Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 101 (2007) 955–963
Food Chemistry
www.elsevier.com/locate/foodchem

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

Yi-Tzu Chen, Kuo-Wei Lin *

Department of Food and Nutrition, Providence University, 200 Chungchi Road, Shalu Town 43301, Taichung County, Taiwan, ROC

Received 3 November 2005; received in revised form 18 January 2006; accepted 27 February 2006

Abstract

The purposes of this study were to investigate the effects of different heating temperatures on the antioxidative activity of yams and on the stability of dioscorin of various yam cultivars (Mingchien, Tainung No. 2 and Keelung). Results from crude yam extracts of all varieties showed decreases in total phenolic and dioscorin contents, DPPH radical-scavenging effect, and ferrous ion chelating capacity with increasing heating temperatures. Following separation and purification, only one protein band with the same molecular weight of 31 kDa (dioscorin) was consistently electrophoresized from three peaks of 25–45 fraction numbers from all yam varieties. Fraction numbers of peaks were reduced and peak area narrowed with increasing heating temperatures and no protein could be extracted at temperatures above 80 °C. The dioscorin protein surface hydrophobicity declined with increasing heating temperatures. Significant correlation of dioscorin content with DPPH·-scavenging effect was noted for all yam species.

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 produce in Taiwan and worldwide production reached 30 million tons in 1998 (Liu et al., 1999). Approximately 15 major different cultivars and four variables are cultivated in different regions of Taiwan. Chinese yam is highly nutritional, attributed to its functional components, such as mucin, dioscin, allantoin, choline, polyphenolases and essential amino acids (Araghinkinam, Chung, White, Eskelson, & Watson, 1996; Bhandari, Kasai, & Kawabata, 2003; Ingrid, Helen, & Ahmad, 1993; Shewry, 2003).

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 fibre 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 et al., 1996). Diosgenin is transformed in 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, Morrison, & Asemota, 2002).

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

* Corresponding author. Tel.: +886 4 26328001x15030/15322; fax: +886 4 26530027.
E-mail address: kwlin@pu.edu.tw (K.-W. Lin).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.02.045

1983). Dioscorin was also reported to have carbonic anhydrase, trypsin inhibitor, dehydroascorbate reductase, and monodehydroascorbate reductase activities (Hou, Chen, & Lin, 1999; Hou, Chen, & Lin, 2000; Hou & Lin, 1997). Purified dioscorin was reported to contain 4 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 a similar DPPH free radical-scavenging ability to glutathione at the same concentration (Hou et al., 2001). Recent researches have also found that dioscorin might be beneficial in controlling high blood pressure and scavenging DPPH and hydroxyl free radicals (Hsu, Lin, Lee, Lin, & Hou, 2002; Iwu et al., 1999).

Freeze-drying of various yam species (Tai-Nung No. 2; Ta-Shan; Ming-Chien) was found to cause higher antioxidative ability than did hot-air drying or drum-drying (Hsu, Chen, Weng, & Tseng, 2003). Chinese yam is commonly consumed in various forms, mainly raw, or as soup or powder. However, functionality of yam and the stability of storage protein dioscorin from various species under different heating temperatures is not yet well documented. Characterizing the functionalities of storage protein (dioscorin) of yam tuber roots from various species will encourage domestic agricultural and possibly pharmaceutical production. Therefore, this study was to evaluate the influences of heating temperatures on the antioxidative properties of yams and on the stability of the purified storage protein (dioscorin) from different species.

2. Materials and methods

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 a farmer association (Rueyfang Town, Taipei County, Taiwan, ROC). 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 modified AACC (1976) with a Megazyme test kit (Total Starch Assay Procedure, Megazyme International Ireland Ltd., Wicklow, Ireland).

2.2. Crude yam extract preparation

Yams from various species were first peeled and then sliced to 1 cm thickness. Approximately 100 g portions of yam slices were heated in 1 l of water at 50, 60, 70, 80, 90, and 100 °C for 10 min. Yams were drained, cooled, weighed, and crude yam extract was prepared, following the modified procedure of Hou, Liu et al. (1999). Raw and heated yams were blended with 50 mM Tris–HCl (pH 8.3) at 1:4 (w/v) for 90 s (at 22,000 rpm). The mixture was transferred to a cold room (4 °C) and stirred at low speed for 4 h. 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 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 heated at different temperatures.

