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Effects of Domestic Processing on Steroidal Saponins in Taiwanese Yam Cultivar (*Dioscorea pseudojaponica* Yamamoto)

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The effects of domestic processing on steroidal saponins and furostanol and spirostanol glycosides in Taiwanese yam cultivar (*Dioscorea pseudojaponica* Yamamoto) were studied. The baking or frying of yam slices was conducted at 150, 180, and 200 °C for 3, 5, and 10 min. Yam slices were steamed or microwave cooked at 2450 MHz with an output power of 850 W for 3, 5, and 10 min. The various saponins were quantified by HPLC with an evaporative light scattering detector (ELSD). Results showed that the contents of saponins were decreased along with increasing cooking temperature and time except for the steaming treatment. None of the steamed yam slices significantly change their initial compositions or quantities of furostanol and spirostanol glycosides. Fried yam slices had the highest loss of saponins, especially at 200 °C for 10 min (93 and 97% reductions for total furostanol and spirostanol glycosides, respectively). After baking for 10 min at 200 °C, the total furostanol and spirostanol glycosides were reduced by 67 and 74%, respectively. There were 12, 44, and 84% decreases for total furostanol glycosides and 10, 35, and 75% reductions for total spirostanol glycosides in yam slices after microwave cooking for 3, 5, and 10 min, respectively. Diosgenin, the aglycone of these saponins, could be found in yams after microwave cooking and baking, but not in steamed and fried yams.

KEYWORDS: Yam; *Dioscorea pseudojaponica* Yamamoto; processing; steroidal saponin; furostanol and spirostanol glycosides; diosgenin

INTRODUCTION

Yams are perennial trailing rhizome plants of the *Dioscorea* genus (belong to the Dioscoraceae family). The tuber of yam (*Dioscorea* spp.), which contains many nutrients, for example, carbohydrates, essential amino acids, vitamin C, minerals, and physiologically active components such as musin (glycoprotein), polysaccharide, and steroidal saponins (*1*−*10*), is consumed as a food and widely used in traditional Chinese medicine (*11*). In Taiwan, it is also one of the popular foods in recent years because of its potential health benefits. Steroidal saponins are the most important bioactive compounds in yam (*7*−*10*). Many biological functions of steroidal saponins have been reported including anticarcinogenic (*12*, *13*), antithrombotic (*14*, *15*), antiviral (*16*), hemolytic (*15*, *17*), hypocholesterolemic (*18*, *19*), and hypoglycemic effects (*20*). Furthermore, diosgenin, the predominant aglycone of the yam steroidal saponin, is also used as a steroid intermediate in the pharmaceutical industry (*11*, *21*).

Two groups of steroidal saponins in *Dioscorea pseudojaponica* Yamamoto, a Taiwanese native variety of yam, were distinguished in our pervious investigation: furostanol glycosides, including 26-*O*-*β*-D-glucopyranosyl-22α-methoxyl-(25*R*)-furost-5-en-3*β*,26-diol 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-{[α-L-rhamnopyranosyl-(1→4)]-*O*-[α-L-rhamnopyranosyl-(1→4)]}-*β*-D-glucopyranoside (**1**), methyl protodioscin (**2**), and methyl protogracillin (**3**), and spirostanol glycosides, including (25*R*)-spirost-5-en-3*β*-ol 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-{[α-L-rhamnopyranosy-(1→4)]-*O*-[α-L-rhamnopyranosyl-(1→4)]}-*β*-D-glucopyranoside (**4**), dioscin (**5**), and gracillin (**6**) (*9*, *10*).

Many papers have presented the effect of processing on saponins for chickpea (*Cicer arietinum*) (*22*), black gram (*Vigna mungo*) (*22*−*24*), mung bean (*Vigna radiate* L.) (*24*), faba bean (*Vicia faba*) (*25*), oat (*26*), kernels of melons (*Cucurbitacea* species) (*27*), pigeon pea (*28*), ginseng roots (*29*, *30*), and so on. There is, however, no report on the influences of domestic processing on saponins of yam. The objectives of this study were to investigate the effects of steaming time, microwave cooking time, baking temperature and time, and frying temperature and time on individual steroidal saponins in Taiwanese yam (*D. pseudojaponica* Yamamoto). In addition, Drumm et al. (*31*) reported that the decrease in saponin content might be due to the hydrolysis of the glycosidic bond between the sapogenin and glycosidic residue during thermal processing. Therefore, we also determined the content of diosgenin after yam processing.

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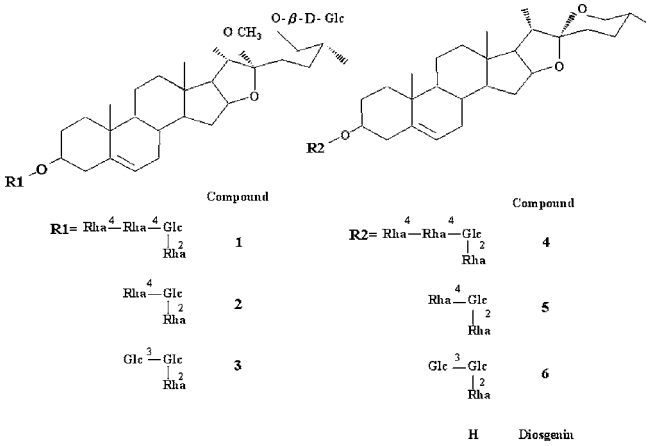

Figure 1. Structures of furostanol glycosides and spirostanol glycosides.

