Amino Acid Composition and Subunit Constitution of Protein Fractions from Cowpea (*Vigna unguiculata* L. Walp) Seeds

Chi-Wah Chan and R. Dixon Phillips*

Center for Food Safety and Quality Enhancement, Department of Food Science and Technology, Agricultural Experiment Station, University of Georgia, Griffin, Georgia 30223

The protein fractions—albumins, globulins, glutelins, and prolamins—of cowpea (*Vigna unguiculata* cv. California Blackeye No. 5) seeds were characterized for their subunit constitutions by SDS–PAGE and by amino acid compositions. The abundance of protein fractions was in the following order: globulins, albumins, glutelins, and prolamins. Four major polypeptides with molecular masses of 65, 60, 59, and 50 kDa were separated by SDS–PAGE for globulins. Three of these polypeptides were found to be covalently bound with carbohydrate by the periodic acid–Schiff reagent test. Albumins contained four major polypeptides with molecular masses of 99, 91, 32, and 30 kDa. The alkali-soluble glutelin was mainly composed of polypeptides in the molecular mass range 62–44 kDa. Polypeptides of molecular mass 105, 62, 59, and 54 kDa were found in the prolamin fraction. The cowpea seeds with 24% protein were found to be rich in lysine and leucine but deficient in the sulfur amino acids. Each of the fractions was distinct with respect to its amino acid composition. The glutelin and prolamin fractions were high in essential amino acids compared with the other two fractions.

Keywords: *Cowpea protein; albumins; globulins; glutelins; prolamins; SDS–PAGE; amino acid profile*

INTRODUCTION

In recent years, characterization of the individual storage proteins of legume seeds has been subject to intensive research (Pernollet and Mosse, 1983). Cowpea (*Vigna unguiculata* L. Walp) is one of the important legumes being studied due to its importance as a food crop of tropical and subtropical regions in Africa, Asia, and South America. On the basis of the classical Osborne (1924) separation, seed proteins were classfied according to their solubility in water (albumins), salt solution (globulins), alcohol (prolamins), and alkali (glutelins). The relative proportion of each fraction in a seed strongly affects the nutritional quality of the total seed protein (Johnson and Lay, 1974). For the past two decades, the major storage protein (globulin fraction) has been isolated and characterized in different studies (Sefa-Dedeh and Stanley, 1979; Carasco *et al.*, 1978; Khan *et al.*, 1980; Murray *et al.*, 1983; Pedalino *et al.*, 1992). However, in addition to globulins, the other storage proteins may play a role in nutritional and functional aspects of cowpea use. For this reason, our study was aimed at both major and minor storage proteins of the cowpea seed characterized. Different storage proteins were characterized as to their electrophoretic properties and amino acid profiles.

MATERIALS AND METHODS

Cowpeas (*V. unguiculata* cv. California Blackeye No. 5) were obtained from Pennington Seed Co., Madison, GA, and held at −20 °C until used. Dry seeds were decorticated mechanically as described by Phillips *et al.* (1988). The decorticated seeds were ground by an electric coffee mill into a fine powder and defatted with hexane. The defatted cowpea flour was stored at 2 °C until used.

Extraction Procedures. A modified procedure for extraction and fractionation of cowpea proteins was adopted from the Carasco *et al.* (1978) study. Defatted cowpea flours were extracted sequentially at room temperature using a meal–solvent ratio of 0.05 (g/mL) by extraction medium I [0.1 M sodium phosphate buffer, pH 8.0, 2% NaCl, 0.2 mM phenylmethanesulfonyl fluoride (PMSF, a protease inhibitor), and 0.02% sodium azide (an antimicrobial agent)], extraction medium II (0.01 N sodium hydroxide, 0.2 mM PMSF, and 0.02% sodium azide), and extraction medium III (70% ethanol). The extraction procedure is shown in Figure 1. Protein fractions were then prepared by prolonged dialysis of extracts against 33 mM sodium acetate buffer, pH 5.0, at 4 °C for 72 h or more. The protein that precipitated after medium I extraction was designated "globulin", and the soluble protein from that extraction was designated "albumin". Alkali-soluble protein and alcohol-soluble protein were designated "glutelin" and "prolamin", respectively. Each of the four fractions was freeze-dried (Freezemobile 5, Virtis Co., Inc., New York) for 72 h or more to obtain dry powders and stored at 2 °C until used.

