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Quantitative analysis of allantoin and allantoic acid in yam tuber, mucilage, skin and bulbil of the *Dioscorea* species

Yi-Chung Fu [a,*], Lin-Huei A. Ferng [b], Pau-Yau Huang [a]

[a] *Department of Food Science, National Chung Hsing University, 250 Kuokuang Road, Taichung City 40227, Taiwan, ROC*
[b] *Department of Food Science, National I-Lan University, No. 1, Sec. 1, Shen-Lung Road, I-Lan 260, Taiwan, ROC*

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Abstract

Allantoin and allantoic acid in yam tubers of the *D. batatas* (Hualien no. 3) and *D. pseudojaponica* Yamamoto (Keelung) were quantitatively analyzed because the compounds were thought to prevent inflammation and ulcers in humans as well as playing an important role in the storage and translocation of nitrogen in higher plants. To expand yam tuber use, yam tuber mucilage can be used as an ingredient in functional foods and as a skin protectant. The objectives of this research are to analyze the contents of allantoin and allantoic acid in *D. batatas* and *D. pseudojaponica* Yamamoto and to compare bubble separation and centrifugation for recovering allantoin and allantoic acid from the mucilage. The total amount of allantoin and allantoic acid of the following *Dioscorea* (yam) in both the pulp and skin, respectively, were (mean ± RSD, mmoles/g solid): tuber of Keelung yam (*D. pseudojaponica* Y.) 0.370 ± 6.8%, 1.130 ± 3.4%; tuber of Hualien no. 3 yam (*D. batata*) 0.278 ± 7.8%, 0.714 ± 9.1%; bulbil of Hualien no. 3 yam (*D. batata*) 0.179 ± 7.9%, 0.297 ± 3.4%. The Keelung yam was much higher in allantoin and allantoic acid content than the Hualien no. 3. The skin of yam tuber is rich in allantoin and allantoic acid. The allantoin and allantoic acid content is 305% (in Keelung yam) and 257% (in Hualien no. 3) higher than that of the pulp. Bubble separation is an appropriate procedure in the practice for maximum recovery of allantoin and allantoic acid in yams. It was found that 80% of allantoin and allantoic acid yield can be recovered from the low starch contents of mucilage of the Keelung yam using bubble separation.

Keywords: Bubble separation; Yam; Mucilage; Allantoin; Allantoic acid

1. Introduction

Allantoin and allantoic acid are important in the nitrogen economy of plants, and research on their metabolism has attracted considerable attention (Fujihara & Tamaguchi, 1978; Matsumoto, Yatazawa, & Yamamoto, 1977a, Matsumoto, Yatazawa, & Yamamoto, 1977b, 1977c; Mothes, 1961; Ory, Gordon, & Singh, 1969; Osuji & Ory, 1987; Schubert & Boland, 1990; Thomas & Schrader, 1981). Allantoin is also a pharmacologically active compound (Anonymous, 1967). It is a common constituent of plants (Drewes & van Staden, 1975), being a component of the pathway of purine catabolism. Based on the wide use and clinical acceptance of allantoin, as well as published reports in the literature (Cajkovac, Oremovic, & Cajkovac, 1991; Fisher, 1981; Garnick, Singh, & Winkley, 1998; Margraf & Covey, 1977; Pinheiro, 1997; Sakuma, Ogawa, Kimura, Yamamoto, & Ogihara, 1998; Willital & Heine, 1994), allantoin has long been known to enhance the efficacy and desirability of cosmetic creams and lotions through its action as a skin protectant. The Merck Index lists the therapeutic applications of allantoin as a topical vulnerary (wound healer) and treatment for skin ulcers

* Corresponding author. Tel.: +886 4 22853922; fax: +886 4 22876211.
E-mail address: frank12@ms18.hinet.net (Y.-C. Fu).

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(The Merck index, 2001). The US FDA's (Food and Drug Administration) Tentative Final Monograph on skin protectant drug products for Over-The-Counter (OTC) human use has classified allantoin as a Category I (safe and effective) active ingredient skin protectant (Federal Register, 1983, 1990). The FDA has approved allantoin skin creams (0.5–2.0%) as non-prescription drug products for: (1) the temporary protection of minor cuts, scrapes, burns and sunburn; (2) preventing and protecting skin and lips against chapping, chafing, cracking and wind-burn, and (3) relieving dryness and softening cold sores and fever blisters. The allantoin is demulcent and works to soothe irritative conditions, both internally and externally. It works to promote the healing of tissues within the body. The allantoin is taken internally to promote cell proliferation. It protects tissues in the stomach, accelerates the healing processes throughout the stomach and bowels, and promotes increased tissue repair throughout the entire gastrointestinal tract.

