Composition of Guayule Leaves, Seed, and Wood

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Major components of guayule leaf, seed, and woody tissue were assayed, with nutritional aspects primarily in mind. Crude protein in the deoiled seed meal was about 39%, whereas deresinated derubberized leaf and wood tissue showed 14–18%. The amino acid composition of these plant residues is reported. Tissue extracts were investigated for carbohydrates and other components. Betaine is ubiquitous in these tissues. Seed oil triglycerides have a fatty acid profile similar to that of the fatty acids in wood resin hydrolysates. Leaf wax, also present as traces in the wood, is mainly docosanyl eicosanoate. Mouse feeding studies showed deresinated leaf meal to be nontoxic but deficient as a sole source protein. The nutritional quality of the seed meal in mouse diets was good, and the seed oil gave no problems as a feed oil replacement. Gravimetric assays for resin and rubber are improved by high-speed homogenizer milling, by the use of 95% aqueous acetone as a resin extractant prior to extraction of rubber with cyclohexane, and by infrared spectroscopy as a method for monitoring rubber quality.

Of the many species of plants known to contain natural rubber, only two of these, *Hevea brasiliensis*, the rubber tree of Southeast Asia, and the guayule shrub *Parthenium argentatum*, have been important sources of commercial rubber. Guayule has potential to become an important source of natural rubber in the United States (National Academy of Sciences, 1977). In 1978 Congress passed Public Law 95-592, the Native Latex Commercialization and Economic Development Act, "to authorize a program of research, development and demonstration of guayule rubber production and manufacture as an economic development opportunity for the Southwestern States".

A typical guayule shrub of the future may contain up to 20% rubber depending upon the success of plant breeding and rubber stimulation studies. This will leave about 80% of the plant material as a source of potential byproducts. Published data on chemical compositions of guayule leaves, woody tissue, resins, and seeds are scarce.

The resins have received the most study, beginning with the work of Alexander (1911) on the isolation of α-pinene and partheniol. Haagen-Smit and co-workers (Haagen-Smit and Siu, 1944; Haagen-Smit and Fong, 1948) proved the structures of parthenyl cinnamate and a number of volatile terpenes present in the leaves. Scora and Kumamoto (1979) resumed this work on the identification of leaf volatiles using GC–MS. The following monoterpenes have now been reported: α-pinene, β-pinene, camphene, sabinene, cadinene, β-myrcene, limonene, terpinolene, β-ocimene, ocimene, and phellandral.

Information on the higher polyisoprenoid compounds is more limited. Romo et al. (1970) and Romo de Vivar et al. (1970) identified two sesquiterpenes as the cinnamic acid and *p*-anisic acid esters of germacrene, which have been named guayulin A and guayulin B, respectively. The same group (Rodriguez-Hahn et al., 1970) identified three tetracyclic triterpene derivatives of cycloartenol which they named argentatins A, B, and C. Research on secondary metabolites of guayule is being undertaken by Rodriguez (1978) in his search for potentially allergenic compounds. Banigan and Meeks (1953) identified α-linoleic acid as the principal fatty acid in hydrolysates of wood resins and also showed the presence of palmitic, stearic, and linolenic acids. Keller et al. (1981) determined relative concentrations of these acids and established the presence of oleic acid in the hydrolysate.

The chief reserve carbohydrates of guayule were reported by Traub and Slattery (1946, 1947) to be levulins and inulin. Pentosans including probably xylan are also present. Despite the abundance of fructose polymers, free fructose consituted only a minor portion of the free sugars. Sucrose was found along with limited evidence for a trace of mannose (Cooil and Slattery, 1948). Other minor components include betaine reported by Murray and Walter (1945) and several flavonoids shown by Banigan (1953) and confirmed by Dominquez (1978). Walter (1944) and Banigan et al. (1951a) reported data on the leaf wax. Earle and Jones (1962) found that the seeds contained 24% oil and about 35% crude protein.

