Protein and Amino Acids of Sweet Potato (*Ipomoea batatas* (L.) Lam.) Fractions

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Sweet potatoes contain nutritionally significant amounts of high-quality protein but usually do not have enough to provide and adequate protein calorie ratio. A method of fractionating sweet potatoes into fiber, starch, chromoplasts, syrup, and protein is presented. Nearly half of the nitrogen contained in the sweet potato may be recovered as a concentrate containing over 80% protein. Amino acid composition of the protein shows it to be limiting in total sulfur-containing amino acids. There is an excess of lysine, suggesting usefulness as a supplement to grain products.

Sweet potato protein is of good quality (Nagase, 1957; Purcell et al., 1972; Yamamoto, 1954), but the level which ranges from 2–11%, dry basis (Cooley, 1948; Crosby, 1964; Juritz, 1921; Murthy and Swaminathan, 1954; Purcell et al., 1972) usually does not provide an adequate protein calorie ratio. Nevertheless, the potentially high yield of sweet potato (Hernandez, 1975) suggests that it could be an important source of protein. Extraction of the protein may be required, because its even distribution within the root precludes easy production of high protein sweet potato products (Purcell et al., 1976).

We report a method for fractionating sweet potato to obtain a protein concentrate. We also report the distribution of protein among the fractions and the amino acid composition of the proteins within each fraction.

MATERIALS AND METHODS

Sweet Potatoes. Cured roots of Centennial and Jewel cultivars were obtained from the North Carolina Agricultural Experiment Station farm near Clayton, North Carolina and from a commercial packer near Wake Forest.

Fractionation. Washed, unpeeled roots were cut into 2-cm cubes, and 500 g was blended for 30 s with 1500 mL of H_2O. The puree was filtered with two layers of cheesecloth. Fibrous material which was retained by the cheesecloth was again blended with 1000 mL of H_2O and filtered. Material retained by the cheesecloth was designated as "fiber". Filtrates were screened with a 120-mesh screen to retain cell walls not trapped by the cheesecloth. Starch grains in the filtrate were sedimented by centrifugation at 1000*g* for 10 min. The starch was resuspended in water and allowed to settle by gravity. Wash water was discarded.

The centrifugal supernatant was heated with stirring to 65 ± 1 °C. Upon addition of 0.5% $CaCl_2 \cdot 2H_2O$ the chromoplasts precipitated. After cooling to 30–40 °C the chromoplast paste was compacted by centrifugation at 10 000*g* for 10 min. Heating the supernatant to 95 °C coagulated the soluble proteins. After cooling to 30–40 °C, proteins were compacted by centrifugation at 10 000*g* for 10 min. The pellet was designated "protein" and the remaining liquid as "syrup".

Chromoplast and protein pellets were separately resuspended in acetone, and filtration removed residual water. Each fraction was extracted with a 1:1 mixture of acetone–ethyl ether until the extracts were colorless. Resulting powders were dried overnight in a forced draft oven at 70 °C. Syrup was freeze-dried. Aliquots of each fraction were used for dry matter and protein determinations.

Nitrogen Analysis. Nitrogen content of each fraction was determined by the Kjeldahl method with copper and selenium catalysts. Protein was calculated as N × 6.25.

Amino Acid Analysis. Amino acid composition of each fraction was determined by automated amino acid analysis (Spackman et al., 1958). After hydrolysis and "clean-up", the samples were injected into a Beckman 119 Amino Acid Analyzer. Tryptophan and cystine–cysteine, however, were determined colorimetrically after enzyme hydrolysis.

Acid Hydrolysis. Samples containing 6–9 mg of protein were hydrolyzed with 6 N HCl in the presence of 4% thioglycholic acid (Purcell et al., 1972; Matsabura and Sasaki, 1969). After hydrolysis, the samples were dried over NaOH in a partially evacuated desiccator, resuspended in 4 mL of 1 N HCl, and passed through an ion exchange clean-up column.

