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Effects of pH on the total phenolic compound, antioxidative ability and the stability of dioscorin of various yam cultivars

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Abstract

The purposes of this study were to investigate the antioxidative activity and the stability of dioscorin of various yam cultivars (Ming-chien (MC), Tainung No. 2 (TN2) and Keelung (KL)) under the influences of varying pH. Total phenolic contents from all yam varieties were the highest at pH 5, but gradually decreased with elevated pH. The DPPH-scavenging effects of all yam varieties under acidic pH environments were superior to those at other pH, and paralleled the trend of total phenolic content. The ferrous ion chelating capacity was found to be the highest for all yams at pH 8. No dioscorin protein could be extracted at pH 4. At pH 5–10, a single dioscorin protein band with molecular weight of 31 kDa was observed and appeared between the fraction numbers of 25–40. The relative protein surface hydrophobicity (RSo) of all yams was influenced by pH.

Keywords: Yam; Dioscorin; Antioxidative ability; Phenolic compound; SDS-PAGE; DPPH free radical scavenging effect; Surface hydrophobicity

1. Introduction

Chinese yam (*Dioscorea* spp.) is a very typical agricultural product in Taiwan and worldwide production reached 30 million tons in 1998 (Liu et al., 1999). Approximately 15 major different cultivars and 4 variables are cultivated in different regions in Taiwan. Chinese yam is a highly nutritional produce attributed to its functional components, such as mucin, dioscin, allantoin, choline, polyphenolases, vitamins and essential amino acids (Bhandari, Kasai, & Kawabata, 2003; Ingrid, Helen, & Ahmad, 1993; Omonigho & Ikenebomeh, 2000; Shewry, 2003). Bhandari and Kawabata (2004) found Nepal wild yam tubers to have significant antioxidant activities, as evaluated by DPPH free radical scavenging, ferrous ion chelating, reducing power, and total antioxidant activity, and consumption of fresh yam tubers for health benefit was thus recommended.

Yam is a major dietary source in certain African countries and Chinese yam is considerably consumed in Taiwan due to its nutritional values and unique taste. General compositions (dry-weight basis) of 75–84% starch, 6–8% crude protein and 1.2–1.8% crude fiber have been reported (Wanasundera & Ravindran, 1994). Yam also possesses certain specific functionalities. Dihydrodioscorine, alkaloidumentorin and dioscoretine extracted from *D. dumetorum* were reported to be hypoglycemic (Iwu et al., 1999). Diosgenin, a steroid sapogenin of yam, has been utilized to manufacture steroid hormones such as cortisone, estrogen and progesterone (Araghinkinam, Chung, White, Eskelson, & Watson, 1996). Diosgenin is transformed in the human intestine into serum dehydroepiandrosterone (DHEA) which is associated with reduced lipid peroxidation, lowered serum triglycerides and LDL and elevated HDL (Araghinkinam et al., 1996; McAnuff, Omoruyi, St. A. Morrison, & Asemota, 2002).

Dioscorin is the major storage protein of the yam tuber, and comprised of approximately 80–85% of total soluble proteins (Conlan et al., 1998; Harvey & Boulter, 1983).

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Dioscorin was also reported to have carbonic anhydrase, trypsin inhibitor, dehydroascorabte reductase, and monodehydroascorbate reductase activities (Hou & Lin, 1997; Hou, Chen, & Lin, 1999; Hou, Chen, & Lin, 2000). Purified dioscorin was reported to contain four subunits with molecular weight of 31 kDa and consisted of more than 98% of total proteins (Wanasundera & Ravindran, 1994). Dioscorin extracted from fresh yam (*D. batatas* Decne) was found to have similar DPPH free radical scavenging ability to glutathione at the same concentration (Hou et al., 2001). Recent research has also found that dioscorin might be beneficial in controlling high blood pressure and for scavenging DPPH and hydroxyl free radicals (Hsu, Lin, Lee, Lin, & Hou, 2002; Iwu et al., 1999). In addition, yam tuber mucilage was found to exhibit antioxidative ability (Hou, Hsu, & Lee, 2002).

Freeze-drying of various yam species (Tai-Nung No. 2; Ta-Shan; Ming-Chien) was found to yield higher antioxidative ability than hot-air drying and drum-drying (Hsu, Chen, Weng, & Tseng, 2003). Chinese yam is commonly consumed in various forms, mainly in raw, soup or powder. Our previous report (Chen & Lin, 2007) had demonstrated the functionality of yam and the stability of storage protein dioscorin from various species under different heating temperatures. However, characteristics of yams and the unique functionality of dioscorin pertaining to antioxidative ability under the influences of varying pH have not been documented yet. Characterizing the functionalities of yam tubers and their purified storage protein (dioscorin) from various species will encourage domestic agricultural and possibly pharmaceutical production. Therefore, this study was to evaluate the influences of pH on the antioxidative properties of yams and on the stability of the purified storage protein (dioscorin) from different species.

2. Materials and method

2.1. Materials

Mingchien (*Dioscorea alata* L. var. *purpurea*) and Tainung No. 2 (*Dioscorea alata* L. var. Tainung No. 2) were purchased from a farmer (Mingchien Shiang, Nantou County, Taiwan, ROC). Keelung yam (D. *japonica* Thunb. var. *pseudojaponica* (Hay.) Yamam) was purchased from the Rueyfang farmer association (Rueyfang Township, Taipei County, Taiwan, ROC). Upon delivery to the Department of Food and Nutrition, Providence University, all yams were stored in a cooler controlled at 16 °C for subsequent experiments.

