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Effect of Variety on Protein Content, Amino Acid Composition and Trypsin Inhibitor Activity of Cowpeas

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ABSTRACT

*Catalogued varieties of cowpea (*Vigna unguiculata *(L.) Walp.) from the International Institute for Tropical Agriculture, Ibadan, Nigeria, were analysed for crude and 'true' protein (nitrogen × 6·25) content, amino acid composition and trypsin inhibitor activity (TIA). Crude protein values ranged from 23% to 31·3% and 'true' protein from 20·7% to 27·3% (values on a dry weight basis). In comparison with the Provisional Amino Acid Scoring Pattern of FAO (1973), the amino acid values for all varieties were found to be low in methionine and higher in lysine, leucine, isoleucine and phenylalanine + tyrosine. Values for threonine were variable with respect to the Pattern. No relationship between protein content and content of methionine was found, despite one being previously reported between protein content and content of sulphur-containing amino acids. All cowpeas contained TIA, with two varieties showing exceptionally high levels.*

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.), a starchy grain legume, is a member of the sub-family *Papilionaceae*, the largest of the three divisions of the

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Leguminosae. The cultivation of cowpea is carried out extensively in Nigeria, the USA, Upper Volta and Uganda, and, together, these countries yield 90% of the world's production. Throughout Africa, cowpeas are a common ingredient of the thick soup which accompanies meals of the staple food. They are also often eaten alone, or in special preparations such as fried or steamed bean cakes, called akara balls and moin-moin, respectively, in Nigeria (Dovlo *et al.*, 1976). In industrialised countries, cowpeas are available in most health food shops and can add variety to the diets of the increasing numbers of vegetarians in these communities. They have the advantage, over other grain legumes, of being relatively quick-cooking.

The proximate composition of cowpeas was reported by Johnson & Raymond (1964) as 11% water, 23% protein, 56·8% carbohydrate, 1·3% fat, 3·9% crude fibre and 3·6% ash. However, the composition varies considerably according to the cultivar and cultivation. Thus, the protein content has been reported to vary between 19% and 26% (on a dry weight basis) and varieties with up to 35% protein are also known to exist (Summerfield *et al.*, 1974). Most protein values given in the literature are for crude protein, with total nitrogen calculated as protein using a nitrogen-to-protein conversion factor (usually 6·25). Legumes contain a considerable proportion of non-protein nitrogen-components, which means that crude protein values overestimate the protein content. Genetic and environmental factors also affect the amino-acid composition of cowpeas. A comparison of the essential amino acid values obtained by Busson (1965), Otoul (1973), Evans & Boulter (1974) and Ologhobo & Fetuga (1982) with the values in the Provisional Amino Acid Scoring Pattern (PAASP) of FAO (1973) showed that cowpeas compare favourably with this Pattern. Although they are a little low in the sulphur-containing amino acids, the high level of lysine usefully complements the amino acid composition of cereals, which are low in this amino acid.

Although a wide range of anti-nutritional factors are found in most legumes (Liener, 1978), the only one of importance in cowpeas is the trypsin inhibitor. Reports vary on the levels of trypsin inhibitor activity (TIA) in cowpeas. Thus, Bressani & Elias (1977) reported that cowpeas contained considerably less than other legumes, while Gatehouse *et al.* (1979) found wide differences between varieties.

Cowpeas, like other starchy legumes grown in tropical and sub-tropical regions of the world, are low-yielding and at risk of being replaced in cultivation by high-yielding varieties of cereals. Since the early 1970s the International Institute of Tropical Agriculture (IITA) at Ibadan, Nigeria, has been involved in a breeding programme to improve the yield of cowpeas. As cowpeas are used directly for human consumption, there is need to monitor changes in the content of both nutrients and anti-nutritional

substances as such breeding programmes progress. Therefore, in this study, catalogued varieties from IITA, as well as two locally-purchased cowpea varieties, were analysed for protein (crude and 'true'), amino-acid composition and TIA.

