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ScienceDirect
Food Chemistry 110 (2008) 670–677
FOOD CHEMISTRY
www.elsevier.com/locate/foodchem

Effects of different storage conditions on steroidal saponins in yam (*Dioscorea pseudojaponica* Yamamoto) tubers

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Received 4 December 2007; received in revised form 15 February 2008; accepted 18 February 2008

Abstract

Effect of storage on steroidal saponins, furostanol and spirostanol glycosides, in yam (*D. pseudojaponica* Yamamoto) tubers was determined. Unpeeled and vacuum-sealed peeled tubers were stored at −18, 4, 17 and 25 °C from 5 to 80 days separately. Furostanol glycosides in unpeeled tubers could be converted by furostanol glycoside 26-*O*-*β*-glucosidase (F26G) to spirostanol glycosides after 4 °C storage for 35 days (chilling injury could be found), or 17 and 25 °C storage for 50 days. The conversion increased with storage times. Peeled tubers stored at 17 and 25 °C for 5 days could experience organoleptic injury, which would enlarge with increasing storage period. After 35 days of storage, large part of vacuum-sealed tubers transformed to juice with stench. In the early 20 days storage, saponins in these tubers were lost rapidly. F26G activity in decreasing order was 25 °C > 17 °C > 4 °C and could be inhibited under vacuum.

Keywords: Furostanol glycoside; Furostanol glycoside 26-*O*-*β*-glucosidase; Spirostanol glycoside; Steroidal saponin; Yam (*Dioscorea* spp.); Storage

1. Introduction

Yam (*Dioscorea* spp.) is one of the principal staple foods in numerous tropical countries (Hariprakash & Nambisan, 1996). It is also widely used in traditional Chinese medicine to promote health and longevity (Liu, Wang, Shyu, & Song, 1995). Because of its health profit, yam has been a popular food in Taiwan for many years. Steroidal saponins, furostanol and spirostanol glycosides, exist in many kinds of yams, for instance, *Dioscorea floribunda* (Hoyer, Sucrow, & Winkler, 1975), *D. composita* (Espejo, Campos, Jung, & Giral, 1982), *D. zingiberensis* (Tang & Jiang, 1987), *D. olfersiana* (Haraguchi, Zaccharias, Young, & Chu, 1994), *D. colletti* var. *hypoglauca* (Hu, Dong, Yao, Kobayashi, & Iwasaki, 1996; Hu, Yao, Kobayashi, & Iwasaki, 1997), *D. pseudojaponica* Yamamoto (Yang, Lu, & Hwang, 2003a) and *D. polygonoides* (Osorio et al., 2005). They were considered to be the major functional compounds in the crops (Hu et al., 1996, 1997; Liu et al., 1995). Many literatures reported that steroidal saponins had notable anti-carcinogenic (Hu, Lin, Liu, & Yang, 2007), anti-thrombotic (Zhang et al., 1999), anti-viral (Aquino et al., 1991), hemolytic (Zhang et al., 1999), hypocholesterolemic (Sauvaire, Ribes, Baccou, & Loubatieŕes-Mariani, 1991) and hypoglycemic (Kato, Miura, & Fukunaga, 1995) capacities. The aglycon part (sapogenin) of the yam steroidal saponins namely diosgenin has been used as starting material for semi-synthesis of steroidal hormones such as progesterone and testosterone (Chen & Wu, 1994).

Afoakwa and Sefa-Dedeh (2001) investigated chemical compositions and quality changes occurring in trifoliate yam (*D. dumetorum* pax) tubers after harvesting. The harvested tubers were stored under cold room condition (4 °C) and tropical ambient condition (28 °C) for 24–72 h. Their results showed that moisture content decreased about 6–10%, starch levels declined from 70.5 to 66.5 g/100 g, and sugar and fibre contents slightly increased within 72 h after harvesting. Moreover, textural properties, namely hardness

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0308-8146/$ - see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2008.02.061

and adhesiveness, also increased considerably during storage. Under the same storage conditions, Afoakwa and Sefa-Dedeh (2002) pointed out that storage could cause decreases in the rheological properties (paste characteristics) of the starch in trifoliate yam tuber within 36 h of harvesting; similarly, α- and β-amylase activities in the tubers more than doubled within 24 h after harvesting. Samples stored at 4 °C showed smaller changes in both rheological properties and amylase activities than those stored at 28 °C in all experiments. Medoua, Mbome, Agbor-Egbe, and Mbofung (2005) investigated the effect of storage under tropical ambient conditions (19–28 °C, RH 60 ~ 85%) for 56 days on the physicochemical characteristics of flours produced from trifoliate yam tubers, and found that water absorption capacity, oil absorption capacity, water-soluble index, hydrophilic–lipophilic index, swelling capacity and least gelatinizing concentration were significantly influenced by tuber storage time.

Dinan, Harmatha, and Lafont (2001) reported that the age, the cultivar or the geographic locality of a plant could significantly affect its saponin content. The storage condition after plant harvesting might influence the saponin level as well. There was, however, no thorough report concerning the effects of various storage conditions on saponins in yam. Yang et al. (2003a) determined three furostanol glycosides and three spirostanol glycosides in *D. pseudojaponica* Yamamoto, which is the Taiwanese native yam cultivar. Wang and Liu (1992) reported that the quality of yam tubers could be maintained well for 26 weeks when they were stored at 17 °C.

In general, there are two selling forms of yam tubers in the Taiwanese market, whole tubers (at ambient temperature, 25 °C under atmosphere condition) and vacuum-sealed peeled tubers (at 4 or −18 °C). In this study, we investigated the changes of steroidal saponins in yam (*D. pseudojaponica* Yamamoto) tubers under different storage conditions. The unpeeled tubers (under atmosphere condition) and vacuum-sealed peeled tubers were stored at −18, 4, 17 and 25 °C from 5 to 80 days, respectively.