Proximate compositions of raw yams were determined following the AOAC (1995) procedures. Total starch content was determined using the Megazyme test kit (Total Starch Assay Procedure, Megazyme International Ireland Ltd., Wicklow, Ireland).

2.2. Crude yam extract preparation

Yam from various species were first peeled and then sliced in 1 cm thickness. Approximately 100 g of yam slices were blended for 4 h in a cold room (4 °C) with citric acid-Na_2HPO_4 buffers adjusted to different pH (4–10) at 1:4 (W/V) ratios. Following centrifuging at 12,500*g* for 30 min (High-Speed Centrifuge, Avanti J-25, Beckman Coulter Inc., Palo Alto, CA, USA), the supernatant was obtained as a crude yam extract.

Analyses including total phenolic content, DPPH radical scavenging effect, ferrous ion chelating effect and protein content were performed on crude yam extract from different cultivars treated by varying pH buffers.

2.3. Total phenolic content

Total phenolic content of yams of different species was determined using the procedure of Kähkönen et al. (1999). Following mixing 0.2 ml of crude yam extract with 1 ml Folin-Ciocalteu's phenol reagent and 7.5% Na_2CO_3 (0.8 ml) at room temperature for 30 min, A_{765} was measured and expressed as gallic acid equivalent (μg gallic acid/g fresh yam weight).

2.4. DPPH radical scavenging effect

The procedure of (Yamaguchi, Takamura, Matoba, & Terao, 1998) for the determination of DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging effect was followed. Crude yam extract of 100 μl was mixed with 400 μl of 100 mM Tris–HCl (pH 7.4) and 500 μl of freshly prepared 80 mM DPPH ethanolic solution. After incubating in a dark place for 20 min, the absorbance at 517 nm was read. Solutions of 100 ppm of ascorbic acid, α-tocopherol, and BHA were used for comparison. Lower absorbance indicates better DPPH-scavenging ability of crude yam extract. The scavenging effect was calculated according to the following formula:

$$\text{DPPH} - \text{scavenging effect } (\%) = [1 - (\text{Sample OD}_{517}/\text{Blank OD}_{517})] \times 100\%$$

2.5. Ferrous ion chelating effect

The procedure of Decker and Weich (1990) was adopted for determining ferrous ion chelating effect of crude yam extract from different yam cultivars. Followed by mixing 100 μl crude yam extract with 925 μl of 50 mM phosphate buffer (pH 7.4) and 25 μl of 2 mM $FeCl_2$, the mixture was allowed to settle for 30 s. Fifty microliters of 5 mM ferrozine was added to the mixture and allowed to react for 10 min. The absorbance of the mixture was determined at 562 nm. Lower absorbance refers to better ferrous ion chelating effect. A solution of 200 ppm EDTA was used for comparison. The chelating effect was calculated as follows:

$$\text{Ferrous ion chelating effect } (\%) = [1 - (\text{Sample OD}_{562}/\text{Blank OD}_{562})] \times 100\%$$

2.6. Protein content

Protein concentration of crude yam extract was determined by the BCA (bicinchoninic acid) method according to the procedure of Pierce Protein Assay (Pierce Biotechnology Inc., Rockford, IL, USA).

2.7. Extraction and purification of dioscorin

The storage protein of yam (dioscorin) was extracted and purified following the procedures of (Hou et al., 1999) and Chen and Lin (2007). Initially, dioscorin protein was fractionated by precipitating crude yam extract in 45–75% ammonium sulfate. The protein precipitate was dissolved in 10 volumes (V/W) of 50 mM Tris–HCl buffer (pH 8.3) and dialyzed overnight (Regenerated Cellulose Tubular Membrane, Cellu.Sep T4 12,000–14,000 MW, Seguin, TX, USA.) to remove ammonium sulfate. The dialyzed protein solution was centrifuged (10,000*g*, 10 min) and the supernatant was collected for gel permeation chromatography. Protein concentration of the supernatant was determined.

Fractionated yam protein solution was mixed with approximately 6–10 ml of DEAE Sephadex A-25 ion exchange, and was shaken for 1 h. The precipitate was collected after centrifuging at 10,000*g* for 10 min and re-dissolved in 50 ml of 50 mM Tris–HCl (pH 8.3) buffer containing 150 mM NaCl. After 1 h shaking and centrifugation at 10,000*g* (10 min), the supernatant was obtained for gel permeation. Protein concentration of the supernatant was also determined.

The Sephadex™ G-75 column (C16/70, Amersham Pharmacia Biotech, Uppsala, Sweden) was first equilibrated by eluting 50 mM Tris–HCl (pH 8.3) at a flow rate of 27 ml/h (Peristaltic pump P-1, Amersham Pharmacia Biotech, Uppsala, Sweden). After protein solution was eluted into column, the eluting buffer was changed to 100 mM Tris–HCl, pH 7.9 (containing 100 mM NaCl) at the same flow rate. The purified dioscorin protein solution was collected every 3.6 ml for a total of 60 fractions. Individually collected fractions were subjected to 280 nm absorption for identifying protein existence. Only identified protein solution from individual fraction was subjected to SDS-PAGE for molecular weight determination. Individual protein fractions were passed through a 2 ml filter (Microcentrifuge Filters, Ultrafree-CL 10,000 MW, Millipore Corporation, Bedford, MA, USA.) and concentrated (4 °C, 4000×*g*, 45 min, Hettich Universal 16R, Tuttlingen, Germany) for protein molecular weight determination. A Mini-PROTEAN® apparatus (Electrophoresis Systems, Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used to conduct a standard SDS-PAGE experiment.