Data on the composition of the various plant materials are a prerequisite for byproduct studies. Our search for promising areas of byproduct interest provided an impetus for developing an improved gravimetric assay for rubber. Preliminary nutritional information is also reported as a basis for further investigations.

EXPERIMENTAL SECTION

Raw Materials. Leaf and woody tissues were obtained from 3-year-old shrubs of strain 593 supplied by Dr. George Hanson of the Los Angeles State and County Arboretum (LASCA). Seeds were collected from 2-year-old mixed variety plants growing on a plot maintained for us by Margarita Mondrus-Engle at California State Polytechnic University, Pomona.

Reference Standards. Pure rubber was prepared by using a sample of rubber containing 3.4% resin supplied through the courtesy of Dr. E. Campos-Lopez, Centro de Investigacion en Quimica Aplicada, Saltillo, Mexico (Campos-Lopez et al., 1978). Five grams of this rubber was dissolved in 500 mL of hexane containing 50 mg of 2,6-di-*tert*-butyl-4-methylphenol (BHT) oxidation inhibitor. The solution was filtered through Sargent No. 500 filter paper to remove insolubles. Deresination was carried out by pouring this clear filtrate slowly with stirring into excess acetone to precipitate the rubber. After vacuum desiccation, an infrared spectrum showed that resin removal was substantially complete. Donald Weihe, Central Research Laboratories, Firestone Rubber Co., Akron, OH, provided a reference sample of leaf wax. A series of straight-chain saturated alcohols C_{18} to C_{24} and acids C_{18} to C_{26} were obtained from Larodan Lipids, Malmo, Sweden, for iden-

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tification of the wax. Research grades of carbohydrates and betaine were used as TLC standards.

Gravimetric Resin and Rubber Analysis. A representative branch was cut into small pieces, dried at 65 °C overnight to <10% moisture, and ground through a 0.04-in. round hole screen in a Weber hammermill. A 2.00-g sample of the tissue was weighed into a 50-mL centrifuge tube and extracted with 20 mL of acetone–water (95:5) in a Tekmar Tissumizer for 1 min at a setting of 80. After 20 min of centrifugation at about 1000g, the supernatant was decanted, and this resin extraction was repeated twice more. The combined acetone extracts were filtered and evaporated to dryness, and the resin was weighed. An infrared spectrum of this resin was inspected at 830 cm^{-1} for the absence of low molecular weight rubber. The woody tissue residue was extracted with 2 × 20 mL of cyclohexane for 1 min each time on the Tissumizer, centrifuging after each extraction. The combined extracts evaporated to dryness yielded the rubber which was weighed. An infrared spectrum provided an estimate of hydroxyl (3400-cm^{-1}) and carbonyl (1700–1730-cm^{-1}) resin contaminants.

Seed Composition. Seeds were threshed and cleaned by using LASCA facilities and methods (Toukdarian, 1980). Clean seeds were hammer milled through a 1.25-mm slotted screen to <18 mesh. A 1035-g quantity of this oily seed meal was batch extracted with hexane to yield 265 g of yellow seed oil and 720 g of dry seed meal. A suspension formed in the oil on standing and was collected, 1.5 g. Thin-layer chromatography indicated that this precipitate is mainly a triglyceride containing a small amount of product corresponding to the leaf wax. TLC and an infrared spectrum and a fatty acid analysis of the seed oil indicated that it is a triglyceride. The composition of the seed meal is summarized in Tables I and II.

Fatty Acid Analysis. Guayule seed oil was transesterified in an acidic methanol solution, and the esters were isolated from other hexane-soluble material by silica column chromatography (Scheerens et al., 1978). The isolated methyl esters were separated on a Perkin-Elmer Model 880 gas chromatograph with a $^1/_8$ in. × 16 ft stainless steel column packed with 15% DEGS on Chromosorb W. The operation was isothermal at 170 °C with an injection port temperature of 200 °C and nitrogen carrier gas at a flow rate of 20 mL/min. The composition of the methyl ester mixture was linoleic, 75.2%, palmitic, 10.2%, stearic, 7.8%, and oleic, 6.8%.