2. Materials and methods

2.1. Yam samples and their preparation

Yam (*D. pseudojaponica* Yamamoto) tubers (white flesh and cortex), cylindrical shape (ca. 4 cm in diameter and ~120 cm long), were randomly harvested (matured) on January 15, 2007 from a farm in Keelung City, Taiwan and divided into two main groups. All tubers were cleaned with distilled water, wiped with sterilized dry cloth and then cut into sticks of 25 cm long and ca. 4 cm in diameter. The sticks in each group were also randomly divided into 87 portions of about 300 g each. A total of 84 portions was used for storage (21 portions of the tuber sticks were randomly sampled for each storage condition) while the other 3 portions were used for control. One group of tuber sticks was further peeled and vacuum-sealed (using polyethylene bags) with a vacuum packaging machine (model: TH-250, Dah Yeou Industrial Co., Ltd., Taichung, Taiwan). The other group was preserved as whole tuber sticks in a normal atmosphere. The cortex was about 2.2% of total tuber weight. After these tubers were stored at −18, 4, 17 and 25 °C for 5, 10, 20, 35, 50, 65 and 80 days separately, 3 portions in each condition were took out randomly and cut the tuber sticks into 4 mm thick slices using a Salad Shooter (National Presto Industries, Eau Claire, WI, USA) (the sticks with cortices were peeled in advance). These slices were lyophilized with a freeze-dryer (Vastech Scientific Co., Ltd., Taipei, Taiwan) and ground to 40 mesh or below for saponin analysis.

2.2. Chemicals and materials

Three furostanol glycosides including 26-*O*-β-D-glucopyranosyl 3β, 22, 26-trihydroxy-(25*R*)-furost-5-ene-3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-{[α-L-rhamnopyranosyl-(1→4)]-*O*-[α-L-rhamnopyranosyl-(1→4)]}-β-D-glucopyranoside (**1**) (MW = 1194), protodioscin (**2**) (MW = 1048) and protogracillin (**3**) (MW = 1064) were obtained through heating 26-*O*-β-D-glucopyranosyl-22α-methoxyl-(25*R*)-furost-5-en-3β, 26-diol-3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-{[α-L-rhamnopyranosyl-(1→4)]-*O*-[α-L-rhamnopyranosyl-(1→4)]}-β-D-glucopyranoside (**4**) (MW = 1208), methyl protodioscin (**5**) (MW = 1062) and methyl protogracillin (**6**) (MW = 1078) in 30% aqueous acetone at 95 °C for 4 h, separately (these solutions were then concentrated to dryness) with the method of Matsurra, Ushiroguchi, Itakura, and Fuwa (1989). Compounds **4**~**6** (furostanol glycosides), and three spirostanol glycosides including (25*R*)-spirost-5-en-3β-ol 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*- {[α-L-rhamnopyranosy-(1→4)]-*O*-[α-L-rhamnopyranosyl-(1→4)]}-β-D-glucopyranoside (**7**) (MW = 1014), dioscin (**8**) (MW = 868) and gracillin (**9**) (MW = 884), were isolated from the yam tubers with our previous reported method (Yang et al., 2003a). Diosgenin standard was purchased from Sigma (St. Louis, MO, USA). Fig. 1 shows the structures of these steroidal saponins and diosgenins. Methanol and *n*-butanol were obtained from Tedia Co. (Fairfield, OH, USA). Deionized water was prepared by Ultrapure™ water purification system (Lotun Co., LTD. Taipei, Taiwan). Crude furostanol glycoside 26-*O*-β-glucosidase (F26G) was prepared from the fresh yam tuber using the method reported by Inoue and Ebizuka (1996).

2.3. Hydrolysis of furostanol glycosides with yam crude F26G in the model system

The method was based on that reported by Inoue and Ebizuka (1996). Sample vials (25 ml) contained 10 mL of 200 μM compound **1**, **2** or **3** (prepared with 50 mM acetate buffer solution, pH 5.0) were added 3 mL of crude F26G followed by reaction at 4, 17 and 25 °C for 1, 2, 3 and 5 days, respectively. These reactions progressed under atmosphere and vacuum (with vacuum sealing) conditions separately. In order to reduce the interference of dissolved

