Characterization of Yam Bean (*Pachyrhizus erosus*) Proteins

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Seed proteins from Mexican yam bean seeds (*Pachyrhizus erosus* L.) were sequentially extracted according to the Osborne classification. Albumins were the major fraction (52.1−31.0%), followed by globulins (30.7−27.5%). The minor protein fraction was prolamins (0.8%). Defatting with chloroform/methanol remarkably affected the distribution of protein solubility classes; albumins were the most affected fraction (4.3−17.5%). Electrophoretic patterns of albumins showed bands at 55, 40, 35, and 31 kDa. After reduction of the globulin fraction exhibited two triplets, one from 35 to 31 kDa and the second from 19 to 21 kDa, these could be compared to the acid and basic polypeptides of 11S-like proteins. Prolamins showed one band at 31 kDa, and glutelins after reduction showed three main bands at 52, 27, and 14 kDa. Trypsin inhibitors were assayed in saline extracts; the values found (1232−2608 IU/g of meal) were lower than those of other legumes. In general, yam bean seed proteins showed an excellent balance of all essential amino acids; albumins contain the highest amount of essential amino acids.

Keywords: *Yam bean; jicama; seed storage proteins*

INTRODUCTION

The lack of information on many basic aspects of underutilized crops hinders their development and their sustainable utilization. One of these is the yam bean plant (genus *Pachyrhizus*), which is believed to have originated in Mexico and Central America, where it was cultivated by the ancient Mayans and Aztecs several centuries ago (*1*). The Mexican jícama (*Pachyrhizus erosus*) is now being rediscovered as a root crop of great economic importance. Its tuber production capacity is reported to be the highest among the tuber-bearing legumes. Yields average 40 ton/ha but may reach 80 ton/ha (*1*, *2*). Although these tuberous roots have been used mostly for their low carbohydrate content, they have, on a dry weight basis, 3−5 times the protein content of other root crops, such as potato. Recently, it was reported that yam bean proteins YBG1 and YBG2 exhibited cysteine protease activities; another yam bean protein, YBG22, was also shown to exhibit high sequence homology with protease inhibitors (*3*).

Available information on the constituents of yam bean seeds is rather limited; the presence of an insecticidal compound called rotenone has been reported, and its insecticidal and fungicidal properties have been extensively studied (*1*). Mature seeds contain high amounts of a good-quality vegetable oil; studies agree that if retonone is removed, the oil has a composition comparable to that of groundnut and cottonseed oil (*1*). However, there are no reports on the characteristics of the storage proteins present in yam bean seeds. Thus, the present work provides some information on the physicochemical and functional properties of the isolated yam bean seed proteins.

MATERIALS AND METHODS

Two samples of Mexican yam bean (*Pachyrhizus erosus*) were obtained from two localities, San Juan from state of Guanajuato and Costa-Costa from the state of Nayarit, Mexico. Prior to analysis, the seeds were milled to a fine powder and kept in refrigerator at 4 °C until used. The seed flour was defatted with two different solvents, hexane and a mixture of chloroform/methanol (2:1 v/v). Flour/solvent (1:10 w/v slurry) was stirred for 24 h; the solvent was eliminated by centrifugation, and the meal was dried at room temperature and stored at 4 °C until used.

Protein Fractionation Procedures. Fractionation of protein was carried out according to the method of Osborne (*4*), with some modifications. Suspensions of meal/water (1:10 w/v) were stirred for 3−4 h at room temperature and centrifuged at 13000*g* for 15 min at 18 °C. The supernatant called albumin fraction a was kept at 4 °C until used. The pellet was resuspended with a solution of 50 mM Tris-HCl, pH 8, containing 0.1 M NaCl and stirred as before. The resulting supernatant was designated globulin 0.1 M NaCl (fraction $g_{0.1}$). The pellet was extracted with 50 mM Tris-HCl, pH 8, containing 0.3 M NaCl. After centrifugation, the supernatant was called fraction globulin 0.3 M NaCl ($g_{0.3}$), and the pellet was resuspended with 70% aqueous 2-propanol (2PrOH), extracted under stirring for 3−4 h, and centrifuged at 13000*g* for 15 min at 18 °C. The resulting supernatant was designated the prolamin fraction (p), and the pellet was resuspended in a solution of 0.1 M NaOH; after centrifugation, the supernatant was designated the glutelin fraction (gl), and the pellet was called residue (*5*, *6*).

A micro-Kjeldahl method (*7*) was used to determine protein content in the protein fractions. The nitrogen to protein conversion factor used was 6.25 (the most common factor used for all legumes).