MATERIALS AND METHODS

Yam Sample. Tubers of a yam (*D. pseudojaponica* Yamamoto) that was identified by the Taiwan Agricultural Research Institute (Taichung, Taiwan) were obtained from Keelung City, Taiwan. The yam tuber with a white cortex and flesh is cylindrical in shape (ca. 4 cm in diameter and ~130 cm long). The moisture of the yam tuber was 72.6% determined according to the AOAC method (*32*).

Chemicals and Standards. Diosgenin was purchased from Sigma Chemical Co. (St. Louis, MO). Steroidal saponin standards, including compounds **1**−**6** (**Figure 1**), were isolated in our laboratory, as described in our previous paper (*9*). The purities of these standards were >95%. Solvents used for the extraction and separation of steroidal saponins and diosgenin, including acetonitrile, *n*-butanol, and methanol, were purchased from Merck Co. (Darmstadt, Germany). Deionized water was prepared by using an Ultrapure water purification system (Lotun Co., Ltd., Taipei, Taiwan) and was degassed under vacuum followed by filtering through a 0.22 μm membrane filter (nylon) prior to use.

Yam Processing. Yam tubers were first cut into sticks of 25 cm long and ca. 4 cm in diameter. The yam sticks were cleaned, peeled, and cut into 4 mm thick slices using a Cucina slicer (model HR7633) (Koninklijke Philips Electronics Co., Suzhou, Jiangsu, China), separately. The yam slices were also randomly collected and divided into 75 portions of 300 g each. A total of 72 portions was used for processing (3 portions of yam slices were randomly sampled for each cooking treatment), whereas the other 3 portions were used as control. The following domestic processing methods were employed.

Steaming. Three portions of yam slices were steamed in each case for 3, 5, and 10 min; a total of nine portions in each group was used.

Microwave Cooking. Three portions of yam slices were cooked in a microwave oven (Galanz Enterprises Co., Ltd., Shunde, Guandong, China) in each case at 2450 MHz for 3, 5, and 10 min with an output power of 850 W; a total of nine portions in each group was used.

Baking. Three portions of yam slices were baked in a baking oven (Chung Pu Baking Machinery, Co., Ltd., Taichung, Taiwan) in each case at 150, 180, or 200 °C for 3, 5, and 10 min; a total of 27 portions was used.

Frying. Three portions of yam slices were fried in each case in 2 L of hydrogenated soybean oil at 150, 180, or 200 °C for 3, 5, and 10 min; a total of 27 portions was used.

These cooked yam slices and controls were lyophilized in the FreeZone 18 L Freeze Dry System (Labconco Co., Kansas City, MO) and ground in the RT08 grinder (Rong-Tsong Co., Taipei, Taiwan) to 40 mesh or below prior to further analysis. The moisture contents of all dried yam powder were about 4% as determined by the Labwave 9000 microwave moisture analyzer (CEM Co., Matthews, NC).

Determination of Yam Saponins and Diosgenin. *Extraction of Yam Saponins and Diosgenin.* The method used was similar to that of our previous paper (*10*). A 40 g sample of freeze-dried yam powder was extracted with 1 L of methanol for 24 h at 25 °C followed by filtration and concentration in a rotary evaporator (Panchun Scientific Co., Kaohsiung, Taiwan) at 30 °C. The residue was suspended in 25 mL of distilled water and partitioned against 25 mL of *n*-butanol three times to yield saponin extract. The extract was washed with 50 mL of distilled water three times, and then the solvent was removed in a rotary evaporator at 45 °C. The dried extract was dissolved in 1 mL of methanol for HPLC analysis.

Table 1. Steroidal Saponins in Yam after Steaming for 3, 5, and 10 min[a]

compound	content of saponins[b] (μg/g of dw) after steaming for 3 min	5 min	10 min	LSD[c] at 0.05 probability
1	42.92 ± 5.02	42.82 ± 3.44	42.54 ± 5.52	2.70
2	48.02 ± 2.94	47.87 ± 2.73	47.62 ± 4.77	2.44
3	28.57 ± 4.27	28.67 ± 3.57	28.42 ± 3.64	0.81
total furostanol glycosides	119.51 ± 12.23	119.36 ± 9.74	118.58 ± 13.93	4.71
4	33.14 ± 4.46	32.77 ± 5.11	32.59 ± 3.82	1.25
5	43.01 ± 5.08	42.39 ± 3.25	42.23 ± 2.99	2.32
6	23.02 ± 3.22	22.47 ± 4.21	22.49 ± 3.96	0.85
total spirostanol glycosides	99.17 ± 12.76	97.63 ± 12.57	97.31 ± 10.77	2.71
total saponins	218.68 ± 24.99	216.99 ± 22.31	215.89 ± 24.70	5.67