Recently, the presence of allantoin in protea seed (Drewes & van Staden, 1975), soybean (Matsumoto et al., 1977a, 1977b, Matsumoto, Yatazawa, & Yamamoto, 1977c), comfrey plants (Teixeira & Duarte, 1985), alfalfa (Cheng et al., 1999) and coffee (Mazzafera & Gonçalves, 1999) has been reported. Roots were considered to be the main site of ureides synthesis (Thomas & Schrader, 1981). The yam (*Dioscorea* spp., *Dioscoreaceae*) is an important pharmaceutical plant that is widely used in the drug industry. The *Dioscorea* rhizome contained ureides (allantoin and allantoic acid) that are considered to present inflammation and ulcers in the human body (Sagara, Suto, Kawaura, & Yoshida, 1988). Ueda and Sasake (1956) determined the weight of allantoin of *D. opposite* Thumb. The anomalously high levels of allantoin found in yams suggest that its purine catabolism is different from that of the other root crops. Ozo, Robinson, and Reeves (1987) showed that the levels of allantoin found in yams were much higher than those in potato, sweet potato and cassava. Ninomiya, Murata, Tada, and Shimoishi (2003) reported that the *Dioscorea* species accumulated allantoin only in tubes.

The yam tuber is rich in starch and mucilage. Recently the applications of the low starch contents of mucilage in nutraceutical and cosmeceutical industries are required. Mucilage coats various tissues, providing lubrication as well as cooling – an activity that relieves gastrointestinal discomfort. So, it can be used as a skin protectant and an ingredient of functional foods. Actually separating starch from yam tuber is difficult because of the presence of viscous polysaccharide polymers (glycoprotein). In this study, the mucilage was recovered using centrifugation and bubble separation methods. This study analyzed the contents of allantoin and allantoic acids in *D. pseudojaponica* Y. (Keelung) and *Dioscorea batatas* (Hualien no. 3) and investigated the effect of different separation methods, centrifugation and bubble separation, on the recovery yield percentages of allantoin and allantoic acid from yam mucilage.

2. Materials and methods

Two varieties of yams, *Dioscorea batatas* (Hualien no. 3) and *Dioscorea japonica* Thunb var. *pseudojaponica* Yamamoto (Keelung) were used in the present study. Allantoin and allantoic acid contents from the pulp, mucilage and skin of both two yam tubers were quantitatively analyzed by the HPLC method. An abundance of aerial bulbils in the leaf axils was produced by Hualien no. 3 yam (*D. batata*). These small bulbils (aerial tubers) are a dark grayish brown, generally subspherical, smooth-surfaced, 1 cm in diameter and can sprout and grow into new vines. Allantoin and allantoic acid contents in the bulbil (Hualien no. 3 only) of yam tubers of *D. batatas* were also quantitatively analyzed with the HPLC method. A low starch content of mucilage was recovered by both the bubble separation and centrifugation methods.

2.1. Raw materials

Tubers of Keelung yam (*Dioscorea japonica* Thunb var. *pseudojaponica* Yamamoto) were obtained from local farmers in the Shihlin (Taipei, Taiwan) region. Hualien no. 3 yam (*D. batata*) was provided by the Taiwan Agricultural Research Institute (Hualien, Taiwan). Yam tubers and bulbil were weighed, peeled, and cut into small pieces. The pulp and skin of yam tuber and bulbil were freeze–dried. In order to obtain a homogeneous sample for analysis, portions of dried samples were ground to a fine powder (75–100 mesh) in a grinding mill. The flour samples were packed into airtight sample bottles and stored in the freezer until being used.

2.2. Proximate compositions of yam flours

The moisture, crude protein, crude fat, ash and crude fiber content of the yam tubers were determined according to AOAC (1995) approved methods 950.01, 976.05, 920.39, 955.03 and 962.09, respectively. The starch levels were calculated using a total starch assay kit (Megazyme International Ireland Ltd, Wicklow, Ireland). Non-starch soluble carbohydrates were extracted using 80% aqueous ethanol and assayed using the method of phenol – sulfuric acid using the methods of Dubois, Gilles, Hamilton, Rebers, and Smith (1956) and Asp (1993). Each determination was made in triplicate. The monosaccharide composition analysis were determined and quantified by high pressure liquid chromatography (HPLC) with an RI detector.

2.3. Methods for mucilage separation

Fig. 1 depicts a flow diagram for separating the mucilage from the yam tuber. Fourteen times (w/w) the amount of water (140 g water/10 g freeze–dried yam flour) was added into freeze–dried flour to form yam slurry. Equal amounts of water (1200 g water/150 g yam slurry) were used in the two methods, A and B, to dilute the viscous yam slurry to form a homogeneous slurry suspension for bubble separation and centrifugation. The single-stage procedure (method A) involved separating the mucilage and starch paste only using bubble separation. For method B, the relative centrifugal force (RCF) effect on mucilage separation and recovery was investigated. A high-speed refrigerated centrifuge (CR21G, Hitachi Koki Co., Ltd., Japan) was used. The water-soluble mucilage and starch paste separation procedure using centrifugation is described as follows. The slurry suspension was centrifuged at different RCFs (6000, 7300*g* RCF) at 4 °C for 30 min. The suspension was separated into a solid fraction and a liquid fraction. The sedimented solid paste was then treated with water and stirred to allow it to resuspend. The resuspended solution was then centrifuged and again separated into solid and liquid fractions. The mucilage collected from the above two methods was freeze–dried and subjected to detailed analysis. The mucilage composition was then compared.