Electrophoresis. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to

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Table 1. Effect of Solvent Extraction on Protein Contents of Yam Bean Seed Flour[a]

	whole seed flour	defatted flour	
		hexane	chloroform/methanol
San Juan	$24.4^{d} \pm 0.7$	$26.4^{bc} \pm 0.8$	$27.6^{ab} \pm 0.3$
Costa-Costa	$26.4^{bc} \pm 0.8$	$28.6^{a} \pm 0.4$	$25.4^{c} \pm 0.8$

[a] Percent, dry basis. Means ± standard deviation of three replicates. Means ot sharing a common superscript letter are significantly different at $p = 0.05$ by Tukey's multiple-range test.

the method of Laemmli (*8*) with or without reduction of the protein by 2-mercaptoethanol (2-ME) in 15% polyacrylamide gels. Protein samples (1 mg/mL) were dissolved in 0.1 M Tris-HCl, pH 6.8, and 2% (w/v) SDS. Electrophoresis was conducted at a constant current of 20 mA for 2−3 h. The gel was stained with an ethanol/acetic acid/water (45:45:1 v/v/v) solution containing Coomassie Brilliant Blue R250 at a final concentration of 0.25% (w/v). Destaining was achieved by washing the gel during 2 h with the same solution but without the dye and then overnight with a solution of acetic acid (5%, v/v). Molecular weights of the protein subunits were calculated by using the 10 kDa protein ladder (Gibco-BRL, Life Technologies, Gaithersburg MD).

Trypsin Inhibitors. Inhibitors were extracted with 100 mM phosphate solution at pH 7.2 containing 150 mM NaCl. A meal/solution suspension (1:10 w/v) was stirred for 2 h at 4 °C; after centrifugation at 13000*g* for 60 min, the supernatant was recovered and the inhibition of trypsin (bovine trypsin, Sigma Chemical Co.) and trypsin-like activities was followed by monitoring the rate of hydrolysis of *N*-α-benzoyl-L-arginine ethyl ester (BAEE) (*9*). Trypsin inhibition was reported as inhibitor units per gram of sample (IU/g), where 1 unit was defined as the increase of 0.01 unit of the absorbance at 253 nm.

Amino Acid Analysis. Amino acid analysis was performed by reversed-phase high-performance liquid chromatography (RP-HPLC). Protein sample (100 mg) was hydrolyzed with 6 N HCl at 150 °C for 24 h. After filtration, 25 μL was used for derivatization with phenyl isothiocyanate, and derivatized samples were analyzed in a C-18 Pico-Tag column (*10*). Data were integrated using a model 19-740 integrator (Waters Chromatography, Millipore Corp.).

RESULTS

Protein Fractionation. The protein content of yam bean seeds is shown in Table 1. The whole flours contained 24.4 and 26.4% protein for San Juan and Costa-Costa, respectively. Defatting with hexane caused an increase in both protein contents, which could be due to fat removal. Defatting with chloroform/methanol had the same effect as hexane on the protein content for San Juan flour (27.6%), but for Costa-Costa it had no effect on the protein content (25.4%). A decrease of protein solubilization has been reported due to protein denaturation by the solvent as well as by the extraction of nitrogenous compounds (*6*, *11*).

Protein fractionation of San Juan yam bean seeds defatted with hexane (Table 2) showed that albumins are the main fraction, contributing half of the protein content in the seed (52.1%). Globulins are the second fraction, yielding a total of 30.7%, and prolamins were the lowest fraction at only 0.8%. A similar fractionation pattern was obtained for Costa-Costa flour. These results show that although yam bean is a legume plant, the protein fractionation pattern does not correspond to those of other legumes such as soybeans, peas, and common beans, in which globulins are the main fraction (*12*, *13*). Yam bean seed protein fractions are actually similar to the pattern reported for amaranth proteins (*7*). An effect of defatting with chloroform/methanol was observed on protein fractionation (Table 2), for which the most dramatic change was observed in the albumin fraction: values changed to 4.3 and 17.5% for San Juan and Costa-Costa, respectively. The insolubilized protein was recovered in the residue. These data show that methanol/chloroform solvent causes more damage than hexane on protein solubilization.

Table 2. Protein Fractionation of Defatted Yam Bean Seed Flour[a] (Grams of Protein/100 g of Protein, Dry Basis)

	hexane		chloroform/methanol	
fraction	San Juan	Costa-Costa	San Juan	Costa-Costa
albumins	52.1^{a}	31.0^{b}	4.3^{ce}	17.5^{cd}
globulins, 0.1 M NaCl	20.1^{c}	15.0^{cd}	11.5^{cde}	12.9^{cde}
globulins, 0.3 M NaCl	10.6^{d}	12.5^{cde}	12.2^{cde}	13.6^{cde}
prolamins	0.8^{g}	0.7^{g}	0.7^{g}	0.8^{g}
glutelins	3.0^{f}	4.4^{f}	5.0^{f}	7.0^{f}
residue	13.3	36.2	65.9	47.6
N recovery (%)	99.9	99.8	99.6	99.4

[a] Means of triplicates. Means not sharing a common superscript letter are significantly different at $p = 0.05$ by Tukey's multiple-range test.