[a] The original contents of compounds **1**–**6** in fresh yam (control) are 43.12 ± 3.57, 48.07 ± 1.86, 28.65 ± 3.32, 32.86 ± 3.79, 43.12 ± 2.38, and 22.42 ± 3.94 μg/g of dw, respectively (119.84 ± 8.75 μg/g of dw for total furostanol glycosides, 98.40 ± 10.11 μg/g of dw for total spirostanol glycosides, and 218.24 ± 18.86 μg/g of dw for total saponins). [b] Values are mean ± SD obtained by triplicate analyses. [c] Least significant difference: difference of two means between treatments including control exceeding this value is significant ($p < 0.05$).

HPLC Analysis of Yam Saponins and Diosgenin. The analyses for saponins and diosgenin were performed with a *PrimeLine* Gradient model 500G HPLC pump system (Analytical Scientific Instruments, Inc., El Sobrante, CA). A subsidiary Alltech ELSD 2000 evaporative light scattering detector (ELSD) (tube temperature, 75 °C; air flow rate, 2.8 L/min) (Alltech Associates Inc., Deerfield, IL) was used to detect steroidal saponins and diosgenin. The analytical conditions outlined by Yang et al. (*9*, *10*) were followed. For saponins, a Luna C18 column (4.6 mm i.d. × 250 mm, 5 μm) (Phenomenex, Torrance, CA) kept at 45 °C with a Colbox column oven (Hipoint Scientific Co., Kaohsiung, Taiwan) and a step gradient solvent system consisting of methanol and deionized water, 62:38 (v/v) in the first 20 min and 71:29 (v/v) from 21 to 65 min at a flow rate of 1 mL/min, were used. For diosgenin, the Luna C18 column kept at 25 °C and an isocratic solvent system, acetonitrile/water (95:5, v/v) at a flow rate of 1 mL/min were employed. The steroidal saponins and diosgenin in extracts were identified by (1) the addition of diosgenin and furostanol and spirostanol glycoside standards to samples for cochromatography; (2) comparison of retention time and mass spectrum (**1**, *m*/*z* 1231 $[M + Na]^+$; **2**, *m*/*z* 1231 $[M + Na]^+$; **3**, *m*/*z* 1101 $[M + Na]^+$; **4**, *m*/*z* 1037 $[M + Na]^+$, 1015 $[M + H]^+$; **5**, *m*/*z* 891 $[M + Na]^+$, 869 $[M + H]^+$; **6**, *m*/*z* 907 $[M + Na]^+$, 885 $[M + H]^+$; diosgenin, *m*/*z* 437 $[M + Na]^+$, 415 $[M + H]^+$), which was resolved on a VG platform II LC-MS (Micromass Co., Cheshire, U.K.). The mobile phase was split at a ratio of 1/50 to the mass detector. The conditions of LC-MS were as follows: ESI^+ mode, cone voltage = 40 eV, source temperature = 200 °C, scan range from *m*/*z* 50 to 1500. A Chem-Win computer software system (Shuen-Hua Co., Taipei, Taiwan) was used for data processing. Triplicate analyses were conducted, and the mean values were determined. The data were also subjected to analysis of variance, and least significant difference test procedures were applied to determine significance between means, at a level of $p < 0.05$.

RESULTS AND DISCUSSION

Steaming. The contents of compounds **1**−**6** in yam were 43.12, 48.07, 28.65, 32.86, 43.12, and 22.42 μg/g of dw, respectively. **Table 1** shows the saponin contents in yams after steaming for 3, 5, and 10 min. There was no pronounced change in saponin contents; moreover, doisgenin also could not be found in these steamed yams. Therefore, furostanol and spirostanol

Table 2. Steroidal Saponins in Yam after Microwave Cooking for 3, 5, and 10 min[a]

compound	content of saponins[b] (μg/g of dw) after cooking time of			LSD[c] at 0.05 probability
	3 min	5 min	10 min	
1	38.09 ± 3.57 (11.12)	27.02 ± 1.99 (37.34)	9.14 ± 2.52 (78.80)	1.58
2	42.38 ± 4.47 (11.84)	28.64 ± 5.54 (40.42)	8.84 ± 2.05 (81.61)	3.63
3	24.45 ± 4.06 (14.66)	11.36 ± 2.24 (60.34)	1.08 ± 0.79 (96.23)	2.84
total furostanol glycosides	104.92 ± 12.1 (12.45)	67.02 ± 9.77 (44.08)	19.06 ± 5.36 (84.10)	5.59
4	30.51 ± 3.91 (7.15)	22.42 ± 4.27 (31.77)	10.27 ± 3.21 (68.75)	0.88
5	39.41 ± 5.12 (8.60)	29.29 ± 5.24 (32.07)	12.88 ± 4.25 (70.12)	2.64
6	19.05 ± 2.94 (15.03)	12.14 ± 3.21 (45.85)	1.23 ± 0.79 (94.51)	2.71
total spirostanol glycosides	88.97 ± 11.97 (9.58)	63.85 ± 12.72 (35.11)	24.38 ± 8.25 (75.22)	4.02
total saponins	193.89 ± 24.07 (11.16)	130.87 ± 22.49 (40.03)	43.44 ± 13.61 (80.10)	9.28