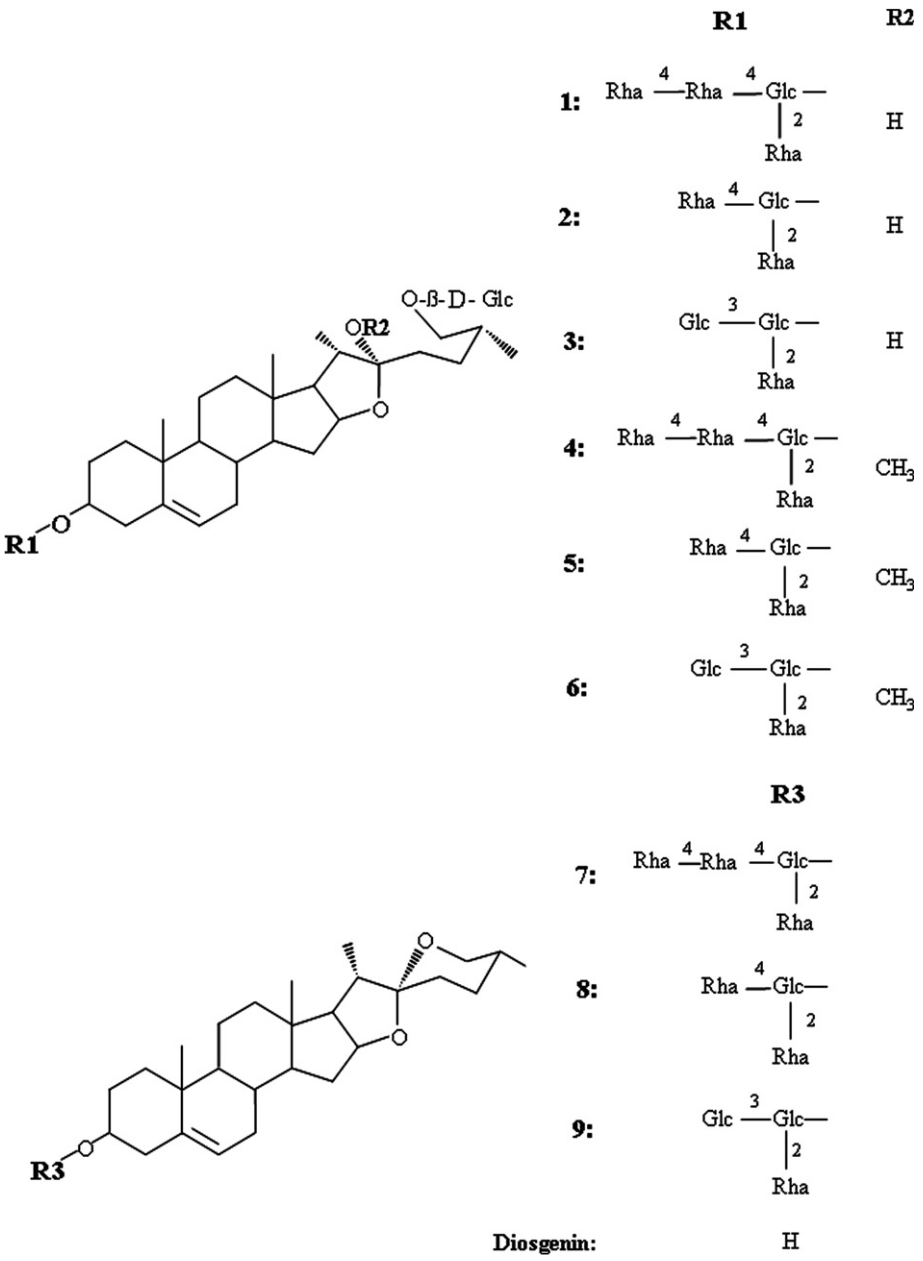

Fig. 1. Structures of furostanol and spirostanol glycosides of the yam tuber (*D. pseudojaponica*).

oxygen, the deionized water was degassed rigorously before use. As we could see, these two reaction conditions would result in different enzyme activities to the furostanol glycosides conversion.

2.4. Extraction of yam saponins

Saponins in yam tuber powder were extracted with the method of Yang, Lu, and Hwang (2003b). A 40 g of the powder was extracted with 1 L of methanol at ambient temperature (25 °C) for 24 h followed by filtration and concentration in a rotary evaporator at 30 °C. The extracted residue was suspended in 25 mL of distilled water and then partitioned against 25 mL of *n*-butanol 3 times to yield saponin extract. After washing the extract with 50 mL of distilled water for 3 times, *n*-butanol was removed using a rotary evaporator at 45 °C. Yam saponins in model system were extracted as following: 10 mL of *n*-butanol was added to each vial to extract the saponins for 3 times after enzyme hydrolysis. The combined extract (30 mL) was washed with 30 mL of distilled water 3 times and then concentrated to dryness. Each dried extract was dissolved in 1 mL of methanol for HPLC analysis.

2.5. HPLC analysis of yam saponins

Yam saponins were analyzed with a *PrimeLine*™ Gradient Model 500G HPLC pump system (Analytical Scientific Instruments, Inc., El Sobrante, CA, USA) and an Alltech 3300 evaporative light scattering detector (ELSD) (Alltech

Associates Inc., Deerfield, Ireland) (tube temperature, 75 °C; air flow rate, 2.8 L/min). The analytical condition was similar to that reported by Yang et al. (2003b). A Luna C18 column (4.6 mm i.d. × 250 mm, 5 μm particle size) (Phenomenex, Torrance, CA, USA) kept at 45 °C in a Colbox column oven (Hipoint Scientific Co., Kaohsiung, Taiwan) and a step gradient solvent system mixing methanol and deionized H_2O, 62/38 (v/v) from 0 to 20 min and 71/29 (v/v) from 21 to 65 min at a flow rate of 1 mL/min, were used to separate saponins. A Chem-Win computer software system (Shuen-Hua Co., Taipei, Taiwan) was employed to process data.

2.6. Statistical analysis

The quantitative analyses of the yam saponins were carried out in triplicate and the mean values were calculated. Statistical analyses of the data were executed by the analysis of variance and least significant difference test (LSD) procedure was used to determine significance between means, at a level of $p < 0.05$.

3. Results and discussion

3.1. Changes of steroidal saponins in yam tubers under various storage conditions

Compounds **1-3** were the natural furostanol glycosides in yams. They could be methylated at position C-22 to form compounds **4-6** while treated with methanol during the procedures of extraction and isolation (Inoue & Ebizuka 1996; Hu et al., 1997). The original contents of compounds **1-3** and **7-9** in the tuber fleshes were 50.42, 53.32, 27.79 μg/g dw and 31.14, 43.13, 20.12 μg/g dw, respectively (see footnote a in Tables 1 and 2). In the investigation, unpeeled and peeled yam tubers were stored under atmospheric and vacuum-sealed conditions separately. During 80 days of storage, the content and composition of furostanol and spirostanol glycosides in peeled and unpeeled tubers at −18 °C, and peeled tubers at 4 °C did not show significant differences in level. The unpeeled tubers stored at 17 and 25 °C could also maintain saponin content and composition until 35 days later. After 50–80 days of storage, all furostanol glycosides in these tubers were reduced significantly as storage period increased, whereas the spirostanol glycosides showed opposite results. The changes of steroidal saponins in the unpeeled tubers stored at 25 °C were larger than those stored at 17 °C. There were no pronounced changes in saponin content and composition when the tubers (with cortices) were stored at 4 °C within 20 days. These tubers presented obvious chilling injury after they were stored for 35 days. The injury became more and more serious as storage time increased. Three furostanol glycosides and three spirostanol glycosides in these tubers also showed significant decreases and increases, respectively after 35–80 days storage. The longer the tubers were stored, the more the changes of steroidal saponins took place.