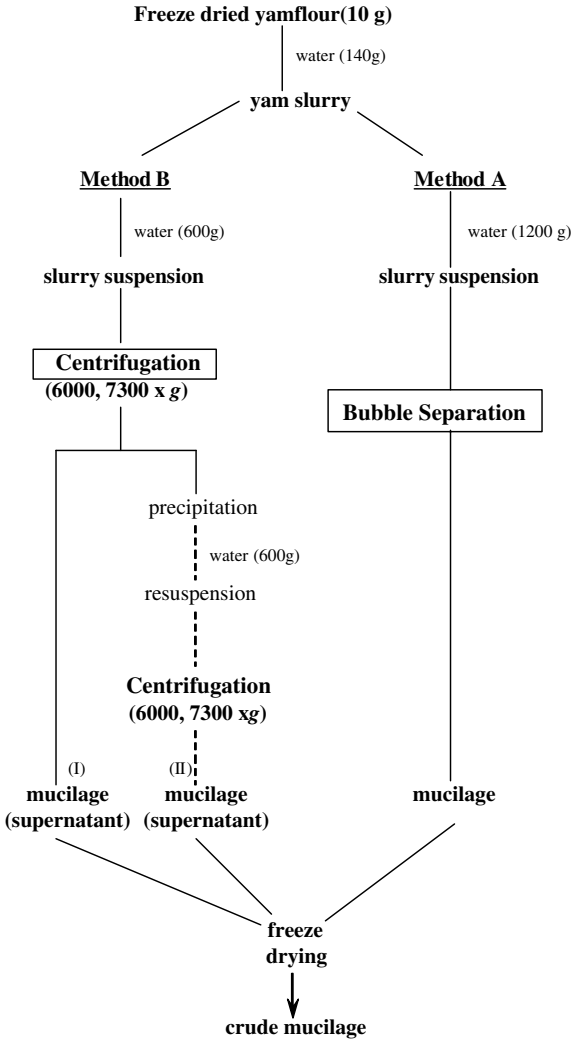

Fig. 1. Flow diagram for the separation of crude mucilage from the tuber of yam.

2.4. Bubble separation method

Fig. 2 shows a schematic diagram of a bubble separation system for obtaining yam tuber slurry suspension and separating the starch and mucilage. This lab-scale bubble separation system employed modified procedures from Fu, Mou, and Yeh (2003) and Fu (2004). This system was composed of a foaming system and a defoaming system.

The foaming system was composed of a yam slurry suspension tank, a glass foam column, a tank for diluted mucilage, a starch reservoir tank and a mucilage reservoir. A feed stream was obtained by pumping the slurry suspension from the reservoir tank into a foam column using a centrifugal pump (Ulvac G-100D, Ulvac Kiko, Inc.). Gas for the purpose of separation was provided by a gas cylinder. Bubbles were produced by introducing gas through a gas sparger that passed through the yam slurry suspension to the surface, resulting in the formation of a stable foam phase. The final bubbles were collected at the column exit and transferred into the next stage of the defoaming system. The remaining diluted mucilage lying at the bottom of the two columns was recirculated and pumped back into the slurry suspension tank.

The other process variables were: gas flow rate (1500 cm^3/min), bubble flow rate (450 cm^3/min), foam column I (100 cm high with a foam height of 55 cm), foam column II (70 cm high with a foam height of 40 cm), and the diameter of both columns (3 cm). A flat gas sparger (perforated ceramic plate 3 cm in diameter, pore size = 0.2 μm) was used. All experiments were conducted at room temperature (25 ± 2 °C).

The defoaming system was composed of a defoaming column (L = 60 cm, D = 6 cm), a vacuum pump and a mucilage reservoir tank. Packed glass wool (L = 35 cm) and a porous plate (6 cm in diameter, pore size = 0.5 mm) were used in the defoaming column. By adjusting the vacuum pump, the bubbles could come in contact with a porous plate under which a lower pressure was applied, placing the bubbles into a pressure gradient ΔP. The pressure gradient was lower than the capillary pressure of the plate pores dampened by the mucilage. Under these conditions, liquid, but not gas or solids, was able to pass through the porous plate. A theoretical investigation of the foam using the pressure

