JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Application of Bubble Separation for Quantitative Analysis of Choline in *Dioscorea* (Yam) Tubers

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A modified assay based on the AACC official method 86−45 (AACC, 2000) for the determination of choline in three cereals and three varieties of *Dioscorea* (yam) tubers was developed. When tested in wheat, rice, and oat flour, choline estimated by the modified method was 34.0−45.3% higher than that of the original AACC method. In a system with higher contents of starch and mucilage, such as *Dioscorea* (yam) tubers, extra procedures in sample preparation needed to be carried out to separate starch and mucilage. The choline contents of the following *Dioscorea* (yam) tubers using the original AACC method and the present modified AACC method through coupling an additional bubble separation procedure, respectively, were (mean ± SD, mg/g solid) Keelung yam (*D. pseudojaponica* Y.) 0.92 ± 0.09 and 2.21 ± 0.12, Yangmingshan yam (*D. alata* L.) 0.77 ± 0.09 and 1.78 ± 0.28, and Ming-Chien yam (*D. purpurea*) 0.44 ± 0.09 and 1.35 ± 0.19. Choline was 231−306% higher than when the original AACC method was used. *Dioscorea* (yam) tubers were much higher in choline content than they were in cereals. Bubble separation is an appropriate procedure in the practice for the maximum assay of choline in yams. It is accurate, rapid, easy to handle, and especially good for recovering choline from a starch and polysaccharide−protein-containing system.

KEYWORDS: Choline; *Dioscorea*; yam; centrifugation; mucilage; bubble separation

INTRODUCTION

Recently, the concept of functional food raises in yam has been studied for use as a functional food. In Eastern countries, different yam species of *Dioscorea* have been widely used for the enhancement of health. The dried slices of yam tuber are frequently used as a superior medical herb substance and functional food for the traditional treatment of several illnesses in China (*1*). In Chinese pharmacopoeia, *Pentsao*, the medicinal uses of *Dioscorea* rhizome include indigestion, anorexia, diarrhea, and diabetes (*2*). It is suggested to have a hypoglycemic effect (*3*) and promote the health of elderly woman (*4*, *5*). Despite the long time consumption of yam tubes and an increasing interest in yams, their nutritional values have not been extensively investigated (*6*−*9*).

Choline is an important nutrient; it is necessary for growth, normal bone development, fat metabolism, and egg production. Choline is an important phospholipid (lecithin and sphingomyelin); it is required for the synthesis of the neurotransmitter acetylcholine, acts as a source of labile methyl groups, and is a component of pulmonary surfactant (*10*). Choline deficiency has been reported, despite it being widely available in food. The most common signs of choline deficiency are fatty liver and hemorrhagic kidney necrosis (*11*). Evidence for free-radical activity in the liver with choline deficiency is reported, and this may be related to the carcinogenesis process (*12*). Choline is ingested mainly in the form of phosphatidyl choline rather than in the free-base form. Choline chloride and choline bitartrate are added to infant formulas and milk products to ensure the presence of adequate choline levels (*11*). Recently, an adequate intake (AI) of choline has been established by the Institute of Medicine, Food, and Nutrition Board (*13*). The AI is used when the recommended dietary allowance (*14*) cannot be determined because sufficient data are lacking. There is comparatively little literature concerning the choline content in yams, despite the fact that the estimation of choline in many plants materials has been reported (*15*, *16*).

The importance of choline as an essential nutrient creates a need for suitable analytical methods. The reineckate method, AACC official method 86−45 (*17*), has been most widely used for analysis of plant materials. The estimation of choline in plant materials is difficult because of the various forms in which it may occur. 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 (*7*, *18*). In a previous study, there was difficulty in fully recovering allantoin and allantoic acid from yam pulp using centrifugation (*19*). With two consecutive centrifugations, the total amount of allantoin and allantoic acid recovered from the mucilage of Keelung yam tuber was less than 30%. We hypothesize that the presence of viscous mucilage

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10.1021/jf048501h CCC: $30.25
Published on Web 02/23/2005

may cause analytical difficulty for the purposes of choline determination.