Table 1
The contents of furostanol glycosides in yam tubers after storing at various temperatures from 5 to 80 days

Compound[a]	Storage day	Content of saponins (μg/g dw)[b] Storage temperature of yam tuber (°C)								LSD[c] at 0.05 probability
		Tuber with cortex & atmosphere storage				Tuber without cortex & vacuum storage				
		−18	4	17	25	−18	4	17	25	
	5	50.51 ± 3.41	50.31 ± 2.72	51.20 ± 3.08	50.65 ± 1.94	50.31 ± 2.58	50.07 ± 2.58	48.34 ± 3.57	49.03 ± 4.12	
	10	49.32 ± 2.54	49.84 ± 2.80	50.11 ± 2.54	50.28 ± 3.16	49.84 ± 1.93	50.28 ± 2.15	40.32 ± 2.25	38.68 ± 3.08	
	20	51.13 ± 3.01	49.50 ± 2.63	49.99 ± 3.31	49.76 ± 2.37	51.13 ± 2.87	49.87 ± 2.87	17.44 ± 2.08	13.65 ± 1.84	
1	35	50.24 ± 3.25	47.03 ± 3.02	50.20 ± 2.06	49.09 ± 2.94	50.24 ± 2.98	50.10 ± 2.98	–	–	1.03
	50	49.83 ± 4.04	43.42 ± 3.22	49.31 ± 4.01	47.29 ± 1.35	50.06 ± 3.61	50.06 ± 3.61	–	–	
	65	49.89 ± 3.32	37.13 ± 4.12	48.53 ± 2.89	46.82 ± 4.11	49.83 ± 3.32	49.83 ± 3.32	–	–	
	80	49.77 ± 3.54	30.41 ± 3.71	47.66 ± 3.42	44.86 ± 3.46	49.68 ± 3.02	49.91 ± 3.02	–	–	
	5	53.41 ± 3.72	53.30 ± 3.65	52.86 ± 3.72	53.28 ± 1.92	53.22 ± 2.86	53.41 ± 1.42	51.82 ± 3.92	51.37 ± 4.04	
	10	54.09 ± 2.77	53.28 ± 2.80	53.37 ± 2.77	54.09 ± 2.77	52.99 ± 3.61	53.25 ± 3.01	42.85 ± 2.25	38.44 ± 3.34	
	20	53.07 ± 4.12	52.91 ± 2.63	54.02 ± 4.12	53.37 ± 3.42	53.10 ± 2.64	53.35 ± 2.82	20.04 ± 1.76	15.15 ± 1.31	
2	35	53.26 ± 2.94	49.25 ± 3.02	53.19 ± 2.98	52.67 ± 5.02	53.41 ± 3.72	54.01 ± 3.53	–	–	1.41
	50	52.96 ± 1.99	44.80 ± 3.22	53.36 ± 1.79	50.92 ± 4.31	53.16 ± 4.52	52.90 ± 3.22	–	–	
	65	53.06 ± 3.68	37.44 ± 4.12	50.36 ± 1.61	48.86 ± 2.90	52.87 ± 3.06	53.41 ± 1.99	–	–	
	80	52.89 ± 4.21	31.51 ± 3.71	49.93 ± 4.09	46.72 ± 4.04	53.18 ± 3.39	53.15 ± 2.76	–	–	
	5	28.04 ± 2.16	27.27 ± 2.42	27.55 ± 1.88	27.60 ± 2.29	28.10 ± 2.14	27.73 ± 3.26	26.19 ± 2.58	25.75 ± 3.07	
	10	27.57 ± 3.24	28.02 ± 3.34	27.60 ± 1.63	27.73 ± 3.18	27.61 ± 3.83	27.94 ± 1.71	19.37 ± 1.75	18.04 ± 1.39	
	20	27.38 ± 1.94	27.47 ± 2.55	27.83 ± 3.46	27.81 ± 1.64	28.02 ± 4.21	27.55 ± 2.09	1.45 ± 0.18	1.01 ± 0.16	
3	35	27.71 ± 1.33	24.65 ± 2.81	27.45 ± 4.05	27.69 ± 3.11	27.66 ± 2.56	27.62 ± 3.20	–	–	1.63
	50	27.27 ± 4.15	19.69 ± 1.94	26.51 ± 3.40	25.53 ± 2.84	27.27 ± 3.75	27.48 ± 2.66	–	–	
	65	27.55 ± 2.42	13.29 ± 1.38	25.72 ± 2.76	24.42 ± 4.13	27.52 ± 3.38	27.55 ± 4.00	–	–	
	80	27.20 ± 3.38	7.94 ± 0.82	24.33 ± 3.61	22.11 ± 3.52	27.39 ± 2.52	27.35 ± 1.57	–	–	

[a] The original contents of compounds **1**∼**3** in yam tuber flesh (control) were 50.42 ± 2.34, 53.32 ± 3.72, 27.79 ± 1.57 μg/g dw, respectively.
[b] Values are mean ± S. D. obtained by trizplicate analyses; – = no determined.
[c] Least significant difference: Difference of two means between treatments including controls exceeding this value is significant ($p < 0.05$).