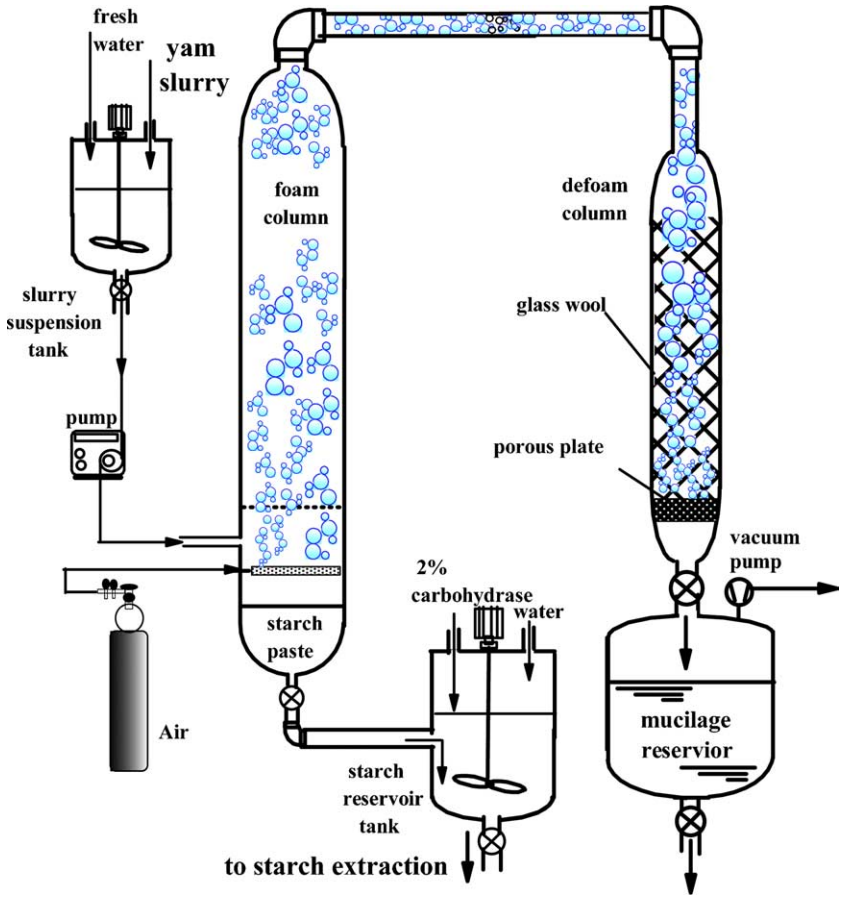

Fig. 2. Apparatus and operate flow chart of the lab-scale bubble separation system.

difference ΔP in the Plateau–Gibbs borders can be seen in Lalchev and Exerowa (1981). This method can accelerate drainage and cause a more rapid destruction of the foam. A defoaming agent is not necessary with this method.

2.5. Allantoin and allantoic acid determination

The freeze–dried samples of pulp, skin, and mucilage (supernatant) of the *Dioscorea* tubers were quantitatively analyzed for allantoin and allantoic acid content. This study used modified procedures of Ozo et al. (1987). 2-g of freeze–dried power was dissolved in 10 ml of water. Ten to 15 times the amount of 95% ethanol (v/v) was added, and the mixture was ultrasonicated for 10 min at 4 °C to be stored for overnight. The supernatant evaporated almost to the point of total dryness on a rotary evaporator. Four ml of distilled water was added and ultrasonicated for 30 min at 4 °C. A one ml sample was taken and centrifuged at 7700*g* for 10 min. The solution was used for determining the content of allantoin and allantoic acid. Allantoin and allantoic acid were analyzed by HPLC. The conditions were as follow: HPLC column: Zorbax ODS (5 μm, 250 × 4.6 mm); Mobile phase: EtOH/$CHCl_3$/H_2O (0.5/0.012/100); flow rate: 0.2 ml/min; UV wavelength: 200 nm; Column temperature: 27 °C. Before injection, the samples were filtered through a 0.22 μm millipore filter. The chromatogram was acquired using ChromatoStation chromatography software (N2000, Zhejiang University, China). Allantoin and allantoic acid concentrations were also obtained with a colorimetric assay (Vogels & van der Drift, 1970). The standards for allantoin and allantoic acid were purchased from Sigma Chemical Company (St. Louis, MO).

3. Results and discussion

3.1. Tuber chemical composition and derived ingredients

The tuber and bulbil proximate analysis results are shown in Table 1. All results are expressed on a dry-weight basis. Both yam varieties contained about 20% solid matter. The starch and protein contents in Keelung yam (*D. pseudojaponica* Y.) tuber were 60.6% and 16.6%, respectively. Those in Hualien no. 3 (*D. batata*) tuber were 78.0% and 11.6%, respectively. Starch is the most important component in a tuber with a sig-

Table 1
Chemical composition of Keelung and Hualien no. 3 yams in % of dry matter

Sample	Crude fat (%)	Crude fiber (%)	Ash (%)	Crude protein (%)	Starch (%)	Soluble carbohydrates (%)	Dry matter (%)
Keelong yam (tuber) (*D. pseudojaponica*)	0.30	2.8	4.2	16.6	60.6	16.2	19.8
Hualien no. 3 yam (tuber) (*D. batata*)	0.20	2.7	3.9	11.6	78.0	4.2	16.8
Hualien no. 3 yam (bulbil) (*D. batata*)	0.44	5.5	5.2	12.7	71.9	4.4	21.5

Reported values are the means with relative standard deviation (SD/mean) <8% ($n > 3$).

nificant protein content. Other authors describe tubers with contents varying from 65% to 80% of starch and 2.1% to 14.3% protein (Alves, Grossmann, & Silva, 1999; Fu et al., 2003; Lii & Tsai, 1985; Tsai & Tai, 1984). The non-starch soluble carbohydrate content in Keelung yams is high in comparison with Hualien no. 3 yams. The starch content in Keelung yams is lower. The highly soluble carbohydrates in Keelung yams cause the slurry very viscous resulting in starch separation difficulty. The yam crude fat, crude fiber, and ash chemical content was 0.20–0.30%, 2.7–2.8%, and 3.9–4.2%, respectively. These values are similar to those reported previously. No other report detailing the chemical composition of aerial bulbils was found. The starch content of Hualien no. 3 yam in aerial bulbil is less than that of the tuber. However, the ash and fiber contents in aerial bulbil are higher.