Leaf Composition. Cleaned leaves were dried at 50 °C and then hammer milled through a 1-mm round hole screen. A 3.38-kg quantity of the ground leaves was extracted with 3 × 7 l of methylene chloride to yield 150 g (4.44%) of dark green resin and 2.98 kg of leaf meal. The resin was redissolved in 1 L of acetone and refrigerated overnight. A wax and some rubber precipitated. The wax was separated from the rubber by extraction into hot ethanol. The ethanol solution was decolorized with charcoal, and 9.0 g of purified wax crystallized on cooling. The composition of this leaf meal is summarized in Tables I and II.

Guayule Wax. The wax isolated from leaves had a mp of 76 °C. An infrared spectrum showed principal absorption at 2950, 2910, 2845, 1726 (ester), 1455, 1405, 1370, 1200, 1160, 940, 907, and 710 cm^{-1}. Elemental analysis was as follows. Anal. Calcd for $C_{42}H_{84}O_2$: C, 81.21; H, 13.63; O, 5.15. Found: C, 81.29; H, 13.35; O, 5.08. Alkaline hydrolysis yielded an alcohol fraction, mp 62–64 °C. TLC on silica gel G with a chloroform–ethanol (98:2) developer and sulfuric acid char detection indicated that the principal alcohol is docosanol (reported mp 71 °C), plus a small amount of higher alcohol. The acid fraction after several recrystallizations yielded a compound, mp 75–76 °C. TLC indicated that this is principally eicosanoic acid (reported mp 76 °C). The predominant ester of guayule wax is therefore docosanyl eicosanoate.

Wood Composition. Defoliated woody parts of the plant including branches, stems, and roots were cut into small pieces, dried at 65 °C, and ground through intermediate and small hammer mills. This woody tissue was solvent extracted batchwise, first with acetone to remove total resins and then with methylene chloride to remove rubber. Resins were divided into a hexane-extractable portion, 4.4%, of the wood and a more polar part, 3.0%. The residual matter, or bagasse, was analyzed as summarized in Table I and II.

Thin-Layer Chromatography. Methanol extracts of residual tissues were examined for carbohydrates by TLC using Merck silica gel G absorbent, 100% methanol or methanol–ethyl acetate (5:7) developers, and various detection reagents such as 1% *p*-anisidine–phthalic acid for reducing sugars. The same developing solvents were used in a search for flavonoids by employing UV fluorescence and ferric chloride spray detection. Iodine–potassium iodide reagent and Dragendorff's reagent were used to detect alkaloids on TLC plates developed with methanol–ammonium hydroxide (100:1). Acetone extracts were examined for glycosides by using an ethyl acetate–ethanol (7:3) developer and iodine vapor detection.

Amino Acid Analysis. Duplicate samples of 100 mg of plant tissue plus 100 mg of sodium thioglycolate were autoclaved for 16–18 h with 6 N HCl. The hydrolysate was evaporated to dryness under vacuum, and the residue was dissolved in sodium citrate buffer at pH 2.2 and filtered (Reid, 1980). An analysis of the buffer solution was run on a Beckman Model 121 automatic amino acid analyzer and results are summarized in Table II.

Mouse Feeding Studies. Ten Charles River CD-1 male mice per treatment were housed one per cage in suspended steel cages. Feed and water were supplied ad libidum to the animals for a 3-week test period. The control diet consisted of whole egg, 34.8%, sucrose, 41.25%, corn oil, 3%, cellulose, 3%, AIN vitamin mix, 1%, AIN mineral mix, 3.5%, chromic oxide, 0.2%, choline chloride, 0.2%, DL-methinoine, 0.1%, and bentonite, 12.75%. For the leaf and seed meal diets, substitution was principally for sucrose and cellulose. The additive values of deresinated leaf meal, deoiled seed meal, and seed oil at several levels are in Table III.