2.3. Total phenolic content

Total phenolic contents of yams of different species were determined using the procedure of Kähkönen et al. (1999). Following mixing of 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 equivalents (μg gallic acid/g fresh yam weight).

2.4. DPPH radical-scavenging effect

The procedure of Yamaguchi, Takamura, Matoba, and Terao (1998) for the determination of DPPH free radical-scavenging effect was followed. Crude yam extract (100 μl), for comparing with 100 ppm ascorbic acid, α-tocopherol and BHT, was individually 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 measured. Solutions of 100 ppm of ascorbic acid, α-tocopherol, and BHA were used for comparison. A lower absorbance indicates a better DPPH·-scavenging ability of crude yam extract. The scavenging effect was calculated according to the following formula:

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

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. Following mixing of 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 settled for 30 s. Fifty microlitres 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 of EDTA was used for comparison. The chelating effect was calculated as follows:

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

Table 1
Proximate compositions of raw yam from various species

	Wet-weight			Dry-weight		
	MC[a]	TN2[a]	KL[a]	MC	TN2	KL
Moisture (%)	$73.42^{A} \pm 0.27$	$68.54^{C} \pm 0.51$	$71.03^{B} \pm 0.31$			
Protein (%)	$5.36^{B} \pm 0.09$	$7.28^{A} \pm 0.12$	$3.60^{C} \pm 0.02$	$20.2^{B} \pm 0.37$	$23.1^{A} \pm 0.38$	$12.4^{C} \pm 0.07$
Fat (%)	$0.25^{A} \pm 0.03$	$0.23^{A} \pm 0.03$	$0.21^{A} \pm 0.04$	$0.92^{A} \pm 0.11$	$0.72^{B} \pm 0.09$	$0.73^{B} \pm 0.14$
Ash (%)	$1.73^{A} \pm 0.02$	$1.76^{A} \pm 0.03$	$0.84^{B} \pm 0.03$	$6.51^{A} \pm 0.09$	$5.60^{B} \pm 0.10$	$2.89^{C} \pm 0.11$
Carbohydrate (%)	$19.7^{C} \pm 0.24$	$21.6^{B} \pm 0.99$	$23.0^{A} \pm 0.37$	$73.9^{B} \pm 0.92$	$68.7^{C} \pm 3.16$	$79.4^{A} \pm 1.29$

[A–C]Means (±SD) in the same row for similar weight basis bearing different letters are significantly different ($P < 0.05$).
[a] MC, Mingchien; TN2, Tainung No. 2; KL, Keelung.

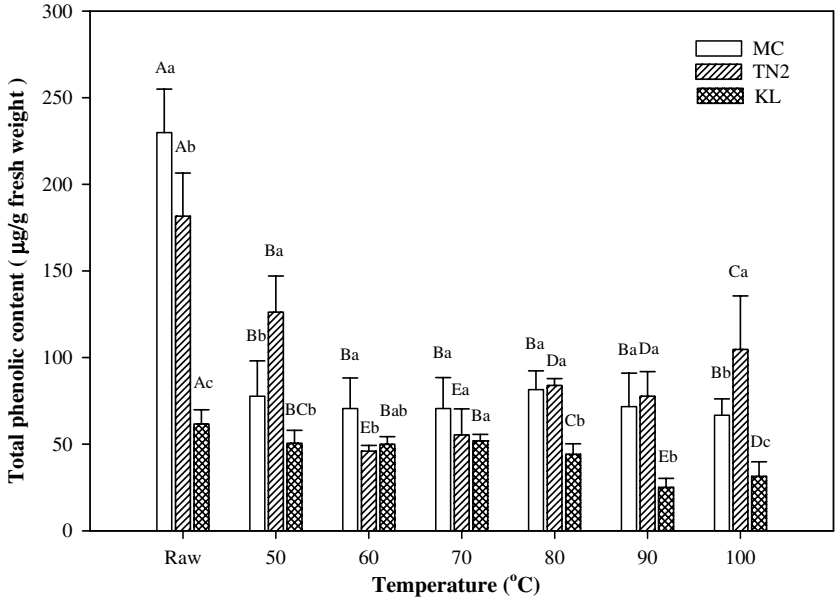

Fig. 1. Changes of total phenolic contents of different yam species prepared by different cooking temperatures. [A–E]Means (±SD) for the same yam at different temperatures bearing unlike letters are different ($P < 0.05$). [a–c]Means (±SD) for yams at the same temperature bearing unlike letters are different ($P < 0.05$).

