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Chemical composition and nutritional evaluation of two little-known species of *Vigna*

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The seeds of *Vigna capensis* and *V. sinensis* have been analysed for proximate and mineral composition, seed protein fractions, seed protein amino acid profiles, fatty acid lipid profiles and antinutritional factors. The crude protein contents of the *Vigna* species investigated appeared to be closer to the levels of commonly consumed pulses. The seeds of the two *Vigna* species analysed were rich in the minerals K, Ca, Mg, P and Fe. The albumins and globulins constituted the major bulk of seed proteins in *V. capensis* and *V. sinensis*. Seed proteins of *V. sinensis* were found to have higher methionine contents when compared with the other sample investigated. In *V. capensis* and *V. sinensis* the contents of the essential amino acids valine, leucine, tyrosine, phenylalanine and lysine were fairly high. Fatty acids such as palmitic, linoleic and linolenic acids were found to be relatively high in both the tribal pulses investigated. Antinutritional factors such as total free phenols, tannins, L-DOPA and haemagglutinating activity were also analysed or assayed.

INTRODUCTION

Food legumes constitute a cheap alternative source of protein and calories, particularly for people who are unable to afford the high cost of dietary protein from animal sources (Noel & Rosario, 1989). The search for novel, high-quality but cheap sources of protein and energy has continued to be a major concern of governments and bodies charged with the responsibility for food and nutrition in many parts of the developing world (Balogun & Fetuga, 1986). In India, information on the chemical composition of seeds of tribal pulses and the wild progenitors of cultivated legumes is relatively scarce. Hence in the present study the mature seeds of *Vigna capensis* and *V. sinensis*, which were originally eaten by the tribal people of north and north-eastern India, were subjected to biochemical investigation with a view to assessing their nutritional potential.

MATERIALS AND METHODS

The seeds of *V. capensis* were procured from Dr D. K. Hore, NBPGR, Bishnupur, Shillong, Meghalaya, India, and the seeds of *V. sinensis* were collected from Jammu Valley, India. The moisture content was measured by drying 50 transversely cut mature and dry seeds in an oven at 80°C for 24 h and expressed on a percentage basis. The seeds were powdered separately in a Willey mill to 60-mesh size. The fine seed powder so obtained was used for further analyses. The crude protein content was calculated by multiplying the percentage of Kjeldahl nitrogen (Humphries, 1956) by the factor 6·25. The remaining proximate constituents were estimated by AOAC (1970) methods. The nitrogen-free extractives were calculated by difference (Muller & Tobin, 1980). The energy content was determined by multiplying the percentages of crude protein, crude fat and nitrogen-free extractives (total crude carbohydrates) by the factors 4, 9 and 4 respectively (Osborne & Voogt, 1978). The total (true) proteins were extracted by the method of Basha *et al.* (1976) with slight modification (ethanol treatment was omitted so as to save the prolamin fraction). The extracted proteins were purified by precipitation with cold 20% trichloroacetic acid (TCA). The seed protein fractions, albumins and globulins, were extracted following the method of Murray (1979). From the residual pellet, the prolamin fraction was extracted by treating it with 70% ethanol (1 : 5, w/v) overnight; after centrifugation the supernatant, containing prolamins, was air-dried and dissolved in 0·1 M NaOH. To the remaining pellet 0·4 N NaOH (1 : 10, w/v) was added, left overnight and centrifuged at 20 000 *g* for 20 min. The supernatant thus obtained was designated as glutelins. The fractions so obtained were estimated (Lowry *et al.*, 1951) after 20% cold

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Table 1. Proximate and mineral composition[a]

	Proximate composition (g/100 g seed flour)			Mineral composition (mg/100 g seed flour)	
	Vigna capensis	*Vigna sinensis*		*Vigna capensis*	*Vigna sinensis*
Moisture	10·20 ± 0·04[b]	7·00 ± 0·04	Na	5·79 ± 0·02	26·1 ± 0·13
Crude protein (Kjeldahl N × 6·25)	22·4 ± 0·40	24·5 ± 0·63	K	3163 ± 2·11	2 583 ± 1·98
Crude lipid	4·23 ± 0·03	2·59 ± 0·03	Ca	428 ± 1	827 ± 1
Crude fibre	6·47 ± 0·05	4·69 ± 0·03	Mg	225 ± 1	217 ± 0·14
Ash	5·01 ± 0·04	4·52 ± 0·02	P	265 ± 0·24	443 ± 0·34
Nitrogen-free extractives (NFE)	61·88	63·70	Fe	14·9 ± 0·18	32·7 ± 0·11
			Cu	2·00 ± 0·03	2·58 ± 0·03
Calorific value (Kcal/100 g DM)	375·23	376·11	Zn	1·78 ± 0·04	1·51 ± 0·02
			Mn	1·34 ± 0·02	1·94 ± 0·04

[a] All values are means of triplicate determinations expressed on dry weight basis.
[b] ± Denotes the standard error.