In an experiment to determine protein quality, protein at an 8% level in the diet was supplied as whole egg, casein, leaf meal, or seed meal. Leaf meal was incorporated at a 57.1% dietary level as the sole source of protein. The seed meal containing 38.9% crude protein was incorporated at a 20.6% dietary level, also as the sole source protein. Semipurified experimental diets were formulated to supply all nutrients in compliance with the National Research Council (1978) requirements for mice. Feed intakes and body weights were measured weekly for 3 weeks.

RESULTS AND DISCUSSION

The major components of guayule leaf, seeds, and woody tissue are reported in Tables I and II. Resin and rubber contents followed expectation for 3-year-old irrigated shrubs of this strain. Crude protein content of the residual matter after extraction of seed oil, leaf resin, wood resin, and rubber was about 39% for seeds, 18% for leaves, and 14% for the woody tissue. Leaf meal contains about 25%

Table I. Composition of Guayule Leaves, Wood, and Seeds

component	leaf, %[a]	wood, %[a]	seed[b]
rubber (CH_2Cl_2 extract)	0.3	6.7	
wood resin (acetone extract)		7.4	
hexane-soluble fraction		4.4	
leaf resin (CH_2Cl_2 extract)	7.8		
wax	0.3	0.06	
seed oil			26.9
residual matter	92[c]	83	73[c]
moisture	8.9	2.4	6.2
crude protein ($N \times 6.25$)	14.0[d]	13.9	38.9
acid detergent fiber	18.0	51.6	
crude fiber	10.7	42.6	24.3
lignin	4.0	17.2	
ash, mineral matter	19.9	4.1	10.1
petroleum ether extractables	0.2	0.2	0.5
total sugars, as invert	3.8	1.4	
reducing sugars	0.8	0.2	
sucrose	2		1.8
levulins[e]	0.5	1	
inulins[e]	0.2	0.2	
betaine	2	1	0.2

[a] Three-year-old shrubs, variety 593, courtesy of LASCA. [b] Two-year-old shrubs, mixed varieties. [c] Leaf meal and seed meal. [d] Values vary from 14 to 22%. [e] Cooil and Slattery (1948).

Table II. Amino Acids in Guayule Leaf Meal, Wood, and Seed Meal

amino acid	leaf meal, %[a]	wood, %[b]	seed meal, %[a]
aspartic acid	1.04	0.78	3.26
threonine	0.41	0.36	1.05
serine	0.35	0.43	1.22
glutamic acid	1.12	0.75	7.46
proline	0.69	1.39	1.52
glycine	0.76	0.40	2.11
alanine	0.59	0.34	1.32
cystine and cysteine	0.18[b]	0.10	0.07
valine	0.61	0.66	1.92
methionine	0.17	0.12	0.58
isoleucine	0.49	0.35	1.54
leucine	0.79	0.55	2.29
tyrosine	0.24	0.39	0.59
phenylalanine	0.46	0.43	1.49
histidine	0.19	0.33	0.83
lysine	0.60	0.48	1.40
arginine	0.46	0.69	2.72
total amino acids[c]	9.15	8.55	31.37
crude protein ($N \times 6.25$)	14.00	14.10	38.90

[a] Deresinated and deoiled. [b] Data courtesy of R. A. Buchanan, formerly with NRRL, USDA, obtained by using young plants of about 4.6% rubber supplied by LASCA. [c] Not including tryptophan and betaine.

Table III. Mouse Feeding Studies[a]

dietary additive	final body[b] wt/mouse, g	total feed[c] intake day^{-1} mouse^{-1}, g
control	30.8	4.51
leaf meal, 10%[d]	29.3	4.57
leaf meal, 20%[d]	28.4	3.99
seed meal, 5%	29.3	4.36
seed meal, 10%	29.1	4.35
seed oil, 1%	29.3	4.29
seed oil, 3%	28.5	4.21

[a] Ten male mice per treatment; one mouse per cage; 3-week values. [b] Average initial body weight was 9 g, weighed weekly. [c] Feed weighed twice weekly. [d] Deresinated.

water-soluble matter, some of which is protein that coagulates on boiling the extract. Woody tissue contains 10% water solubles, none of which is protein. Protein quality is estimated principally in terms of the content of the essential amino acids. In this regard guayule leaf protein compares favorably with alfalfa leaf protein (Buchanan, 1978) which varies from 26% prebud to 16% full bloom (Kohler et al., 1978).