The present paper describes a modified method based on the original principle of the AACC official method 86−45 for estimations of choline in plant materials. Fu et al. (*18*, *20*) used the bubble separation method to separate most of starch and viscous mucilage from yam tubers. Centrifugation was also thought to be a good method for separating starch and mucilage. This study also investigated the effect of an additional bubble separation or centrifugation procedure for separating starch and mucilage prior to applying the modified method on the determination of choline in *Dioscorea* (yam) tubers.

MATERIALS AND METHODS

Three varieties of yams, *Dioscorea alata* L (Yangmingshan yam), *D. purpurea* (Ming-Chien), and *Dioscorea japonica* Thunb var. *pseudojaponica* Yamamoto (Keelung) were used in the present study. Tubers of Keelung yam (*Dioscorea japonica* Thunb var. *pseudojaponica* Yamamoto) and Yangmingshan yam (*D. alata* L.) are both Taiwanese native varieties and were obtained from local farmers in the Shihlin and Yan gmingshan (Taipei, Taiwan) region, respectively. Ming-Chien yam (*D. purpurea*) was provided by the Taiwan Agricultural Research Institute (Taichung, Taiwan). Wheat, oat, and rice flour samples were purchased from supermarkets.

Yam tubers were weighed, peeled, cut into small pieces, and freeze-dried. 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. Wheat, oat, and rice flour samples were freeze-dried.

Proximate Analysis of Yam Tubers. The moisture, crude protein, crude fat, ash, and crude fiber content of the yam tubers were determined according to AOAC (*21*) approved methods 950.01, 976.05, 920.39, 955.03, and 962.09, respectively. Starch levels were calculated using a total starch assay kit (Megazyme International Ireland, Ltd., Wicklow, Ireland). Nonstarch soluble carbohydrates were extracted with 80% aqueous ethanol and assayed by the method of phenol−sulfuric acid using the methods of Dubois et al. (*22*) and ASP (*23*). Each determination was made in triplicate.

Methods for Crude Mucilage Separation. Figure 1 depicts the flow diagram for the separation of crude mucilage from the tuber of yam. To form yam slurry, 14 times (w/w) the amount of water (700 g of water/ 50 g of freeze-dried yam flour) was added into freeze-dried flour. Equal amounts of extra water (6 kg of water/ 0.75 kg of yam slurry) was added by two methods, A and B, to dilute the viscous yam slurry to form homogeneous slurry suspension for bubble separation and centrifugation. The single-stage procedure (method A) involved only bubble separation for separating mucilage and yam paste. In method B, the effect of the relative centrifugal force (RCF) on the separation and recovery of mucilage was investigated. A high-speed refrigerated centrifuge (CR21G, Hitachi Koki Co., Ltd., Japan) was used. The sequence of the procedure for separation of the water-soluble mucilage and starch paste by centrifugation is described as follows. The slurry suspension was centrifuged at different relative centrifugal forces (3500*g*, 6000*g*, and 7300*g* RCF) at 4 °C for 30 min. The suspension was separated into a solid and liquid fraction. The sedimented solid paste was then treated with water and stirred to allow it to resuspend. The resuspension 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 composition of the crude mucilage was then compared.