Electrophoretic Patterns. There were no differences in electrophoretic patterns of the protein fractions from San Juan (Figure 1) and Costa-Costa (data not shown) flours defatted with hexane. Albumins (Figure 1A, lane a) show a main band at 55 kDa and three minor bands at 40, 35, and 31 kDa. This pattern was changed after reduction with 2-ME, giving a triplet around 20 kDa and one more band at 14 kDa (Figure 1B, lane a). In general, globulins extracted with 0.1 and 0.3 M NaCl show the same pattern in the absence of the reducing agent (Figure 1, lanes $g_{0.1}$ and $g_{0.3}$, respectively); bands were located at 81, 59, and 40 kDa, the only difference being the band at 38 kDa found in globulins extracted with 0.1 M NaCl. The solubility of these components may depend on the ionic strength of the extracting agents as reported by Gueguen and Barbot (*13*). After reduction, globulins 0.1 M give a triplet from 35 to 31 kDa and three more bands at 19−21 kDa. These subunits might be related to the polypeptide chains constitutive of the 12.7S-type globulin from amaranth and soybean globulins (*5*, *14*, *15*).

The same pattern was found for globulins 0.3 M (Figure 2B, lane $g_{0.3}$) with the only difference being that only one band was observed in the region of 19−21 kDa. The components detected in the relatively high to moderate molecular weight region (81 to 40 kDa) have been reported by other workers for pea convicilin (*16*) and soybean conglycinin (*17*). SDS-PAGE of yam bean prolamins in the absence of 2-ME showed only one band at 31 kDa, which remains after reduction (parts A and B of Figure 1, respectively, lane p). The glutelin pattern without reduction (Figure 1A, lane gl) shows a pattern similar to that of the globulins, but after reduction glutelins show bands at 52, 27, and 14 kDa (Figure 1B, lane gl). Defatting with chloroform/methanol had little effect on the electrophoretic patterns (Figure 2); the major change was seen in the globulin fraction. This fraction shows fewer bands with and without reduction (Figure 2, lanes a and b).

Trypsin Inhibitors. We found trypsin inhibitor activity in the saline extract of yam bean seeds. There were no significant differences in the activities among the two flours and the two defatting agents; the values

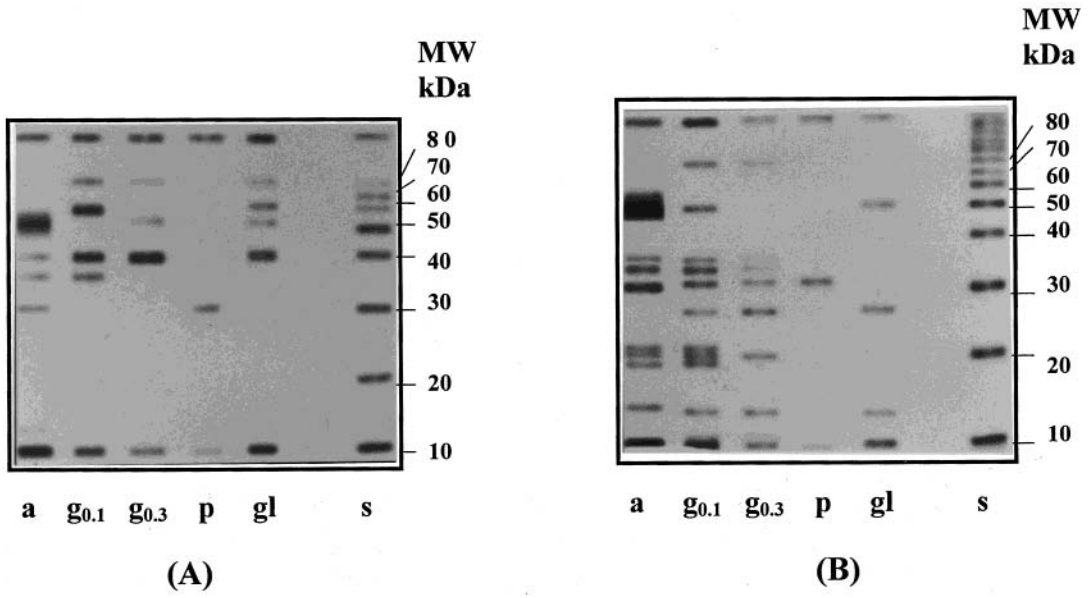

Figure 1. Electrophoretic patterns of protein fractions from hexane-defatted San Juan yam bean seed flours: (A) nonreduced proteins; (B) reduced proteins; (lane a) albumins; (lane $g_{0.1}$) 0.1 M globulins; (lane $g_{0.3}$) 0.3 M globulins; (lane p) prolamins; (lane gl) glutelins; (lane s) 10 kDa ladder molecular weight standard.