Soxhlet extraction of residual matter with methanol removed about 20% from the leaves and 9% from the wood. Thin-layer chromatography of the leaf extract indicated the presence of sucrose, glucose, and fructose. An extract of the seed meal by HPLC showed 1.8% sucrose and no other sugars. A test for starch was negative. Betaine has previously been identified in the leaves and wood (Murray and Walter, 1945; Banigan et al., 1951b). TLC using ammonium hydroxide–methanol (1:100) developer and Dragendorff's reagent or potassium iodide–iodine reagent detection confirmed the presence of betaine, R_f 0.25, in leaves, wood, and seeds. A second alkaloid detecting spot appeared at R_f 0.67 in leaf extract and a third spot near the origin in seed extract, both in lesser quantities than betaine. Polyphenols were found in a methanol extract of seed meal separated by TLC and detected by fluorescence and ferric chloride spray. No glycosides were detected on TLC of an acetone extract of the seeds by using acidic salicylaldehyde spray reagent.

Guayule seeds present the possibility of an edible food crop over the years the plants are in the field before harvest. The plant is a prolific seed producer, but the seeds are very small. In a preliminary study, Earle and Jones (1962) reported that guayule seeds contained a triglyceride oil and the seed meal is about 35% protein. The seeds contain 27% oil, and the protein content of our deoiled seed meal was 38.9%. A gas chromatography analysis of the methyl esters of the fatty acids from the oil showed the presence of linoleic, palmitic, stearic, and oleic acids, which also occur in the wood resin.

Hernandez (1976) fed nonderesinated leaf meal to rats at 15.4% and 31% levels in isonitrogenous diets supplemented with alfalfa. All of the animals on guayule rations lost weight during a 3-week feeding period. Morphological examination of the rats on guayule rations indicated lesions and other pathological changes in organs and other tissues. This is not surprising in view of the variety and quantity of terpenes ingested, as much as 2.5% in the 31% diet.

When deresinated guayule leaf meal was fed to weanling mice at 10% and 20% additive levels containing 34.8% whole egg as a source of protein and substituting principally for sucrose, the amount of feed consumed in 3 weeks and final body weights were comparable to those of controls (Table III). The mice ate well and grew normally, although slightly less than controls who received more sugar in their diets. However, when deresinated leaf meal was added at a 57.1% level as a sole source protein, all 10 mice died within 7 days. The mice refused to eat a sufficient quantity of the diet after the first day to survive.

Mice ate well and gained weight comparable to controls when fed deoiled seed meal at 5% and 10% additive levels (Table III). When the seed meal was added to a mouse diet at a 20.6% level as a sole source protein, there was no mortality during a 3-week feeding period. This demonstrated that the nutritional quality of guayule seed meal is good. Also, when guayule seed oil was fed to mice at 1% and 3% additive levels, feed intake and growth were normal for 3 weeks with no problems (Table III). Guayule seeds are nutritious and represent a potential edible interim crop for special uses.

Gravimetric assays for resin and rubber are improved by high-speed homogenizer milling, as with the Tekmar Tissumizer used here. Deresination tests on homogenizer milled samples of woody tissue have revealed several solvents superior to acetone, traditionally used for guayule resin extraction. Among the solvents studied, 95% aqueous acetone, 95% aqueous ethanol, and 95% aqueous 2-propanol were superior to 100% acetone. The aqueous acetone proved outstanding for removal of both hydroxyl and carbonyl group containing resins from woody tissue. Such enhanced resin removal has improved gravimetric assays for the rubber, providing a cleaner rubber as observed in an IR spectrum. IR spectrum also showed there was no significant rubber loss to these resin solvents which is in line with the Meeks et al. (1950) report that guayule contains about 1% low molecular weight acetone-soluble rubber.