Bubble Separation Method. Figure 2 shows the schematic diagram of a bubble separation system for obtaining slurry suspension of yam tubers and separating starch and mucilage (*20*, *24*). This system was composed of a foaming and defoaming system. The foaming system was composed of a yam slurry suspension tank, a glass foam column (L = 100 cm, and D = 3 cm), and a starch reservoir tank. A feed stream was obtained by pumping slurry suspension from the reservoir tank into the foam column using a centrifugal pump (Ulvac G-100D,

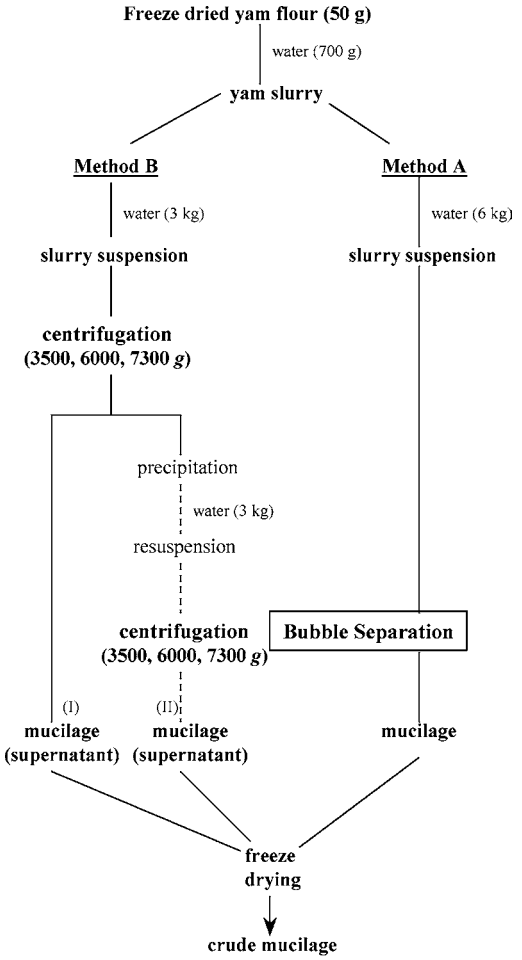

Figure 1. Flow diagram for the separation of crude mucilage from the tuber of yam.

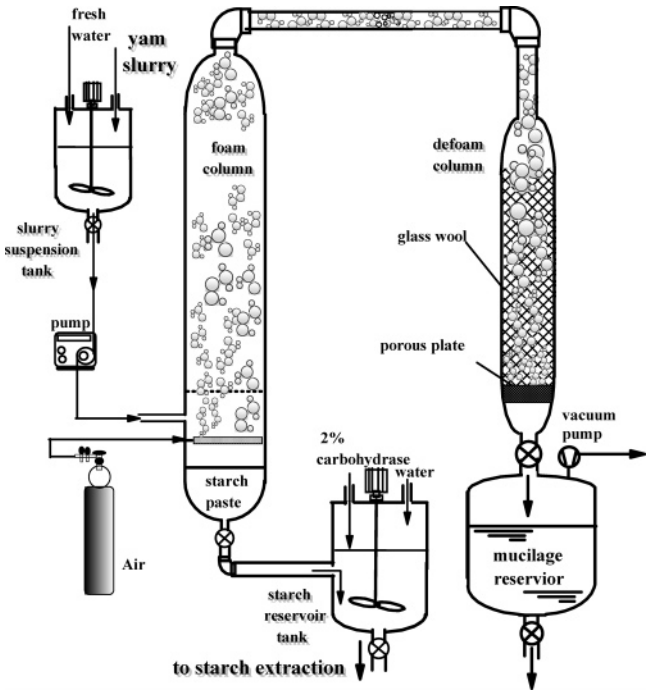

Figure 2. Apparatus and operating flowchart of the lab-scale bubble separation system.