[a] The original contents of compounds **1**–**6** in fresh yam (control) are 43.12 ± 3.57, 48.07 ± 1.86, 28.65 ± 3.32, 32.86 ± 3.79, 43.12 ± 2.38, and 22.42 ± 3.94 μg/g of dw, respectively (119.84 ± 8.75 μg/g of dw for total furostanol glycosides, 98.40 ± 10.11 μg/g of dw for total spirostanol glycosides, and 218.24 ± 18.86 μg/g of dw for total saponins). [b] Values are mean ± SD obtained by triplicate analyses; numbers in parentheses are the percent decrease of control values. [c] Least significant difference: difference of two means between treatments including controls exceeding this value is significant ($p < 0.05$).

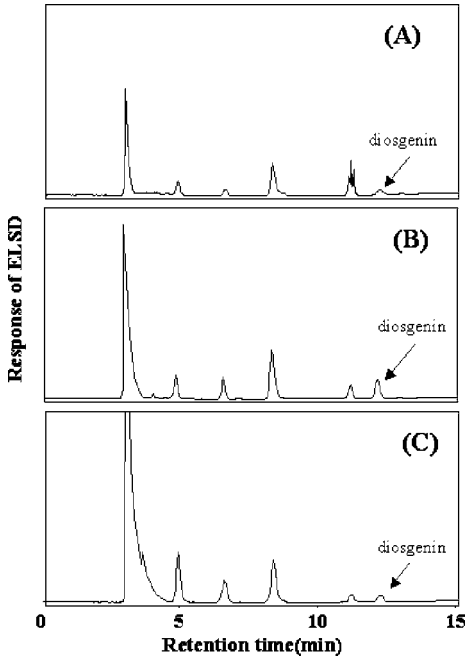

Figure 2. HPLC chromatograms of the extracts of yam (*Dioscorea pseudojaponica* Yamamoto) cooked in microwave oven for various times: (**A**) 3 min; (**B**) 5 min; (**C**) 10 min. HPLC conditions: column, Luna C-18 (4.6 mm i.d. × 250 mm, 5 μm); mobile phase, acetonitrile/ water = 95:5 (v/v); flow rate, 1 mL/min; detection, evaporative light scattering detector (ELSD) (tube temperature, 75 °C; gas flow rate, 2.8 mL/min).

glycosides in yam could endure steaming treatment. Önning et al. (*26*) indicated that incubation of oat saponins, avenacosides A and B, in buffer solutions (pH 4−7, 0.2 mg/600 μL) at 100 °C for 3 h gave no reduction in their contents. Badifu (*27*) boiled the kernels of melons (*Cucurbitacea* species) grown in Nigeria at 98 ± 1 °C for 10 min and found that saponins were reduced by 59.4−74.5%. He illustrated that solubilization of the saponin into the boiling water could have contributed to the decrease. Jood et al. (*22*) cooked eight varieties chickpea (*Cicer arietinum*) and four varieties of black gram (*Phaseolus mungo*) in boiling water (3 times the weight of dry seeds) until they became soft. The losses of saponin were 11−15% for chickpea and 7−14% for black gram. Cooking decreased saponin slightly when it followed soaking (37 °C for 12 h in plain water). Sharma and Sehgal (*25*) verified that saponin in faba bean (*Vicia faba*) could leach out to water during soaking at 37 °C for 12 h and lost about 23%. Cooking of the soaked seeds in boiling water until they became soft could reduce saponin content by about 35%. Shi et al. (*33*) also indicated that portions of saponins in edible legumes would be dissolved in water and lost in soaking, washing, and blanching liquors. By contrast, steaming treatment should be able to retain saponin in foods well.

Microwave Cooking. Table 2 presents the saponin contents in yams after microwave cooking for 3, 5, and 10 min. Each furostanol (compounds **1**−**3**) and spirostanol glycoside (compounds **4**−**6**) was decreased significantly in the initial processing stage, when yam slices were cooked for 3 min. The longer the periods of cooking, the higher was the reduction. The maximum reductions in total furostanol and spirostanol glycoside contents were observed when yam slices were cooked for 10 min. There were 12.45, 44.08, and 84.10% losses for total furostanol glycosides and 9.58, 35.11, and 75.22% losses for total spirostanol glycosides in yam slices after microwave cooking