Table 2
The contents of spirostanol glycosides in yam tubers after storing at various temperatures from 5 to 80 days

Compound[a]	Storage day	Content of saponins (μg/g dw)[b] Storage temperature of yam tuber (°C)								LSD[c] at 0.05 probability
		Tuber with cortex & atmosphere storage				Tuber without cortex & vacuum storage				
		−18	4	17	25	−18	4	17	25	
	5	31.23 ± 3.04	31.20 ± 3.15	30.41 ± 1.68	31.25 ± 2.44	31.09 ± 3.24	31.24 ± 2.99	30.05 ± 3.01	29.22 ± 2.43	
	10	31.05 ± 2.52	31.47 ± 2.64	31.22 ± 2.26	30.90 ± 3.87	31.26 ± 2.52	31.09 ± 3.26	22.88 ± 2.14	20.59 ± 2.04	
	20	31.19 ± 2.83	31.11 ± 2.06	31.13 ± 3.48	31.61 ± 2.57	30.94 ± 2.83	31.14 ± 2.76	5.34 ± 0.21	2.82 ± 0.10	
7	35	31.21 ± 3.39	33.90 ± 3.37	31.22 ± 3.04	32.28 ± 3.32	30.91 ± 3.39	31.25 ± 3.02	–	–	1.21
	50	30.98 ± 2.81	37.06 ± 2.92	32.45 ± 4.02	33.77 ± 3.58	31.17 ± 2.81	30.84 ± 2.79	–	–	
	65	31.08 ± 2.60	42.46 ± 2.57	32.42 ± 2.51	34.26 ± 2.99	31.09 ± 2.60	31.21 ± 2.33	–	–	
	80	31.06 ± 2.18	47.97 ± 2.49	33.65 ± 2.46	35.84 ± 3.41	31.11 ± 2.18	31.03 ± 3.82	–	–	
	5	43.02 ± 2.86	43.10 ± 3.13	43.40 ± 4.02	42.99 ± 3.11	43.25 ± 1.84	43.19 ± 3.33	41.32 ± 4.10	40.68 ± 2.09	
	10	43.35 ± 3.30	43.17 ± 2.67	43.08 ± 3.62	42.23 ± 2.06	43.07 ± 2.95	42.89 ± 2.92	33.10 ± 3.55	31.73 ± 3.71	
	20	43.09 ± 4.16	43.26 ± 3.71	42.51 ± 2.97	43.08 ± 3.47	42.91 ± 3.70	43.32 ± 2.90	15.32 ± 1.28	11.44 ± 1.04	
8	35	42.96 ± 2.25	46.41 ± 3.84	43.26 ± 3.00	43.69 ± 2.40	43.10 ± 2.90	43.03 ± 2.90	–	–	1.32
	50	42.86 ± 3.81	50.02 ± 2.99	43.09 ± 4.04	45.27 ± 3.28	43.31 ± 2.90	43.21 ± 2.90	–	–	
	65	43.02 ± 2.53	56.16 ± 5.12	45.51 ± 2.66	46.76 ± 4.21	43.07 ± 2.90	43.01 ± 2.90	–	–	
	80	43.18 ± 1.59	60.98 ± 4.81	45.87 ± 2.75	48.58 ± 3.82	43.00 ± 2.90	43.09 ± 2.90	–	–	
	5	19.85 ± 2.53	20.30 ± 2.20	20.22 ± 3.00	20.27 ± 1.63	20.01 ± 3.52	20.19 ± 3.14	19.27 ± 1.88	18.10 ± 1.75	
	10	20.27 ± 1.55	19.95 ± 3.02	20.23 ± 2.43	20.16 ± 1.84	19.90 ± 2.44	20.05 ± 2.67	12.34 ± 1.30	10.03 ± 0.99	
	20	20.15 ± 1.61	20.36 ± 2.42	20.03 ± 1.99	20.10 ± 2.53	20.21 ± 2.52	19.99 ± 1.31	ND	ND	
9	35	20.31 ± 2.49	22.63 ± 1.60	20.35 ± 2.17	20.20 ± 3.41	20.07 ± 1.99	20.34 ± 1.65	–	–	1.06
	50	19.93 ± 1.76	26.71 ± 1.74	21.12 ± 2.54	21.97 ± 2.65	20.18 ± 3.24	20.11 ± 2.88	–	–	
	65	20.09 ± 2.30	32.00 ± 2.81	21.86 ± 1.05	22.88 ± 1.78	19.89 ± 2.27	20.02 ± 2.73	–	–	
	80	20.14 ± 2.17	36.33 ± 3.05	22.90 ± 1.38	24.77 ± 2.35	20.10 ± 2.46	19.88 ± 3.56	–	–	

[a] The original contents of compounds **7**∼**9** in yam tuber flesh (control) were 31.14 ± 2.19, 43.13 ± 2.90, 20.12 ± 1.73 μg/g dw, respectively.
[b] Values are mean ± S. D. obtained by trizplicate analyses; ND = not detected; – = no determined.
[c] Least significant difference: Difference of two means between treatments including controls exceeding this value is significant ($p < 0.05$).

Fig. 2 shows some chromatograms of yam tuber extracts for different storage time at 4 °C under atmosphere condition. All data are showed in Tables 1 and 2.

Inoue and Ebizuka (1996) reported that glucoses at position C-26 on furostanol glycoside structures could be excided by F26G (the number, type and bond form of carbohydrate group at position C-3 could be kept) and converted to their corresponding spirostanol glycosides in the rhizomes of crape ginger (*Cotus speciosus*). Kalinowska, Zimowski, Pączkowski, and Zdzislaw (2005) illustrated