MATERIALS

Description of materials

Table 1 describes the cowpea varieties selected for the present study. The catalogued varieties were bred at IITA and cultivated at the Plant Environment Laboratory, University of Reading. Cowpea Nos 23 and 24 were purchased locally.

Sample preparation

All cowpeas were ground whole, using a cyclone sample mill (Tecator Ltd, 71 Whiteladies Rd, Bristol BS8 2NT) and stored in air-tight containers at $-18°C$. Samples were thawed to room temperature before analysis, without opening the container.

Analytical methods

(a) Moisture
Samples (approximately 1 g) were accurately weighed in aluminium dishes and dried in a forced-air oven at $100°C \pm 2°C$ for 16 h, cooled in a desiccator and re-weighed.

(b) Protein

(i) Crude protein. Cowpea samples were analysed for crude protein by the short Kjeldahl method (Method No. 2) of Marshall & Walker (1978), using a Technicon thermostated aluminium digestor block (Technicon Instruments Co. Ltd, Hamilton Close, Basingstoke, Hampshire, RG21 2YE), holding 20 Kjeldahl tubes (250 ml). A Technicon Autoanalyser, using the nitroprusside–salicylate–hypochlorite reagent system was used for the determination of ammonia.

(ii) *True protein.* True protein, as used here, is the nitrogen precipitated with 10% trichloroacetic acid (TCA) and converted to protein by multiplying by the nitrogen-to-protein conversion factor of 6·25. For each analysis, 10 ml of 10% TCA were added to approximately 0·8 g of

TABLE 1
Description of Cowpea Varieties

Cowpea No.	*IITA cat. No.*	*Approximate length of seed (cm)*	*Description of seed*
1	TVu 662	0·5	Brown, black pigmentation, white eye.
2	TVu 2027	0·6	Cream, black eye, round.
3	TVu 1469	0·5	Brown-beige, dark brown pigmentation, black eye.
4	TVu 201-1D	0·6–0·7	Mottled beige-brown and red-brown, black eye, round.
5	TVu 4248	0·5–0·9	Cream, square, dark eye.
6	TVx 3-5G	0·4	Dark brown, square.
7	TVu 3671	0·9	Green-cream, kidney shaped.
8	TVu 1035	0·5–0·6	Red-brown, kidney shaped.
9	TVu 4057	0·9–1·0	Mottled, green-cream and brown-cream, flattened kidney shape.
10	TVx 12-01E	0·6–0·8	Mottled, dark red-brown and cream, round, kidney shaped.
11	K 2809	0·4–0·6	As above.
12	4S 3438	0·7–0·8	Mottled cream and dark brown, red eye.
13	TVx 1836-19E	0·7	Green-cream, square, prominent brown eye.
14	TVx 2112	0·5–0·7	Beige, shrunken, prominent black eye, kidney shaped.
15	TVu 4557	0·7–0·8	Mottled green-cream and light brown, square, kidney shaped.
16	TVu 4186	0·7–0·8	Light green-cream, regular shape.
17	TVu 76	0·5–1·0	Brown-cream, prominent eye.
18	TVx 1193-059D	0·7–0·8	Red-brown, flat kidney shape.
19	TVu 2321	0·7–0·9	Mottled dark and light beige, square kidney shaped.
20	TVu 57	0·5–0·6	Mottled dark brown and black, some round, some square.
21	TVu 354	0·5–0·7	As above.
22	TVu 4552	0·9–1·0	Mottled yellow-cream and light brown.
23	[a]	1·0	Purchased in Uxbridge, Middlesex. Cream with typical black eye.
24	[a]	1·0	Purchased in Reading, Berkshire. As above.

[a] No IITA catalogue number.

accurately-weighed, ground cowpea sample in a 100 ml polypropylene bottle and left at 4°C for 30 min for precipitation to occur. The sample was then washed with 30 ml of 10% TCA into a Whatman No. 541 filter paper. The sample was then washed twice with 30 ml of 10% TCA. Between each washing, the liquid was allowed to drain away completely. The dried filter

paper and residue were then digested by the short Kjeldahl method (Marshall & Walker, 1978) and analysed for nitrogen content. Correction for the nitrogen content of filter paper was made by analysing two reagent blanks containing filter paper and reagents, under the same conditions as for cowpea samples.