3.2. Mucilage monosaccharide composition

The monosaccharide composition of the bubbles recovered from the mucilage was analyzed by HPLC following acid hydrolysis. As expected, the predominant monosaccharide detected in the mucilage sample was mannose (Table 2). However, others units like glucose, galatose, arabinose and xylose were also found at levels under 5%. The high mannose content level agrees with the results reported by Misaki, Ito, and Harada (1972). According to these results, a significantly low level of glucose (1.5–1.9%) was detected. This can be interpreted as being due to possible starch contamination. The soluble carbohydrates extracted from the mucilage can be regarded as a very pure mannan according to Aspinall's definition (Aspinall, 1959). This low level of starch contamination also presents an excellent separation of starch and mucilage from the tubers using the bubble separation process.

3.3. Allantoin and allantoic acid determination

Several methods for allantoin determination are known. Allantoin is converted to allantoic acid by mild alkaline hydrolysis, followed by acid hydrolysis of the latter to urea and glyoxylic acid. The concentration of the latter substance is then determined colometrically or spectrophotometrically as phenylhydrazone. A detailed evaluation of this method was undertaken by Young and Conway (1942). Allantoic acid is commonly found with allantoin and interferes with the standard estimation of it because it also yields glyoxylic acid on degradation. So, according to the method of Young and Conway (1942), the sum of allantoin and allantoic acid is measured with the expression "allantoin" also including allantoic acid.

When Ninomiya et al. (2003) investigated fresh tubers of *Dioscorea opposite* "Tsukuneimo", the amount of ureides containing allantoin and allantoic acid was first spectrophotometrically determined by adding phenylhydrazine hydrochloride, using the method of Kaczmarek and Walicka (1958). Allantoin content was determined by a HPLC method (Yamamoto et al., 1998). The amount of allantoic acid was then calculated by substracting the amount of allantoin assessed by HPLC from that of ureides determined by spectrophotometry. Allantoin and allantoic acid were detected in the xylem sap of coffee by HPLC under analytical conditions of 235 nm UV detection and HOAc at 0.2 ml/min (Mazzafera & Gonçalves, 1999). But the separation of these two components was still unsatisfactory. As observed for allantoic acid, a very tiny allantoic acid peak on the tail was found with the analytical conditions they used. Based on our study, with careful selection of the buffer ($EtOH/CHCl_3/H_2O$ (0.5/0.012/100)), UV absorption spectra (200 nm) and flow rate (0.2 ml/min), it was actually possible to separate the allantoin and allantoic acid by HPLC under the conditions used here (Fig.

Table 2
Monosaccharide composition of crude mucilage separated from Keelung and Hualien no. 3 yams using the bubble separation method

	Glucose (%)	Galactose (%)	Mannose (%)	Arabinose (%)	Xylose (%)	Others (%)
Keelong yam[a] (tuber) (*D. pseudojaponica*)	1.5	2.1	95.4	0.8	–	<0.2
Hualien no. 3 yam[a] (tuber) (*D. batata*)	1.9	2.7	93.9	0.6	0.1	<0.8

Relative standard deviation (SD/mean) was less than 4%.