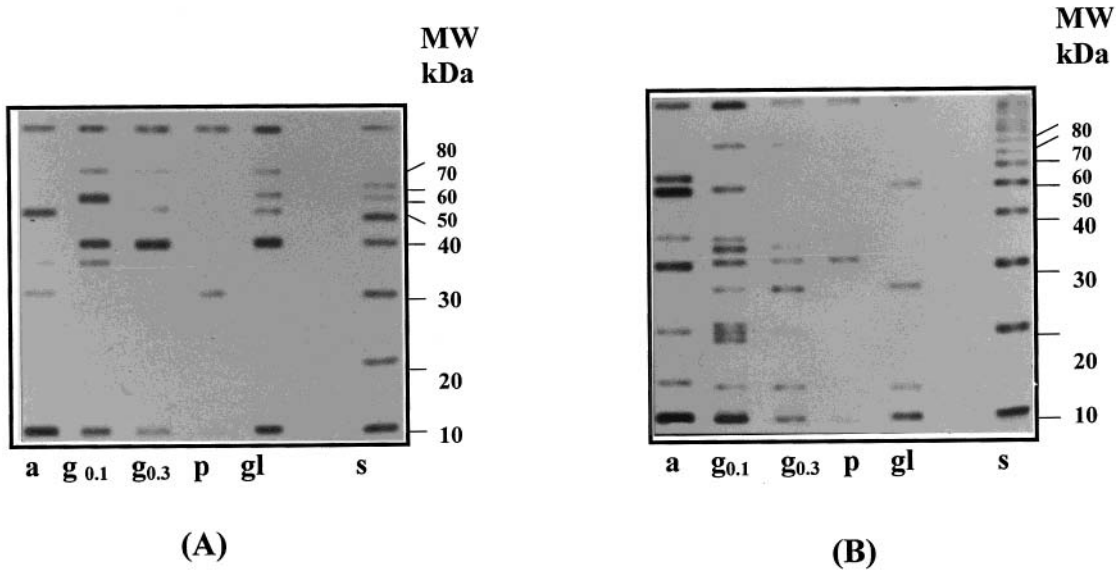

Figure 2. Electrophoretic patterns of protein fractions from chloroform/methanol-defatted San Juan yam bean seed flour: (A) nonreduced proteins; (B) reduced proteins; (lane a) albumins; (lane $g_{0.1}$) 0.1 M globulins; (lane $g_{0.3}$) 0.3 M globulins; (lane p) prolamins; (lane gl) glutelins; (lane s) 10 kDa ladder molecular weight standard.

Table 3. Trypsin Inhibitor Activity in Yam Bean Seed Flour[a] (IU/Gram of Flour)

	hexane	chloroform/methanol
San Juan	$1260^{bc} \pm 0.5$	$1232^{c} \pm 0.9$
Costa-Costa	$1288^{b} \pm 1.5$	$2608^{a} \pm 1.1$

[a] IU, inhibitor unit. Means ± standard deviation of four replicates. Means not sharing a common superscript letter are significantly different at $p = 0.05$ by Tukey's multiple-range test.

found were from 1232 to 2608 IU/g of meal (Table 3). This activity is lower than that of other leguminous seeds such as lentil (7000 IU/g) but higher than that found in amaranth flour (820 IU/g) (A. Chagolla-López, personal communication). This was not surprising because legume seeds contain abundant levels of this kind of inhibitor, as was found in dioscorin, a major tuber storage protein of yam (*Discorea batatas* Decne), which showed both carbonic anhydrase and trypsin inhibitor activities (*18*), and several other seeds as reported by Benjakul et al. (*19*). Many storage proteins have been reported to have functional properties other than being storage sinks, and most of them were shown to have other activities such as acyl transferase activity, which is involved in tuber tissue response to wounding, and protease inhibitors and seed lectins, which are known to be active in plant defense mechanisms (*3*, *18*).