TCA precipitation. The purified total seed proteins were acid-hydrolysed with 6 N HCl at 110°C for 24 h *in vacuo*. After flash evaporation the dried residue was dissolved in citrate buffer (pH 2·2). Aliquots were analysed in an LKB Biochrome automated amino acid analyser, model 4151–Alpha Plus. The different amino acids recovered are presented as g/100 g protein. The total lipids were extracted from the seed flour according to the method of Folch *et al.* (1957) using chloroform and methanol mixture in the ratio of 2 : 1 (v/v). Methyl esters were prepared from the total lipids by following the method of Metcalfe *et al.* (1966). Fatty acid analysis was performed by gas chromatography (Shimadzu, model-R1A), an instrument equipped with a flame ionization detector and a glass column (2 mm × 3 m) packed with 1% diethylene glycol succinate on Chromosorb W (silanised 80/100 mesh). The carrier gas was nitrogen, at a flow rate of 32 ml min^{-1}. The column temperature was 190°C. Peaks were identified by comparison with authentic standards, quantified by peak area integration and expressed as weight per cent of total methyl esters; the relative weight per cent of each fatty acid was determined from integrated peak areas. All the minerals except phosphorous were analysed by atomic absorption spectrophotometry (Issac & Johnson, 1975). Phosphorus content in the triple acid-digested extract was determined colorimetrically (Dickman & Bray, 1940). The antinutritional factors such as tannins (Burns, 1971), total free phenols (Bray & Thorne, 1954) and the non-protein amino acid, L-DOPA (3,4-dihydroxyphenylalanine) (Brain, 1976) were quantified. The haemagglutinating activities of albumin and globulin fractions of seed proteins (Liener, 1976) were also assayed.

RESULTS AND DISCUSSION

The crude protein contents of *V. capensis* and *V. sinensis* investigated in the present study appear to be closer to the levels of commonly consumed pulses such as green gram, black gram, pigeonpea and chickpea (IARI, 1971; Gupta & Wagle, 1978; Jambunathan & Singh, 1980, 1981; Sotelo *et al.*, 1987; Luz Fernandez & Berry, 1988). The crude lipid content of *V. capensis* is higher when compared with the other *Vigna* species such as *V. calcaratus, V. sublobata* and *V. glabrescens* (Rajaram & Janardhanan, 1992). Similarly, the crude lipid content of *V. sinensis* is more or less equal to that of *V. unguiculata* (Omueti & Singh, 1987). The crude fibre content of *V. capensis* is found to be higher when compared with the other commonly cultivated pulses such as chickpea, horse gram, peas, red gram and black gram (Premakumari *et al.*, 1984). The ash content of *V. capensis* and *V. sinensis* is more or less equal to that of *V. unguiculata* (Johnson & Raymond, 1964; Kachare *et al.*, 1988), *Phaseolus vulgaris* (Tezoto & Sgarbieri, 1990) and *Cicer arietinum* (Singh *et al.*, 1991). The contents of total carbohydrate and calorific values of the samples investigated in this study seem to be higher compared with the most common pulses consumed in India. The data on mineral analysis (Table 1) reveal that the seeds of the samples investigated appear to be a rich source of potassium, calcium, magnesium, phosphorus and iron when compared with recommended dietary allowance (RDA) values (NRC/NAS, 1980).

In the samples investigated, albumins and globulins constitute the major seed proteins (Table 2), whereas the values for the protein fractions of *V. capensis* appear to be similar to those of *V. sesquipedalis*

Table 2. Data on seed total (true) proteins and protein fractionation

Fraction	(g/100 g seed flour)[a]	
	Vigna capensis	*Vigna sinensis*
Total protein (true protein)	18·8 ± 0·04[b]	19·3 ± 0·1
Albumins	5·14 ± 0·02	5·20 ± 0·03
Globulins	11·2 ± 0·1	11·5 ± 0·1
Prolamins	0·81 ± 0·01	0·81 ± 0·02
Glutelins	1·67 ± 0·02	1·73 ± 0·04

[a] All values are means of triplicate determinations expressed on dry weight basis.
[b] ± Denotes the standard error.

Table 3. Amino acid profiles of acid-hydrolysed, purified total seed proteins

	Vigna capensis (g/100 g protein)	*Vigna sinensis* (g/100 g protein)	FAO/WHO (1973) requirement pattern (g/100 g protein)
Glutamic acid	15·7	15·0	
Aspartic acid	12·1	10·9	
Serine	3·91	3·58	
Threonine	2·57	4·02	4·0
Proline	6·13	3·36	
Alanine	5·69	4·30	
Glycine	3·18	3·01	
Valine	5·16	5·50	5·0
Cystine	Trace	Trace	3·5
Methionine	1·16	2·02	
Isoleucine	5·31	2·36	4·0
Leucine	7·93	8·03	7·0
Tyrosine	2·17	2·42	6·0
Phenylalanine	5·36	5·39	
Lysine	6·19	6·62	5·5
Histidine	5·13	2·30	
Tryptophan	ND[a]	ND	1·0
Arginine	6·01	7·06	

[a] ND, not detected.