(c) *Amino acid analysis*

Amino acid composition of the cowpea protein was determined by ion-exchange chromatography after acid hydrolysis, according to the method of Spackman *et al.* (1958), as described by Walker (1979). The amino acid analyser used was a Jeol JLC-6AH (Jeol (UK) Ltd, Jeol House, Grove Park, Colindale, London NW9 0JN). For the hydrolysis of the protein, a cowpea sample (20–30 mg), was weighed into a hydrolysis tube made from a screw-threaded glass joint (Fisons Catalogue No. 5Q 28, Fisons Scientific Apparatus Ltd, Bishop Meadow Road, Loughborough, Leicestershire LE11 0RG) with a sealed end, plastic cap and rubber ring (Fisons Cat. Nos QC 28/13 and QR 28/11, respectively). Hydrochloric acid (5 ml of 6M) was added and nitrogen bubbled through the mixture for 20 min prior to sealing the tube with a Teflon disc and placing in an oven at 110°C ± 1°C for 24 h.

All calculations for amino-acid analysis were carried out using a computer program written in BASIC. Methionine values used for calculations were the sum of the methionine and methionine sulphoxide peaks (Walker, 1983). Values for nitrogen recovery were expressed as a percentage of total nitrogen (by Kjeldahl analysis) in the sample analysed. Finally, all the values expressed as g/16 g N were corrected to 100% nitrogen recovery.

(d) *Trypsin inhibitor activity*

The method of Smith *et al.* (1980) was used to determine the TIA of cowpea varieties. As a certain amount of variability was noted in TIA measurement in preliminary experiments, each cowpea variety was subjected to three replicate analyses. A single replicate, prepared from a fresh extract on the day, was analysed on each of three consecutive days: the order in which samples were analysed being varied according to a randomised block design.

The method of Smith *et al.* (1980) involved the extraction of the inhibitor(s) from the sample at pH 9·5 and mixing unfiltered sample solutions with bovine trypsin to form a stable complex. The activity of the remaining trypsin was then measured by offering it benzoyl-DL-arginine-*p*-nitroanilide (BAPNA) under standard conditions. The absorbance of the yellow-coloured *p*-nitroaniline released by the action of trypsin was measured spectrophotometrically at 410 nm. Within 40–60% inhibition of trypsin, the relationship between the absorbance and concentration of

residual trypsin activity is a straight line (Smith *et al.*, 1980). Therefore, within these limits, the amount of pure trypsin inhibited per unit weight of sample was calculated from a single level of residual trypsin.

RESULTS AND DISCUSSION

The contents of moisture, crude and true protein and TIA of the cowpea varieties are presented in Table 2. The range of crude protein values was 23·0% to 31·3% and with a SED (standard error of the difference between means of any two varieties analysed) of 0·43, showed significant differences between the varieties at $P < 0{\cdot}05$. Figure 1 shows the groupings that emerge when structured *t*-tests are used as a means of performing a one dimensional cluster analysis. The interpretation of data involving an unstructured set of treatments (varieties) such as this has long been a problem. In many cases experimenters choose, wrongly, to use multiple comparison techniques. These have increasingly been criticised (for example, Carmer & Walker, 1982) as failing to provide the information required. The techniques concentrate on the search for significant *differences*, whereas the aim of this study is to discover the *similarities* between varieties on the basis of a number of different measurements. Statisticians are turning to a study of cluster analysis as a solution to the problem (Caliński & Corsten, 1985) and the method employed here is a simplified version of one of these techniques which has been shown to give good results (Othman, F. pers. comm.).