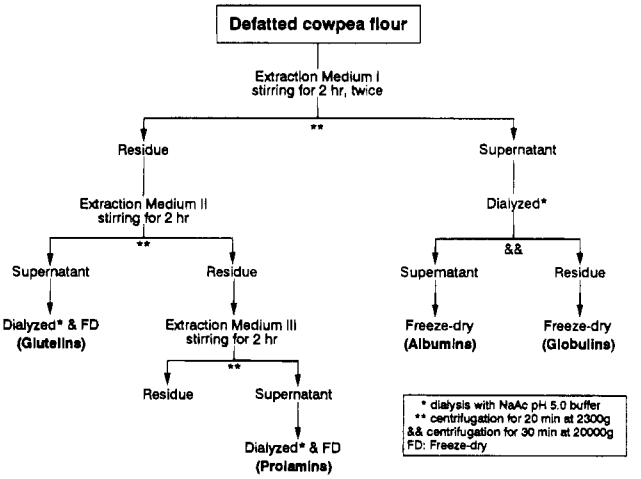

Figure 1. Scheme for protein extraction from cowpea flour.

Protein Content. Protein in four cowpea protein fractions and the cowpea flour was analyzed for nitrogen content according to the Kjeldahl method (AOAC, 1984).

Electrophoresis. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was carried out in a vertical slab gel apparatus (Bio-Rad Protean I) according to the modified method of Laemmli (1970). Protein samples were

0021-8561/94/1442-1857$04.50/0

dissolved in 65 mM Tris-HCl, pH 6.8, and 2% SDS. Reduction of disulfide bridges was performed by β-mercaptoethanol (5% v/v) at 100 °C for 3 min. Electrophoresis was conducted at a constant current of 35 mA for stacking gel (4% polyacrylamide) and 50 mA for resolving gel (10 or 12% polyacrylamide) in 5–6 h. After electrophoresis, the gel was fixed with trichloroacetic acid (12.5% w/v) for 30 min and stained either overnight by addition of Coomassie Brilliant Blue R-250 (Sigma Chemical Co., St. Louis, MO) in methanol/water/acetic acid (5/4/1 by vol) or by silver stain (Morrissey, 1981). Destaining of Coomassie-stained gels was achieved by successive incubation in acetic acid/water/methanol (1.5/17.5/1 by vol) until a desired background was obtained. Destaining of silver-stained gels was according to the procedure of Morrissey (1981). The molecular weights of protein subunits were calculated using the following standard proteins: myosin (200 000), β-galactosidase (116 250), phosphorylase B (97 400), serum albumin (66 200), ovalbumin (45 000), carbonic anhydrase (31 000), and trypsin inhibitor (21 500) (Sigma).

Glycoprotein Analysis. These studies were performed under the same conditions and with the same apparatus as described in SDS–PAGE. A 10% gel was used loaded with the same protein samples; the gel was cut into halves after the run. One half was stained with Coomassie Brilliant Blue R-250 as described above; the other half was stained by periodic acid–Schiff reagent (Zacharius, 1969; Doerner and White, 1990).

Amino Acid Analysis. Freeze-dried proteins (albumins, globulins, glutelins, and prolamins) and defatted cowpea flour samples were hydrolyzed according to a modified method of Phillips (1983), under argon using 6 N HCl with 0.5% phenol at 110 °C for 24 h. Each sample after hydrolysis was diluted and adjusted to pH 2.1, the same as the amino acid standard (amino acid standard H, Pierce, Inc., Rockford, IL). The sample solution was passed through a 0.22-μm Teflon filter and was ready for HPLC injection.