Ulvac Kiko, Inc.). The gas to be used for the separation was provided by a gas cylinder. Bubbles were produced by the introduction of gas through the gas sparger (perforated ceramic plate 3 cm in diameter,

Table 1. Proximate Analysis of Tubers of Keelung, Yangmingshan, and Ming-Chien Yams[a]

sample	crude fat (%)	crude fiber (%)	ash (%)	crude protein (%)	starch (%)	soluble carbohydrates (%)
Keelung yam[b] (*D. pseudojaponica*)	0.300 ± 0.012	2.80 ± 0.08	4.20 ± 0.20	16.60 ± 0.66	60.6 ± 3.1	16.2 ± 0.61
Yangmingshan yam[b] (*D. alata* L.)	0.28 ± 0.013	2.70 ± 0.07	3.31 ± 0.13	13.20 ± 0.61	77.4 ± 3.3	3.41 ± 0.12
Ming-Chien yam[b] (*D. purpurea*)	0.29 ± 0.012	2.10 ± 0.10	4.51 ± 0.22	12.21 ± 0.56	75.6 ± 2.27	5.62 ± 0.25

[a] Reported values are the means with relative standard deviation (SD/mean) < 5% ($n = 3$). [b] Moisture content of Keelung, Yangmingshan, and Ming-Chien yams are 80.2, 79.6, and 81.6%, respectively.

pore size = 0.2 μm), which passed through the yam slurry suspension to the surface, resulting in the formation of a stable foam phase. The bubbles were collected at the column exit and passed into the next stage of the defoaming system. The remaining material (starch paste) left in the foam column is drained and collected in a starch reservoir tank.

The defoaming system was composed of a defoaming column (L = 60 cm, and 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. After the vacuum pump was adjusted, the bubbles can come in contact with a porous plate under which a lower pressure was applied resulting in the bubbles having a pressure gradient of ΔP. The latter was to be made lower than the capillary pressure of the plate pores dampened by the mucilage. Under these conditions, liquid but not gas or any solids is able to pass through the porous plate. Theoretical investigation of the foam through the creation of a pressure difference ΔP in the Plateau−Gibbs borders can be seen in Lalchev and Exerowa (*25*). This method can accelerate drainage, a more rapid destruction of the foam, and it is not necessary to add any defoaming agent. The process optimization of the design was demonstrated (*7*, *20*, *24*). All experiments were conducted at room temperature (25 ± 2 °C).

Choline Determination. This modified method is based on the reineckate technique, AACC official method 86−45 (*17*) and involves hydrolysis of samples in 20% HNO_3, neutralizing with NaOH, filtering and adjusting pH levels to 13, precipitation by addition of ammonium reineckate solution, washing and drying of the precipitate, dissolution in acetonitrile, and measuring at 526 nm. The experiment for choline determination was conducted as follows: a 2-g freeze-dried power sample was digested by simmering in 100 mL of 20% nitric acid for 5 h. After hydrolysis, the digest filtered through a sintered glass funnel of medium porosity (0−15 μm) under suction. A known volume of filtrate was adjusted to pH 13 by adding sodium hydroxide pellets and then 10 N NaOH. The volume of the solutions in the beakers was noted, and 15-mL samples in triplicates were transferred into 30-mL centrifuge tubes. The centrifuge tube was then chilled before adding 4 mL of freshly prepared 5% ammonium reineckate solution. The centrifuge tube was again chilled overnight at 4 °C. The reineckate precipitate was centrifuged out at 18675*g* for 40 min, and the supernatant was discarded. The choline reineckate precipitate was washed 3 times with 3-mL of 1-propanol, dried in a desiccator containing sulfuric acid for 4 h, and dissolved in 4 mL of acetonitrile. The solution was centrifuged to remove the insoluble material, and the concentration of choline reineckate was quantified by spectrophotometric measurement (Model U-2001, Hitachi, Japan) at 526 nm after 20−30-fold dilution with 50% ammonium hydroxide.

Sigma grade choline chloride was recrystallized 4 times from hot anhydrous ethanol, dried over sulfuric acid, and used as the standard. Computation of choline at a reference to the standard curve was constructed from measurements of known concentrations of choline reineckate. Methyl red color standard was used to set the spectrophotometer prior to each measurement. Add 40 mL of 6.25 mg % aqueous methyl red solution to 460 mL of 0.1 M citrate buffer at pH 3.7.