The true protein contents also varied significantly at $P < 0{\cdot}05$, and the SED for all values was 0·58—showing that the method was less precise than the estimation of crude protein. The range of values for true protein was 20·7% to 27·3% which was lower than the values for crude protein (as expected). The range of mean values was narrower for true protein than crude protein, which can be accounted for by considerable variation in the non-protein nitrogen. The groupings of the cowpea varieties shown in Fig. 2 are broadly similar to those in Fig. 1. Values in Table 2 show that non-protein nitrogen calculated as crude protein varied from 1·5% to 4·4%. Values for true protein are not often quoted in the literature, but values for crude protein in Table 2 were within the ranges reported by other workers (e.g. Bliss, 1973; Boulter *et al.*, 1973).

The TIA values for all cowpeas showed highly significant differences between varieties ($P < 0{\cdot}001$) and were lower than those quoted by Smith *et al.* (1980) for some unheated soyabean samples, which showed levels *ca.* 30 mg per g. Nevertheless, two cowpea varieties, Nos 2 and 16, showed higher levels than the others (12·6 and 13·9 mg trypsin inhibited per g, respectively. Although this is the first report of a high level of TIA in cowpea

TABLE 2
Moisture, Crude and True Protein Contents and Trypsin Inhibitor Activity (TIA) of Cowpeas
(Each value is the mean of two replicate analyses, % protein is presented on a moisture-free basis)

Cowpea No.	*Moisture* (%)	*Protein* (% $N \times 6{\cdot}25$[a])		*NPN* (*as crude protein*)	*TIA*[c]
		Crude	*True*[b]		
1	11·6	31·3	27·3	4·0	6·1
2	ND	ND	ND	ND	12·6
3	13·2	26·7	23·8	2·9	5·1
4	11·1	25·7	22·1	3·6	ND
5	10·1	25·0	22·0	3·0	7·1
6	12·3	26·3	23·5	2·8	7·4
7	10·9	25·6	22·1	3·5	8·8
8	11·1	28·2	24·8	3·4	7·9
9	11·9	27·5	23·8	3·7	9·0
10	11·4	28·0	24·2	3·8	ND
11	12·7	24·4	22·8	1·5	7·9
12	10·3	29·0	26·2	3·6	8·3
13	11·9	27·5	25·2	2·3	6·2
14	10·3	29·3	24·9	4·4	ND
15	11·6	26·1	23·9	2·2	ND
16	10·0	26·7	23·5	3·2	13·9
17	11·5	28·5	25·2	3·3	7·7
18	12·1	27·5	24·6	2·9	4·8
19	12·2	26·5	24·0	2·5	5·0
20	12·1	27·2	24·3	2·9	4·6
21	11·8	27·0	24·0	3·0	4·6
22	11·2	27·5	23·9	3·6	ND
23	10·0	23·0	21·0	2·0	ND
24	9·6	23·1	20·7	2·4	6·0
SED	0·25	0·43	0·58		0·56
(df)	(23)	(23)	(23)		(34)

[a] Nitrogen-to-protein conversion factor.
[b] Obtained by precipitating with 10% trichloroacetic acid.
[c] mg of trypsin inhibited/g sample (mean of three replicate analyses and uncorrected for moisture content).
df, degrees of freedom; ND, not determined; SED, standard error of difference of two means ($\sqrt{2s^2/n}$, where s^2 = residual mean square obtained from analysis of variance and n = number of replicates).