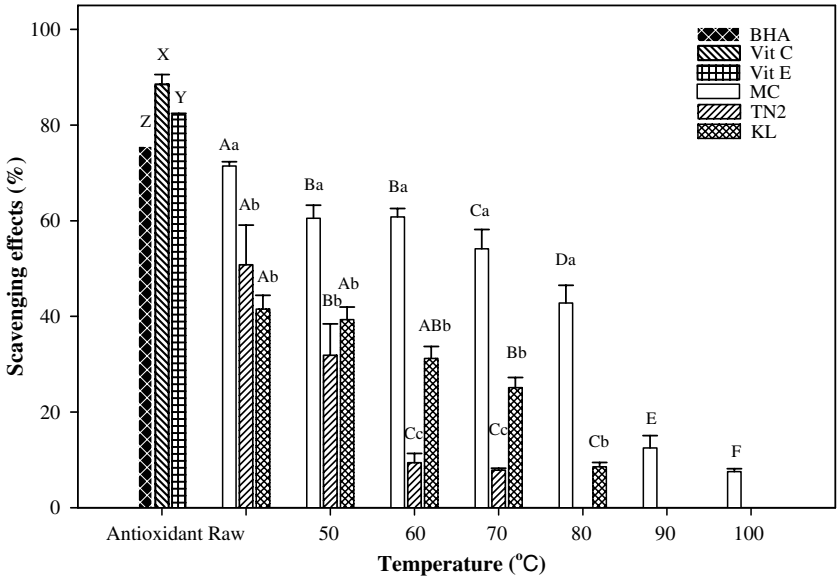

Fig. 2. Changes of DPPH radical-scavenging effect of different yam species prepared by different cooking temperatures. [A–F]Means (±SD) for the same yam at different temperatures bearing unlike letters are different ($P < 0.05$). [X–Z]Means (±SD) for the antioxidants bearing unlike letters are different ($P < 0.05$). [a–c] Means (±SD) for yams at the same temperature bearing unlike letters are different ($P < 0.05$).

2.6. Protein content

Protein concentrations of crude yam extract of varying yam species were determined by the bicinchoninic acid (BCA) method according to the procedure of the 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, Liu et al. (1999) with modification. 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 of 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 the supernatant protein solution was eluted from the 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 fraction were subjected to 280 nm absorbance measurement for identifying protein existence. Only identified protein solutions from

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

	Temp.	Phenol content	DPPH·-scavenging	Chelating effect
MC				
Temp.	1			
Phenol content	−0.6364	1		
DPPH·-scavenging	−0.9456***	0.5096	1	
Chelating effect	−0.6793	0.9958**	0.5360	1
Dioscorin content	−0.9904***	0.6419	0.9535**	0.6785
TN2				
Temp.	1			
Phenol content	−0.4812	1		
DPPH·-scavenging	−0.8851**	0.8157*	1	
Chelating effect	−0.9743***	0.4513	0.8783*	1
Dioscorin content	−0.9563***	0.5175	0.9039**	0.9878***
KL				
Temp.	1			
Phenol content	−0.8980**	1		
DPPH·-scavenging	−0.9755***	0.9108**	1	
Chelating effect	−0.7823*	0.6863	0.7007	1
Dioscorin content	−0.9189**	0.8825**	0.9177**	0.8617*

* Significant at $P < 0.05$.
** Significant at $P < 0.01$.
*** Significant at $P < 0.001$.