2.8. Protein surface hydrophobicity

All fractions containing dioscorin protein were combined and concentrated in microcentrifuge filters and an aliquot of concentrated protein solution was taken for protein surface hydrophobicity determination (Boyer, Joandel, Ouali, & Culioli, 1996; Galazka, Ledward, Sumner, & Dickinson, 1997). Concentrated dioscorin protein solution was re-dissolved in 100 mM Tris–HCl (pH 7.9, containing 100 mM NaCl) to the concentration range of 10–60 μg/ml. Two milliliters of dioscorin protein solution at varying concentrations were mixed with 10 μl of 5 mM ANSA (8-anilino-1-naphthalene-sulfonic acid) solution (in 0.05 M phosphate buffer). Following 20 min incubation in a dark place, the fluorescence reading was taken (Fluorophotometer, Model F-4500, Hitachi, Tokyo, Japan) using excitation wavelength at 385 nm and emission wavelength at 470 nm. Regression of fluorescence reading against protein concentration resulted in a straight line and the slope of the line was designated as protein surface hydrophobicity (So). The relative surface hydrophobicity (RSo) was calculated as the ratio of So of pH-treated sample to So of control. Control was set as the absorbance of pH 8 because of the pH value (8.3) of extraction medium Tris-buffer. Higher RSo reflects more protein hydrophobic bindings with fluorescent probe than the control and may indicate more protein denaturation.

2.9. Statistical analysis

Data collected were statistically analyzed as a completely randomized design of SAS and analysis of variance of GLM (general linear model). Mean comparisons for treatment effect at different heating temperatures were performed using the Duncan's multiple range test method for significant main effects at $P < 0.05$.

3. Results and discussion

Carbohydrate and crude protein contents of raw yams of different yam species ranged from 68.7–79.4% to 12.4–20.2% (dry-weight basis), respectively, as reported

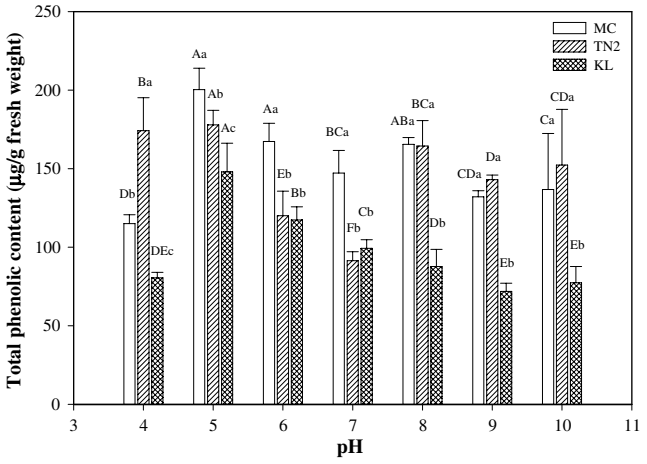

Fig. 1. Changes of total phenolic contents of different yam species treated by varying pH. $^{A–F}$Means (±SD) for the same yam at different pHs bearing unlike letters are different ($P < 0.05$). $^{a–c}$ Means (±SD) for yams at the same pH bearing unlike letters are different ($P < 0.05$).

previously (Chen & Lin, 2007). Total phenolic contents (μg/g, as gallic acid) of all yams extracted at varying pH are shown in Fig. 1. Total phenolic contents for all yams were the highest at pH 5 and the lowest at pH 4. MC and TN2 yams exhibited gradual decreasing trends with increasing pH in total phenolic content except for MC

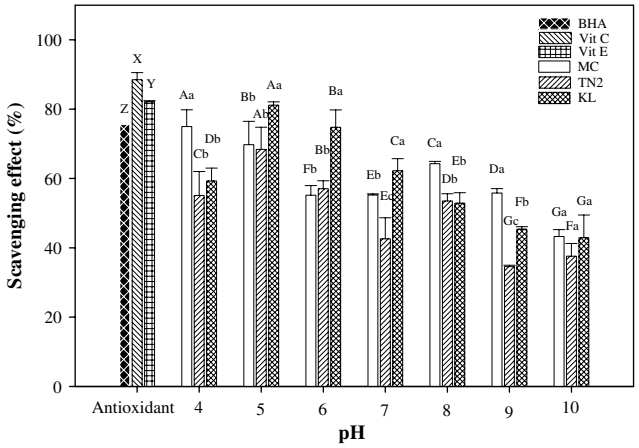

Fig. 2. Changes of DPPH radical scavenging effect of different yam species treated by varying pH. $^{A–G}$Means (±SD) for the same yam at different pHs bearing unlike letters are different ($P < 0.05$). $^{a–c}$Means (±SD) for yams at the same pH bearing unlike letters are different ($P < 0.05$). $^{X–Z}$Means (±SD) for antioxidants bearing unlike letters are different ($P < 0.05$).

