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Proximate and Amino Acid Composition of Cowpea (*Vigna unguiculata*, Walp) Protein Concentrate Prepared by Isoelectric Point Precipitation

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ABSTRACT

A cowpea protein concentrate (CPC) was extracted from decoated seeds, by the method of isoelectric point precipitation using dilute hydrochloric acid. Proximate analysis revealed that the CPC had a protein content (N × 6·25) as high as 82%. Amino acid analysis indicated that the profile of amino acids in CPC was similar to that found in the raw material. The potential use of CPC to supplement foods that are low in protein is discussed.

INTRODUCTION

In various parts of the tropics, leguminous seeds constitute an important component of the staple diet. Among the legume seeds which are widely cultivated and consumed in Africa and South America, is the cowpea (*Vigna unguiculata*) (Boulter *et al.*, 1975). The mean crude protein content of various varieties of cowpea falls somewhere between 22% and 24%, but it may vary from 21% up to 34% in certain breeds (Evans & Boulter, 1974). There is some evidence that cowpea may be regarded as a major contributor to dietary protein in those regions of the world where this grain legume constitutes a staple food (Olayide *et al.*, 1972; Luse & Okwuraiwe, 1975). Luse and Okuraiwe (1975) observed that the most significant role which legumes could play in tropical nutrition was to supply high quality protein to children. Yet Aykroyd and Doughty (1964) noted that even in those regions

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of the world where the average *per capita* consumption of cowpea was high, that of children appeared negligible.

Among the most important factors considered to be responsible for limiting the consumption of cowpea are: low protein digestibility and the flatulence caused by its ingestion (Elias *et al.*, 1976). Thus, cowpea must be adequately processed, especially when it is intended for use as a component of the diet of young children. None of the methods of preparation of cowpea for consumption has been found to address the flatus factor, including the traditional and other processing methods reported by Onayemi and Potter (1976), Elias *et al.* (1976), Luse and Okwuraiwe (1975) and Onayemi *et al.* (1976). However, Molina *et al.* (1976) have developed a technique for protein extraction from the flatulence-causing starch in cowpea. The principle of the latter technique was used in this study in an attempt to produce a condensed form of protein which may find some use as a supplement to low-protein cereal foodstuffs.

MATERIALS AND METHODS

Materials

Cowpea seeds were purchased locally and stored in a cold room (4°C) until they were used shortly thereafter.

Methods

Preparation of cowpea protein concentrate

Batches of cowpea were soaked briefly in distilled water and decoated manually. The cotyledons were subsequently air-dried and then pulverized in a Mikro pulverizer to an average particle size of 250 microns (60 mesh screen). Protein extraction was carried out in batches of 2·13 kg, according to the one-stage isoelectric point precipitation technique developed by Molina *et al.* (1976). An outline of the procedure used is shown in Fig. 1. Each batch of 2·13 kg (there were altogether seven batches) of the decoated or peeled cowpea meal (PCM) was suspended in 46·6 litres of distilled water to give approximately 4·6 g PCM per 100 ml water. The suspension was first adjusted to pH 9·0 with 5% (w/v) sodium hydroxide solution to enhance protein solubilization. The yellowish slurry thus obtained was then dispensed into 500 ml conical flasks and shaken at medium speed for 1 h with the aid of a mechanical shaker, at ambient temperature. The slurry was subsequently pooled and centrifuged at 4700*g* using a Sorvall Dupont TZ-28 model centrifuge, fitted with a continuous flow adapter rotor head. The

effluent supernatant, which contained the solubilized protein fraction, was recovered as well as the pelleted starch residue. The effluent was then adjusted to pH 4·0 using 3M hydrochloric acid and centrifuged at 18 800*g*. The resultant pellets from all the batches, or cowpea protein concentrate (CPC), were bulked and then transferred to Pyrex dishes and allowed to freeze overnight at −20°C. This was followed by drying *in vacuo* (250–150 millitorrs) at 5 to 10°C. The dry product was then stored in cellophane bags in a cold room until chemical analysis was performed on it. The chemicals used throughout the study were reagent grade.

Chemical analysis

Proximate analysis was performed on the whole cowpea meal, PCM, cowpea seed coats, and CPC in accordance with the procedures of the AOAC (1975).

Amino acid analysis was performed on CPC and PCM in duplicate by hydrolysing each sample with 6N HCl under vacuum at 105°C for 22 h. The hydrolysates were evaporated to dryness and made up to volume with a pH 7·2 citrate buffer (Onayemi & Potter, 1976). Aliquots of the hydrolysates were then subjected to ion-exchange column chromatography, using an Hitachi automated amino acid analyser, in accordance with the method of Spackman *et al.* (1958). Tryptophan and cysteine were not determined.