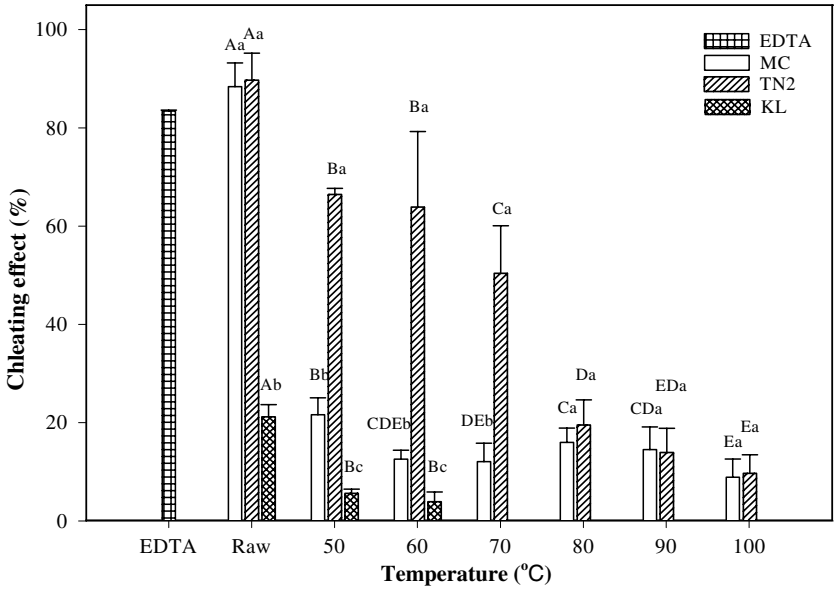

Fig. 3. Changes of ferrous ion-chelating effect of different yam species prepared by different cooking temperatures. $^{A–E}$Means (±SD) for the same yam at different temperatures bearing unlike letters are different ($P < 0.05$). $^{a–c}$Means (±SD) for yams at the same temperature bearing unlike letters are different ($P < 0.05$).

individual fractions were subjected to SDS–PAGE for molecular weight determination. Individual protein fractions were placed in 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® III 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 for protein surface hydrophobicity determination (Boyer, Joandel, Ouali, & Culioli, 1996; Galazka, Ledward, Sumner, & Dickinson, 1997). Concentrated dioscorin protein solutions were re-dissolved in 100 mM Tris–HCl (pH 7.9, containing 100 mM NaCl) to the concentration range of 10–60 μg/ml. Two millilitres 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 of incubation in a dark place, the fluorescence value was determined (Fluorophotometer, Model F-4500, Hitachi, Tokyo, Japan), using an excitation wavelength of 385 nm and emission wavelength of 470 nm. Regression of fluorescence value against protein concentration resulted in a straight line and the slope of the line is designated as protein surface hydrophobicity (So). The relative surface hydrophobicity (RSo) is calculated as the ratio of So of heated sample to So of raw sample. A higher RSo reflects more protein hydrophobic bindings with fluorescent probes than the control (raw) and indicates more possible protein denaturation.

2.9. Statistical analysis

Data collected were statistically analyzed by a completely randomized design of SAS (1988) and analysis of variance of general linear model (GLM). Mean compari-