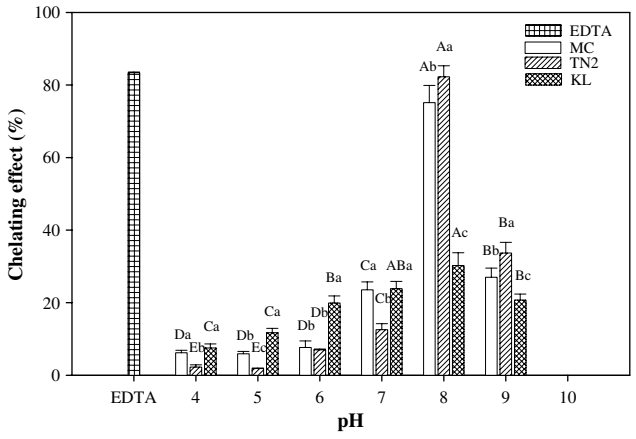

Fig. 3. Changes of chelating effect on ferrous ion of three yam species treated by varying pH. $^{A–E}$Means (±SD) for the same yam at different pHs bearing unlike letters are different ($P < 0.05$). $^{a–c}$ Means (±SD) for yams at the same pH bearing unlike letters are different ($P < 0.05$).

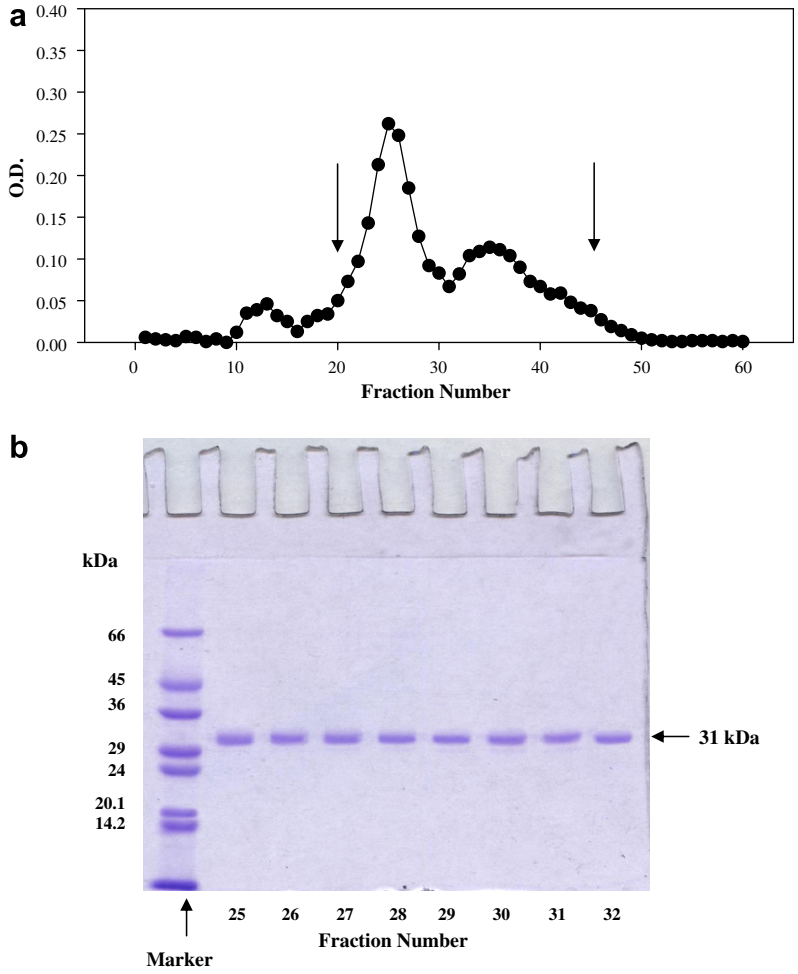

Fig. 4. (a) Gel filtration chromatogram (b) gel electrophoretogram of purified storage protein of Mingchien yam treated at pH 8. Arrows indicate the fraction range detected by 280 nm absorbance and collected for SDS-PAGE protein identification.

yam at pH 8. KL yam, on the other hand, was in a dual bell-shape pattern with two high peaks occurring at pH 5 and 8. Generally, MC yam contained the highest total phenolic content of the yams at the same pH medium. Therefore, extraction of phenolic compounds appeared to be pH dependent, which was in agreement with previous finding (Lemańska et al., 2001). Liu et al. (1999) reported higher total phenolic content for MC purple yam (*D. alata* L. var. *purpurea*) than other white yams, which was possibly due to high anthocyanin concentration. Anthocyanin pigments are relatively unstable but greatest stability is found under acidic environment (von Elbe & Schwartz, 1996).

Results of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging effect revealed that all yam species displayed different degree of DPPH-scavenging effects across all pH (Fig. 2). MC yam was shown to contain the highest DPPH-scavenging effect (74.96%) at pH 4, which was comparable to that of BHA (75.56%), and gradually dropped with increasing alkalinity. KL yam showed the highest DPPH-scavenging effect (81.15%) at pH 5 among all yams, which was similar to that of Vitamin E (88.5%), and then followed the same trend as MC yam. Results indicated better DPPH-scavenging effect at acidic environments for all yam species.

Yams of different species showed the highest ferrous ion chelating effect at pH 8 (Fig. 3), and ferrous ion chelating effect of TN2 (82.27%) being similar to 100 ppm EDTA (83.38%) was significantly higher than other yams, but gradually decreased with descending pH. Dioscorin of all yams have similar pIs ranging from 4.61 to 6.55 (Chen & Lin, 2007) resulting in more carboxylic groups for ferrous ion chelation at neutral to alkaline environments.