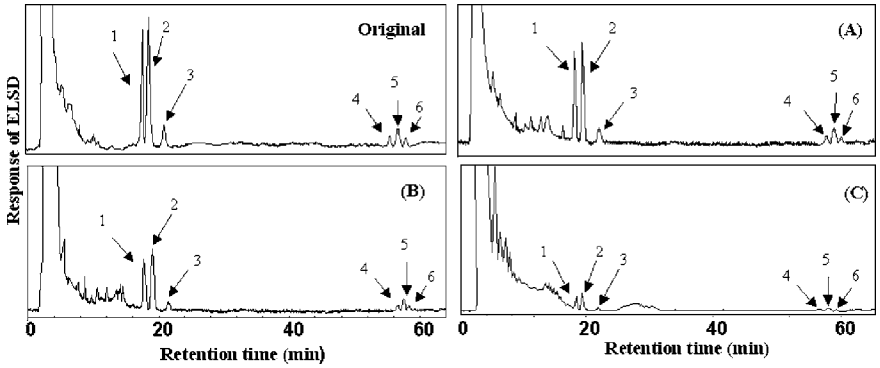

Figure 3. HPLC chromatograms of the extracts of yam (*Dioscorea pseudojaponica* Yamamoto) baked at various temperatures for 10 min: (**A**) 150 °C; (**B**) 180 °C; (**C**) 200 °C. HPLC conditions: column, Luna C-18 (4.6 mm i.d. × 250 mm, 5 μm); mobile phase, methanol/water = 62:38 (v/v) in the first 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).

Table 3. Steroidal Saponins in Yam after Baking at Various Temperatures for 3, 5, and 10 min[a]

compound	baking time (min)	content of saponins[b] (μg/g of dw) after toasting at 150 °C	180 °C	200 °C	LSD[c] at 0.05 probability
1	3	42.12 ± 3.95 (2.32)	40.92 ± 4.72 (5.10)	35.57 ± 6.04 (17.51)	
	5	41.25 ± 3.02 (4.34)	35.73 ± 4.44 (17.14)	29.56 ± 3.97 (31.44)	1.84
	10	38.03 ± 3.82 (10.64)	28.12 ± 3.96 (34.79)	18.84 ± 4.10 (56.31)	
2	3	46.67 ± 3.24 (2.91)	44.98 ± 2.94 (6.43)	40.89 ± 4.53 (14.94)	
	5	45.27 ± 2.75 (5.82)	38.76 ± 3.18 (19.36)	32.21 ± 3.42 (32.99)	1.25
	10	41.28 ± 4.14 (14.13)	26.33 ± 3.41 (45.23)	17.69 ± 3.25 (63.20)	
3	3	27.28 ± 2.95 (4.78)	24.08 ± 5.02 (15.95)	21.59 ± 4.11 (24.64)	
	5	26.37 ± 3.41 (7.96)	19.95 ± 4.15 (30.37)	14.07 ± 2.34 (50.89)	1.94
	10	23.51 ± 3.23 (17.94)	10.64 ± 3.35 (62.86)	2.61 ± 0.82 (87.40)	
total furostanol glycosides	3	116.07 ± 10.14 (3.15)	109.98 ± 12.68 (8.23)	98.05 ± 14.68 (18.18)	
	5	112.89 ± 9.18 (5.80)	94.44 ± 11.77 (21.09)	75.84 ± 9.73 (36.72)	3.38
	10	102.82 ± 11.19 (14.20)	65.09 ± 10.72 (45.69)	39.14 ± 8.17 (67.34)	
4	3	31.33 ± 3.12 (4.95)	29.13 ± 2.89 (11.35)	25.38 ± 5.03 (22.76)	
	5	30.64 ± 3.84 (7.04)	27.18 ± 3.26 (17.29)	19.77 ± 4.24 (39.84)	1.42
	10	28.85 ± 3.83 (12.47)	20.93 ± 1.94 (36.31)	10.63 ± 3.41 (67.65)	
5	3	40.82 ± 4.13 (5.33)	38.05 ± 4.23 (11.76)	33.07 ± 5.36 (23.31)	
	5	37.95 ± 2.68 (11.99)	33.86 ± 5.10 (21.47)	24.29 ± 4.97 (43.67)	1.67
	10	36.18 ± 4.05 (16.09)	25.78 ± 4.17 (40.21)	13.54 ± 3.62 (68.60)	
6	3	21.53 ± 2.75 (3.97)	18.46 ± 3.73 (17.66)	14.41 ± 3.59 (35.73)	
	5	19.68 ± 3.18 (12.22)	17.00 ± 4.66 (24.17)	10.95 ± 3.13 (51.16)	1.78
	10	17.06 ± 3.26 (23.91)	8.08 ± 2.06 (63.96)	1.67 ± 0.93 (92.55)	
total spirostanolglycosides	3	93.68 ± 10.0 (4.80)	85.64 ± 10.85 (12.97)	72.86 ± 13.98 (25.96)	
	5	88.27 ± 9.7 (10.29)	78.04 ± 13.02 (20.69)	55.01 ± 12.34 (44.10)	3.38
	10	82.09 ± 11.14 (16.57)	54.79 ± 8.17 (44.32)	25.84 ± 7.96 (73.74)	
total saponins	3	209.75 ± 20.14 (3.89)	195.62 ± 23.53 (10.36)	170.91 ± 28.66 (21.69)	
	5	201.16 ± 18.88 (7.83)	172.48 ± 24.79 (20.97)	130.85 ± 22.07 (40.04)	6.22
	10	184.91 ± 22.33 (15.27)	119.88 ± 18.89 (45.07)	64.98 ± 16.13 (70.23)	