Amino Acid Composition. Amino acid composition of whole yam bean flour is given in Table 4. Both samples, San Juan and Costa-Costa showed, very good quality, having amounts comparable to (or higher than) the amounts of all essential amino acids suggested by FAO/WHO/UNU (*20*). Interestingly, yam bean seed storage proteins have high levels of lysine and methionine, which are deficient in common cereals and legumes, respectively. The amino acid compositions of the different protein fractions for San Juan flour are given in

Table 4. Amino Acid Composition of Yam Bean Seed San Juan Flour (Grams of Amino Acid/100 g of Crude Protein)

			FAO/WHO/UNU ref pattern	
amino acid	San Juan	Costa-Costa	children	adult
isoleucine	5.7	5.4	2.8	1.3
leucine	8.0	7.6	6.6	1.9
lysine	7.7	7.8	5.8	1.6
methionine	1.9	1.8	2.5[a]	1.7[a]
cysteine	3.1	4.0		
phenylalanine	5.7	5.7	6.3[b]	1.9[b]
tyrosine	4.0	4.1		
threonine	3.9	4.5	3.4	0.9
tryptophan	nd[c]	nd	1.1	0.5
valine	6.8	6.3	3.5	1.3
histidine	0.9	1.1	1.9	1.6
arginine	4.4	4.4		
alanine	3.55	3.5		
aspartic acid	7.9	8.3		
glutamic acid	18.7	17.3		
glycine	4.9	4.8		
proline	5.2	5.2		
serine	5.6	5.6		

[a] Requirements for methionine + cysteine. [b] Requirements for phenylalanine + tyrosine. [c] nd, not determined.

Table 5. Essential Amino Acids of Protein Fractions from Yam Bean San Juan Seeds (Grams of Amino Acid/100 g of Crude Protein)

		globulins[a]		
amino acid	albumins	0.1 M	0.3 M	glutelins
isoleucine	3.2	6.1	9.9	1.1
leucine	7.7	6.1	7.0	5.5
lysine	8.3	4.7	1.9	3.5
methionine	2.1	2.7	3.0	3.1
phenilalanine	5.3	7.1	12.3	6.6
tyrosine	5.1	5.7	2.8	4.8
threonine	5.5	3.2	4.1	4.7
valine	4.1	2.8	2.1	2.7
histidine	5.3	3.2	1.5	4.4

[a] Globulins extracted with 0.1 and 0.3 M NaCl.

Table 5. Globulins had high amounts of essential amino acids, whereas glutelins were the poorest in terms of essential amino acids.

DISCUSSION

Plant storage proteins are of great nutritional value to mankind, and they also have diverse functional roles such as contribution to seed development, supply of amino acids to developing seedling, insecticidal properties, and antiproteolytic properties (*21*). The major protein fraction of yam bean seeds, according to the Osborne classsification, was albumins; this fraction usually accounts for the biologically active proteins. For amaranth proteins, two classes of albumins have been reported (*22*, *23*): one fraction that is soluble in water and the other soluble in saline solutions. We found that water soluble proteins present an electrophoretic pattern similar to that of proteins extracted with 0.1 or 0.3 M NaCl; further characterizations of these fractions should be carried out to determine if there was some cross-contamination during the extractions steps. We also found that the albumin fraction is very sensitive to the defatting agent. Chloroform/methanol caused insolubilization of the protein; in general, it seems that hexane causes lower denaturation of proteins. Trypsin inhibitor activities were apparently not affected by the defatting agent; these activities in yam bean are lower than those found in other legume seeds. Protease inhibitors in plants may be important in regulating and controlling endogenous proteinases, in serving as storage proteins, and in acting as protective agents against insect and/or microbial proteinases (*18*). Nowadays, proteinase inhibitors are drawing much attention as antimicrobial agents, and finding new materials containing these proteins is important. Interestingly, the amino acid composition of whole yam bean seed flours shows a better balance of essential amino acids than other seeds (Table 6) (*24*), and therefore these seeds could be a good source of high nutritional quality protein.

Table 6. Amino Acid Composition of Yam Bean and Other Seed Flour (Grams of Amino Acid/100 g of Crude Protein)

amino acid	yam bean	corn[b]	beans[b]
isoleucine	5.5	3.5	4.4
leucine	7.8	12.3	7.9
lysine	7.7	3.0	6.9
methionine	1.8	2.0	1.0
cysteine	3.5	2.3	0.7
phenylalanine	5.7	4.4	5.4
tyrosine	4.0	3.3	2.6
threonine	4.2	3.3	4.2
valine	6.5	4.3	5.2
histidine	1.0	3.0	4.8

[a] Mean of San Juan and Costa-Costa seed flours. [b] Paredes-López et al (*24*).

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