Results of gel filtration chromatography and SDS gel electrophoresis of MC yam extract treated by pH 8 buffer, and followed by ammonium sulfate precipitation and anionic exchange purification are shown in Fig. 4a and b. Results from 280 nm absorption indicated that protein was eluted between the fraction numbers of 20–45, and only one single protein band with a molecular weight of 31 kDa consistently appeared in each fraction. Similar outcomes were observed for TN2 and KL yam extracts (data not shown). These findings agreed with previous reports (Harvey & Boulter, 1983; Hou et al., 1999; Hou et al., 1999, 2001) for the molecular weight of storage protein,

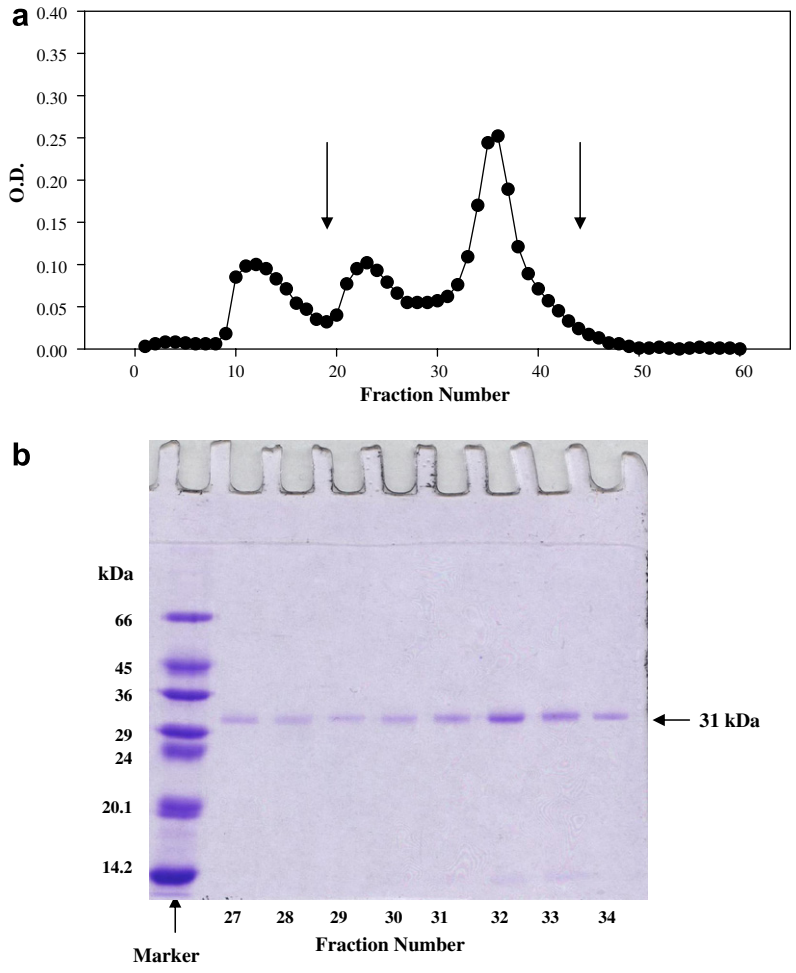

Fig. 5. (a) Gel filtration chromatogram (b) gel electrophoretogram of purified storage protein of Mingchien yam treated at pH 10. Arrows indicate the fraction range detected by 280 nm absorbance and collected for SDS-PAGE protein identification.