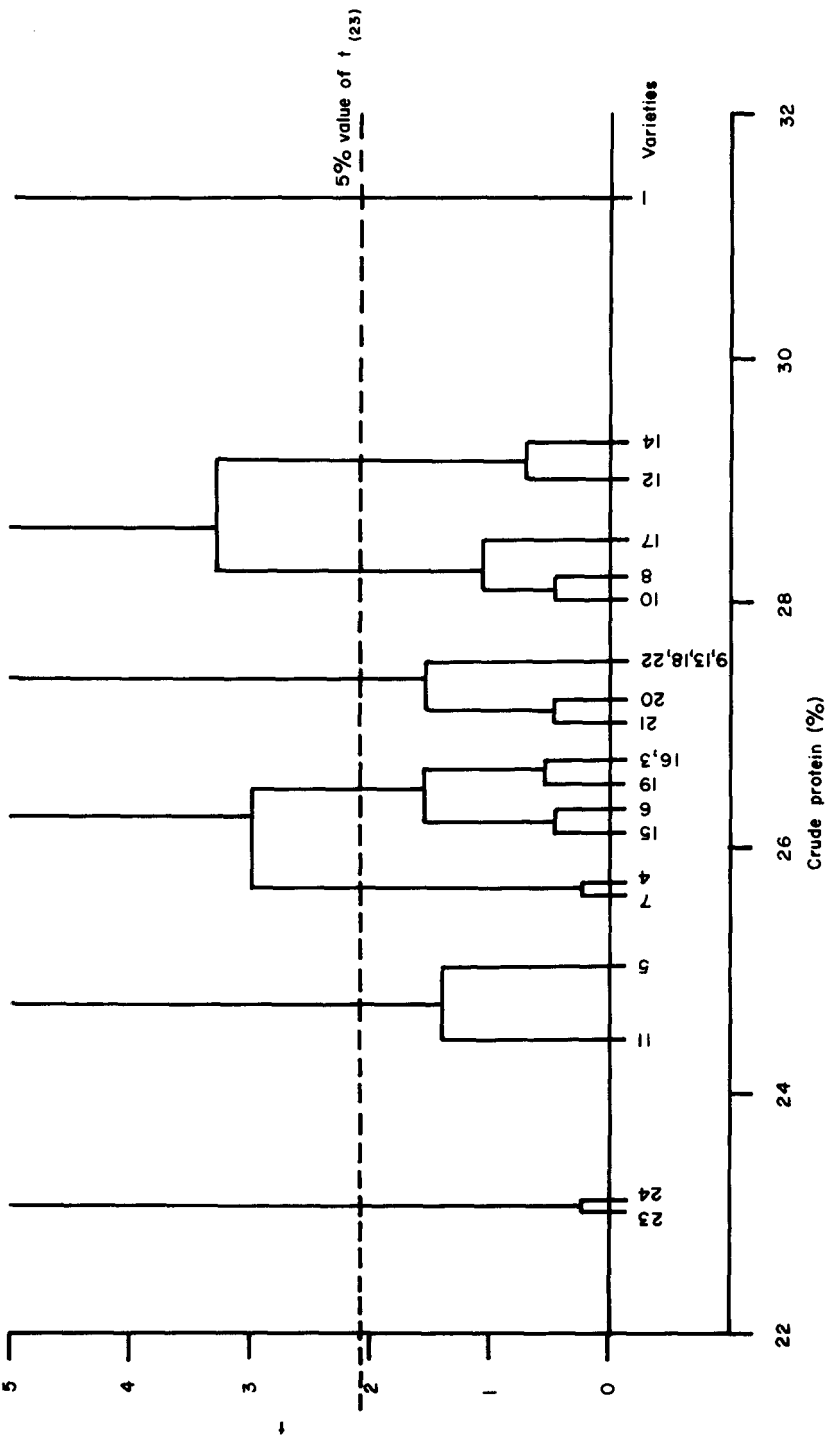

Fig. 1. Statistical groupings of cowpea varieties for crude protein.