[a] The original contents of compounds **1**–**6** in fresh yam (control) are 43.12 ± 3.57, 48.07 ± 1.86, 28.65 ± 3.32, 32.86 ± 3.79, 43.12 ± 2.38, and 22.42 ± 3.94 μg/g of dw, respectively (119.84 ± 8.75 μg/g of dw for total furostanol glycosides, 98.40 ± 10.11 μg/g of dw for total spirostanol glycosides, and 218.24 ± 18.86 μg/g of dw for total saponins). [b] Values are mean ± SD obtained by triplicate analyses; numbers in parentheses are the percent decrease of control values. [c] Least significant difference: difference of two means between treatments including controls exceeding this value is significant ($p < 0.05$).

Table 4. Diosgenin in Yam after Baking at Various Temperatures for 3, 5, and 10 min

baking time (min)	content of diosgenin[a,b] (μg/g of dw) after toasting at 150 °C	180 °C	200 °C
3	1.04 ± 0.46 f (29.27)[c]	3.02 ± 1.14 de (31.76)	3.56 ± 0.87 cd (18.07)
5	2.02 ± 0.91 ef (27.81)	5.47 ± 1.23 b (29.13)	5.17 ± 1.32 b (14.31)
10	3.14 ± 1.01 de (22.72)	10.89 ± 3.02 a (26.96)	4.73 ± 1.69 bc (7.50)

[a] Values are mean ± SD obtained by triplicate analyses. [b] Values bearing different letters are significantly different ($p < 0.05$). [c] Numbers in parentheses are the conversion ratios (percent) from lost yam saponins to diosgenin (hypothesizing the saponins could be transformed completely).

for 3, 5, and 10 min, individually. Some papers have mentioned that processing with the microwave oven would degrade chemical compounds of food (*34*, *35*). Ginsenoside degradation was also investigated during microwave heating by Ren et al. (*30*). Compared to spirostanol glycosides, furostanol glycosides seemed to be removed more easily in microwave cooking. Felon et al. (*36*) expressed that the ingredients of food could be dissolved into its juice and flowed away under microwave heating. Furostanol glycosides are more hydrophilic than spirostanol glycosides (*9*); therefore, furostanol glycosides might be well dissolved into yam juice and lost more readily during microwave cooking.

The hydrolysis of the glycosidic bond between the sapogenin and the glycosidic residue could take place in microwave processing. The contents of diosgenin in all microwave-cooked yam slices were 1.07, 4.02, and 2.19 μg/g of dw (the conversion ratios from lost yam saponins to diosgenin were 18.07, 18.01, and 4.96% individually, hypothesizing that the saponins could be transformed completely) after heating for 3, 5, and 10 min, respectively. The longer the yam was cooked, the more the diosgenin was degraded. **Figure 2** shows the changes of diosgenin after cooking for 3, 5, and 10 min. The results proved that structural decomposition and possible leaching out with yam juice caused yam saponins loss during microwave processing.

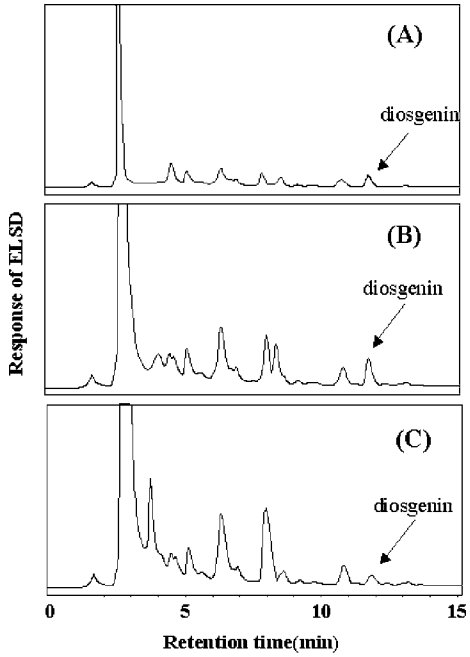

Figure 4. HPLC chromatograms of the extracts of yam (*Dioscorea pseudojaponica* Yamamoto) baked at 180 °C for various times: (**A**) 3 min; (**B**) 5 min; (**C**) 10 min. HPLC conditions were the same as in **Figure 2**.

