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American groundnut (*Apios americana*) protein isolate: Amino acid profile

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ABSTRACT

Protein from *Apios americana* (termed *Apios*) seeds, a neglected North American legume, was extracted using ultrafiltration and protein micellar mass methodologies. The goal was to enhance the utilization of *A. americana* seeds as a food ingredient in food systems. The protein content increased from 23.4% (seed flour) to 74.1% (seed isolate). Amino acid composition of the extract was determined using ion-exchange column chromatography. The amino acid levels increased and the most abundant amino acids in the protein isolate were glutamic and aspartic acids. Leucine was the most predominant essential amino acid. Other essential amino acids present in higher amounts were phenylalanine, valine, threonine and lysine in descending order of abundance.

INTRODUCTION

American groundnut (*Apios americana* Medikus) is a bean- and tuber-bearing legume which is native to eastern North America where it ranges from Canada to southern Florida[11]. Its sweet, starchy tubers were once much esteemed by Native Americans[2] and were reported to be a significant factor in the survival of the pilgrims through their first few winters in New England[12]. Another bean- and tuber-bearing pulse, African yam bean (AYB), is an important staple of populations in Africa. AYB is a predominant legume in the diets of Southeastern Nigerians. The protein content of AYB varies from 21-29%[8] with its methionine and lysine levels equal to or better than those of soybeans[4,10]. The potential of American groundnut for domestication as a food crop has been discussed[3]. Studies reported a crude protein content of 16.5% for the tubers[13,14] and 25.6% for the seeds[15]. The total and free amino acid contents of American groundnut seeds and tubers were investigated. Results of the study[15] showed that aspartic and glutamic acids were the predominant amino acids in both seeds and tubers. The objectives of this study were to: a) isolate the protein from groundnut seeds for use as a food ingredient; b) determine the amino acid composition of the isolate; c) improve on the utilization of *Apios* seeds. The extraction procedure used was based on the protein micellar mass (PMM) process developed by Murray *et al.*[7]. This method has been shown to preserve protein native structure[1], as the general methodology of acid solubilization or alkali precipitation of protein was not used.

MATERIALS AND METHODS

Source of material and sample preparation

Apios seeds were obtained from populations grown

at Louisiana Agricultural Experiment Station farm. Approximately 500g seeds were used for analyses. Seeds were ground in a hammer mill (48mesh screen), mixed thoroughly in a ball mill and stored for further analysis. Lipid extraction of samples was carried out using the methodologies previously described[6,14]. Defatted samples were stored frozen for further analysis.

Protein separation

The *Apios* protein was extracted from the defatted meal using the PMM process[7]. Samples were mixed for 1hr at room temperature in a series of buffers ranging from pH 5.5 to 6.5 with either 0.1M NaCl/0.1M NaH_2PO_4 or 0.01M NaCl/0.01M NaH_2PO_4. The pH values were adjusted with phosphoric acid. These conditions were selected to cover both pH and ionic strength ranges that would largely accomplish the removal of the antinutritional factors without inflicting any undue damage to the protein. After mixing for 1hr, the sample was centrifuged (Sorvall Centrifuge, Norwalk, CT) at 10,000rpm for 30min to remove the seed and hull debris. The supernatant, containing the solubilized protein, was filtered through a pre-moistened cheese cloth to remove any remaining debris.

The supernatant was processed by ultrafiltration using standard instrument procedures (Membrex Inc., Fairfield, NJ). The extract was concentrated through a 20,000 molecular weight cut-off Membrex UltraFilic™ membranes in a Benchmark® Gx vortex flow filtration system (Membrex Inc., Fairfield NJ) under a pressure of 20-30psi. Other operation conditions were temperature 25°C and flow rate 200 ml/min. The retentate was recycled through the module and permeate was drawn as clarified protein extract. The solubilized protein isolate was diluted to 15 times its volume with distilled water and left overnight (approximately 16 hr) in cold storage (4ºC). During this time, insoluble protein micelles settle at the bottom of the dilution vessel. The protein was collected by centrifugation of the micellar suspension at 10,000rpm for 30min. The extracted protein was frozen and freeze-dried.