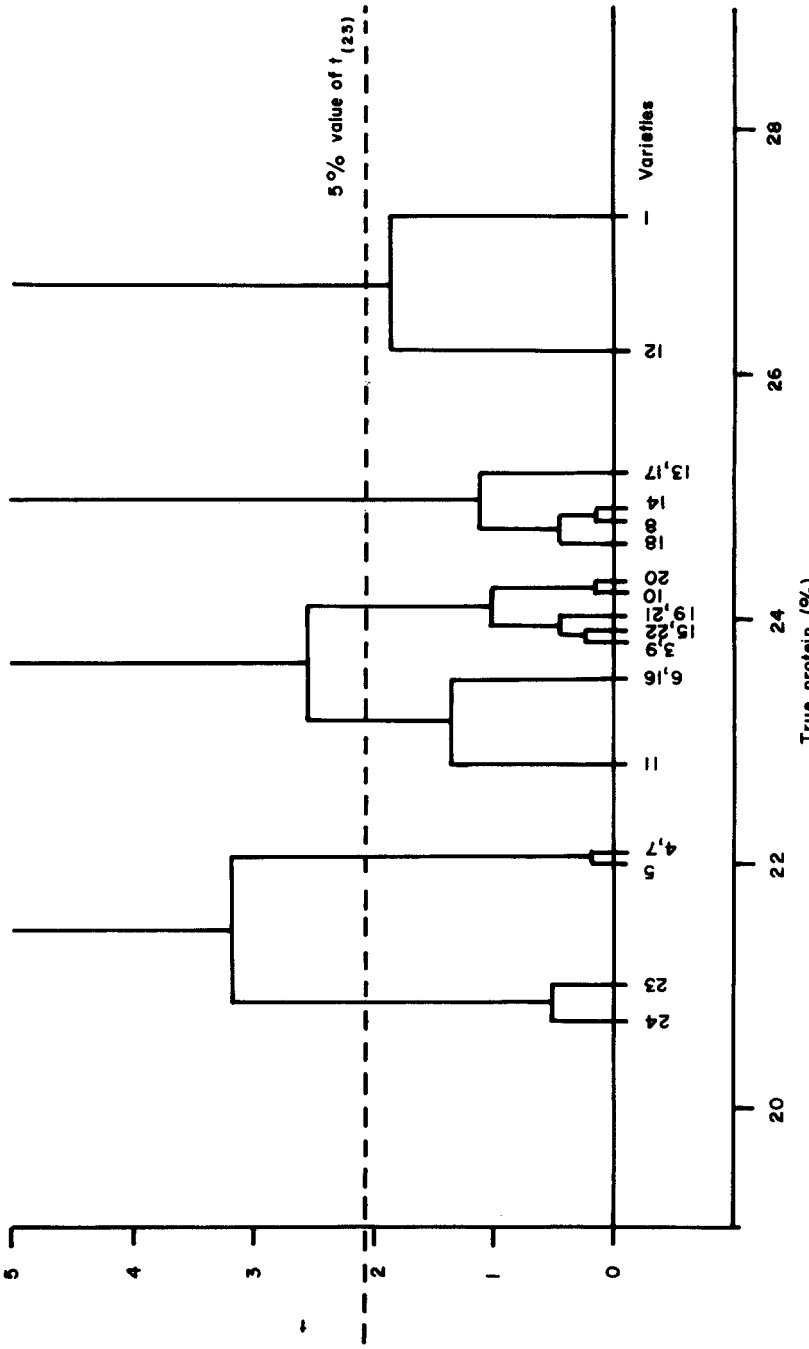

Fig. 2. Statistical grouping of cowpea varieties for true protein.

No. 16, cowpea No. 2 was considered an unusual variety by Gatehouse *et al.* (1979), because it contained a higher level of trypsin inhibitor (22·7 Trypsin Inhibitor Units $\times 10^3$) than the seven other cowpeas varieties analysed by them. The TIA value for cowpea No. 2 obtained in the present work, substantiated the data of Gatehouse *et al.* (1979). These authors concluded that the high level of trypsin inhibitor in cowpea No. 2 conferred resistance on this variety to infestation with the insect species, *Callosobruchus maculatus* during storage. If it is indeed the presence of relatively high levels of trypsin inhibitors which effects insect resistance during storage, then cowpea No. 16 should also show comparable insect resistance to cowpea No. 2.

The essential amino-acid composition, amino acid scores (based on the PAASP; FAO, 1973) and the limiting amino acids of some of the cowpea varieties are presented in Table 3. Values for cystine ranged from 0·03 to 0·5, but were considered too variable by this method for inclusion in this table. Non-essential amino acids are shown in Table 4. Only the essential amino acids, isoleucine and valine, and the non-essential amino acid, proline, showed no significant difference between varieties using the *F*-value obtained from Analysis of Variance. For the remaining amino acids there are significant differences between varieties.