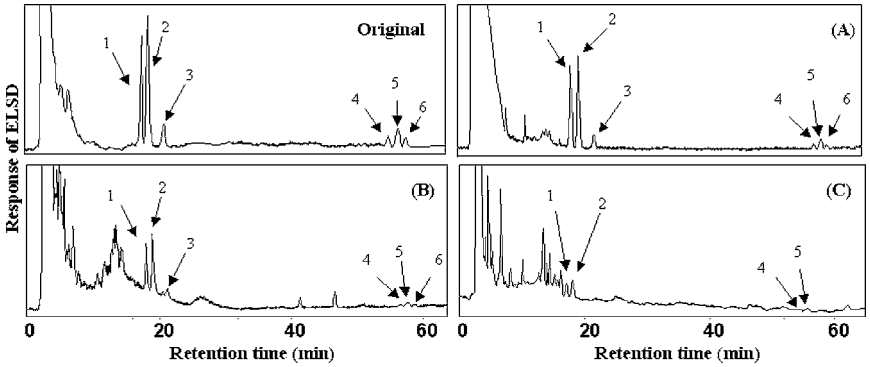

Figure 5. HPLC chromatograms of the extracts of yam (*Dioscorea pseudojaponica* Yamamoto) fried at 200 °C for various times: (**A**) 3 min; (**B**) 5 min; (**C**) 10 min. HPLC conditions were the same as in **Figure 3**.

Table 5. Steroidal Saponins in Yam after Frying at Various Temperatures for 3, 5, and 10 min[a]

compound	frying time (min)	content of saponins[b] (μg/g of dw) after frying at 150 °C	180 °C	200 °C	LSD[c] at 0.05 probability
1	3	38.10 ± 2.97 (11.64)	33.47 ± 3.74 (22.38)	26.47 ± 4.85 (38.61)	
	5	32.42 ± 3.41 (24.81)	29.45 ± 5.10 (31.70)	17.23 ± 3.52 (60.04)	2.08
	10	25.62±2.87 (40.58)	17.46 ± 1.97 (59.51)	3.19 ± 1.02 (92.60)	
2	3	42.19 ± 3.04 (12.23)	37.68 ± 3.51 (21.61)	34.27 ± 4.53 (28.71)	
	5	37.94 ± 3.78 (21.07)	31.42 ± 1.97 (34.64)	20.35 ± 3.87 (57.67)	1.81
	10	29.83 ± 4.15 (37.94)	16.07 ± 3.32 (66.57)	5.06 ± 1.44 (89.47)	
3	3	24.02 ± 2.77 (16.16)	19.16 ± 2.42 (33.12)	13.09 ± 4.21 (54.31)	
	5	18.66 ± 3.54 (34.87)	14.24±3.24 (50.30)	6.23 ± 2.61 (78.25)	2.19
	10	9.48± 2.87 (66.91)	2.87 ± 0.78 (89.98)	ND	
total furostanol glycosides	3	104.31 ± 8.78 (12.96)	90.31 ± 9.67 (24.64)	73.83 ± 13.59 (38.39)	
	5	89.02 ± 10.73 (25.72)	75.11 ± 10.31 (37.32)	43.81 ± 10.00 (63.44)	5.10
	10	64.93 ± 9.89 (45.82)	36.40 ± 6.07 (69.63)	8.25 ± 2.46 (93.11)	
4	3	28.11 ± 4.12 (14.46)	23.22 ± 3.17 (29.34)	21.35 ± 4.02 (35.03)	
	5	25.67 ± 3.11 (21.88)	19.01 ± 2.74 (42.15)	15.29 ± 3.17 (53.47)	1.76
	10	20.48 ± 1.94 (37.67)	10.68 ± 2.5 (67.50)	1.27 ± 0.74 (96.14)	
5	3	35.14 ± 4.42 (18.51)	34.15 ± 6.03 (20.80)	33.56 ± 4.42 (22.17)	
	5	34.03 ± 3.04 (21.08)	22.58 ± 4.12 (47.63)	20.23 ± 5.17 (53.08)	2.51
	10	25.19 ± 3.97 (41.58)	10.97 ± 3.22 (74.56)	1.93 ± 0.87 (95.52)	
6	3	17.44 ± 3.48 (22.21)	14.01 ± 3.16 (37.51)	12.34 ± 4.52 (44.96)	
	5	13.01 ± 2.79 (41.97)	9.94 ± 2.57 (55.66)	1.24 ± 0.79 (94.47)	2.76
	10	2.69 ± 0.84 (88.00)	0.96 ± 0.42 (95.72)	ND	
total spirostanolglycosides	3	80.69 ± 12.02 (18.00)	71.38 ± 12.36 (27.46)	67.25 ± 12.96 (31.66)	
	5	72.71 ± 8.94 (26.11)	51.53 ± 9.43 (47.64)	36.76 ± 9.13 (62.64)	5.86
	10	48.36 ± 6.75 (50.85)	22.61 ± 6.15 (77.02)	3.20 ± 1.61 (96.75)	
total saponins	3	185.00 ± 20.80 (15.23)	161.69 ± 22.03 (25.91)	141.08 ± 26.55 (35.36)	
	5	161.73 ± 19.67 (25.89)	126.64 ± 19.74 (41.97)	80.57 ± 19.13 (63.08)	10.46
	10	113.29 ± 16.64 (48.09)	59.01 ± 12.22 (72.96)	11.45 ± 4.07 (94.75)	

[a] The original contents of compounds **1**–**6** in fresh yam (control) are 43.12 ± 3.57, 48.07 ± 1.86, 28.65 ± 3.32, 32.86 ± 3.79, 43.12 ± 2.38, and 22.42 ± 3.94 μg/g of dw, respectively (119.84 ± 8.75 μg/g of dw for total furostanol glycosides, 98.40 ± 10.11 μg/g of dw for total spirostanol glycosides, and 218.24 ± 18.86 μg/g of dw for total saponins). [b] Values are mean ± SD obtained by triplicate analyses; numbers in parentheses are the percent decrease of control values; ND, not detected. [c] Least significant difference: difference of two means between treatments including controls exceeding this value is significant ($p < 0.05$).