RESULTS AND DISCUSSION

Chemical Composition of Tubers and Derived Ingredients. The results of the proximate analysis of tubers are shown in **Table 1**. All results are expressed on a dry-weight basis. Three varieties of yam tubers contain about 20% solid matter. Starch is the most important component of tuber with a significant protein content ranging from 12.2 to 16.6%. Other authors describe tubers with contents varying from 65 to 80% of starch and 2.1 to 14.3% of protein (*7*, *26*−*28*). The nonstarch soluble carbohydrate content in Keelung yams is significantly higher than those of Yangmingshan and Ming-Chien yams; on the contrary, the starch content in Keelung yams is lower. The highly soluble carbohydrates cause the yam slurry of Keelung yams to be very viscous, resulting in starch separation difficulty. The analysis of the chemical composition of crude fat, crude fiber, and ash in yam tubers revealed amounts of 0.28−0.3, 2.1−2.8, and 3.3−4.5%, respectively. These values are similar to those reported previously.

Choline Determination. The method commonly used for the estimation of choline in plant material, AACC method 86−45, involves the precipitation of choline as the reineckate salt in a colorimetric reaction, where choline is chemically measured as choline reineckate (*17*). This method involves Soxhlet extraction of a sample with methanol for 24 h, hydrolysis of choline compounds by boiling in a saturated solution of barium hydroxide, $Ba(OH)_2$, precipitation of choline as the reineckate salt at about pH 10, solubilization of this precipitate in acetone, and measurement of absorbance at 526 nm.

Ackerman and Salmon (*29*) showed that hydrolysis of a mixed plant and animal tissue sample with barium hydroxide resulted in inconsistent and low values for choline as compared to direct hydrolysis with 25% nitric acid, followed by choline reineckate precipitation from 0.1 to 0.2 N sodium hydroxide. Plant flour samples were digested with 20% HNO_3 for 5 h in this experiment. It was found that hydrolysis for 5 h with 25% HNO_3 did not increase the yield of choline when compared to a 20% HNO_3, and more consistent results were obtained with a longer hydrolysis time. Some researchers have shown that the sensitivity of this method could be increased by measuring the absorbance of choline reineckate in acetone solution at 327 nm (*30*, *31*). Argoudelis and Tobias (*32*) examined the ultraviolet spectrum of choline reineckate in a number of solvents and concluded that acetonitrile was the best solvent. We found that choline reineckate in acetonitrile exhibits intense absorption maxima at 526 nm. In comparison to the AACC official method 86−45, analysis procedures of plant protein source samples showed a digestion of choline compounds in 20% nitric acid for 5 h instead of in a saturated solution of barium hydroxide and measuring absorbance of choline reineckate in acetonitrile solution at 526 nm instead of in acetone solution at 327 nm.

The procedure that we used resulted in higher recovery and much more consistent values for total choline in plant protein sources than what the AACC method 86−45 did (**Table 2**). In wheat, rice, and oat flour, the total choline estimated by our

Table 2. Effect of Methods of Estimation on the Choline Content of Plant Materials

	estimation method[a] (mg/g solid)		
	AACC	this method (modified AACC	
sample	barium hydroxide in acetone solution (hydrolysis) (526 mm)	nitric acid in acetonitrile solution (hydrolysis) (526 mm)	increases (%)
wheat flour	0.86 ± 0.17	1.25 ± 0.09	45.3
rice flour	0.79 ± 0.11	1.13 ± 0.12	43.0
oat flour	1.03 ± 0.13	1.38 ± 0.09	34.0
Keelung yam flour (*D. pseudojaponica*)	0.92 ± 0.09	1.07 ± 0.07	16.3
Yangmingshan yam flour (*D. alata* L.)	0.77 ± 0.09	0.87 ± 0.09	13.0
Ming-Chien yam flour (*D. purpurea*)	0.44 ± 0.09	0.51 ± 0.09	15.9

[a] Mean ± SD ($n = 3$)

modified AACC procedures were 34.0−45.3% higher than that measured by the AACC method 86−45. However, the choline content in yams showed a lower value (13.0−16.3%) than that of cereal flours. About half of this increase was due to the extraction and hydrolysis steps, and the rest was due to the procedure for the precipitation of choline reineckate in the acetonitrile solution. Similar results were also found by Atwal et al. (*33*) in which samples were spectroscopically measured at 303 nm rather than 526 nm.