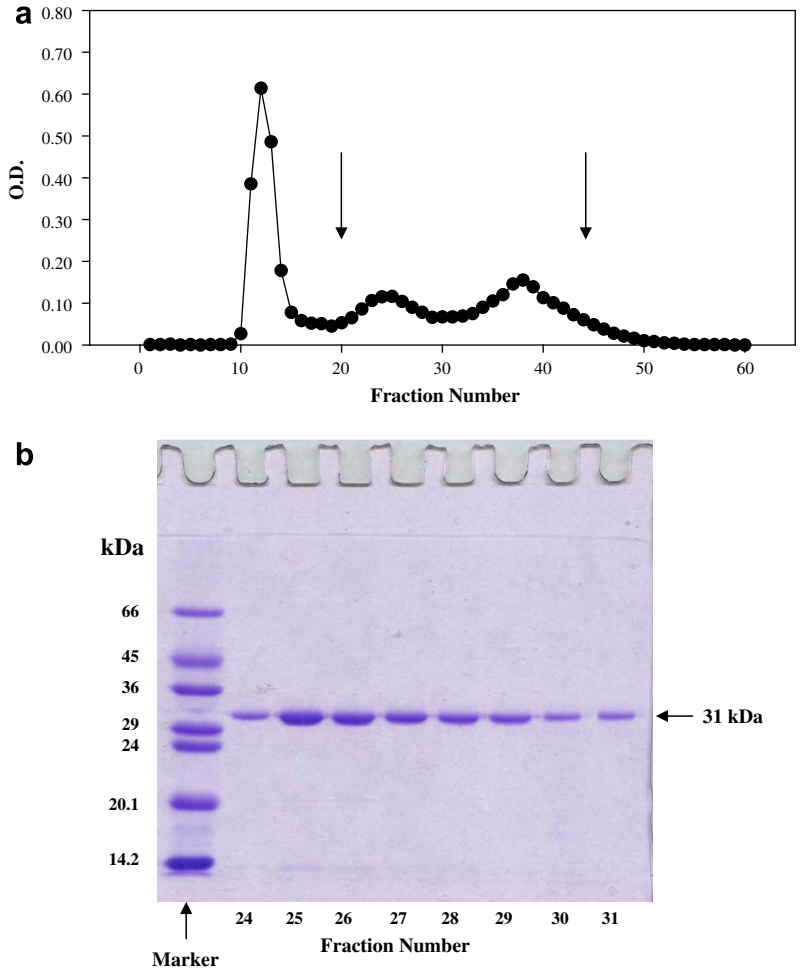

Fig. 6. (a) Gel filtration chromatogram (b) gel electrophoretogram of purified storage protein of Mingchien yam treated at pH 5. Arrows indicate the fraction range detected by 280 nm absorbance and collected for SDS-PAGE protein identification.

Table 1
Total protein contents of Keelung (KL) and Mingchien (MC) yams at individual purification steps treated by various pH

	pH						
	4	5	6	7	8	9	10
KL							
Crude extract[a]	$8.48^{E} \pm 0.35$	$15.01^{A} \pm 0.41$	$15.48^{A} \pm 1.33$	$13.12^{B} \pm 0.31$	$10.25^{D} \pm 0.26$	$13.07^{B} \pm 0.12$	$11.08^{C} \pm 0.19$
Ammonium sulfate fractionation[a]	$2.26^{D} \pm 0.10$	$6.92^{A} \pm 0.25$	$6.05^{B} \pm 0.51$	$5.56^{C} \pm 0.11$	$5.34^{C} \pm 0.12$	$6.09^{B} \pm 0.41$	$7.11^{A} \pm 0.68$
Ion exchange fractionation[b]	$46.44^{E} \pm 2.18$	$293.89^{A} \pm 3.48$	$175.67^{BC} \pm 18.35$	$154.33^{C} \pm 4.38$	$191.22^{B} \pm 24.65$	$319.78^{A} \pm 7.01$	$120.33^{D} \pm 89.09$
Gel filtration[b]	ND[c]	$240.10^{A} \pm 48.23$	$212.01^{A} \pm 27.82$	$137.49^{B} \pm 41.62$	$237.72^{A} \pm 15.23$	$150.32^{B} \pm 17.46$	$142.33^{B} \pm 22.44$
MC							
Crude extract[a]	$4.50^{E} \pm 0.23$	$14.83^{A} \pm 0.15$	$11.82^{C} \pm 0.19$	$10.62^{C} \pm 0.08$	$9.35^{D} \pm 0.16$	$12.33^{B} \pm 0.34$	$14.61^{A} \pm 0.54$
Ammonium sulfate fractionation[a]	$0.19^{F} \pm 0.15$	$2.98^{B} \pm 0.02$	$3.67^{A} \pm 0.13$	$2.25^{C} \pm 0.08$	$2.03^{D} \pm 0.05$	$1.29^{E} \pm 0.18$	$3.02^{B} \pm 0.33$
Ion exchange fractionation[b]	$86.66^{E} \pm 0.50$	$320.66^{A} \pm 29.50$	$155.11^{D} \pm 4.16$	$210.44^{B} \pm 17.00$	$199.33^{BC} \pm 10.28$	$143.00^{D} \pm 7.63$	$193.55^{BC} \pm 10.52$
Gel filtration[b]	ND[c]	$155.96^{C} \pm 61.26$	$179.29^{B} \pm 25.25$	$209.77^{A} \pm 49.49$	$178.69^{B} \pm 44.37$	$127.07^{D} \pm 19.46$	$134.00^{D} \pm 33.95$

[A–F] Means (±SD) within the same row bearing unlike letters are significantly different ($P < 0.05$).
[a] mg/g fresh yam.
[b] μg /g fresh yam.
[c] ND = Not detected.

and this protein was confirmed as dioscorin. Fraction number of MC yam treated by pH 10 buffer (Fig. 5a and b) was narrowed and the band intensity of dioscorin protein in each fraction lowered. Apparently, treating MC yam in pH 5 buffer resulted in low dioscorin protein extraction as exhibited by low optical absorption (Fig. 6a). Appearance of 31 kDa protein band and the patterns of fraction number were consistent for KL and TN2 yams (data not shown). Nevertheless, dioscorin protein could not be extracted and purified for all yams cultivars when they were treated in more acidic medium (pH 4) from the observation of no detectable optical absorption at 280 nm and no protein band in gel electrophoretograms (data not shown). The same tendency for KL and TN2 yams was noted.

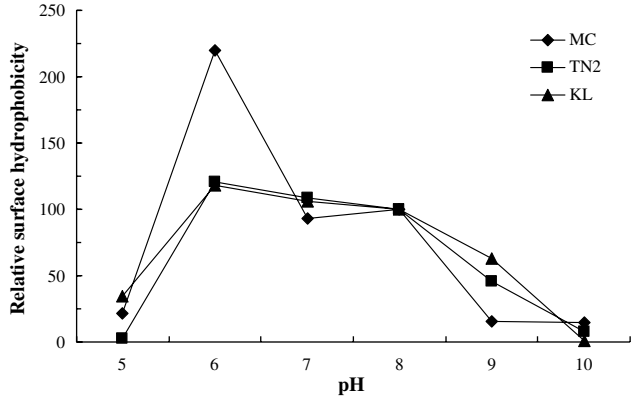

Fig. 7. Relative surface hydrophobicity curve of dioscorin of yams treated by varying pH. $^{A–C}$ Means (±SD) bearing unlike letters are significantly different ($P < 0.05$).