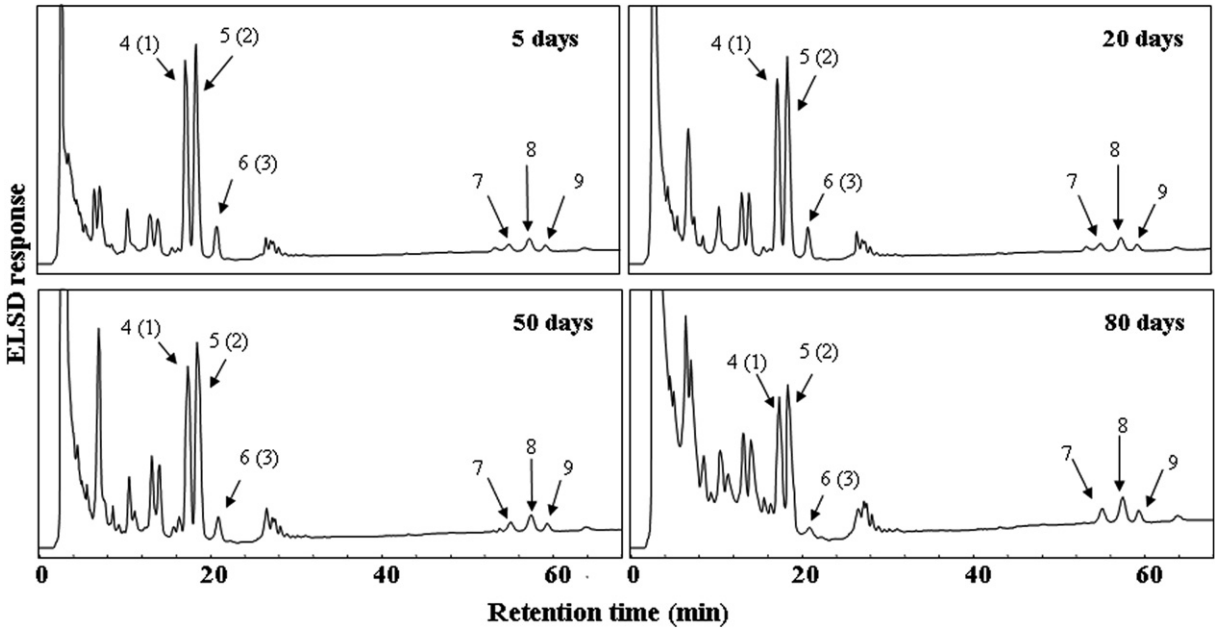

Fig. 2. HPLC chromatograms of saponins extracts of yam tubers after 4 °C storage for various times under atmosphere condition. HPLC conditions: column, Luna C-18 (4.6 mm i.d. × 250 mm, 5 μm); mobile phase, MeOH/ H_2O = 62/38 (v/v) from 0 to 20 min and 71/29 (v/v) from 21 to 65 min; flow rate, 1 ml/min; detection, evaporative light scattering detector (ELSD) (tube temperature, 75 °C; gas flow rate, 2.8 mL/min).

that bidesmosidic furostanol glycosides, contained in freshly harvested rhizomes of crape ginger, could be converted to monodesmosidic spirostanol glycosides in stored rhizomes. We found that furostanol and spirostanol glycosides coexisted in freshly harvested yam tubers. The furostanol glycosides in the long-term stored yam tubers could also be converted to spirostanol glycosides regardless of 4, 17 or 25 °C storage under atmospheric condition (Tables 1 and 2). The chilling injured tubers (4 °C storage) presented the highest conversion. The decreased moles of furostanol glycosides were nearly equal to the increased moles of spirostanol glycosides in these tubers. Scandalios (1993) indicated that the production of activated oxygen species (AOS) might contribute to the formation of chilling injury. Wise and Naylor (1987) illustrated that AOS would damage membrane lipids, proteins and nucleic acid and disorder homeostasis of the organism further. Moreover, enzyme activities (e.g. superoxide dismutase, catalase, duaiacol peroxidase and so on) and chemical compositions (e.g. carotenoids, flavonols and phenolic compounds, etc) could be changed in the chilling injured plants (Asada, 1994; Walker & Mckersie, 1993). Yam crude F26G still had enzyme activity (in the chilling injured yam tubers) at low temperature (4 °C); nevertheless, this enzyme activity seemed to be inhibited in vacuum-sealed peeled tubers.