It must be noted that, in the case of yam samples, after 5% ammonium reineckate solution was added, the reineckate precipitate was a little bit sticky and different from the precipitate from the cereal flour samples. After centrifugation, the choline reineckate precipitate could not dissolve easily in acetonitrile and it formed some small coagulated particulates. This problem is more significant in yam samples than in other cereal samples. It was recommended by the AACC method that, to prevent caking and consequent incomplete extraction, pumice could be mixed with the sample. Pumice was added 2 or 3 times (w/w), but it was found that pumice caused no improvement in the extraction of choline and the small coagulated particulates still remained. Yam tubers are rich in starch and soluble carbohydrates, especially in the case of Keelung yam. After acid hydrolysis, more monosaccharide (mannose) was contained in yam samples in comparison with other cereal samples. Also, a large amount of unhydrolyzed particles such as starches, proteins, and fibers would still remain in the hydrolysate. These unhydrolyzed particles may coagulate with choline reineckate resulting in extraction difficulty and consequently causing the incomplete extraction.

For solving the problem mentioned above, the procedures were modified as follows for all of the cereals and yams samples: before filtering, the hydrolysates was first neutralized with NaOH and then filtered with suction through a sintered glass tube. After this, filtration samples were then added with sodium hydroxide pellets and it was adjusted to pH 13 to precipitate choline as the reineckate salt. The remaining procedures were the same. This extra procedure may help to remove the undissolved particulates and resolve the coagulation problem.

Bubble Separation and Centrifugation for Separating Mucilage and Starch. Because the same modified method was used for choline extraction, the choline content in yams had increased less (13.0−16.3%) than that (34.0−45.3%) of the cereal flours. It seems reasonable to speculate that the higher content of mucilage in the starch system may consequently cause incomplete extraction of choline. A system that can successfully separate starch and mucilage may solve this problem and improve choline extraction.

It is difficult to separate starch from yam tubers because of the presence of viscous polysaccharide polymers (glycoprotein). The settling of starch granules is often hindered by the presence of various components such as mucilage. Fu et al. (*7*) has 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 yield of soluble carbohydrates and protein in separated mucilage was 98.8 and 74.1%, respectively (*7*). Centrifugation is also commonly used in chemical analysis. For separating mucilage and starch, centrifugation may also be a useful method.

A total of 50 g of freeze-dried yam flour was extracted with 6700 g of water using centrifugation and bubble separation. The procedures are shown in **Figure 1**. In **Table 3**, values for total choline estimated by bubble separation were 2−2.5 times higher than those estimated directly from flour. This showed that 95.5, 94.4, and 91.1% of the total choline was separable and remained in the mucilage of Keelung, Yangmingshan, and Ming-Chien yams, respectively. Less than 6% of choline was detected in the separated starch paste. Although the total choline of yam samples estimated by our modified AACC procedures was already 13.0−16.3% higher than that of the original AACC method 86−45 (in **Table 2**), an even higher content of choline can be assayed if an additional bubble separation procedure can be applied to separate starch and mucilage prior to applying the modified method.