Three rubber solvents were investigated for their ability to remove rubber from deresinated woody tissue in a 20-h Soxhlet extraction following an 8-h resin extraction with acetone. Benzene extracted 9.1%, cyclohexane 6.5%, and hexane 6.1% rubber from the 593 strain deresinated shrub. An infrared spectrum of the benzene-extracted rubber showed the presence of aromatic compounds not in the cyclohexane- or hexane-extracted rubbers. We also found that benzene extracts partially oxidized rubber as evidenced by hydroxyl and carbonyl peaks in infrared spectra. These materials may account for the high rubber assay using benzene. Cyclohexane, our preferred rubber solvent, gave the best yield of spectrally clean rubber. Gravimetric assays by Soxhlet extraction gave consistently lower rubber values, about 0.5% than extraction by homogenizer milling. In one instance the rubber remaining in the tissue after 20 h of Soxhlet extraction was recovered in two 60-s millings with cyclohexane by a Tekmar Tissumizer. Apparently this homogenizer completes the breakup of the woody tissue cells to free the rubber.

An experiment was carried out to determine if homogenizer milling in solution results in desolublizing structural changes in the rubber such as gel formation. A solution of pure rubber and polystyrene in benzene–cyclohexane was milled in the Tekmar Tissumizer for 5 min at about 20 000 rpm. The solution was centrifuged at 1000*g* for 20 min before and after milling to precipitate possible gel. Infrared spectra of the rubber–polystyrene films before and after milling were identical. There was no quantitative difference in the area of the rubber peak at 830 cm^{-1} compared to the area of the polystyrene peak at 740 cm^{-1}. Although milling natural rubber is known to decrease molecular weight, high-speed milling in solution does not appear to cause cross-linking or gel formation to decrease rubber solubility. However, high-speed milling with the Tissumizer for longer periods does seem to partially break down lignin and possibly cellulose, with increased amounts of hydroxyl-, carbonyl-, and aromatic-containing fragments in aqueous acetone and dioxane solvents.

Guayule wax has been of interest because of its desirable properties of hardness and high melting point, 76 °C (Walter, 1944). Banigan et al. (1951a) determined other properties of this apparent mixture of long-chain esters and showed that the wax occurs principally in the leaves and floral parts. The sharp melting point of this purified wax is due to the predominance of the C_{42} ester, docosanyl eicosanoate, in the mixture. This ester mixture is chemically similar to jojoba wax, hydrogenated jojoba oil, mp 68–70 °C. The infrared spectra of guayule wax and jojoba wax are identical. A TLC on silica gel using carbon tetrachloride development showed guayule wax, R_f 0.58, comparable with jojoba wax, R_f 0.54. Jojoba wax is a mixture of C_{20} and C_{24} straight-chain alcohols and C_{18} to C_{22} straight-chain carboxylic acids, compared to the principal alcohol C_{22} and the principal acid C_{20} of guayule wax. A TLC analysis indicates that the leaves contain about 0.3% of this wax and the woody parts about 0.06%. A hectare yield of leaves on 3–4-year-old shrubs would provide only 12 kg of guayule wax. Jojoba wax can be an economical substitute in view of its nearly identical chemical composition and availability.

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Studies on Taramira Seed (*Eruca sativa* Lam.) Proteins

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The total extractable proteins of the defatted Taramira meal (*Eruca sativa* Lam.) have been fractionated into a number of components by gel filtration, ion-exchange chromatography, and polyacrylamide gel electrophoresis. A major globulin fraction (F1) which constituted 40% of total proteins has been isolated to homogeneity. Protein F1 was a typical storage glycprotein containing 8% total carbohydrates. Its molecular weight was 2.5×10^5 as determined by gel filtration. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis gave four subunits with molecular weights of 60 000, 35 000, 26 600, and 18 000, respectively. The apparent viscosity of the F1 protein was 0.038 dL/g, and the absorption maximum was at 280 nm. The amino acid acid composition of total protein and F1 protein has been determined.