Enzyme Hydrolysis. Samples containing 60 mg of protein were weighed into 50-mL Erlenmeyer flasks, suspended in 5 mL of 0.02 M phosphate buffer (pH 4.5) and mixed with 2 mL of the same buffer containing 4 mg of amyloglucosidase (Sigma Chemical Company) and 0.5 mL of toluene as a preservative. The mixtures were incubated 16 h at 34 °C, then neutralized with 0.1 mL of 0.4 N NaOH and 5 mL of 0.2 M phosphate buffer, pH 7.5. Proteins were hydrolyzed by addition of 3 mg of pronase (Sigma *Streptomyces griseus* protease) and incubation for 24 h at 34 °C. After incubation, samples were heated to 121 °C cooled, acidified to pH 2 with 0.5 mL of HOAC, and 0.1 mL of 3 N HCl. Samples were centrifuged and the supernatant passed through a "clean-up" column.

Ion-Exchange Cleanup. Acid washed Dowex 50W resin, 200–400 mesh, 0.35 g, was suspended in 1 mL of 1 N HCl and poured into a 6-mm tube plugged with glass wool. The sample was applied when the acid no longer covered the top of the resin. After the sample had passed into the column, the column was washed three times with 1 mL of 0.5 N HOAC and twice with 1-mL portions of water. Amino acids were eluted with a mixture of 4 mL of 20% triethylamine in 20% aqueous acetone. The amino acids were dried over H_2SO_4 at 0.5 atm and taken up in 4-mL sample dilution buffer.

Measurement of Tryptophan. Tryptophan in the enzyme hydrolyzate was determined according to Spies (1968). Blanks consisting of 1 mL samples in 9 mL 21 N H_2SO_4 were made for each sample. Absorbance at 590 nm was subtracted from absorbance of the respective samples. Samples were compared to a reference consisting of Diamino benzaldehyde in 21 N H_2SO_4. The amount of tryptophan was estimated from net absorbance and a standard curve.

Determination of Cystine–Cysteine. Total cystine and cysteine were determined by modification of the

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Table I. Protein Content and Recovery of Protein and Dry Matter in Each Fraction (Means and Ranges for Two Cultivars from Two Locations)

	% protein dry basis	% total recovered in fraction	
		Protein	Dry matter
Fiber	3.0 (2.7-3.5)	9.3 (8.1-10.7)	25.8 (20.4-27.8)
Starch	0.2 (0.1-0.24)	0.4 (0.2-0.6)	24.5 (21.1-27.6)
Chromoplast	42.6 (39.3-66.0)	19.1 (15.7-23.8)	5.1 (3.8-7.0)
Protein	83.4 (80.7-88.0)	48.4 (44.4-50.5)	5.0 (3.8-6.4)
Syrup	4.7 (3.9-6.0)	22.7 (18.7-27.1)	39.5 (34.2-46.6)

method of Manning et al. (1971). A 2-mL aliquot of the enzyme hydrolyzate was mixed with 1 mL of 2.5% sodium borohydride in 0.2 M PO_4 buffer, pH 7.5, and held at 40 °C. After 30 min 1 mL of acidulant, 10.4 g of NaH_2PO_4 per 100 mL of 0.1 N HCl, and 1 mL of acetone were added, and the mixture was purged with N_2 for 6 min. The mixture was neutralized with 1 mL of 0.1 N NaOH and color was developed by addition of 1 mL of 0.2 M PO_4 buffer (pH 7.5) containing 2 mg of 5,5′ dithiobis(2-nitrobenzoic acid) (DTNB). Volume was adjusted to 7.0 mL, samples were held at 25 °C for 40 min, and absorbance was measured at 420 nm. Controls were made for each sample, consisting of all reagents except DTNB. All samples were read against a blank made by using distilled water instead of sample. Absorbance of controls were subtracted from absorbance of samples and the amount of cysteine was estimated by use of a standard curve.

Statistical Procedures. Analysis of variance (AOV) for each amino acid was calculated to determine fraction effects upon content. AOV-derived least-significant differences (LSD) were used to compare fraction means.

RESULTS AND DISCUSSION

The fiber fraction contained an average of 25.8% of the total dry matter (Table I). Microscopic examination showed that the fraction consisted mainly of cell walls and vascular fibers. There were a few unruptured cells, starch grains, and chromoplasts. Further blending and washing caused only small changes in the composition of this fraction. The major source of protein in this fraction was probably the cell walls.