Protein and amino acids determination

Analysis of isolate for percent protein was carried out as described in the Kjeldahl methodology outlined in the AOAC Official Methods of Analysis[9] in a conventional micro Kjeldahl distillation apparatus. Total and free amino acids were determined with an ion-exchange column chromatography using standard instrument procedures (Beckman Instruments Inc., Palo Alto, CA). For total amino acids, samples were hydrolyzed in vacuum-sealed tubes at 110°C for 24 hr prior to analysis. Sample sizes were 0.07g and 5.0g for total and free amino acids, respectively. All analyses were done in duplicates. For each replicate, two injections were made onto the column with the results averaged. The analyzer (automated Beckman 116 Amino Acid Analyzer) was standardized with amino acids from Beckman Instruments Inc. (Palo Alto, CA).

RESULTS AND DISCUSSION

Protein level

The procedure used to separate protein from whole *Apios* seeds was based on the process developed by Murray *et al*.[7]. The protein content of *Apios* seeds isolate was higher when compared to that of the seed flour. An earlier study[15] reported a protein content of 23.4% (N x 5.7) for the seed flour while this study reports a protein content of 74.1% (N x 5.7) for *Apios* seed isolate. An increase in amino acid levels was also observed in the isolate. The increase in protein and amino acid content may be due to the isolation procedures used. The protein extract was separated from other food components using the PMM and ultrafiltration processes, thereby presenting the protein and amino acids in a concentrated form. PMM method has been shown to preserve the native state of the protein extract[1] while providing a medium for optimal removal of phytic acid and phenolic compounds[5].

Amino acid composition

Levels of total and free amino acids in the sample are shown in TABLE 1. Glutamic acid was the most abundant amino acid in the seed isolate. This confirmed earlier report[15] which indicated that glutamic acid predominated in seeds of American groundnut. The next most prominent amino acid was aspartic acid. Other amino acids present in substantial amounts were leucine, histidine, proline and alanine in descending order of abundance. The most predominant essential amino acid was leucine. Other essential amino acids present in higher amounts were phenylalanine, valine, threonine and lysine in descending order of abundance. Although the amino acid profile of *Apios* isolate was similar to that of the seed flour reported by Wilson *et al*.[15], the amino acid levels were higher in the isolate than in the whole seed flour. Free amino acids in American groundnut seed protein isolate were much lower than proteina-

ceous amino acids. The sum of the free amino acids represented approximately 2% of total amino acids. In *Apios* protein isolate, free amino acid levels followed this descending order of abundance: glutamic acid ≈ aspartic acid > arginine > leucine > threonine > serine.

TABLE 1 : Total and free amino acid composition of *Apios americana* seed protein isolate (g amino acid/100g protein)[a]. Amino acid notations are shown.

Amino Acids	One-letter symbol	One-letter symbol help	Total	Free
Essential				
Cysteine	C	*C*ysteine	2.65±0.04	0.04±0.08
Isoleucine	I	*I*soleucine	9.85±0.02	0.08±0.07
Leucine	L	*L*eucine	21.84±0.0	0.62±0.04
Lysine	K	before *L*	9.95±0.01	0.11±0.07
Methionine	M	*M*ethionine	0.99±0.06	ND
Phenylalanine	F	*F*enylalanine	14.46±0.01	0.03±0.04
Threonine	T	*T*hreonine	11.56±0.02	0.43±0.08
Tryptophan	W	t*W*o rings	2.06±0.08	0.02±0.09
Tyrosine*	Y	t*Y*rosine	6.86±0.04	0.03±0.09
Valine	V	*V*aline	14.18±0.02	0.06±0.02
Nonessential				
Alanine	A	*A*lanine	15.00±0.01	0.21±0.07
Arginine	R	a*R*ginine	7.18±0.02	0.95±0.08
Aspartic Acid	D	aspar*D*ic acid	31.84±0.01	1.20±0.03
Glutamic	E	glu*E*tamic acid	48.38±0.02	1.29±0.01
Acid				
Glycine	G	*G*lycine	14.22±0.03	0.06±0.05
Histidine	H	*H*istidine	18.88±0.01	0.20±0.02
Proline	P	*P*roline	16.10±0.02	0.28±0.06
Serine	S	*S*erine	12.80±0.01	0.40±0.07

[a]**Mean ± c.v.; ND = Not detected; *Essential only in certain cases**

CONCLUSION

Findings of this investigation revealed that processing methodologies such as ultrafiltration and protein micellar mass increased the protein content and amino acid levels in *Apios* beans protein isolate. This study may open new field of application for the American groundnut. A future study that compares the amino acid composition and functionality of Apios beans protein isolate to those of soy protein isolates will provide insights on its value and application as a protein ingredient in foods of mixed components.

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