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NUTRITIONAL COMPOSITION AND ANTINUTRITIONAL FACTORS OF LITTLE-KNOWN SPECIES OF *VIGNA*

[COMPOSICIÓN NUTRICIONAL Y FACTORES ANTINUTRICIONAL DE ESPECIES POCO CONOCIDAS DE *VIGNA*]

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SUMMARY

The seeds of *Vigna trilobata, V. radiata* var. *sublobata, V. umbellata, V. unguiculata* subsp. *cylindrica, V. aconitifolia, V. vexillata* and *V. bourneae* were collected from different geographical regions in Western Ghats, Tamil Nadu. They were analyzed for their proximate and mineral composition, vitamins (niacin and ascorbic acids), fatty acid profiles, amino acid profiles of total seed proteins, *in vitro* protein digestibility (IVPD) and certain antinutritional factors. The crude protein ranged from 18.24 to 26.12%, crude lipid 3.18 to 6.48%, total dietary fibre 3.42 to 7.48%, ash 3.10 to 4.12% and carbohydrates 59.44 to 72.06%. Energy values of the seeds were 1584.87 – 1644.34 kJ100g^{-1}DM, which were comparable to those of other legumes. The fatty acid profiles of all the species of *Vigna* revealed that the seed lipids contained higher concentrations of palmitic, oleic, linoleic and linolenic acids. The essential amino acid profiles of total seed proteins compared favourably with FAO/WHO (1991) requirements, except that there were deficiencies of sulphur containing amino acids in all the species of *Vigna*. The IVPD of the different *Vigna* species ranged from 70.38 to 79.12%. Antinutritional substances like total free phenolics, tannins, L-DOPA (3, 4-dihydroxyphenylalanine), phytic acid, hydrogen cynide, trypsin inhibitor activity, oligosaccharides and phytohaemagglutinating activity were also investigated. The antinutritional factors that were detected were thought to have little nutritional significance if the beans are properly processed.

Key words: *Vigna* spp,; amino acid profiles; fatty acid profiles; IVPD; antinutrient.

RESUMEN

Las semillas de *Vigna trilobata, V. radiata* var. *sublobata, V. umbellata, V. unguiculata* subsp. *cylindrica, V. aconitifolia, V. vexillata* and *V. bourneae* se colectaron de diferentes regiones geográficas en el oeste de Ghats Tamil Nadu. Se analizó su composición proximal y mineral, vitaminas (niacina y ácido ascórbico), perfiles de ácidos grasos, perfiles de aminoácidos de la proteína total de la semillas, digestibilidad de la proteína *in vitro* (IVPD) y algunos factores antinutricionales. La proteína cruda tuvo un rango entre 18.24 a 26.12%, lípidos totales de 3.8 a 6.48%, fibra dietética total de 3.42 a 7.48%, cenizas de 3.10 a 4.12% y carbohidratos de 59.44 a 72.06%. Los valores de energía de la semilla estuvieron entre 1584.87 a 1644.34 kJ100g^{-1}MS, los cuales fueron comparables con otras leguminosas. Los perfiles de ácidos grasos de todas las especies de *Vigna* revelaron altas concentraciones de ácido oleico, linoleico y linolenico. Los perfiles de aminoácidos esenciales del total de proteína de la semilla se compararon favorablemente con los requerimientos de la FAO/WHO (1991), con excepción de ciertas deficiencias de aminoácidos azufrados en todas las especies de *Vigna* El IVPD de las diferentes especies de *Vigna* tuvo un rango de 70.38 a 79.12%. Sustancias antinutricionales como el total de fenoles libres, taninos, L-DOPA (3-4 dihidroxifenilalanina), ácido fitico, cinacina hidrogenada, actividad inhibidora de la tripsina, oligosacáridos y actividad fitohematoaglutinadora también se determinaron. Los factores antinutricionales que fueron detectados, se presume que presentan una pequeña significancia si los frijoles son procesados correctamente.

Palabras clave: *Vigna* spp.; Perfil de aminoácidos; perfil de ácidos grasos; IVPD; antinutricional.

