou *et al*., 2002) and dioscorin (Hou *et al*., 2001) from yam (*D. batatas* Decne). Moreover, Hsu *et al*. (2002) demonstrated that dioscorin of yam (*D. alata* cv. Tainong No. 1) and its peptic hydrolysates were capable of inhibiting ACE using *N*-[3-(2-furyl)acryloyl]-Phe-Gly-Gly (FAPGG) as substrate, that is a target for pharmacological agents used in the treatment of hypertension. Recently Lee *et al*. (2003) reported that the tuber mucilage of yam (*D. batatas* Decne) (YTM) was extracted and purified. This purified YTM appearing as a single protein band with molecular weight of above 250 kDa by SDS-PAGE inhibited ACE using FAPGG as substrate.

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

Chen P.-Y., Tu Y.-X., Wu C.-T., Jong T.-T., and Chang C.-M. J. (2004), Continuous hot pressurized solvent extraction of 1,1-diphenyl-2-picrylhydrazyl free radical scavenging compounds from Taiwan yams (*Dioscorea alata*). J. Agric. Food Chem. **52**, 1945–1949.

Chung S.-K., Osawa T., and Kawakishi S. (1997), Hydroxyl radical-scavenging effects of species and scavengers from brown mustard (*Brassica nigra*). Biosci. Biotechnol. Biochem. **61**, 118–123.

Conlan R. S., Griffiths L. A., Turner M., Fido R., Tatham A., Ainsworth C., and Shewry P. (1998), Characterisa-

tion of the yam tuber storage protein dioscorin. J. Plant Physiol. **153**, 25–31.

Davis B. J. (1964), Disk electrophoresis – II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. **121**, 404–427.

Harvey P. J. and Boulter D. (1983), Isolation and characterization of the storage protein of yam tubers (*Dioscorea rotundata*). Phytochemistry **22**, 1687–1693.

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

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

Hsu F.-L., Lin Y.-H., Lee M.-H., Lin C.-L., and Hou W.-C. (2002), Both dioscorin, the tuber storage protein of yam (*Dioscorea alata* cv. Tainong No. 1), and its peptic hydrolysates exhibited angiotensin converting enzyme inhibitory activities. J. Agric. Food Chem. **50**, 6109–6113.

Laemmli U. K. (1970), Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227**, 680–685.

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

Lowry O. H., Rosebrough N. J., Farr A. L., and Randall R. J. (1951), Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**, 265–275.

Nagai T., Nagashima T., Suzuki N., and Inoue R. (2005), Antioxidant activity and angiotensin I-converting enzyme inhibition by enzymatic hydrolysates from bee bread. Z. Naturforsch. **60c**, 133–138.

Okada Y. and Okada M. (1998), Scavenging effect of water soluble proteins in broad beans on free radicals and active oxygen species. J. Agric. Food Chem. **46**, 401–406.

Satin M. (1998), Functional properties of starches. Spotlight tropical starch misses market. AGSI report, Agriculture21, FAO-Magazine, p. 11.

Tsai S. S. and Tai F. J. (1984), Studies on the mucilage from tuber of yam (*Dioscorea alata* Linn.). I. Isolation and purification of the mucilage. J. Chinese Agric. Chem. Soc. **22**, 88–94.