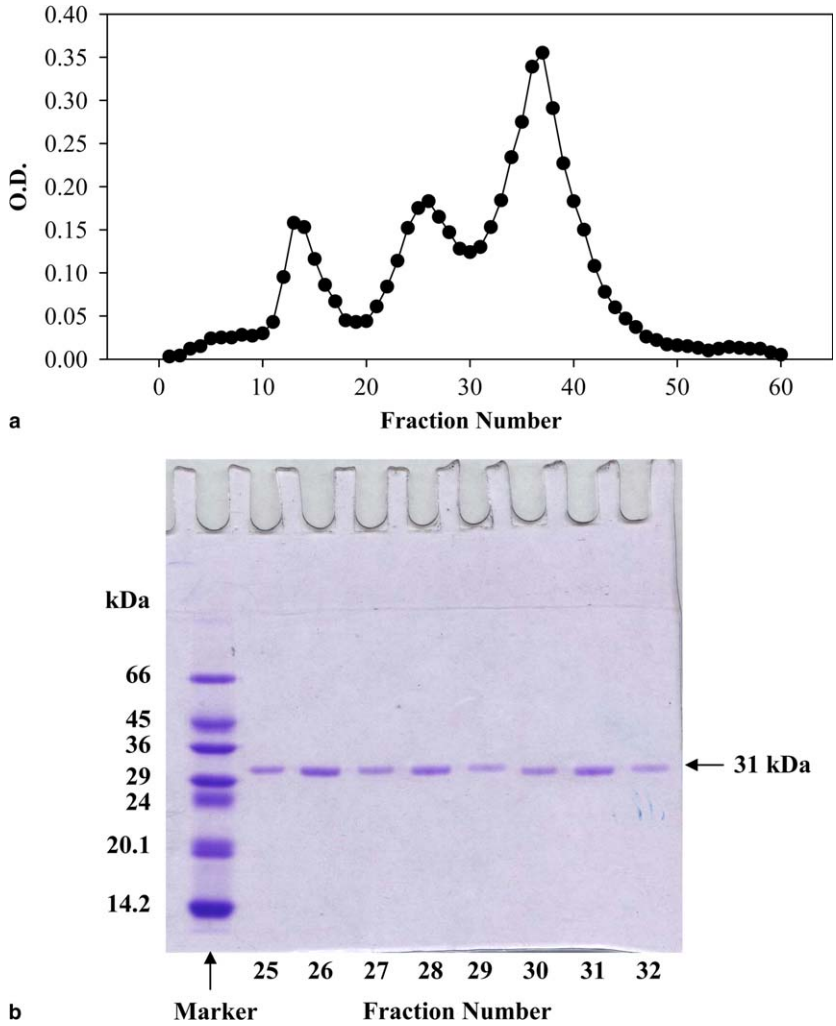

Fig. 4. (a) Gel filtration chromatogram (b) gel electrophoretogram of purified storage protein of fresh Mingchien yam. Arrows indicate the fraction range detected by 280 nm absorbance and collected for SDS–PAGE protein identification.

sons for treatment effects at different heating temperatures were performed using Duncan's multiple range test for significant main effects at $P < 0.05$.

3. Results and discussion

Carbohydrate and crude protein contents of raw yams of different species ranged from 68.7% to 79.4% and 12.4% to 20.2% (dry-weight basis), respectively (Table 1). Raw yams contained higher total phenolic contents than did cooked yams (see Fig. 1). Raw Mingchien (MC) yam had highest total phenolic contents among all yams, but showed the greatest decline, followed by cooking at 50 °C and statistically they were unchanged at higher cooking temperatures. On the other hand, raw Keelung (KL) yam remained the lowest, regardless of temperature effects. All treatments showed a decreasing pattern with increasing cooking temperatures, while total phenolic compounds of Tainung No. 2 (TN2) yams rose at temperature above 70 °C.

Results of 1,1-diphenyl-2- picrylhydrazyl (DPPH) free radical-scavenging effect revealed that DPPH·-scavenging effects significantly declined with increasing cooking temperatures (see Fig. 2). Raw yams of all species had the highest DPPH·-scavenging effects, but they were all lower than commercial antioxidants. DPPH·-scavenging effect of MC yams appeared to be more resistant to heating temperatures, while those of TN2 and KL yams disappeared above 70 and 80 °C, respectively. Hsu et al. (2003) reported higher DPPH free radical-scavenging effect for MC yam than for Tai-Nung No. 2 and Ta-Shan yams under the same drying conditions. Heating temperature resulted in a significant decline in ferrous ion-chelating capacity among various yams (see Fig. 3). Both raw MC and TN2 yams had chelating capacities comparable to 200 ppm EDTA, while KL yam lost its chelating effect above 60 °C. At higher heating temperatures, TN2 and KL yams paralleled in ferrous ion-chelating capacity. MC yam appeared to have higher antioxidative ability than TN2 and KL yams.

Pearson correlation coefficients between antioxidative ability, dioscorin content and heating temperature for different yam cultivars are shown in Table 2. Regardless of yam cultivars, temperature had negative impacts on phenolic compound, dioscorin content and antioxidative ability. Highly significant correlation of DPPH·-scavenging effect