Aliquots of the hydrolysates were then subjected to ion-exchange column chromatography, using a Waters system (Millipore Corp.). The Waters system was equipped with a Waters system interface module, Model 510 pumps, a temperature control module, a postcolumn reaction system, a Model 440 absorbance detector, system controller software [Baseline 810] (Millipore), and a sodium cation-exchange column (Pickering Laboratories, Mountain View, CA). The buffer system contained solvent A (pH 3.28, 0.2 N Na) and solvent B (pH 7.40, 1.0 N Na) (Pickering Laboratories). Ninhydrin (Trione, Pickering Laboratories) was used for the postcolumn derivatization of amino acids, and wavelengths of 436 and 546 nm were used for sample detection. The column was held at 50 °C and the postcolumn reaction coil at 120 °C. Amino acids were eluted by a gradient control from 100% A to 100% B at 0.3 mL/min, with a linear gradient that began at 10 min and was completed at 32 min. Ninhydrin was pumped at a constant flow rate of 0.2 mL/min. Tryptophan and cysteine were not determined.

Statistical Analysis. Numerical data from duplicate determinations were analyzed using analysis of variance (ANOVA) and significance difference test procedures of the Statistical Analysis System (SAS, 1986).

RESULTS AND DISCUSSION

The protein content of the defatted cowpea flour was found to be 23.8% (N × 6.25). Similar findings were reported by different investigators (Bliss, 1972; Bressani, 1985). Variations in protein content of cowpea are due to genetic and environmental factors (Bliss, 1972). The protein content of four cowpea protein fractions and residues was determined immediately after extraction in triplicate. The amount of each component was used as a basis for the calculation of its content in cowpea seed proteins (Table 1). The globulin fraction was the major cowpea seed protein with 66.6% of the total, followed by albumins with 24.9%. Different studies also reported that globulins were the major seed protein,

Table 1. Distribution of Proteins in Different Protein Fractions

fraction	concn[a]	SD[b]
albumins	24.9	0.23
globulins	66.6	0.62
glutelins	4.7	0.67
prolamins	0.7	0.03
unextractable protein	3.1	0.82

[a] Standard deviation obtained from triplicate determination. [b] Percent total protein (Kjeldahl) DSB.

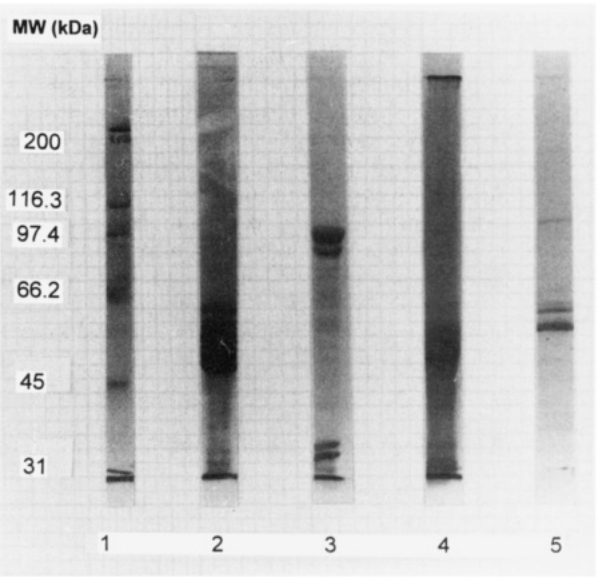

Figure 2. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) pattern of Bio-Rad molecular weight standard proteins (lane 1), cowpea globulins (lane 2), albumins (lane 3), glutelins (lane 4), and prolamins (lane 5).

ranging between 48.2 and 90%. The albumin fraction varied from 2.5 to 28% (Azimov, 1970; Boulter *et al.*, 1973; Murray *et al.*, 1983; Kachare, 1986). Del Rosario et al. (1981) reported that glutelin and prolamin proteins were composed of 5.16–6.74 and 0.8–1.05% of total N, respectively. These values were similar to those of our study. These variations in the reported values of relative proportions of various fractions are similar to those reported by Chavan *et al.* (1989). Such variations can be attributed to genetic differences, methods of protein extraction and determination, and protein content of the sample. This also indicates that an increase in protein content in certain cultivars is associated with an accumulation of proteins which are not soluble in the common solvents employed for extraction.