In comparison with the PAASP of FAO (1973), the essential amino acid composition of cowpeas (Table 3) was found to be low in methionine and higher in lysine, leucine, isoleucine and phenylalanine + tyrosine. Values for threonine were variable, some being higher and others lower than the FAO (1973) pattern. Only one variety, No. 16, was slightly lower in valine than the FAO pattern.

Lysine varied between 6·7 to 8·1 g/16 g N, showing highly significant varietal differences. These values are comparable with, if not a little higher than, those reported in the literature (e.g. Litzenberger, 1973; Evans & Boulter, 1974). The wide variation between varieties indicates potential for increasing the lysine content of cowpea by breeding. Legumes of high lysine content complement low-lysine cereals, the lysine content of which can only be altered with difficulty in breeding programmes.

The methionine content of cowpea varieties varied significantly (at $P < 0{\cdot}001$), within the range 1·5 to 2·3 g/16 g N; the high end of the range showed higher levels than previous reports. Thus, Evans & Boulter (1974) reported 1·35 to 1·7 g/16 g N for nine varieties, while Summerfield *et al.* (1974) reported 0·35 to 0·90 g/16 g N. Even at the higher levels reported here, cowpeas are low in methionine with respect to the PAASP. However, cowpeas are normally eaten in conjunction with cereals, which are relatively high in methionine, which would bring the essential amino acid composition closer to the FAO (1973) pattern. Although threonine was the second

TABLE 3
Essential Amino Acid Content of Cowpeas
(Each value is the mean of two replicate analyses)

Cowpea No.	*Essential amino acids (g/16 g N^1)*							
	Ileu	*Leu*	*Lys*	*Met*	*Phe*	*Tyr*	*Thr*	*Val*
FAO (1973)[2]	4·0	7·0	5·5	3·5[3]	6·0		4·0	5·0
1	4·3	7·8	6·7	1·8	5·7	2·8	3·9	5·0
3	4·6	7·8	7·0	2·0	5·7	3·1	3·9	5·1
5	4·8	8·5	8·0	2·0	6·0	3·1	4·5	5·6
6	4·6	8·1	6·6	2·3	5·9	3·1	4·0	5·5
7	4·5	7·8	6·7	1·8	5·6	2·7	4·1	5·3
8	4·5	8·4	8·1	2·1	6·2	3·0	4·3	5·5
9	4·3	7·9	6·7	2·1	5·7	2·5	4·1	5·3
10	4·5	7·8	6·7	1·9	5·8	2·5	3·8	5·4
11	4·7	8·3	8·0	2·3	6·2	3·6	4·2	5·7
13	4·7	8·0	7·4	1·5	5·8	2·2	4·0	5·6
14	4·3	7·6	7·1	1·7	5·6	2·4	4·3	5·1
15	4·4	7·9	7·1	2·1	5·6	2·8	4·0	5·2
16	4·2	7·7	7·1	1·8	5·6	2·9	4·0	4·9
17	4·4	7·9	7·2	1·7	5·8	2·5	4·1	5·2
19	4·4	7·9	7·2	2·2	5·7	2·4	4·1	5·4
20	4·3	7·6	8·0	1·6	5·7	2·7	3·6	5·4
21	4·6	8·1	7·5	1·6	6·0	2·6	4·1	5·5
22	4·6	7·8	7·4	1·5	5·7	2·6	4·2	5·3
23	4·4	7·9	7·1	2·0	5·6	2·9	4·0	5·3
24	4·5	7·8	6·8	1·9	5·6	2·9	3·9	5·2
Range	4·2–4·8	7·6–8·5	6·6–8·1	1·5–2·3	5·5–6·2	2·2–3·6	3·6–4·5	4·9–5·7
SED	0·21	0·20	0·24	0·15	0·13	0·26	0·03	0·23
F-value	1·1	2·7 *	12·3 ***	217 ***	5·2 ***	3·5 **	114 ***	1·5