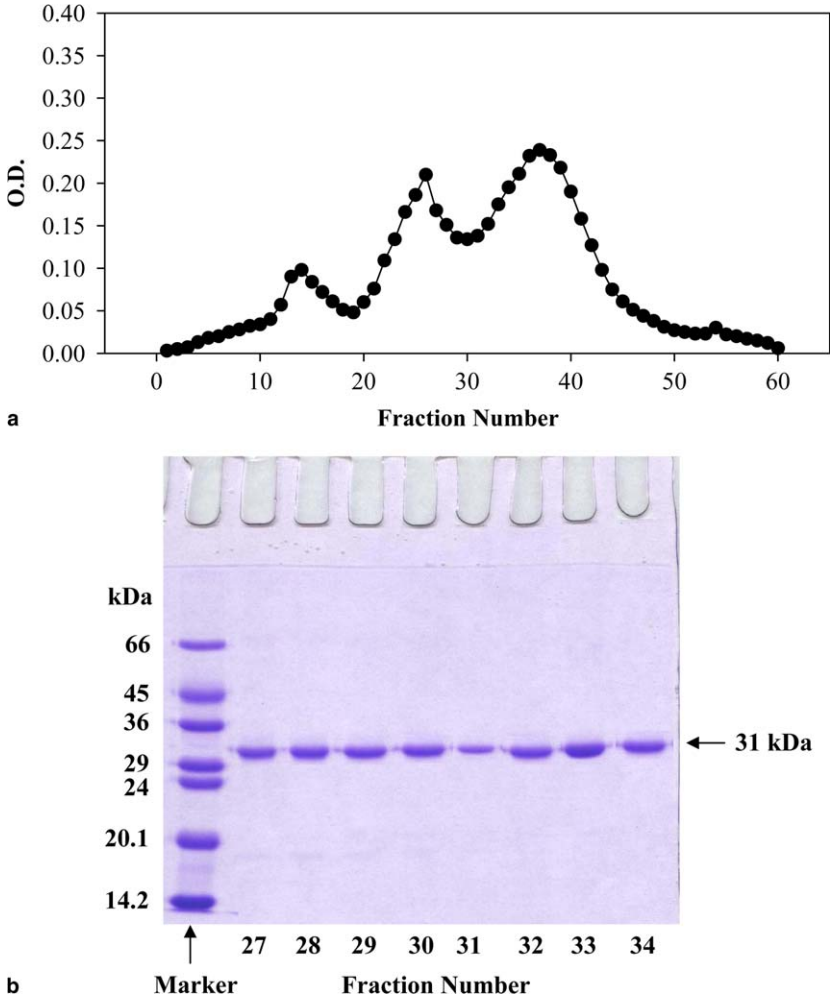

Fig. 5. (a) Gel filtration chromatogram (b) gel electrophoretogram of purified storage protein of water-cooked (50 °C) Mingchien yam. Arrows indicate the fraction range detected by 280 nm absorbance and collected for SDS–PAGE protein identification.

with dioscorin content was noted for all yam species. Total phenolic content and dioscorin content of TN2 and KL yams were highly correlated with DPPH free radical-scavenging effect and ferrous ion-chelating effect.

The gel filtration chromatogram and gel electrophoretogram of fresh, unheated MC yam extract are shown in Figs. 4a and b, respectively. Results from the 280 nm absorbance indicated that protein was eluted between fractions 25 and 45, and only one single protein band with a molecular weight of 31 kDa consistently appeared in each fraction. Similar results were observed for TN2 and KL yam extracts (data not shown). These findings accorded with previous reports (Harvey & Boulter, 1983; Hou, Liu et al., 1999; Hou et al., 2001) for the molecular weight of storage protein, and this protein band was confirmed as dioscorin. Five subunits of dioscorin protein were found using the isoelectric focussing procedure and the isoelectric points of individual dioscorin subunit from various yams were between 4.61 and 6.55 (Chen, 2005). These results were slightly different from previous reports of 5.2–6.8 (Wanasundera & Ravindran, 1994) and 5.68–6.8 (Conlan et al., 1998). However, these differences could possibly be due to the species, cultivation conditions, and harvest periods (Liu et al., 1999).

Heating MC yam at 50 °C resulted in few changes with regard to fraction number (see Fig. 5a), although declining protein concentration (as reflected by the absorbance area) was observed. The intensity and molecular weight of dioscorin appeared to be unchanged under mild heating (see Fig. 5b). Heating at 60 °C caused a significant drop in protein solubility, resulting in a narrow fraction range and lower optical density (see Fig. 6a). Dioscorin protein could only be eluted in the range of 35–45 fraction numbers when MC yam was heated to 80 °C (see Fig. 7a), and the intensity of dioscorin protein was hardly detectable (see Fig. 7b). Continued heating to higher temperatures ($\geqslant$90 °C) caused complete denaturation of dioscorin and disappearance of dioscorin in the column chromatogram and gel electrophoretogram (data not shown). Similar tendencies were found for TN2 and KL yams, except that dioscorin proteins from both yam species were not extractable at temperatures above 80 °C (data not shown).