INTRODUCTION

Inadequate availability and consumption of protein foods in developing countries like India are a major concern as large segments of population of these countries suffer from protein malnutrition. The little known legume crops could make a useful contribution to world's food population because some

of them are well adapted to adverse environmental conditions, exhibit resistance to pests and pathogens and have good nutritional qualities. Hence the identification and introduction of such, hitherto, little known grain legumes, including wild legumes consumed by different tribal sects throughout the tropical regions as well as their genetic improvement of the quality and quantity of protein would be a great contribution (Maikhuri *et al*., 1991; Arinathan *et al*., 2009; Kala *et al*., 2010). Information on the chemical composition of these potential protein sources and their possible utilization as human food is inadequate. However, some of these species are actually consumed by several human groups (like Mayan people) in the American continent since centuries ago. Hence in the present study the mature seeds of different *Vigna* species, which were eaten by the tribal people of the Western Ghats, Tamil Nadu, were subjected to biochemical investigation with a view to assessing their nutritional potential.

MATERIALS AND METHODS

Collection of seeds

Seven species of *Vigna* were gathered as mature pods in individual species from natural strands of various geographical regions, Western Ghats, Tamil Nadu, viz, *Vigna trilobata* (L.) Verdc; *V. aconitifolia* (Jacq.) Marechal and *V. vexillata* (L.) A.Rich from Siruvani reserve forest, Coimbatore district; *Vigna radiata* (L.) Wilczek var. *sublobata* (Roxb.) Verdc. and *V. bourneae* Gamble from Seithur reserve forest and *Vigna unguiculata* (L.) Walp. subsp. *cylindrica* (L.) Eselt. from Ayyanarkoil reserve forest, Virudhunagar district and *Vigna umbellata* (Thumb.) Ohwi & Ohashi from Petchiparai reserve forest, Kanniyakumari district. The collected plants were identified taxonomically with the help of various floras. After thoroughly drying in the sun, the pods were thrashed to remove seeds. The seeds, after thorough cleaning and removal of broken seeds and foreign materials, the mature seeds were stored in airtight plastic jars at room temperature (25°C).

Proximate composition

The moisture content (%) was determined by drying 50 transversely cut seeds in an oven at 80°C for 24h. The air-dried samples were powdered separately in a Wiley mill (Scientific Equipment, Delhi, India) to 60-mesh size and stored in screw-capped bottles at room temperature for further analysis.

The nitrogen content was estimated by the micro-Kjeldahl method (Humphries, 1956) and the crude protein content was calculated (N x 6.25). Crude lipid content was determined using Soxhlet apparatus. The ash content was determined by heating 2g of the dried sample in a silica dish at 600°C for 6h (AOAC, 2005). Total dietary fibre (TDF) was estimated by the non-enzymatic-gravimetric method (Li and Cardozo, 1994). To determine the TDF, duplicate 500 mg ground samples were taken in separate 250mL beakers. To each beaker 25 mL water was added and gently stirred until the samples were thoroughly wetted, (i.e. no clumps were noticed). The beakers were covered with Al foil and allowed to stand 90 min without stirring in an incubator maintained at 37°C. After that, 100 mL 95% ethanol were added to each beaker and allowed to stand for 1h at room temperature (25±2°C). The residue was collected under vacuum in a pre-weighed crucible containing filter aid. The residue was washed successively with 20 mL of 78% ethanol, 10 mL of 95% ethanol and 10 ml acetone. The crucible containing the residue was dried $\geq$2 h at 105°C and then cooled $\geq$ 2h in a desiccator and weighed. One crucible-containing residue was used for ash determination at 525°C for 5h. The ash-containing crucible was cooled for $\geq$ 2h in a desiccator and weighed. The residue from the remaining duplicate crucible was used for crude protein determination by the micro-Kjeldahl method as already mentioned. The TDF was calculated as follows.

$$TDF\% = 100 \times \frac{Wr - [(P+A)/100]\,Wr}{Ws}$$

Where *Wr* = mg residue, *P* = % protein in the residue; *A* = % ash in the residue, and *Ws* = mg sample.

The nitrogen free extract (NFE) was obtained by difference (Muller and Tobin, 1980). The energy value of the seed (kJ) was estimated by multiplying the percentages of crude protein, crude lipid and NFE by the factors 16.7, 37.7 and 16.7, respectively (Siddhuraju *et al.*, 1996).