(Rajaram & Janardhanan, 1990). The data on amino acid profiles (Table 3) indicate that *V. sinensis* seed proteins possess higher methionine values than the other sample investigated. The contents of tyrosine and phenylalanine of *V. capensis* are equal to that of *V. umbellata* var. Kl. the other amino acids (isoleucine, leucine, lysine and arginine) of *V. capensis* are higher when compared with the tribal pulse. *V. umbellata* var. Kl (Rajaram & Janardhanan, 1990). The essential amino acids valine, tyrosine and phenylalanine of *V. capensis* are higher when compared with the WHO requirement pattern (FAO/WHO, 1973). The levels of the essential amino acids threonine, valine, tyrosine, phenylalanine and lysine of *V. sinensis*, presently investigated, are more or less comparable with, or higher than, that of the WHO requirement pattern (Table 3).

The data on the fatty acid composition of the total lipids of the samples investigated (Table 4) indicate that palmitic, oleic, linoleic and linolenic acids are the major fatty acids, as in soybean, black gram and green gram (Salunkhe *et al.*, 1982). The linolenic acid content of *V. sinensis* is more or less equal to that of horse gram, whereas the palmitic and oleic acid contents of

Table 4. Fatty acid profiles of seed lipids[a]

Fatty acid (%)	*Vigna capensis*	*Vigna sinensis*
Palmitic acid (C16 : 0)	18·9	20·8
Stearic acid (C18 : 0)	6·33	5·32
Oleic acid (C18 : 1)	12·1	10·4
Linoleic acid (C18 : 2)	41·1	39·5
Linolenic acid (C18 : 3)	20·3	23·9
Others (unidentified)	1·21	0·09

[a] Average values of two determinations.

Table 5. Data on antinutritional factors

Component	*Vigna capensis*	*Vigna sinensis*
Total free phenols (g/100 g)	1·04[a] ± 0·03	0·37[a] ± 0·01
Tannins (g/100 g)	0·46[a] ± 0·02	0·34[a] ± 0·03
L-DOPA (g/100 g)	0·58[a] ± 0·02	0·45[a] ± 0·02

[a] Denotes mean of triplicate determinations expressed on dry weight basis.

V. sinensis seem to be equal to that of *Cajanus cajan* (Salunkhe *et al.*, 1982). Similarly the linoleic acid content of *V. capensis* is relatively high when compared with *V. unguiculata* and *Phaseolus vulgaris* (Omogbai, 1990).

Although legumes provide 20% of all plant protein in human diets and are even more important in the diets of livestock, their usefulness is decreased by anti-nutritional or toxic compounds associated with the large content of protein in their seeds (Nowacki, 1980). Some of the antinutritional factors, such as protease inhibitors, lectins, tannins, goitrogens, cyanogens, amylase inhibitors and antivitamin factors, constitute the heat-labile antinutritional factors (Liener, 1980), whereas toxic amino acids, alkaloids and cyanogenic glucosides are heat-stable antinutritional factors (Nowacki, 1980). The content of total free phenols in the samples investigated (Table 5) appears to be lower when compared with *V. sesquipedalis* (Rajaram & Janardhanan, 1990). The contents of tannins in *V. capensis* and *V. sinensis* (Table 5) are lower than those in domesticated legumes such as black gram, chickpea, cowpea and green gram (Khan *et al.*, 1979; Rao & Deosthale, 1982). Low levels of tannins are nutritionally significant because tannins are known to inhibit the activities of digestive enzymes (Jambunathan & Singh, 1980).

The contents of the non-protein amino acid L-DOPA in the tribal pulses investigated (Table 5) seem to be relatively low when compared with the earlier values reported in different species of the genus, *Mucuna* (Janardhanan & Lakshmanan, 1985; Rajaram & Janardhanan, 1991; Mary Josephine & Janardhanan, 1992; Arulmozhi & Janardhanan, 1992). Both albumins and globulins of the samples investigated agglutinate erythrocytes of the ABO system (Table 6) without any specificity as in the case of *Dolichos lablab* (Kaushik,

Table 6. Phytohaemagglutinating activity

Protein fraction	Human blood group of erythrocytes	Haemagglutinating activity[a]	
Albumins	A	+	+
Albumins	B	+	+
Albumins	O	+	+
Globulins	A	++	++
Globulins	B	++	++
Globulins	O	++	++

[a] Results of two independent experiments: +, some clumping, pellet disperses partially; ++, no dispersion of pellet; —, no clumping, pellet disperses easily.

1984), *Psophocarpus tetragonolobus* (Kotaru *et al.*, 1987) and *P. scandens* (Kortt, 1988).

On the basis of the above findings, it is concluded that the tribal pulses investigated seem to be a good source of proteins, essential amino acids, essential fatty acids and minerals. The adverse effect of most of the antinutritional factors detected in the present study can be eliminated by moist-heat treatment or a cooking process, since they are heat-labile.

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