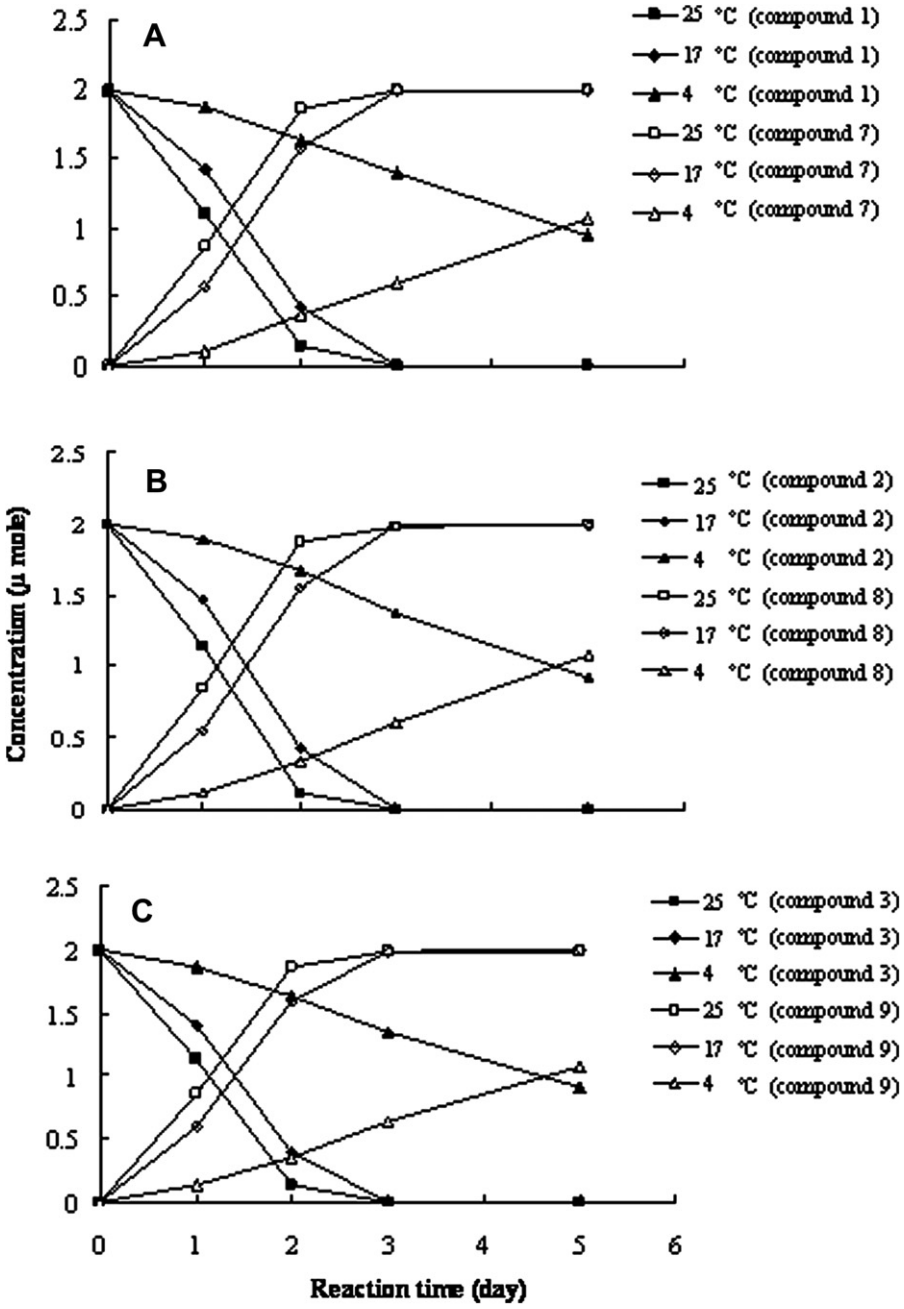

Fig. 3. Changes of saponins after yam crude F26G hydrolysis at different temperature from 1 to 5 days under atmosphere condition (A) compounds **1** and **7**; (B) compounds **2** and **8**; (C) compounds **3** and **9**.

After 5 days of storage at 17 and 25 °C, the vacuum-sealed peeled tubers appeared organoleptic injury. The injury of these tubers was too serious to extract saponins and determine their contents quantitatively after 20 days of storage (the textures of tubers almost lost and transformed to juice with stench). Platenius (1943) evidenced that anaerobic respiration at low level of oxygen resulted in the accumulation of toxic end products of incomplete oxidation that would injure tissues of vegetables. All furostanol and spirostanol glycosides showed significant reduction when the vacuum-sealed peeled tubers were stored at 17 and 25 °C for 5 days. The reduction was aggravated with increasing storage period. Compared to 25 °C stored tuber, the reduction of steroidal saponins in the 17 °C stored one was lower (Tables 1 and 2). These saponins would be dissolved into the transformed juice and flowed away. These furostanol and spirostanol glycosides could be determined definitely in the juice (data not showed).

3.2. Changes of steroidal saponins of yam tubers in model systems under varied conditions

In order to understand the yam F26G activity under atmosphere and vacuum-sealed conditions, we used a model system referred to the method of Inoue and Ebizuka (1996) for evaluation. Fig. 3 shows that compounds **1**, **2** and **3** under atmosphere condition could be converted to compounds **7**, **8** and **9** in all test temperatures. Furostanol glycosides could be converted to their corresponding spirostanol glycosides completely when they were incubated at 17 and 25 °C for 3 days. Yam F26G also showed conversion activity in the model system at 4 °C, though its conversion ability was lower than those at 17 and 25 °C. It is interesting to notice that all furostanol glycosides in the model systems could maintain their original configurations under vacuum-sealed condition, regardless of reaction temperature (data not showed). Therefore, the yam F26G activity could be inhibited in the vacuum condition. Cabezas (1985) mentioned that deficiency in oxygen supply could influence β-glucosidase activity.

4. Conclusions

The content and composition of steroidal saponins in yam tuber seemed to closely relate with their qualities. The larger changes were occurred in the injured yam tubers. The changes were caused by F26G which could convert furostanol glycosides to their corresponding spirotanol glycosides. Its activity was influenced by temperature and could be inhibited in the vacuum condition. A detailed study of the role of air/oxygen to the conversion activity will be explored in the future work.

Acknowledgments

This research was supported by the National Science Council, Taiwan (Project No. NSC 96-2313-B-040-002) and the Chung Shan Medical University, Taiwan (Project No. CSMU-96-OM-A-088).

References

Afoakwa, E. O., & Sefa-Dedeh, S. (2001). Chemical composition and quality changes associated with trifoliate yam *Dioscorea dumetorum* tubers after harvest. *Food Chemistry, 75*, 85–91.

Afoakwa, E. O., & Sefa-Dedeh, S. (2002). Changes in rheological properties and amylase activities occurring in trifoliate yam *Dioscorea dumetorum* starch after harvest. *Food Chemistry, 77*, 285–291.

Aquino, R., Conti, C., DeSimone, F., Orsi, N., Pizza, C., & Stein, M. L. (1991). Antiviral activity of constituents of *Tamus communis*. *Journal of Chromatography, 3*, 305–309.

Asada, K. (1994). Production and action of active oxygen species in photosynthetic tissues. In C. H. Foyer & P. M. Mullineaux (Eds.), *Causes of photooxidative stress and amelioration of defense system in plants*. Boca Raton, FL: CRC Press (pp. 77–104).

Cabezas, J. A. (1985). Diagnostic potential of serum and urine glycosidases in acquired diseases. *Pure and Applied Chemistry, 57*, 547–556.

Chen, Y., & Wu, Y. (1994). Progress in research and manufacturing of steroidal sapogenins in China. *Journal of Herbs, Spices and Medicinal Plants, 2*, 59–70.