Analysis of minerals and vitamins

Five hundred milligrams of the ground legume seed was digested with a mixture of 10 mL concentrated nitric acid, 4mL of 60% perchloric acid and 1 mL concentrated sulphuric acid. After cooling, the digest was diluted with 50 mL of deionised H_2O, filtered through Whatman No. 42 filter paper and filtrates were made up to 100 mL in a glass volumetric flask with deionised H_2O. All minerals except phosphorus were analyzed from a triple acid-digested sample by atomic absorption spectrophotometry, ECIL (Electronic Corporation of India Ltd., India) (Issac and Johnson, 1975). The phosphorus content in the triple acid digested extract was determined colorimetrically (Dickman and Bray, 1940).

Ascorbic acid and niacin were extracted and estimated as per the method given by Sadasivam and Manickam (1996). For the extraction of ascorbic acid, 3g air-dried powdered sample were ground with 25 mL of 4% oxalic acid and filtered. Bromine water was added drop by drop to 10 mL of the filtrate until it turned orange-yellow to remove the enolic hydrogen atoms. The excess of bromine was expelled by blowing in air. This filtrate was made up to 25 mL with 4% oxalic acid and used for ascorbic acid estimation. Two milliliters of the extract was made up to 3 mL with distilled H_2O in a test tube. One milliliter of 2% 2, 4-dinitrophenyl hydrazine reagent and a few drops of thiourea were added. The contents of the test tube were mixed thoroughly. After 3hr incubation at 37°C, 7 mL of 80% H_2SO_4 were added to dissolve the osazone crystals and the absorbance was measured at 540nm against a reagent blank.

For the extraction of niacin, 5g air-dried powdered sample was steamed with 30 mL concentrated H_2SO_4 for 30min. After cooling, this suspension was made up to 50 mL with distilled H_2O and filtered. Five milliliters of 60% basic lead acetate was added to 25 mL of the filtrate. The pH was adjusted to 9.5 and centrifuged (10,000g for 10 min at room temperature) to collect the supernatant. Two milliliters of concentrated H_2SO_4 was added to supernatant. The mixture was allowed to stand for 1hr and centrifuged (10,000g for 10 min at room temperature). The 5 mL of 40% $ZnSO_4$ was added to the supernatant. The pH was adjusted to 8.4 and centrifuged (10,000g for 10 min at room temperature) again. Then the pH of the collected supernatant was adjusted to pH 7 and used as the niacin extract. For estimation, 1 mL extract was made up to 6 mL with distilled water in a test tube, 3 mL cyanogen bromides was added and shaken well, followed by addition of 1 mL of 4% aniline. The yellow colour that developed after 5min was measured at 420nm against a reagent blank. The ascorbic acid and niacin contents present in the sample were calculated by referring to a standard graph and expressed as milligrams per 100 grams of powdered samples.

Lipid extraction and fatty acid analysis

The total lipids was extracted from the seeds according to the method by Folch *et al.*, (1957) using chloroform and methanol mixture in ratio of 2: 1 (v/v). Methyl esters were prepared from the total lipids by the method of Metcalfe *et al*., (1966). Fatty acid analysis was performed by gas chromatography (ASHMACO, Japan; Model No: ABD20A) using an instrument equipped with a flame ionization detector and a glass column (2m X 3mm) packed with 1% diethylene glycol succinate on chromosorb W. The temperature conditions for GC were injector 200°C and detector 210°C. The temperature of the oven was programmed from 180°C and the carrier gas was nitrogen at a flow rate of 30 mL/min. Peaks were identified by comparison with authentic standards, quantified by peak area integration and expressed as weight percentage of total methyl esters; the relative weight percentage of each fatty acid was determined from integrated peak areas.