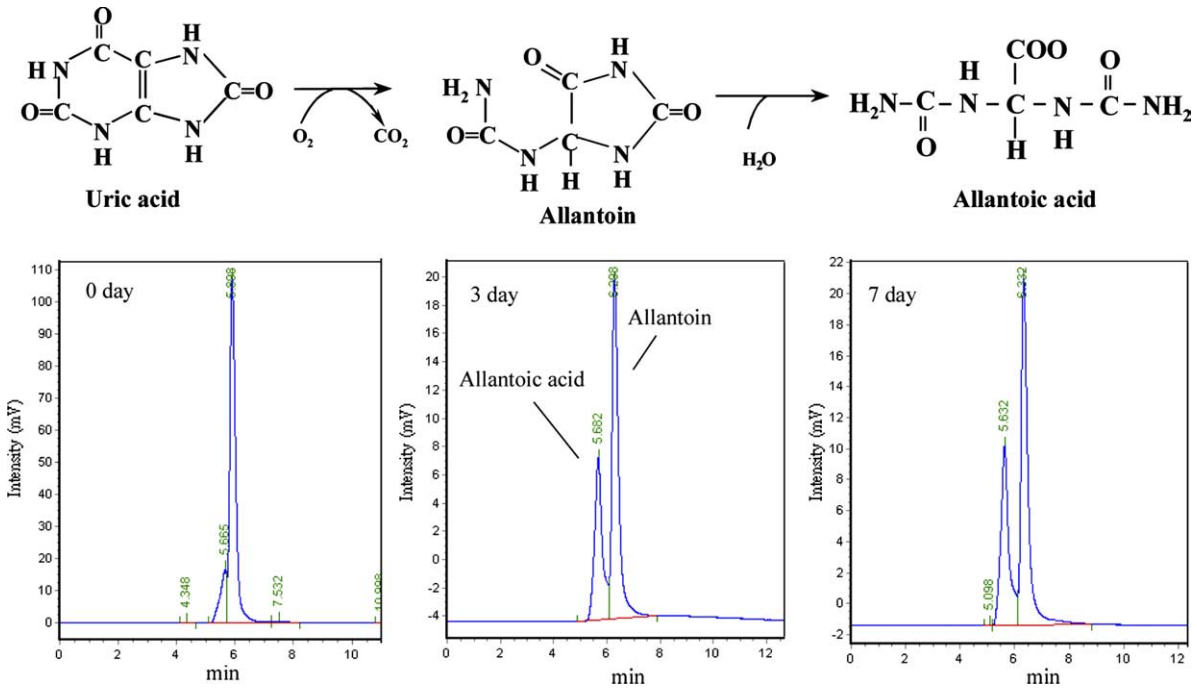

Fig. 3. Catabolism of uric acid and HPLC profile obtained at 200 nm for the detection of allantoic acid and allantoin with various storage times.

3). Fig. 3 depicts the HPLC profile obtained at 200 nm for the detection of the mixture of 500 mg/l allantoic acid and 25 mg/l allantoin. The important point to notice is that the retention time of these two peaks were so close that they could not be separated until the flow rate was reduced to 0.2 ml/min. Without reducing the

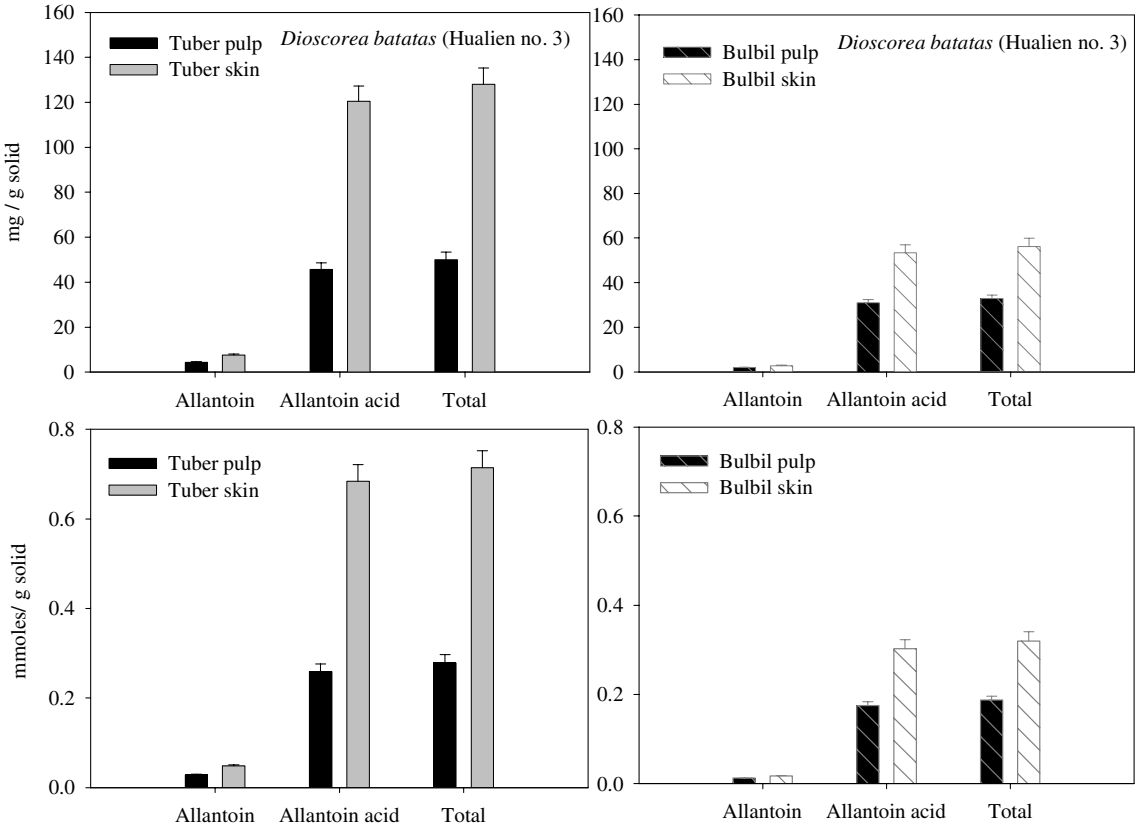

Fig. 4. Concentration of allantoin and allantoic acid in the pulp (tuber), skin (tuber) and bulbil of Hualien no. 3 yam.