Protein concentrations of KL and MC yams during extraction and purification stages are shown in Table 1. All yams treated at high acidic medium (pH 4) resulted in lower protein concentrations at all purification stages; no protein (including dioscorin) was eluted and eventually disappeared in gel electrophoresis (data not shown). The same phenomena were noted for TN2 yam (data not shown). Generally, KL yam contained higher dioscorin concentrations at all pH than other yams.

Fig. 7 shows the relative surface hydrophobicity (RSo) of purified dioscorin protein from different yam species subjected to varying pH. No dioscorin protein was extracted at pH 4 and the relative surface hydrophobicity curve was established in the range of pH 5–10. The changes in relative surface hydrophobicity (RSo) of dioscorin protein displayed a similar pattern for all yam species, and RSo was the highest at pH 6 but declined with increasing pH. Decrease in RSo indicated less fluorescent probe bindings with protein hydrophobic groups and possibly that less denaturation occurred to dioscorin protein. Apparently, dioscorin protein was relatively stable in neutral to mild alkaline media as indicated by the results of protein extractability and surface hydrophobicity.

Pearson correlation coefficients (Table 2) showed negative correlation ($P < 0.01$) between pH and DPPH-free radical scavenging effect for all yam species, while pH was positively correlated ($P<0.05$) to ferrous ion chelating effect of MC and TN2 yams. Under the influence of pH, total phenolic content appeared to affect significantly ($P < 0.05$) the DPPH-scavenging effect of all yam varieties. Yen and Hung (2000) found that total phenolics of Hsiantsao (*Mesona procumbens* Hemsl.) decreased with increasing alkali concentrations, and a positive relationship

Table 2
Correlation coefficient of pH, phenol content, DPPH scavenging, chelating effect and dioscorin content of different yam species

	pH	Phenol content	DPPH scavenging	Chelating effect	Dioscorin content
MC					
pH	1				
Phenol content	−0.0715	1			
DPPH scavenging	−0.7044**	0.6337*	1		
Chelating effect	0.2648*	0.1882	0.1283	1	
Dioscorin content	0.3468*	0.4353	−0.3744	0.2770	1
TN2					
pH	1				
Phenol content	−0.2501*	1			
DPPH scavenging	−0.7364**	0.4420*	1		
Chelating effect	0.3447*	0.0050	−0.1059	1	
Dioscorin content	0.1763	−0.1511	0.1964	0.5281*	1
KL					
pH	1				
Phenol content	−0.5105**	1			
DPPH scavenging	−0.6851**	0.8477**	1		
Chelating effect	0.0411	0.0389	−0.0106	1	
Dioscorin content	0.2383	0.5144	0.2911*	0.4191*	1

* Significant at $P < 0.05$.
** Significant at $P < 0.01$.

between the DPPH-scavenging effect and phenolic content was observed ($r = 0.73$).

Many researchers have shown specific functionalities of yams and the storage protein dioscorin. The present study further demonstrated the antioxidative ability to be pH-dependent and species-dependent as well. Specific yams behaved differently in their antioxidative ability. Current results appeared to be very useful for consumers and processors in light of antioxidative ability of yams and the properties of dioscorin. In terms of profitability, yam species with good antioxidative ability should gain more attention from farmers and processors and be bred, cultivated, and processed.

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References

AOAC (1995). Cereal foods. *Official methods of analysis of AOAC international* (16th ed.). Washington, DC: AOAC International.

Araghinkinam, M., Chung, S., White, T. N., Eskelson, C., & Watson, R. R. (1996). Antioxidant activity of dioscorea and dehydroepiandrosterone (DHEA) in older humans. *Life Sciences, 59*(11), 147–157.

Bhandari, M. R., Kasai, T., & Kawabata, J. (2003). Nutritional evaluation of wild yam (*Dioscorea* spp.) tubers of Nepal. *Food Chemistry, 82*(4), 619–623.

Bhandari, M. R., & Kawabata, J. (2004). Organic acid, phenolic content and antioxidant activity of wild yam (*Dioscorea* spp.) tubers of Nepal. *Food Chemistry, 88*(2), 163–168.

Boyer, C., Joandel, S., Ouali, A., & Culioli, J. (1996). Determination of surface hydrophobicity of fast and slow myosins from rabbit skeletal muscles: implication in heat-induced gelation. *Journal of the Science and Food Agriculture, 72*(3), 367–375.

Chen, Y. T., & Lin, K. W. (2007). Effects of heating temperature on the total phenolic compound, antioxidative ability and the stability of dioscorin of various yam cultivars. *Food Chemistry, 101*(3), 955–963.

Conlan, S., Griffiths, L. A., Turner, M., Fido, R., Tatham, A., Ainsworth, C., et al. (1998). Characterisation of the yam tuber storage protein dioscorin. *Journal of Plant Physiology, 153*(1), 25–31.

Decker, E. A., & Weich, B. (1990). Role of ferritin as a lipid oxidation catalyst in muscle food. *Journal of Agricultural and Food Chemistry, 38*(3), 674–677.