SDS–PAGE Analysis. The four fractions of proteins from cowpea seed were separated by SDS–PAGE as shown in Figure 2. The major storage seed protein, the globulin fraction, was shown to have four major polypeptides with molecular masses of about 65, 60, 56, and 50 kilodaltons (kDa) and a number of minor polypeptide classes with molecular masses distributed over a range of 42–28 kDa. The 60-, 56-, 50-, and 29-kDa polypeptides contained covalently bound carbohydrate by analysis with periodic acid–Schiff reagent. The concentration of carbohydrate was found to be higher in 56- and 29-kDa polypeptides on the basis of intensity of staining. Subunits of two or three major vicilin proteins (the predominant cowpea globulin) are reported to range from 63 to 49 kDa (Carasco *et al.*, 1978; Khan *et al.*, 1980; Murray *et al.*, 1983; Pedalino *et al.*, 1992). Gly-

Table 2. Amino Acid Composition of Cowpea (*V. unguiculata* Cv. California Blackeye No. 5) Compared with Reported[a] Values (Values Are Amino Acid Residues as a Percentage of Those Estimated)

amino acid	cowpea flour[b]	akidi black[c]	Olaludi[a]	cowpea white[a]	cowpea brown[c]	Aremu[d]	Prima[e]	blackeye 1239[f]	egg[g]
asparatic	12.04 ± 0.47	12.75	11.36	13.11	12.59	13.40	11.13	12.54	10.00
threonine	3.76 ± 0.34	3.91	3.83	3.80	3.92	4.12	4.00	4.08	4.95
serine	5.49 ± 0.20	5.71	5.88	6.30	5.66	6.03	5.64	5.72	7.67
glutamic	19.29 ± 0.12	19.82	19.04	19.46	19.50	18.56	17.99	20.98	12.84
proline	4.57 ± 0.80	4.26	3.33	3.55	4.02	4.94	3.78	3.89	4.00
glycine	4.04 ± 0.07	4.14	4.31	4.47	4.21	4.12	4.62	4.63	3.36
alanine	4.12 ± 0.13	4.67	4.51	4.47	4.52	5.46	4.39	4.96	5.89
valine	5.89 ± 0.05	5.41	5.07	4.62	5.57	4.56	5.87	5.99	7.26
methionine	1.16 ± 0.37	1.20	1.28	1.25	1.29	1.12	1.61	1.69	3.26
isoleucine	4.83 ± 0.06	4.59	4.72	4.08	4.82	3.87	5.14	4.71	6.31
leucine	8.25 ± 0.05	8.10	7.95	7.48	7.94	8.02	8.61	8.02	8.86
tyrosine	3.08 ± 0.22	3.44	3.55	3.26	3.29	3.21	3.30	3.55	4.20
phenylalanine	6.42 ± 0.11	5.77	6.19	5.86	5.84	3.83	5.82	6.27	5.70
lysine	7.11 ± 0.40	6.24	7.33	6.97	7.13	7.51	7.23	5.23	6.81
histidine	3.83 ± 0.19	2.94	3.54	2.91	2.59	3.34	3.53	2.54	2.43
arginine	6.10 ± 0.12	7.06	8.12	8.40	7.10	7.94	7.33	5.22	6.46

[a] Bliss (1974); Carasco et al. (1978); Aremu (1990); Ene-Obong and Carnovale (1992). [b] Values, with standard deviation, obtained form duplicate analyses. [c] Ene-Obong and Carnovale (1992). [d] Aremu (1990). [e] Carasco et al. (1978). [f] Bliss (1972). [g] USDA (1976).