flow rate it would be easy to mistake the total amount of allantoin and allantoic acid for allantoin only. This occurred when Wang, Chiang, Du, and Chou (1995) determined the allantoin in yam (*D. opposite Thunb*). It is also interesting to note that the concentration for these two components changes with storage time. Fig. 3 shows the HPLC profiles 0, 3 and 7 days after the solution was prepared. The peaks of allantoic acid and allantoin differed in size and separation. So the allantoin would be in its more stable form of allantoic acid in an aqueous solution. However, if the total amount of allantoin and allantoic acid is the same, it would be more appropriate to represent it in total mmoles per g solid than in total weight (mg). According to Beer's law, the extinction coefficients for allantoin and allantoic acid when they are analyzed are quite different. Mistaking either allantoin for allantoic acid or allantoic acid for allantoin may result in significant errors. It appears that at levels under 200 nm, the extinction coefficient of allantoin is much larger than that of allantoic acid. Allantoin and allantoic acid concentrations were also obtained with a colorimetric assay (Vogels & van der Drift, 1970). However, higher values were obtained for total allantoin and allantoic acid with HPLC. In general, the HPLC determinations were found to be 38% higher than the colorimetric values (data not shown).

Fig. 4 shows the concentration of allantoin and allantoic acid in the pulp (tuber), skin (tuber) and bulbil of Hualien no. 3 yam. The results showed that less of the total amount of allantoin and allantoic acid was found (40% in the skin and 64% in the pulp) in the bulbil than in the tuber. Fig. 5 shows the concentration of allantoin and allantoic acid in the pulp, mucilage and skin of Keelung yam tuber. The total amount of allantoin and allantoic acid in the skin displayed a much higher value (305%) than that of the pulp. Eighty percent of the two components in the mucilage were recovered from the pulp using the bubble separation method. Compared with these two varieties, the two components in Keelung yam showed a higher value (133% in the pulp and 158% in the skin) than in Hualien no. 3 yams.

3.4. Bubble separation and centrifugation for recovering mucilage

It is difficult to separate starch from the tubers. The settling of starch granules is often hindered by the presence of various components like mucilage. Fu et al. (2003) developed a continuous bubble separation process for separating and recovering starch and mucilage from the pulp of yam tubers in the absence of undesirable chemical additives or treatments. It has been shown that excellent separation can be achieved for a system containing starch and polysaccharide–protein-containing complexes. Very low amounts of starch (0.8%) remained in the mucilage and the yields of soluble

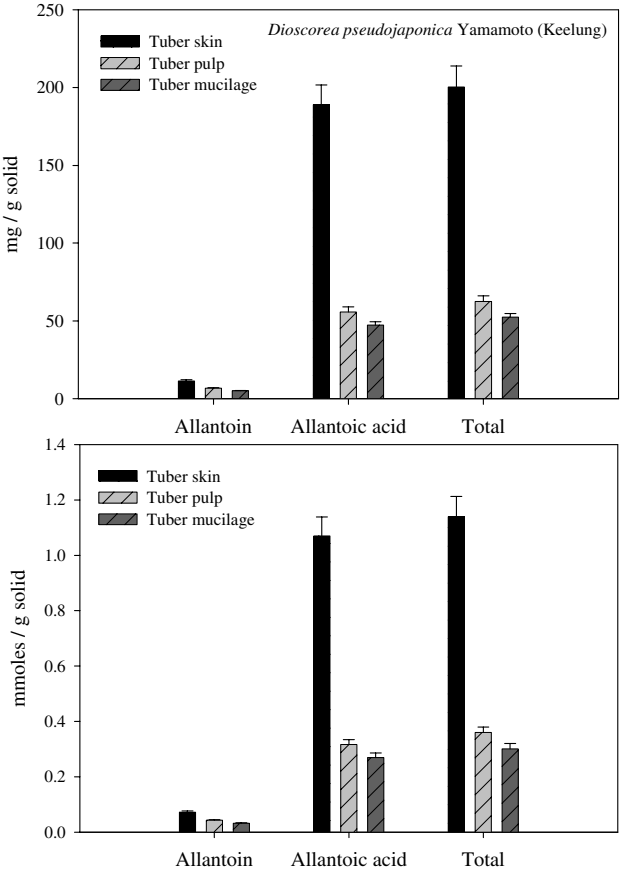

Fig. 5. Concentration of allantoin and allantoic acid in the pulp, mucilage and skin of Keelung yam tuber.

carbohydrates and protein in separated mucilage were 98.8% and 74.1%, respectively (Fu et al., 2003). A very low amount of starch (0.8%) remained in the mucilage. In Fig. 5, 80% of the total amounts of allantoin and allantoic acid was recovered by bubble separation and remained as starch-free mucilage.