Dinan, L., Harmatha, J., & Lafont, R. (2001). Chromatographic procedures for the isolation of plant steroids. *Journal of Chromatography A, 935*, 105–123.

Espejo, O., Campos, L. J., Jung, H., & Giral, F. (1982). Spirostanic diosgenin precursors from *Dioscorea composita* tubers yams. *Phytochemistry, 21*, 413–416.

Haraguchi, M., Zaccharias, D. S. A., Young, M. C. M., & Chu, E. (1994). Steroidal prosapogenins from *Dioscorea olfersiana*. *Phytochemistry, 36*, 1005–1008.

Hariprakash, C. S., & Nambisan, B. (1996). Carbohydrate metabolism during dormancy and sprouting in yam (*Dioscorea*) tubers: Changes in carbohydrate constituents in yam (*Dioscorea*) tubers during dormancy and sprouting. *Journal of Agricultural and Food Chemistry, 44*, 3066–3069.

Hoyer, G. A., Sucrow, W., & Winkler, D. (1975). Diosgenin saponins from *Dioscorea floribunda*. *Phytochemistry, 14*, 539–542.

Hu, C. C., Lin, J. T., Liu, S. C., & Yang, D. J. (2007). A spirostanol glycoside from wild yam (*Dioscorea villosa*) extract and its cytostatic activity on three cancer cells. *Journal of Food and Drug Analysis, 15*, 310–315.

Hu, K., Dong, A., Yao, X. S., Kobayashi, H., & Iwasaki, S. (1996). Antineoplastic agents I. Three spirostanol glycosides from rhizomes of *Dioscorea collettii* var. *hypoglauca*. *Planta Medica, 62*, 573–575.

Hu, K., Yao, X., Kobayashi, H., & Iwasaki, S. (1997). Antineoplastic agents II. Four furostanol glycosides from rhizomes of *Dioscorea collettii* var. *hypoglauca*. *Planta Medica, 63*, 161–165.

Inoue, K., & Ebizuka, Y. (1996). Purification and characterization of furostanol glycoside 26-O-β-glucosidase from *Cotus speciosus* rhizomes. *FEBS Letters, 378*, 157–160.

Kalinowska, M., Zimowski, J., Pączkowski, C., & Zdzislaw, A. (2005). The formation of sugar chains in triterpenoid saponins and glycoalkaloids. *Phytochemistry Review, 4*, 237–257.

Kato, A., Miura, T., & Fukunaga, T. (1995). Effects of steroidal glycosides on blood glucose in normal and diabetic mice. *Chemical and Pharmaceutical Bulletin, 18*, 167–168.

Liu, S. Y., Wang, J. Y., Shyu, Y. T., & Song, L. M. (1995). Studies on yams in (*Dioscorea* spp.) Taiwan. *Journal of Chinese Medicine, 6*, 111–126.

Matsurra, H., Ushiroguchi, T., Itakura, Y., & Fuwa, T. (1989). A furostanol glycoside from (*Allium chinese G.* Don). *Chemical and Pharmaceutical Bulletin, 37*, 1390–1391.

Medoua, G. N., Mbome, I. L., Agbor-Egbe, T., & Mbofung, C. M. F. (2005). Physicochemical changes occurring during post-harvest hardening of trifoliate yam (*Dioscorea dumetorum*) tubers. *Food Chemistry, 90*, 597–601.

Osorio, J. N., Martinez, O. M. M., Navarro, Y. M. C., Watanabe, K., Sakagami, H., & Mimaki, Y. (2005). Polyhydroxylated spirostanol saponins from the tubers of *Dioscorea polygonoides*. *Journal of Natural Products, 68*, 1116–1120.

Platenius, H. (1943). Effect of oxygen concentration on the respiration of some vegetables. *Plant Physiology, 18*, 671–684.

Sauvaire, Y., Ribes, G., Baccou, J. C., & Loubatieŕes-Mariani, M. M. (1991). Implication of steroid saponins and sapogenins in the hypocholesterolemic effect of fenugreek. *Lipids, 26*, 191–197.

Scandalios, J. G. (1993). Oxygen stress and superoxide dismutases. *Plant Physiology, 101*, 7–12.

Tang, S. R., & Jiang, Z. D. (1987). Three new steroidal saponins from the aerial part of *Dioscorea zingiberensis*. *Acta Botanica Yunnanica, 9*, 233–238.

Walker, M. A., & Mckersie, B. D. (1993). Role of the ascorbate-glutathione antioxidant system in chilling resistance tomato. *Journal of Plant Physiology, 141*, 234–239.

Wang, J. Y., & Liu, S. Y. (1992). Studies on the tuber storage of three yam (*Dioscorea alata* L.) genotypes. *Journal of Agricultural Research of China, 41*, 169–177.

Wise, R. R., & Naylor, A. W. (1987). Chilling-enhanced photooxidation. The peroxidative destruction of lipids during chilling injury to photosynthesis and ultrastructure. *Plant Physiology, 82*, 272–277.

Yang, D. J., Lu, T. J., & Hwang, L. S. (2003a). Isolation and identification of steroidal saponins on Taiwanese yam cultivar (*Dioscorea pseudojaponica* Yamamoto). *Journal of Agriculture and Food Chemistry, 51*, 6438–6444.

Yang, D. J., Lu, T. J., & Hwang, L. S. (2003b). Simultaneous determination of furostanol and spirostanol glycosides in Taiwanese yam (*Dioscorea* spp.) cultivars by high-performance liquid chromatography. *Journal of Food and Drug Analysis, 11*, 10–15.

Zhang, J., Meng, Z., Zhang, M., Ma, D., Xu, S., & Kodama, H. (1999). Effect of six steroidal saponins isolated from *Anemarrhenae rhizoma* on platelet aggregation and hemolysis in human blood. *Clinica Chimica Acta, 289*, 79–88.