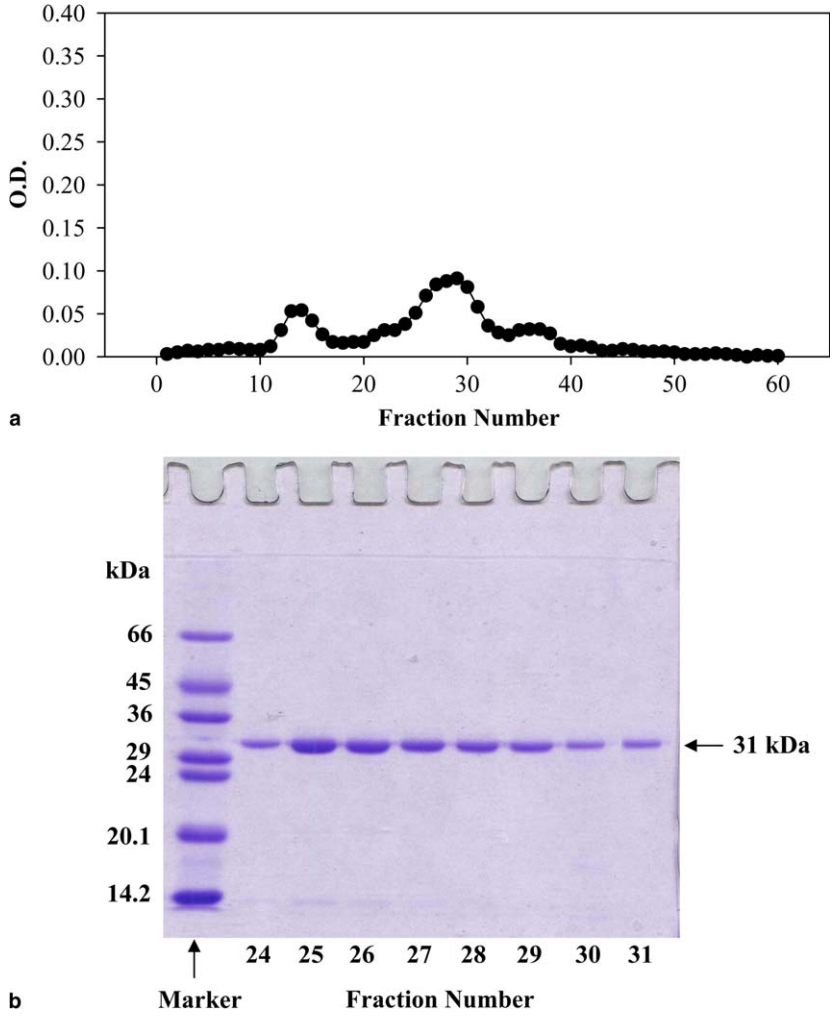

Fig. 6. (a) Gel filtration chromatogram (b) gel electrophoretogram of purified storage protein of water-cooked (60 °C) Mingchien yam. Arrows indicate the fraction range detected by 280 nm absorbance and collected for SDS–PAGE protein identification.