RESULTS AND DISCUSSION

The protein extraction scheme (Fig. 1) involved the adjustment of the aqueous suspension of PCM to pH 9·0 to enhance protein solubilization. Molina *et al.* (1976) reported that protein extraction was favoured either by acid pH (below 3·0) or by a pH over 5·0, and that there was no statistical difference between the efficiency of protein extraction between pH values varying from 8·0 to 11·0. The pH of minimum solubility of the bulk of proteins in *V. unguiculata* was found to be 4·0, a value which was identical with that reported for *V. sinensis* (Molina *et al.*, 1976). This value was assumed to be the mean isoelectric point for the group of proteins found in *V. unguiculata*, and protein recuperation was carried out at this pH in all operations.

Table 1 shows the various fractions obtained from the process of CPC preparation by isoelectric point precipitation of cowpea protein. Approximately 66% of the total protein present in the raw material (i.e. PCM) was recovered in the CPC. This percentage recovery was lower than the values of up to 87% reported by Molina *et al.* (1976). Most of the protein that was not recovered was found in the final effluent (17·9%), with smaller quantities in the starch residue (9·5%) and the seed coat (3·4%).

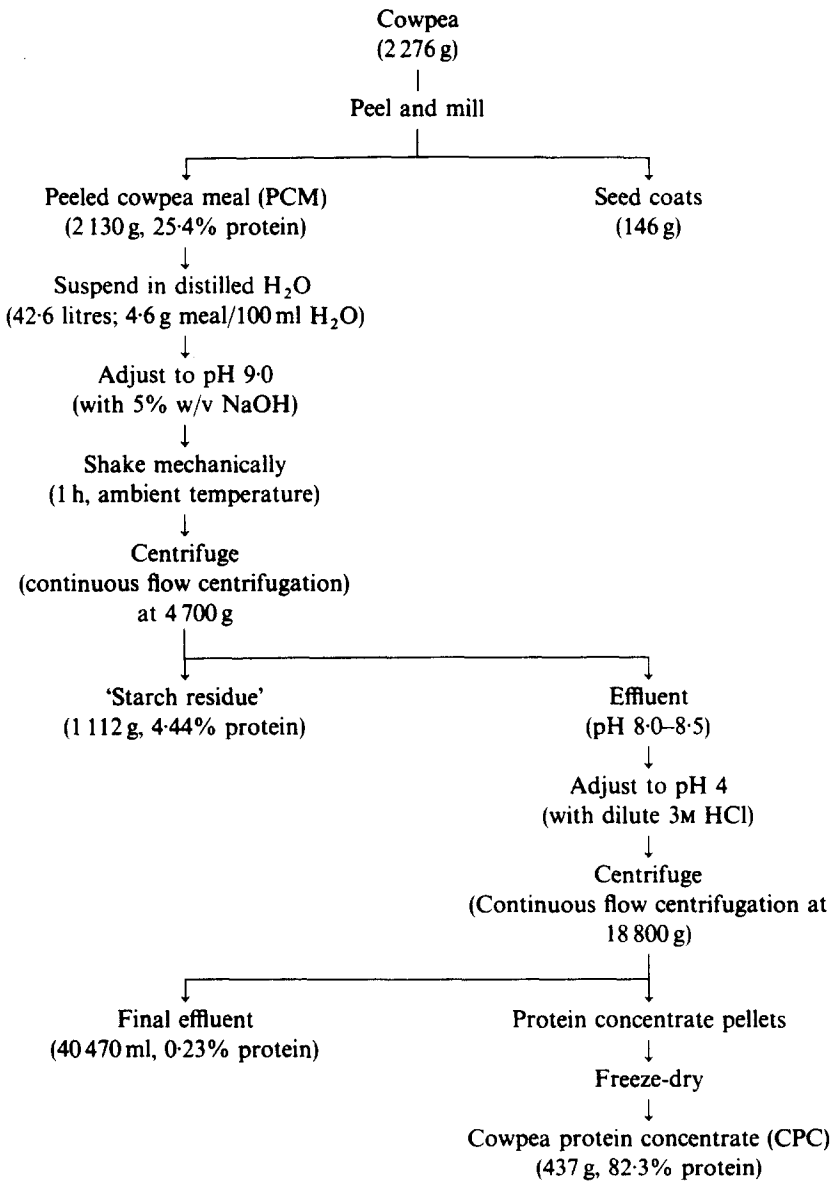

Fig. 1. Procedure for the production of a cowpea protein concentrate from peeled cowpea meal.