Table 3. Amino Acid Composition of the Four Protein Fractions from Cowpea Seed (*V. unguiculata* Cv. California Blackeye No. 5) (Values[a] Are Amino Acid Residues as a Percentage of Those Estimated)

amino acid	globulins	albumins	glutelins	prolamins
asparatic	11.67 ± 0.77a	11.79 ± 0.65a	11.76 ± 0.10a	9.11 ± 0.80b
threonine	3.39 ± 0.36b	5.33 ± 0.60a	3.91 ± 0.04b	4.23 ± 0.02ab
serine	5.22 ± 0.16a	4.82 ± 0.71a	5.66 ± 0.03a	5.16 ± 0.05a
glutamic	16.51 ± 1.68a	12.11 ± 0.67b	17.24 ± 0.04a	11.07 ± 0.48b
proline	3.33 ± 0.30a	3.98 ± 0.68a	3.08 ± 0.34a	3.39 ± 0.52a
glycine	4.48 ± 0.19c	7.57 ± 0.01a	4.30 ± 0.29c	5.55 ± 0.24b
alanine	6.81 ± 0.22c	8.88 ± 0.35b	4.26 ± 0.28d	9.82 ± 0.12a
valine	5.21 ± 0.28b	5.04 ± 0.41b	6.34 ± 0.63ab	6.62 ± 0.29a
methionine	1.12 ± 0.07a	1.02 ± 0.34a	1.41 ± 0.11a	1.73 ± 0.27a
isoleucine	4.67 ± 0.28a	4.48 ± 0.78a	5.23 ± 0.03a	4.84 ± 0.38a
leucine	8.65 ± 0.11a	7.07 ± 0.75b	9.09 ± 0.09a	9.61 ± 0.38a
tyrosine	3.39 ± 0.46a	4.40 ± 0.88a	4.47 ± 0.40a	4.81 ± 0.31a
phenylalanine	5.76 ± 0.43ab	3.70 ± 0.43c	7.06 ± 0.67a	5.55 ± 0.08b
lysine	8.16 ± 0.30ab	9.17 ± 0.83a	7.58 ± 0.10b	8.99 ± 0.03ab
histidine	3.76 ± 0.14a	2.96 ± 0.43a	3.72 ± 0.66a	3.65 ± 0.09a
arginine	7.87 ± 0.32a	7.69 ± 0.95a	4.89 ± 0.71b	5.87 ± 0.08ab

[a] Values, with standard deviation, obtained form duplicate analyses. Means within a row sharing a common letter were not statistically different ($P < 0.05$).

cosylation sites were found both in 54- and 52-kDa polypeptides (Carasco *et al.*, 1978) and in 63- and 58-kDa polypeptides (Khan *et al.*, 1980) in different studies; however, the presence of glycosylation in the 29-kDa polypeptide was not reported.

The most dominant albumin polypeptides were those of molecular mass 99, 91, 32, and 30 kDa, with less prominent polypeptides of molecular mass 28 kDa and a molecular mass range of 83–52 kDa. In electrophoretic studies on cowpea protein, albumins were shown to have a similar pattern of subunit distribution (Khan *et al.*, 1980; Murray *et al.*, 1983; Pedalino *et al.*, 1992). In previous studies, albumins were found to have major polypeptides of molecular masses of 100–105 and 32 kDa, which very much resembled our dominant polypeptides with molecular masses of 99 and 32 kDa (Khan *et al.*, 1980; Murray *et al.*, 1983).

Glutelin was separated into several bands with molecular masses 101, 68, 31, and 29 kDa and a molecular mass range of 62–44 kDa. Four dominant bands of molecular masses 105, 62, 59 and 54 kDa were found in the prolamin fraction.

Amino Acid Analysis. Table 2 shows the amino acid contents of cowpea flour versus other reported values in different varieties. The level of amino acids fell within the range reported for different varieties of *V. unguiculata* (Bliss, 1972; Carasco *et al.*, 1978; Aremu, 1990; Ene-Obong and Carnovale, 1992). As reported by other studies, cowpea seed protein had a relatively high concentration of aspartic and glutamic acid residues and were also rich in lysine and leucine and deficient in the sulfur amino acid methionine. In comparison of the FAO reference pattern of essential amino acids (not shown), most essential amino acids have much higher values except for methionine. Similar findings were reported by Bressani *et al.* (1961) and Ene-Obong and Carnovale (1992). As shown in Table 2, chicken whole egg even had a lower content of phenylalanine and lysine with respect to the cowpea protein. However, cowpea protein is deficient in sulfur-containing amino acids, which lowers the quality of protein.