Baking. Figure 3 displays the HPLC chromatograms of the extracts of yam baked at 150, 180, and 200 °C for 10 min. **Table 3** is the saponin contents in yams after baking at 150, 180, and 200 °C for 3, 5, and 10 min. We found that the contents of furostanol and spirostanol glycosides in yam were reduced with increasing baking temperature and time. The highest and lowest losses of saponins in yam slices occurred with baking at 200 °C for 10 min (about 67% for total furostanol glycosides and 74% for total spirostanol glycosides removed) and 150 °C for 3 min (about 2% for total furostanol glycosides and 5% for total spirostanol glycosides lowered), respectively. By comparison with controls, when yam slices were baked at 150 °C for 10 min or at 180 °C for 3 min and above, their total furostanol glycosides could be decreased significantly; on the other hand, the total spirostanol glycosides were lowered markedly, whereas these slices were baked at not less than 150 °C for 5 min or at 180 °C for 3 min. Önning et al. (*26*) heated avenacoside A (oat saponins) in buffer solutions (0.2 mg/600 μL) at pH 5, 6, and 7 at 140 °C for 10−180 min and found that its breakdown was increased as time prolonged. After heating for 180 min, 19, 12, and 13% (at pH 5, 6, and 7) of avenacoside A was lost, respectively. Gahlawat and Sehgal (*37*) illustrated that roasting (70 °C for 2 h) of weaning food (prepared with 70 g of wheat or barely and 30 g of green bean) resulted in a 44.8−48.6% decrease in saponin. Badifu (*27*) toasted the kernels of melons (*Cucurbitacea* species) at 100 and 125 °C in the free-air oven for 25 min and found about 22−44% saponins reduction. He also pointed out that parts of saponin could be damaged by heat, resulting in structural changes. Sharma and Sehgal (*25*) stated that loss during cooking might perhaps signify the thermolabile nature of saponins. Khokhar and Chauhan (*38*) also mentioned that saponins reduction may be due to the formation of a poorly extractable complex between them and sugar or amino acids. The higher the temperature and time used,

the more the saponins would be broken and complexes would be produced.

Diosgenin could be found in all baked yam slices. **Table 4** shows the contents of diosgenin in yams after baking at 150, 180, and 200 °C for 3, 5, and 10 min. **Figure 4** presents the variations of diosgenin after cooking at 180 °C for 3, 5, and 10 min. In general, their amounts were higher than found for yams cooked with the microwave oven, especially after heating for 5 min. The conversion ratios from lost yam saponins to diosgenin (hypothesizing the saponins could be transformed completely) would be decreased as temperature and time increased.

Frying. Figure 5 shows the HPLC chromatograms of saponin extracts from yam fried at 200 °C for 3, 5, and 10 min. **Table 5** is the saponin contents in yams after frying at 150, 180, and 200 °C for 3, 5, and 10 min. The amounts of furostanol and spirostanol glycosides in yam were found to decrease with rising frying temperature and time. Frying caused the highest furostanol and spirostanol glycoside losses in all treatments. As yam slices were fried at 200 °C for 10 min, compounds **3** and **6** were not detected and about 93% of total furostanol glycosides and 97% of total spirostanol glycosides would be lost. The losses of furostanol and spirostanol glycosides during frying may be due to the destruction of the molecule or production of the poorly extractable complexes and attributed to the leaching of these saponins into the frying oil. Drumm et al. (*31*) commented that some saponins, including steroid (such as furostanol and spirostanol glycosides) and triterpenoid glycosides from nature, were not highly water-soluble and could be leached from the tissue during processing. The lipophilicity of spirostanol glycosides was higher than that of furostanol glycosides; therefore, spirostanol glycosides may leach more during frying. Solubilization of saponin into medium during processing could also contribute to the reduction (*26*). Furthermore, diosgenin could not be detected in any of the fried yam slices. It is supposed that the produced diosgenin might be leached into the frying oil entirely for its higher lipophilicity, and other reactions similar to the losses of steroidal saponins as described above might also happen.

Conclusion. When yam was cooked by steaming for 3−10 min, its furostanol and spirostanol glycosides did not change distinctly. With microwave cooking, baking, and frying, each saponin in yam would be lowered with increasing temperature and time. The highest saponin reduction was observed in fried yam, especially at 200 °C for 10 min. These results could provide information for the influence of different ways to prepare yam on the saponins. Besides, we also study the diosgenin content for different cooking procedures to reveal the degradation of saponins. Other products and pathways of degradation from saponins during cooking will be explored in the future.

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