There was little difference between the protein contents of whole cowpea meal (24·9%) and PCM (25·4%) (Table 2). The seed coat contained 12·0% protein and was found to constitute, on average, only $6{\cdot}4 \pm 0{\cdot}4\%$ (mean $\pm$ standard error of the mean for three determinations) of the dry matter of the whole seed. Thus, seed coat removal prior to protein extraction

TABLE 1
Recovery of Protein in Various Fractions Obtained during the Production of Cowpea Protein Concentrate

Fraction	*Weight of fraction (g)*	*Total protein in fraction (g)*	*Protein in fraction as a percentage total protein*
Cowpea meal	2276	520·0	100·0
Seed coat	146	17·7	3·4
Starch residue	1112	49·4	9·5
Final effluent (ml)	40470	93·1	17·9
Protein concentrate	437	344·3	66·2
Total		504·5	97·0

accounted for 3·4% of the total seed protein. The major constituent of CPC was found to be protein, representing 82·3% of the dry matter (Table 2). The ether extract and ash found in CPC, when expressed as a percentage of the dry matter, were markedly higher than the corresponding values in the whole meal, PCM or seed coat. Conversely, CPC contained less crude fibre or NFE than the other components.

It is remarkable that more than a three-fold increase in the protein content of PCM (25·4%) was obtained as a result of processing it to CPC (82·3%) (Table 2). The latter value was higher than the range of 66–70% reported by Molina *et al.* (1976) who also found a higher percentage protein recovery (87%). The high concentration of protein in CPC in the present work might have been achieved at the expense of total protein recovery.

TABLE 2
Proximate Compositions[a] of Whole Meal, Peeled Meal, Seed Coat and Protein Concentrate of Cowpea

Component	*Whole cowpea meal*	*Peeled cowpea meal*	*Cowpea seed coat*	*Cowpea protein concentrate (CPC)*
Moisture	12·1	11·3	7·1	4·0
Protein	24·9	25·4	12·0	82·3
Ether extract	1·6	1·9	1·2	4·6
Fibre	3·0	1·4	30·2	0·4
Ash	4·2	4·3	4·0	5·9
NFE[b]	66·3	67·0	52·6	6·8

[a] Per cent of dry weight.
[b] Nitrogen-free extract by difference.

TABLE 3
Amino Acid Compositions[a] of Peeled Cowpea Meal and Cowpea Protein Concentrate

Amino acid	*Peeled cowpea meal (PCM)*	*Cowpea protein concentrate (CPC)*
Lysine	6·84	7·43
Histidine	3·04	3·28
Arginine	7·23	7·29
Aspartic acid	12·2	11·9
Threonine	3·75	3·69
Serine	5·49	5·55
Glutamic acid	16·9	16·3
Proline	4·50	4·63
Glycine	3·75	3·23
Alanine	4·97	3·86
Valine	4·15	4·43
Methionine	1·02	0·93
Isoleucine	3·52	2·75
Leucine	7·30	11·2
Tyrosine	2·92	3·13
Phenylalanine	3·49	4·10
Protein %	25·4	82·3

[a] Gram/100 g protein (each figure represents the mean of duplicate determinations).

Table 3 shows the amino acid contents of PCM and CPC. The levels of amino acids in both components fell within the range reported for nine varieties of *V. unguiculata* (Evans & Boulter, 1974). The exceptions were the low phenylalanine and isoleucine in PCM and CPC, and markedly high leucine in CPC. Furthermore, the levels of methionine in PCM (1·02 g/100 g protein) and, to a large extent, in CPC (0·93 g/100 g protein), fell outside the range of 1·35–1·70 g/16 gN reported by Evans and Boulter (1974), but the PCM value was close to the 1·08 g/16 gN found by Molina *et al.* (1976). The values of amino acids reported by the latter workers for *V. sinensis* peeled meal were higher compared with values found in this and other reports (Evans & Boulter, 1974; Elias *et al.*, 1976; Onayemi & Potter, 1976), except for methionine, serine, threonine and tyrosine. For instance, Molina *et al.* (1976) reported a value of 9·99 g leucine/16 gN which was markedly higher than the 7·30 g/100 g protein shown in Table 3. Onayemi and Potter (1976) and Evans and Boulter (1974), reported 6·3–8·0 (from 9 determinations), and 7·30–8·73 g leucine/16 gN (for 9 varieties of *V. unguiculata*), respectively. The low levels

of phenylalanine, methionine and isoleucine (Table 3) could be due to partial destruction of these amino acids in PCM and CPC during acid hydrolysis (Bouter *et al.*, 1975).