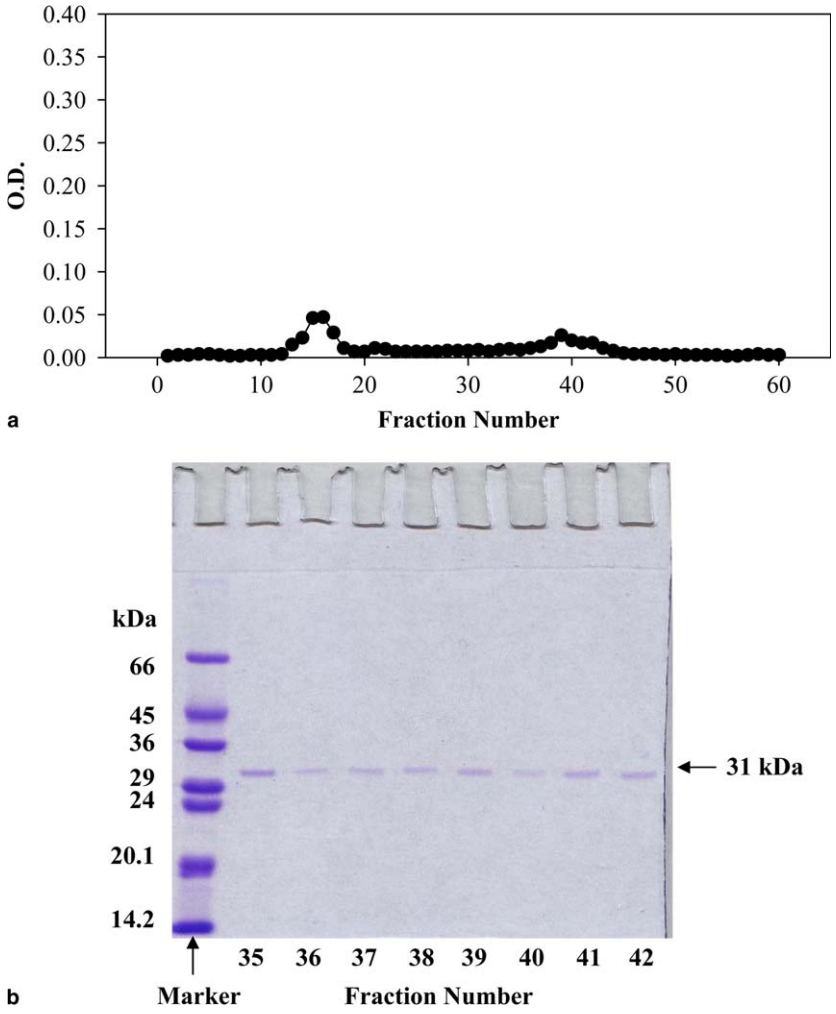

Fig. 7. (a) Gel filtration chromatogram (b) gel electrophoretogram of purified storage protein of water-cooked (80 °C) Mingchien yam. Arrows indicate the fraction range detected by 280 nm absorbance and collected for SDS–PAGE protein identification.

Fig. 8 shows the relative surface hydrophobicity (RSo) of dioscorin protein from different yam species heated to various temperatures. Dioscorin protein showed a decreasing pattern in RSo with increased heating temperatures. MC yam had the lowest RSo at any heating temperature, indicating less fluorescent probe binding with protein hydrophobic groups and possible less denaturation of dioscorin protein.

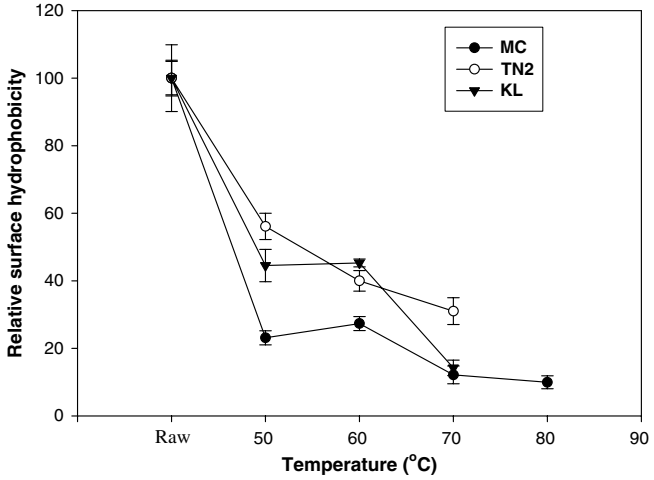

Fig. 8. Relative surface hydrophobicity curve of dioscorin of different yam cooked at different temperatures.

Acknowledgements

This research was funded in part by the National Science Council (Project No. NSC 91-2313-B-126-007), Executive Yuan, Taiwan, ROC and appreciation is acknowledged. Authors are indebted to Prof. C.T. Chang, Department of Food and Nutrition, Providence University, for his technical consultation.

References

AACC. (1976). *AACC method 76-11*. Approved methods of the American Association of Cereal Chemists. St. Paul, MN.

AOAC. (1995). Cereal foods. In B. Doris (Ed.), *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.

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. (2005). Effects of heating temperature and pH on the antioxidative properties and the stability of dioscorin of various yam cultivars. MS thesis, Department of Food and Nutrition, Providence University, Taichung County, Taiwan, ROC.

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 monodehydroascorbate 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., 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.

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.

SAS. (1988). The GLM procedure. In *SAS/STAT® User's guide*. Edition Release 6.03 (pp. 549–640). Cary, NC: SAS Institute, Inc.

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

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.