Galazka, V. B., Ledward, D. A., Sumner, I. G., & Dickinson, E. (1997). Influence of high pressure on bovine serum albumin and its complex with dextran sulfate. *Journal of Agricultural and Food Chemistry, 45*(9), 3465–3471.

Harvey, P. J., & Boulter, D. (1983). Isolation and characterization of the storage protein of yam tubers (*Dioscorea rotundata*). *Photochemistry, 22*(8), 1687–1693.

Hou, W. C., Chen, H. J., & Lin, H. Y. (1999). Dioscorins, the major tuber storage proteins of yam (*Dioscorea batatas* Decne), with dehydroascorbate reductase and monodehydoascorbate reductase activities. *Plant Science, 149*(2), 151–156.

Hou, W. C., Chen, H. J., & Lin, H. Y. (2000). Dioscorins from different *Dioscorea* species all exhibit both carbonic anhydrase and trypsin inhibitor activities. *Botany Bulletin of Academia Sinica, 41*, 191–196.

Hou, W. C., Hsu, F. L., & Lee, M. H. (2002). Yam (*Dioscorea batatas*) tuber mucilage exhibited antioxidant activities *in vitro*. *Planta Medica, 68*(12), 1072–1076.

Hou, W. C., Lee, M. H., Chen, H. J., Liang, W. L., Han, C. H., Liu, Y. W., et al. (2001). Antioxidant activities of dioscorin, the storage protein of yam (*Dioscorea batatas* Decne) tuber. *Journal of Agricultural and Food Chemistry, 49*(10), 4956–4960.

Hou, W. C., & Lin, H. Y. (1997). Dehydroascorbate reductase and monodehydroascorbate reductase activities of trypsin inhibitors, the major sweet potato (*Ipomoea batatas* [L.] Lam) root storage protein. *Plant Science, 128*(2), 151–158.

Hou, W. C., Liu, J. S., Chen, H. J., Chen, T. E., Chang, C. F., & Lin, Y. W. (1999). Dioscorin, the major tuber storage protein of yam (*Dioscorea batatas* Decne) with carbonic anhydrase and trypsin inhibitor activities. *Journal of Agricultural and Food Chemistry, 47*(5), 2168–2172.

Hsu, C. L., Chen, W., Weng, Y. M., & Tseng, C. Y. (2003). Chemical composition, physical properties, and antioxidant activities of yam flours as affected by different drying methods. *Food Chemistry, 83*(1), 85–92.

Hsu, F. L., Lin, Y. H., Lee, M. H., Lin, C. L., & 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. *Journal of Agricultural and Food Chemistry, 50*(21), 6109–6113.

Ingrid, I. M., Helen, N. A., & Ahmad, M. H. (1993). Biochemical composition and storage of Japanican yams (*Dioscorea* spp.). *Journal of the Science of Food and Agriculture, 62*(3), 219–224.

Iwu, M. M., Okunji, C. O., Ohiaeri, G. O., Akah, P., Corley, D., & Tempesta, M. S. (1999). Hypoglycaemic activity of dioscoretine from tubers of *Dioscrea dumetorum* in normal and alloxan diabetic rabbits. *Planta Medica, 56*(3), 264–267.

Kähkönen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S., et al. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry, 47*(10), 3954–3962.

Lemańska, K., Szymusiak, H., Tyrakowska, B., Zieliński, R., Soffers, A. E. M. F., & Rietjeans, I. M. C. M. (2001). The influence of pH on antioxidant properties and the mechanism of antioxidant action of hydroxyflavones. *Free Radical Biology and Medicine, 31*(7), 868–881.

Liu, S. Y., Chang, T. W., Lin, Y. K., Chen, S. F., Wang, J. Y., Zu, G. L., et al. (1999). Studies on the varietal characters, production potential, phytochemical properties, and antioxidant effect of *Dioscorea* spp. *Journal of Agricultural Research of China, 48*(4), 1–22.

McAnuff, M. A., Omoruyi, F. O., St. A. Morrison, E. Y., & Asemota, H. N. (2002). Plasma and liver distributions in streptozotocin-induced diabetic rats fed sapogenin extract of the Jamaican bitter yam (*Dioscorea polygonoides*). *Nutrition Research, 22*, 1427–1434.

Omonigho, S. E., & Ikenebomeh, M. J. (2000). Effect of temperature treatment on the chemical composition of pounded white yam during storage. *Food Chemistry, 71*(2), 215–220.

Shewry, P. R. (2003). Tuber storage proteins. *Annals Botany, 91*(1), 1–15.

von Elbe, J. H., & Schwartz, S. J. (1996). Colorants. In O. W. Fennema (Ed.), *Food Chemistry* (3rd ed., pp. 651–722). New York: Marcel Dekker, Inc.

Wanasundera, P. K. J. P. D., & Ravindran, G. (1994). Nutritional assessment of yam (*Dioscorea lata*) tubers. *Plant Foods for Human Nutrition, 46*, 33–39.

Yamaguchi, T., Takamura, H., Matoba, T., & Terao, J. (1998). HPLC method for evaluation of the free radical-scavenging activity by foods by using 1,1-diphenyl-2-picrylhydrazyl. *Bioscience, Biotechnology, and Biochemistry, 62*(6), 1201–1204.

Yen, G. C., & Hung, C. Y. (2000). Effects of alkaline and heat treatment on antioxidative activity and total phenolics of extracts from Hsian-tsao (*Mesona procumbens* Hemsl.). *Food Research International, 33*(6), 487–492.