Amino acid analysis

The total seed protein was extracted by a modified method of Basha *et al.,* (1976). The extracted proteins were purified by precipitation with cold 20% trichloroacetic acid (TCA). A protein sample of 30mg was hydrolysed by 6N HCL (5mL) in an evacuated sealed tube, which was kept in an air oven maintained at 110°C for 24 hr. The sealed tube was broken and the acid removed completely by repeated flash evaporation after the addition of deionized H_2O. Dilution was effected by means of citrate buffer pH 2.2 to such an extent that the solution contained 0.5mg protein mL^{-1}. The solution was passed through a millipore filter (0.45µM) and derivatized with O-phthaldialdehyde by using an automated pre-column (OPA). Amino acids were analysed by a reverse – phase HPLC (Method L 7400, HITACHI, Japan) fitted with a denali C_{18} 5 micron column (4.6X 150mm). The flow rate was 1 mL min^{-1} with fluorescence detector. The cystine content of protein sample was obtained separately by the Liddell and Saville (1959) method. For the determination of tryptophan content of proteins, aliquots containing known amounts of proteins were dispersed into glass ampoules together with 1 mL 5M NaOH. The ampoules were flame sealed and incubated at 110°C for 18 hr. The tryptophans content of the alkaline hydrolysates were determined colorimetrically using the method of Spies and Chambers (1949) as modified by Rama Rao *et al.,* (1974). The contents of the different amino acids were expressed as $g100g^{-1}$ proteins and were compared with FAO/WHO (1991) reference pattern. The essential amino acid score (EAAS) was calculated as follows:

$$EAAS = \frac{\text{EAAS (g/100g of total protein)}}{\text{EAAS (g/100g reference pattern)}} \times 100$$

Analysis of antinutritional compounds

The antinutritional compounds, total free phenolics (Bray and Thorne, 1954), tannins (Burns, 1971), the non-protein amino acid, L-DOPA (3, 4-dihydroxyphenylalanine) (Brain, 1976), phytic acid (Wheeler and Ferrel, 1971), hydrogen cyanide (Jackson, 1967) were quantified. Trypsin inhibitor activity was determined by the enzyme assay of Kakade *et al.,* (1974) by using benzoil-DL-arginin-*p*-nitroanilide (BAPNA) as a substrate. One trypsin

inhibitor unit (TIU) has been expressed as an increase of 0.01 absorbance units per 10ml of reaction mixture at 410nm. Trypsin inhibitor activity has been defined in terms of trypsin units inhibited per mg protein.

Extraction, TLC separation and estimation of Oligosaccharides

Extraction of oligosaccharides was done following the method of Somiari and Balogh (1993). Five grams each raw seed flours of all the samples were extracted separately with 50 mL of 70% (v/v) aqueous ethanol and kept on an orbital shaker at 130 rpm for 13 hr and then filtered through Whatman No. 1 filter paper. Residue was further washed with 25mL of 70% (v/v) ethanol. The filtrates obtained were pooled and vacuum-dried at 45°C. The concentrated sugar syrup was dissolved in five ml of distilled water.

Separation of oligosaccharides was done by TLC. Thirty grams of cellulose-G powder were dissolved in 45 mL of double distilled water and shaken well until the slurry was homogeneous. TLC plates were coated with the slurry and air-dried. Spotting of the sugar samples was done by using micropipettes. 5 µl aliquots of each sample were spotted thrice separately. The plates were developed by using a solvent system of n-propanol, ethyl acetate and distilled H_2O (6:1:3), and dried (Tanaka *et al.*, 1975). The plates were sprayed with α –naphthol (1%, w/v). Plates were dried in a hot-air oven. The separated spots were compared with standard sugar spots. A standard sugar mixture containing raffinose, stachyose and verbacose (Sigma Chemical). Separated sugars that appeared were verbascose, stachyose and raffinose. The sugar spots were scrapped eluted in 2 mL of distilled H_2O kept overnight at room temperature and filtered through Whatman No. 1 filter paper. The filtrates were subjected to quantitative estimation.

The eluted individual oligosaccharides were estimated by the method of Tanaka *et al.*, (1975). One mL of the eluted and filtered sugar solution was treated with one ml of 0.2 M thiobarbituric acid and one ml of concentrated HCL. The tubes were boiled in a water bath for exactly 6 min. After cooling, the oligosaccharide contents were quantified in a Elico UV-Spectrophotometer model SL 150 at 432 nm. Average values of triplicate estimations were calculated and the content of oligosaccharides was expressed on dry weight basis.

Quantitative determination of phytoheamagglutinating (Lectin) activity.

Lectin activity was determined by the method of Almedia *et al.* (1991). One g of air-dried seed flour was stirred with 10 mL of 0.15N sodium chloride solution for 2hr and the pH was adjusted to 4.0. The contents were centrifuged at 10,000 X g for 20 min. and the supernatants were collected separately. The protein content was estimated by the Lowry *et al.* (1951) method.