The starch sedimented by centrifugation contained some chromoplasts and nuclei. These were removed by subsequent suspension and sedimentation of the starch. These nonstarch components represented an insignificant part of the chromoplast fraction and were discarded due to great dilution. Starch could not be recovered quantitatively by the procedure used because some of the smaller grains would not settle before nuclei and chromoplasts. Average recovery of dry matter in the starch was 24.5% (Table I). Protein concentration of this fraction averaged 0.2%, some of which may have been from nonstarch particulates.

The chromoplast fraction contained all of the carotene not removed with the starch. All microstructure was destroyed, but occasional small starch grains which had not been removed by centrifugation were present. The amount and composition of this fraction were more variable than those of the other fractions (Table I). It contained an average of 19.1% protein and around 30% lipids (data not tabulated).

The protein concentrate, ranging from 80–88% protein contained about half of the total protein (Table I). It is believed to be mainly cytoplasmic proteins.

Table II. Amino Acid Composites of Various Sweet Potato Fractions as Means of Two Cultivars from Two Locations

	g amino acid/16 g of N recovered				
	Fiber	Chromoplasts	Protein	Syrup	$LSD_{0.05}$
Tryptophan	0.6	1.1	1.4	0.3*	0.8
Lysine	4.7*[a]	7.0	8.3	2.4*	2.0
Histidine	1.0*	2.1	2.7	0.8*	0.6
Arginine	1.2*	6.6	8.5	1.1*	2.2
Aspartic acid	22.6	15.6	12.3	27.0*	13.3
Threonine	6.8	6.0	4.5	3.2	N.S.
Serine	6.9*	6.7*	4.0	2.9	1.8
Glutamic acid	11.5	13.7	6.4	10.9	N.S.
Proline	5.7*	5.8*	3.3	1.8	1.5
Glycine	5.5*	6.2*	3.4	2.2	1.2
Alanine	6.5*	7.1*	3.7	2.5	1.4
Half-cystine	.03*	.06*	0.3	.07*	.11
Valine	9.1*	8.4*	5.6	2.6*	2.6
Methionine	0.7*	2.8*	1.7	0.5*	0.9*
Isoleucine	6.0	7.5*	3.8	1.8	2.5
Leucine	9.2*	9.8*	5.0	2.6	2.8
Tyrosine	2.9*	5.4*	4.0	0.8*	1.0
Phenylalanine	6.6	7.3	5.8	2.4*	2.8
% of N recov.	82.0	99.3	79.0	48.4	

[a] (*) significantly different (0.05) than concentrate.

Table III. Lysine and Histidine Contents of Fractions from Centennial and Jewel Cultivars

	Lysine, g/16 g of N		Histidine, g/16 g of N	
	Centennial	Jewel	Centennial	Jewel
Fiber	3.37	6.06	0.84	1.11
Chromoplast	8.48	5.56	2.79	1.47
Protein	6.75	9.77	2.54	2.84
Syrup	2.72	2.01	0.73	0.81
$LSD_{0.05}$	2.88		0.79	

The syrup fraction contained 34–47% of the total dry matter (Table I) and 19–27% of the protein. Concentration of protein (N × 6.25) appeared to be less in Centennial than in Jewel.

Amino acid composition of the fractions varied significantly (Table II), but differences due to location were not significant. There were significant cultivar–fraction interactions for histidine and lysine (Table III). The chromoplast fraction of Centennial contained significantly more histidine and lysine than the same fraction from Jewel. The protein concentrate fraction of Centennial contained significantly less lysine than that of Jewel.

The syrup fraction contained the lowest amount of essential amino acids. Aspartic and glutamic acid accounted for 38% of this fraction (Table II). All essential amino acid levels were below those of the FAO reference protein (Burton, 1965). The fiber fraction contained more amino acids with lipophilic groups, i.e., alanine, valine, isoleucine, leucine, and phenylalanine, than the protein concentrate; this suggests that much of the protein in the fiber fraction is contained in cell walls. For nutritional purposes, the fiber fraction is deficient in tryptophan and sulfur-containing amino acids. Amino acid composition of the chromoplast fraction suggests that the protein is even more lipophilic than the protein of the fiber fraction. Tryptophan and sulfur-containing amino acids limit the nutritional value of this fraction.