Centrifugation was also thought to be a good method for separating starch and mucilage. Ten grams of freeze–dried yam flour was extracted with 1340 g of water using centrifugation and bubble separation. The procedures are shown in Fig. 1. Fig. 6 also shows the effect of centrifugation on the recovery of allantoin and allantoic acid in the separated supernatant (mucilage). The slurry suspension was centrifuged twice and the effects of the centrifugation on the extraction of those two components were studied. Two RCFs, 6000 and 7300*g* for 30 min, were used. Freeze–dried supernatant was used for allantoin and allantoic acid determination. The recovered yields of allantoin and allantoic acid obtained from two consecutive centrifugations of 6000 and 7300*g* were 23.0% and 10.0%, respectively (Fig. 6). These were much smaller than that obtained from the bubble separation method (80%). These experiments involved increasing the number of centrifugations (resuspension and resedimentation), but the results (data not shown)

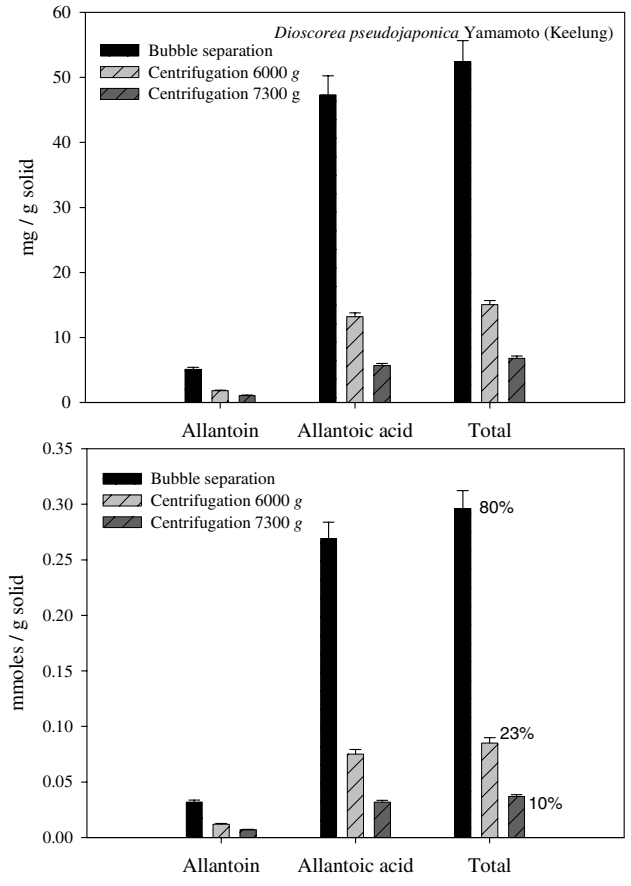

Fig. 6. Concentration of recovered allantoin and allantoic acid from the mucilage of Keelung yam using the bubble separation and centrifugation method. The percentages indicate the recovered molar ratio compared with the total amount of those in the pulp.

showed that less than 7% yield could be obtained. Actually, in previous studies we found that centrifugation destroyed the structure of glycoprotein, resulting in further separation of carbohydrates and proteins in different parts of the starch paste and mucilage (Fu, Chen, & Lai, 2004). These experiments have shown that there is difficulty in fully extracting proteins and soluble carbohydrate from the pulp of yam tubers through using centrifugation. It was found that only 2–8% of the total amount of polysaccharides were recovered.

Recently, applications of the low starch contents of mucilage in the nutraceutical and cosmeceutical industries have been in demand. Centrifugation results in damage to the glycoprotein structure and differences in the proportions of protein. Thus, an attempt to fully recover allantoin and allantoic acid from the mucilage by separating starch using centrifugation may not be feasible. The highest allantoin and allantoic acid contents can be recovered by bubble separation because of the excellent separation of starch and mucilage. Allantoin is active in skin-softening (keratolytic effect) and rapid cell regeneration through precipitating proteins on the skin. The possibility of using yam tuber mucilage in skin-care products has been studied (Lai, 2003). It provides an alternative method for recovering low starch contents of mucilage with high yields of allantoin and allantoic acid and is especially good for recovering allantoin and allantoic acid from a starch and polysaccharide–protein-containing system.

4. Conclusions

The yam tuber is rich in starch and mucilage, however, it is difficult to separate the starch from the tuber because of the viscous soluble carbohydrates-protein (glycoprotein) present in the yam. To expand yam tuber use, low starch contents from the mucilage can be used as an ingredient in functional foods and a skin protectant. The objectives of this research were to analyze the contents of allantoin and allantoic acid in *D. batatas* (Hualien no. 3) and *D. pseudojaponica* Yamamoto (Keelung yam) and to compare bubble separation and centrifugation for recovering allantoin and allantoic acid from the separated mucilage.

In general, it is possible to separate allantoin and allantoic acid by HPLC under the conditions used in this study. The HPLC determinations were more accurate than the colorimetric method. Thirty eight percent higher values were obtained with HPLC. These experiments showed that the Keelung yam tuber was much higher in allantoin and allantoic acid content than the Hualien no. 3 yam. The skin of yam is rich in allantoin and allantoic acid, which are 305% (in Keelung yam) and 257% (in Hualien no. 3) higher than those of the pulp. There is difficulty in fully recovering allantoin and allantoic acid from yam pulp using centrifugation. With two consecutive centrifugations, the total amount of allantoin and allantoic acid recovered from the mucilage of Keelung yam tuber was less than 30%. Bubble separation is an appropriate procedure in the practice for maximum recovery of allantoin and allantoic acid in yams. Eighty percent of the total amount of allantoin and allantoic acid was recovered using bubble separation.

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