Table 3 shows the effect of the RCF of centrifugation on the choline contents in the tubers of Keelung, Yangmingshan, and Ming-Chien yams. The slurry suspension was centrifuged twice, and the effects of the centrifugation on the extraction of choline were studied. Three relative centrifugal forces, 3500*g*, 6000*g*, and 7300*g*, for 30 min were used. Freeze-dried supernatant and residue (starch paste) were used for choline determination. For all three varieties of these yams, the total amounts of choline obtained from two consecutive centrifugations of 3500*g*, 6000*g*, and 7300*g* were larger than that obtained from the flour system but smaller than that obtained from bubble separation. The difference in relative centrifugal forces influences the total amount of choline obtained from the two consecutive centrifugation procedures. In comparison with choline obtained from bubble separation, with two consecutive centrifugations, only 54.1−74.4% of the choline was separated from the pulp of Keelung, Yangmingshan, and Ming-Chien yams. The higher the relative centrifugal forces, the lower the amount of choline extracted by the system. The effects of

Table 3. Effect of RCF and Bubble Separation on the Measurement of Choline in the *Dioscorea* (Yam) Tubers of Keelung, Yangmingshan, and Ming-Chien

			Keelung yam[a] (mg/g solid) (*D. pseudojaponica* Y.)	Yangmingshan yam[a] (mg/g solid) (*D. alata* L.)	Ming-Chien yam[a] (mg/g solid) (*D. purpurea*)
	flour		1.07 ± 0.07	0.87 ± 0.09	0.51 ± 0.09
bubble separation		crude mucilage	2.11	1.68	1.23
		starch paste	0.10	0.10	0.12
		total	2.21 ± 0.12	1.78 ± 0.28	1.35 ± 0.19
centrifugation	7600*g*	supernatant (mucilage)	0.85	0.67	0.47
		precipitate (starch paste)	0.41	0.34	0.26
		total	1.26 ± 0.28	1.01 ± 0.18	0.73 ± 0.18
	6000*g*	supernatant (mucilage)	0.98	0.73	0.52
		precipitate (starch paste)	0.53	0.47	0.35
		total	1.51 ± 0.12	1.20 ± 0.28	0.87 ± 0.18
	3500*g*	supernatant (mucilage)	1.03	0.84	0.58
		precipitate (starch paste)	0.62	0.54	0.43
		total	1.65 ± 0.08	1.38 ± 0.19	1.01 ± 0.19

[a] Mean ± SD ($n = 3$).

Table 4. Comparison of Choline Contents in Keelung, Yangmingshan, and Ming-Chien *Dioscorea* (Yam) Tubers Using the One-Stage Bubble Separation and Two-Stage Centrifugation/Bubble Separation Methods

		Keelung yam[a] (mg/g solid) (*D. pseudojaponica* Y.)	Yangmingshan yam[a] (mg/g solid) (*D. alata* L.)	Ming-Chien[a] (mg/g solid) (*D. purpurea*)
bubble separation		2.21 ± 0.12	1.78 ± 0.28	1.35 ± 0.19
centrifugation and bubble separation	1st, centrifugation at 6000*g* (0 °C, 30 min)	0.98	0.73	0.52
	2nd, bubble separation	1.18	0.93	0.79
	total	2.16 ± 0.22	1.66 ± 0.24	1.31 ± 0.16

[a] Mean ± SD ($n = 3$).

Table 5. Choline Content of Some Plant Materials

sample	choline (mg/g solid)[a]	reference
Dioscorea (yam) flour		
Keelung yam[b] (*D. pseudojaponica* Yamamoto)	2.21 ± 0.12	this paper
Yangmingshan yam[b] (*D. alata* L.)	1.78 ± 0.28	this paper
Ming-Chien yam[b] (*D. purpurea*)	1.35 ± 0.19	this paper
rice flour	1.13 ± 0.12	this paper
wheat flour	1.25 ± 0.09	this paper
wheat flour	1.34 ± 0.00	Atwal et al. (*33*)
wheat flour	0.19 ± 0.09	Wilson and Lorenz (*34*)
whole wheat	0.34 ± 0.11	Wilson and Lorenz (*34*)
oat flour	1.38 ± 0.09	this paper
oat flour	1.44 ± 0.18	Atwal et al. (*33*)
durum flour	1.24 ± 0.11	Atwal et al. (*33*)
rye flour	0.70 ± 0.10	Atwal et al. (*33*)
soybean flour	2.86 ± 0.07	Atwal et al. (*33*)

[a] Mean ± SD ($n = 3$). [b] Using the bubble separation method.

centrifugation reduced the amount of choline extracted by the system with increases in the relative centrifugal forces. At 7600*g* RCF, the extracted choline was the lowest in this study.