The amino acid compositions (Table 3) of PCM and CPC were similar, and therefore the extraction procedure had little effect except for leucine. The leucine level in CPC (11·2 g/100 g protein) was comparable with the 10·1 g/16 gN found in the one-stage protein concentrate (Molina *et al.*, 1976), but markedly higher than the 7·30 g/100 g protein in PCM. As a result of the apparently low isoleucine and high leucine, the leucine:isoleucine ratio increased to 4·08 in CPC from 2·07 in PCM. From the amino acid profiles reported (Molina *et al.*, 1976) for the one-stage concentrate and peeled meal, the ratios were calculated and found to be 2·07 and 1·61, respectively. The high leucine:isoleucine ratio in CPC might have resulted from a probable underestimation of leucine. Since the primary objective was to produce CPC as a protein supplement to cereal diets, a high leucine:isoleucine ratio would be undesirable because it might lead to amino acid imbalance in cereals that are already high in leucine and low in tryptophan and isoleucine.

Preliminary bioassays were performed on CPC using rats. A group of 7 animals was fed moist heat-treated CPC as the sole source of dietary protein (8% protein) for 21 days. One group of rats fed casein and another moist heat-treated PCM, served as controls. The results revealed that CPC was better digested (89·1 ± 1·0%, representing mean ± standard error of the mean), compared with PCM (79·9 ± 2·2%). The protein efficiency ratio (PER) for CPC was 1·76 ± 0·11 compared with 1·94 ± 0·24 and 2·50 ± 0·0 for PCM and casein, respectively. When various combinations of CPC and processed normal dent corn were fed to rats as described above, the combination in which each component contributed 50% of the dietary protein gave the best results. The protein and food efficiency ratios were 2·44 ± 0·18 and 0·28, respectively, compared with 2·50 ± 0·0 and 0·28 for casein. These biological data support the suitability and potential use of CPC as a protein supplement and the good growth observed in the experimental animals failed to confirm the possibility that a high leucine:isoleucine ratio might reduce the utilization of CPC protein for growth.

The wet protein extraction procedure is long and tedious, and involves freeze-drying, a very slow and expensive process. Further investigations are required before the process can find use on a wider scale. In recognition of some of the difficulties involved in the process, Molina *et al.* (1976) have indicated the need to explore alternative procedures such as the dry process. There is also the need to investigate the possible long term effects of consuming concentrates such as CPC by animals and man.

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REFERENCES

AOAC (1975). In *Official Methods of Analysis* (9th edn), ed. W. Storwitz. Association of Official Analytical Chemists, Washington, DC.

Aykroyd, W. R. & Doughty, J. (1964). *Legumes in Human Nutrition*, ed. W. R. Aykroyd & J. Doughty. FAO Nutritional Studies No. 19, FAO, Rome, pp. 1–138.

Boulter, D., Evans, I. M., Thompson, A. & Yarwood, A. (1975). The amino acid composition of *Vigna unguiculata* (Cowpea) meal in relation to nutrition. In *Nutritional Improvement of Food Legumes by Breeding*, ed. M. Milner. Protein Advisory Group of the United Nations Systems, pp. 205–15.

Elias, L. G., Hernandez, M. & Bressani, R. (1976). The nutritive value of precooked legume flours processed by different methods. *Nutr. Rept. Int.*, **14**, 385–403.

Evans, I. M. & Boulter, D. (1973). Chemical methods suitable for screening for protein content and quality in cowpea (*Vigna unguiculata*) meals. *J. Sci. Food Agric.*, **25**, 311–22.

Luse, R. A. & Okwuraiwe, P. E. (1975). Role of legumes in tropical nutrition. In *Proceedings of IITA Collaborators' Meeting on Grain Legume Improvement*, 9–13 June 1975. International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria, pp. 97–104.

Molina, M. R., Argueta, C. E. & Bressani, R. (1976). Protein–starch extraction and nutritive value of the black-eyed pea (*Vigna sinensis*) and its protein concentrate. *J. Food Sci.*, **41**, 928–32.

Olayide, S. O., Olatunbosun, D., Idosogie, E. O. & Abiagom, J. D. (1972). National food balance sheet. In *A Quantitative Analysis of Food Requirements, Supplies, and Demands in Nigeria: 1968–1985*. Federal Dept of Agriculture, Lagos, Nigeria, pp. 19–24.

Onayemi, O. & Potter, N. N. (1976). Cowpea powders dried with methionine: Preparation, storage stability, organoleptic properties, nutritional quality. *J. Food Sci.*, **41**, 48–53.

Onayemi, O., Pond, W. G. & Krook, L. (1976). Effects of processing on the nutritive value of cowpeas (*Vigna sinensis*) for the growing rat. *Nutr. Rept. Int.*, **13**, 299–305.

Spackman, D. H., Stein, W. H. & Moore, S. (1958). Automatic recording apparatus for use in the chromatography of amino acids. *Anal. Chem.*, **30**, 1190–206.