*, **, ***, significant at $P < 0{\cdot}05$, $P < 0{\cdot}01$ and $P < 0{\cdot}001$, respectively; 1, corrected to 100% N recovery; 2, Provisional Amino Acid Scoring Pattern; 3, includes value for cystine; SED, standard error of difference of two means; *F*-value, assessment of overall variety differences obtained from analysis of variance.

limiting amino acid for six of the 23 cowpea varieties in Table 3, it was only marginally limiting in comparison to the FAO (1973) pattern. Values for threonine lower than these have been reported by other workers, e.g. 3·09 to 3·88 g/16 g N (Evans & Boulter, 1974) and 3·3 to 3·6 g/16 g N (Khan *et al.*, 1979).

Mossé & Baudet (1977) demonstrated a straight-line relationship between the contents of lysine + histidine + arginine and crude protein content for

TABLE 4
Non-Essential Amino Acids of Cowpeas

Cowpea No.	*Non-essential amino acids (g/16 g N^1)*							
	Ala	*Arg*	*Asp*	*Glu*	*Gly*	*His*	*Pro*	*Ser*
1	4·4	7·4	11·2	17·0	4·2	3·2	4·5	5·0
3	4·5	7·2	11·1	16·9	4·2	3·2	4·4	4·7
5	4·7	8·0	12·9	17·9	4·3	3·8	4·5	5·4
6	4·4	7·1	11·1	17·0	4·2	3·0	3·8	5·0
7	4·4	6·8	12·1	16·9	4·1	2·9	4·4	5·0
8	4·8	7·7	12·3	18·7	4·5	4·0	4·9	5·6
9	4·4	7·0	11·2	17·5	4·4	3·1	4·4	5·2
10	4·2	7·0	11·6	17·5	4·2	3·0	4·5	4·8
11	4·8	7·6	11·8	17·2	4·6	3·8	4·4	5·3
13	4·4	7·1	11·0	16·8	4·1	3·4	4·7	4·6
14	4·4	7·0	11·1	17·0	4·2	3·2	4·5	5·2
15	4·3	7·0	11·1	16·8	3·9	3·4	4·7	5·1
16	4·1	6·9	11·6	17·1	3·8	3·3	4·6	5·1
17	4·4	6·8	11·3	16·7	4·2	3·3	4·8	5·1
19	4·5	6·8	11·5	17·7	4·3	3·5	4·6	5·0
20	4·2	7·2	10·7	16·2	4·1	4·7	3·3	4·1
21	4·5	5·4	11·7	17·7	4·4	3·3	4·0	4·9
22	4·2	7·0	11·4	16·8	4·1	3·1	4·3	4·7
23	4·4	7·2	11·3	17·0	4·1	3·3	3·2	4·7
24	4·2	7·5	11·3	16·8	4·0	3·3	3·6	4·6
Range	4·1–4·8	5·4–8·0	10·7–12·9	16·2–18·7	3·8–4·6	2·9–4·7	3·2–4·9	4·1–5·6
SED	0·07	0·16	0·24	0·21	0·06	0·15	0·39	0·20
F-value	15·6	12·1	12·1	15·6	22·0	14·6	1·3	4·1
	***	***	***	***	***	***		**

Abbreviations as for Table 3.

broad beans (*Vicia faba* L.). Therefore, using the data presented here, plots of paired data for individual cowpea varieties were made for crude or true protein against: (a) lysine, (b) histidine, (c) arginine, (d) lysine + histidine + arginine and in no case did the plots reveal any relationship which justified further analysis by statistical techniques such as linear regression. For the sulphur-containing amino acids, Evans & Boulter (1974) reported a strong negative correlation between the contents of methionine + cystine and crude protein of cowpea meals. In this present work, values for methionine showed no association with crude or true protein values. Thus it would appear that breeding programmes aimed at improving the protein content of cowpeas would not necessarily have a negative effect on the content of methionine alone.

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