Amino acid compositions of the four protein fractions are given in Table 3. In general, a characteristic amino acid profile of high glutamic and aspartic acid content in the cowpea flour was common in all four protein fractions as discussed above. However, each of the fractions was distinct with respect to its amino acid composition. Among the four fractions, the contents of serine, proline, isoleucine, methionine, tyrosine, and histidine were not significantly different ($P < 0.05$). The albumin protein contained more threonine and tyrosine but less phenylalanine and leucine (essential amino acids) than the globulin protein. These relationships also agreed with the data reported by Carasco *et al.*

Table 4. Distribution of Classified Amino Acids in Cowpea Protein Fractions

classified distribution of amino acids	cowpea protein fraction (%)			
	globulin	albumin	glutelin	prolamin
hydrophobic[a]	35.55	34.17	36.47	41.56
uncharged polar[b]	16.48	22.12	18.34	19.75
acidic[c]	28.18	23.90	29.00	20.18
basic[d]	19.79	19.82	16.19	18.51

[a] Hydrophobic amino acids including Ala, Ile, Leu, Met, Phe, Pro, and Val. [b] Uncharged polar amino acids including Gly, Ser, Thr, and Tyr. [c] Acidic amino acids including Asp and Glu. [d] Basic amino acids including Lys, Arg, and His.

(1978). The glutelin protein contained a higher content of essential amino acids including valine, isoleucine, leucine, and phenylalanine. Relatively high contents of valine, leucine, and lysine were also found in the prolamin protein. Meaningful comparison between fractions can also be made in terms of the proportions of different classes of amino acids (Table 4). Among these fractions, albumins contained the highest amounts of basic and uncharged-polar amino acids, whereas the content of hydrophobic amino acid was lowest. The higher proportions of aspartic and glutamic acids characterized cowpea globulins and glutelins. In addition, glutelins were low in basic amino acid content. Prolamins in cowpea had higher amounts of hydrophobic and lower amounts of uncharged-polar amino acids than other protein fractions. Similar results were reported in the study of black gram protein fractions (Padhye and Salunkhe, 1979). In addition, the low content of aspartic acid residue together with the relatively high proportion of hydrophobic amino acids found in the prolamin protein may explain part of the reason for its alcohol-soluble properties. However, the name "prolamins" did not quite fit in our study as the characteristics of relatively high contents of proline and glutamine were not true in this case.

ACKNOWLEDGMENT

We acknowledge the support of the USAID Bean/Cowpea Collaborative Research Support Program (CRSP), State, and Hatch funds in conducting this research.

LITERATURE CITED

AOAC. *Official Methods of Analysis*, 14th ed.; Association of Official Analytical Chemists: Washington, DC, 1984.

Aremu, C. Y. Proximate and amino acid composition of cowpea (*Vigna unguiculata*, Walp) protein concentrate prepared by isoelectric point precipitation. *Food Chem.* **1990**, *37*, 61–68.

Azimov, B. Comparative study of proteins in the seeds of some of Tadzhikistan leguminous plants. *Izv. Akad. Nauk Tadzh. SSR, Otd. Biol. Nauk* **1970**, *1*, 30–38.

Bliss, F. A. Cowpeas in Nigeria. In *Proceedings, Symposium Nutritional Improvement of Food Legumes by Breeding*; Milner, M., Ed.; PAG/FAO: Rome, 1972; pp 151–158.

Boulter, D.; Evans, I. M.; Derbyshire, E. Proteins of some legumes with reference to environmental factors and nutritional value. *Qual. Plant. Plant Foods Hum. Nutr.* **1973**, *23*, 239–250.

Bressani, R. Nutritive value of cowpea. In *Cowpea Research, Production and Utilization*; Singh, S. R., Rachie, K. O., Ed.; Wiley: New York, 1985; pp 353–359.

Bressani, R.; Elisa, L. G.; Navarrete, D. A. Nutritive value of Central American beans. IV. Essential amino acid content of samples of black beans, red beans, rice beans, and cowpea of Guatemala. *J. Food Sci.* **1961**, *26*, 525–528.