Nutritional value of the protein concentrate is more limited due to deficiency of sulfur-containing amino acids than soy protein. The abundance of lysine suggests that this fraction may be more valuable in supplementing grain protein than soy.

With steadily increasing yield of sweet potatoes and trends toward mechanization, sweet potato may eventually compete with corn as a source of commerical starch. The protein could be retained and used in human diets.

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Lipid Distributions in Green Leaf Protein Concentrates from Four Tropical Leaves

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Protein concentrates were prepared from the green leaves of four tropical plants: chaya, sorghum × sudan, cassava, and sauropus; and the lipid classes, sterols, and fatty acids of those concentrates were studied. About three-fourths of the green protein lipids were neutral lipids, one-fifth to one-fourth were glycolipids, and less than one-twentieth were phospholipids. After saponification of the total lipids about one-third were fatty acids, less than one-third were nonsaponifiables, and about one-third were "residuals" not extracted by hexane. Sterols were identified as cholesterol, stigmasterol, campesterol, β-sitosterol, and isofucosterol. TLC profiles revealed about 15 neutral lipids and about 11 glycolipids. The distributions of fatty acids in the neutral lipid, glycolipid, and phospholipid fractions were different. The glycolipid fraction was the richest source of linolenic acid. Fatty acid distributions were determined for free fatty acids, monogalactosyl diglycerides, acylated monogalactosyl diglycerides, digalactosyl diglycerides, and acylated sterol glucosides. The presence of acylated galactosyl lipids indicated the presence of glycolipid-hydrolyzing and acyl-transferring enzymes in the expressed leaf juices.

Leaf protein concentrates (LPC's) are currently attracting worldwide attention as a novel protein food to supplement diets which are deficient both in quality and quantity of protein (Pirie, 1971, 1975, 1976; Singh, 1975; Kohler et al., 1976). Extensive research conducted on leaves from temperate zones has resulted in two industrial processes for the preparation of LPC, viz., the VEPEX process (Hollo and Koch, 1970) and the Pro-Xan process (Kohler et al., 1968). Leaves from tropical zones, on the other hand, remain an unexploited source of valuable protein. Although research has been conducted in several tropical countries on leaf proteins (Byers, 1961; Singh, 1964; Nazir and Shah, 1966; Joshi, 1971; Martin et al., 1977; Nagy et al., 1978), no industrial process is currently producing LPC from tropical leaves. Two primary factors for considering leaves indigeneous to tropical regions as LPC sources are: (1) a high potential for good yields of protein because of year-round availability of high amounts of sunlight and the agronomic potential of multiple cropping and (2) a need for production of more protein in developing tropical countries to offset the imbalance between population growth and protein supplies (United Nations World Food Conference, 1974).

The green juice expressed from succulent leaves contain soluble proteins that are coagulable into different fractions by differential heat treatment. Juice heated between 50 and 64 °C yields the first protein coagulum. This green protein curd when pressed and dried is known as "green LPC" or "green-fraction LPC" (Bickoff et al., 1975). Practically all chlorophylls and most of the expressed lipids are coprecipitated with this first protein coagulum (Hudson and Karis, 1973). The coprecipitated lipids, which might range from 10 to 30% by weight of the green LPC (Byers, 1971; Edwards et al., 1975; Vander Zanden, 1974; Pirie, 1975), impart both positive and negative attributes to the LPC. On the positive side, these lipids enhance the nutritional quality of the green LPC by contributing important fatty acids, viz., oleic, linoleic, and linolenic acids (Lima et al., 1965; Hudson and Karis, 1973; Betschart and Kinsella, 1975). Negatively, because more than half the fatty acids in LPC are doubly and triply unsaturated (Buchanan, 1969), they are apt to oxidize during storage (Hudson and Karis, 1976; Hudson and Warwick, 1977). Additionally, reaction of unsaturated fatty acids with amino acid residues during preparation and storage of LPC has been implicated as one cause of decreased nutritive value (measured by enzymatic digestibility) (Henry and Ford, 1965; Pirie, 1966; Shah et al., 1967; Buchanan, 1969).

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