Taramira (*Eruca sativa* Lam.), which belongs to the family Cruciferae, is commonly grown as an oilseed crop in northwest India. The seeds contain 20–25% protein and 30–35% oil. The oil is used for edible purposes but is considered inferior to rapeseed oil for industrial purposes (Vaughan, 1970). The cake (residue left after extraction of the oil) is mainly fed to cattle.

In view of the worldwide protein shortage, rapeseed has received a great deal of attention as a potential source of protein for edible purposes because of its balanced amino acid composition (Ballester et al., 1970; Appelqvist and Ohlson, 1972). Proteins from rapeseed have been fractionated and some of their properties studied (Bhatty et al., 1968; Lonnerdal and Janson, 1972; MacKenzie, 1975; Schwenke and Raab, 1979; Schwenke et al., 1980). However, a literature survey showed that no work has been reported on the study of the nature of proteins from Taramira seeds. In the present investigation, Taramira proteins have been isolated and partially characterized.

MATERIALS AND METHODS

Materials. Taramira seed (*Eruca sativa*) var. ITSA was obtained from the Department of Plant Breeding, Punjab Agricultural University, Ludhiana. Reagents used were of analytical grade.

Preparation of Taramira Meal. Taramira seeds were crushed in a mechanical grinder and defatted by repeated extraction with hexane at room temperature (25 °C) for 48 h. The defatted material was then ground to a powder of 85 mesh. The nitrogen content of the flour was 9.8% and the moisture content 6%. Nonprotein nitrogen (estimated from the 10% Cl_3AcOH extract of the defatted meal) accounts for 7–8% of the total nitrogen content of defatted meal.

Extraction of Proteins from Defatted Meal. Water, 0.1 M NaCl, and 1 M NaCl were employed as the extraction solvents for nitrogen solubility studies. Defatted flour (2 g) was dispersed in the solvent (15 mL), and the pH of the suspension was adjusted to the desired value by the addition of 1 M HCl or 1 M NaOH. The extraction was carried out with mechanical shaking for 2 h at room temperature (25 °C). The extract was clarified by centrifugation at 5000 rpm for 15 min. The recovered pellet was then reextracted by shaking for 30 min with 10 mL of the solvent in the same manner. Both extracts and the supernatants were combined and the pH was noted. Extracts of pH range 1–12 were thus prepared. In each case, aliquots of 5 mL were taken for nitrogen estimation by the micro-Kjeldahl method. The nitrogen extracted was expressed as the percentage of the total meal nitrogen.

Ammonium Sulfate Fractionation. To 10 g of the meal 100 mL of 1 M NaCl solution was added, and the contents were shaken for 2 h at room temperature. This extract was centrifuged at 5000*g* for 15 min. The supernatant was dialyzed extensively with 1 M NaCl solution for 48 h and diluted to about 1% protein concentration. Solid $(NH_4)_2SO_4$ was added to the protein solution divided into 8 parts with a 10-mL volume in each case so as to obtain 10–80% $(NH_4)_2SO_4$ concentration. The $(NH_4)_2SO_4$ was mixed thoroughly for 1 h at room temperature and centrifuged at 5000*g* for 15 min to remove the precipitates. The protein in the supernatant was estimated by the method of Lowry et al. (1951) in each case.

Gel Filtration. Two milliliters of 1 M NaCl extract containing nearly 100 mg of protein was chromatographed on a Sephadex G-200 column (60 × 2.2 cm) equilibrated with 0.01 M sodium borate buffer, pH 8.2, containing 1 M NaCl solution. The proteins were eluted at a flow rate of 0.4 mL/min, and 5-mL fractions were collected. Protein in the column effluents was determined by a UV spectrophotometer at 280 nm. A Sephadex G-150 column (60 × 2.2 cm) equilibrated with same buffer was also used for gel filtration studies.

DEAE-cellulose Ion-Exchange Chromatography. DEAE-cellulose was activated by washing first with 1 M NaOH and distilled H_2O and then with 1 N HCl and distilled H_2O. Washed DEAE-cellulose was equilibrated

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