Carasco, J. F.; Croy, R. R. D.; Derbyshire, E.; Boutler, D. The isolation and characterization of the major polypeptides of the seed globulin of cowpea (*Vigna unguiculata* L. Walp.) and their sequential synthesis in developing seeds. *J. Exp. Bot.* **1978**, *29*, 309–323.

Chavan, J. K., Kadam, S. S., Salunkhe, D. K., Eds. Cowpea. In *CRC Handbook of World Food Legumes*; CRC Press: Boca Raton, FL, 1989; Vol. II, p 1.

Del Rosario, R. R.; Lozano, Y.; Noel, M. G. The chemical and biochemical composition of legume seeds 2. Cowpea. *Philipp. Agric.* **1981**, *64*, 49–57.

Doerner, K. C.; White, B. A. Detection of glycoproteins separated by nondenaturing polyacrylamide gel electrophoresis using the periodic acid-schiff stain. *Anal. Biochem.* **1990**, *187*, 147–150.

Ene-Obong, H. N.; Carnovale, E. A comparison of the proximate, mineral and amino acid composition of some known and lesser known legumes in Nigeria. *Food Chem.* **1992**, *45*, 169–175.

Johnson, V. A.; Lay, C. L. Genetic improvement of plant proteins. *J. Agric. Food Chem.* **1974**, *22*, 558–566.

Kachare, D. P. Studies on the nutritional and protein quality of cowpea (*Vigna unguiculata* L. Walp.). M.Sc. thesis, mahatma Phulθ Agricultural University, Rahuri, India, 1986; 86 pp.

Khan, R. I.; Gatehouse, J. A.; Boulter, D. The seed proteins of Cowpea (*Vigna unguiculata* L. Walp.) *J. Exp. Bot.* **1980**, *31*, 1599–1611.

Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680–685.

Morrissey, J. H. Silver stain for proteins in polyacrylamide gels: A modified procedure with enhanced uniform sensitivity. *Anal. Biochem.* **1981**, *117*, 307–310.

Murray, D. R.; Mackenzie, K. F.; Vairihos, F.; Peoples, M. B.; Atkins, C. A.; Pate, J. S. Electrophoretic studies of the seed proties of cowpea, Vigna unguiculaata (L.) Walp. *Z. Pflanz.* **1983**, *109*, 363–370.

Osborne, T. B. *The Vegetable Proteins*; 2nd ed.; Longmans Green: London, 1924; 125 pp.

Padhye, V. W.; Salunkhe, D. K. Biochemical studies on black gram (Phaseolus mungo L.) seeds: amino acid composition and subunit constitution of fractions of the proteins. *J. Food Sci.* **1979**, *44*, 606–610.

Pedalino, M.; Paino D'urzo, M.; Delle Donne, G.; Grillo, S.; Rao, R. The structure of cowpea (Vigna unguiculata L. Walp) seed storage proteins. *Seed Sci. Technol.* **1992**, *20*, 223–231.

Pernollet, J.-C.; Mosse, J. Structure and location of legume and cereal seed storage proteins. In *Seed Proteins*; Daussant, J., Mosse, J., Vaughan, J., Eds.; Academic Press: New York, 1983; pp 155–191.

Phillips, R. D. A scheme for the rapid preparation of protein hydrolyzates for amino acid analysis. *J. Food Sci.* **1983**, *48*, 284–285.

Phillips, R. D.; Chinnan, M. S.; Branch, A. L.; Miller, J.; McWatters, K. H. Effects of pretreatment on functional and nutritional properties of cowpea meal. *J. Food Sci.* **1988**, *53*, 805–809.

SAS. *SAS User's Guide: Statistics*, version 5 ed.; SAS Institute: Cary, NC, 1986.

Sefa-Dedeh, S.; Stanley, D. Cowpea Proteins. 1. Use of response surface methodology in predicting cowpea protein extractability. 2. Characterization of water extractable proteins, *J. Agric. Food Chem.* **1979**, *27*, 1238–1243.

Zacharius, R. M. Glycoprotein staining following electrophoresis on acrylamide gels. *Anal. Biochem.* **1969**, *30*, 148–152.

Received for review May 16, 1994. Accepted May 27, 1994.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts*, July 15, 1994.