On the basis of these experiments, it can be summarized that, the lower the relative centrifugal force, the more choline will be suspended in the mucilage. Using our modified method, the choline can be successfully extracted in the mucilage (supernatant) if the starches can first be separated. The experiments were also conducted with incremental increases in centrifugation operations (resuspension and resedimentaion), but the results (data not shown) showed that less than a 5% increase in the yield of total choline could be further extracted. Actually, in previous studies (*7*, *18*), we found that centrifugation destroyed the structure of glycoprotein, resulting in further separation of carbohydrates and proteins in different parts of starch paste and mucilage. These experiments have shown that there is difficulty in fully extracting proteins and soluble carbohydrate from the pulp of the yam tuber by using centrifugation.

Table 4 shows the total choline content using a single-stage bubble separation and two-stage centrifugation and bubble separation procedures. A single-stage procedure involved only bubble separation for separating mucilage and yam paste. For Keelung yam, the total amount of choline obtained by the single stage and two stage were 2.21 and 2.16 g, respectively; while

those of Yangmingshan yam were 1.78 and 1.66 g, respectively, and Ming-Chien yam were 1.35 and 1.31 g, respectively. Therefore, the second procedure of bubble separation in the two-stage method not only successfully separated the starch and mucilage but also improved choline extraction. The total amount of choline separated by the two-stage method is very close to that obtained from the single-stage bubble separation method. The choline content in some plant material rich in starch is showed in **Table 5**. These values based on the modified method were used for the determination of choline content in three cereals and are also in good agreement with literature values.

Therefore, without separating starch and viscous mucilage, it is difficult to fully extract choline in yam samples. Centrifugation results in damage to the glycoprotein structure, and differences in the proportion of protein and soluble carbohydrates recovered from the suspension is dependent on the relative centrifugal forces used in centrifugation. This is also true for choline determination. An attempt to fully detect choline by separating starch using centrifugation may not be feasible, and centrifugation may only be used for analytical purposes. This may have problems supporting quantitative analysis. In short, experiments were performed to show that separating starch and mucilage helped the consequent choline extraction. The highest choline contents can be measured by stand-alone bubble separation because of the excellent separation of starch and mucilage. It provides an alternative method for the determination of choline, and it is especially good for recovering choline from a starch and polysaccharide−protein-containing system.

CONCLUSION

The method reported here for the estimation of choline in some plant materials resulted in higher recovery, much more consistent values, and much less time consumption than the AACC method 86−45. In a system with higher contents of starch, mucilaginous, and fibrous materials, such as *Dioscorea* (yam) tubers, extra sample preparation procedures needs to be carried out to separate starch and mucilage. Bubble separation could separate most of the water-soluble carbohydrates (predominantly mannan) and proteins from starch to be able to fully assay choline. These experiments have shown that there is difficulty in fully estimating choline from the pulp of yam tuber using centrifugation. After two consecutive centrifugations, only 54.1−74.4% of the total choline was separated from the pulp of Keelung, Yangmingshan, and Ming-Chien yams. Bubble separation is an appropriate procedure in the practice for maximum assay of choline in yams. It is especially good for recovering choline from a starch and polysaccharide−protein-containing system.

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Received for review September 8, 2004. Revised manuscript received January 20, 2005. Accepted January 24, 2005.

JF048501H