Human blood (blood groups A, B and O) was procured from the blood bank of Jothi Clinical Laboratory, Tuticorin. Blood erythrocyte suspensions were prepared by washing the blood samples separately with phosphate-buffered saline and centrifuged for 3min at low speed (3,000 g for 10 min at room temperature). Supernatants were removed with Pasteur pipettes. The washing procedure was repeated three times. The washed cells were diluted by one drop of cells with 24drops of phosphate – buffered saline.

The determination of lectin was done by the method of Tan *et al.,* (1983). Clear supernatant (50µl) was poured into the depression (pit) on a microtitration plate and serially diluted 1:2 with normal saline. The human blood erythrocyte (A, B and O blood groups) suspensions (25µl) were added to each of the twenty depressions. The plates were incubated for 3hours at room temperature. After the incubation period, the titer values were recorded. One Haemagglutinating unit (HU) is defined as the least amount of heamagglutinin that will produce positive evidence of agglutination of 25µl of a blood group erythrocyte after 3hr incubation at room temperature. The phytoheamagglutinating activity was expressed as heamagglutinating units (HU)/mg protein.

Determination of *in vitro* protein digestibility (IVPD).

This was determined using the multi-enzyme technique (Hsu *et al.*, 1977). The enzymes used for IVPD were purchased from Sigma Chemical Co., St. Louis, MO, USA. Calculated amounts of the control (casein) and sample were weighed out, hydrated in 10ml of distilled water and refrigerated at 5°C for 1hr. The samples containing protein and enzymes were all adjusted to pH 8.0 at 37°C. The IVPD was determined by the sequential digestion of the samples containing protein with a multi-enzyme mixture [trypsin (porcine pancreatic trypsin–type IX with 14190 BAEE unites per mg protein), α-chymotrypsin (bovine pancreatic chymotrypsin–type II, 60 units per mg powder) and peptidase (porcine intestinal peptidase–grade III, 40 units per g powder)] at 37°C followed by protease (type IV from *Streptomyces griseus*) at 55°C. The pH drop of the samples from pH 8.0 was recorded after 20min of incubation. The IVPD was calculated according to the regression

equation $Y = 234.84 – 22.56 X$, where Y is the % digestibility and X the pH drop.

Statistical analysis

The data were subjected to a one-way analysis of variance (ANOVA) and the significance if difference between means at 5% was determined by Duncan Multiple Range Test (DMRT) using SPSS software (version 11.5; SPSS Inc., Chicago IL, USA). Results were expressed as means values ± standard deviations of three separate determinations.

RESULTS AND DISCUSSION

The crude protein content of *Vigna* species (Table 1) investigated in the present study, *Vigna radiata* var. *sublobata*, *V. aconitifolia* and *V. vexillata* are found to be higher than pulse crops commonly consumed in India such as *Vigna subterranean* and *Glycine max* (Oluwole and Taiwo, 2009; Martin *et al*., 2010). The crude lipid content of *Vigna radiata var. sublobata*, *V. aconitifolia*, *V. umbellata*, *V. unguiculata* subsp. *cylindrica*, *V. vexillata* and *V. bourneae* is found to be more or less equal to the values of other species like *Vigna radiata* (Noel and Rosario, 1989); *V. trilobata* (Siddhuraju *et al*., 1992); *V. sesquipedalis*, *V. calacaratus*, *V. glabrescens* and *V. sublobata* (Rajaram and Janardhanan, 1990, 1992); *V. capensi*s and *V. sinensis* (Mohan and Janadhanan, 1993) and *V. umbellata* (Mohan and Janardhanan, 1994). The total dietary fibre content of *V. trilobata*, *V. radiata* subsp. *sublobata* and *V. aconitifolia* is found to be higher when compared with the other commonly cultivated pulses such as *Cicer arietinum*, *Macrotyloma uniflorum*, *Pisum sativum*, *Cajanus cajan* and *Vigna mungo* (Premakumari *et al*., 1984). The ash content of the investigated tribal pulses would be important to the extent that it contains the nutritionally important mineral elements. It appears that all the currently investigated tribal pulses have a high range of carbohydrates (59.44 – 72.06%) because of their low fat content when compared with *Arachis hypogaea* and *Glycine max*, which have less carbohydrate content at 26.1% and 20.9% respectively (Narasinga Rao *et al*., 1989). All the investigated *Vigna* species have higher energy value than those of *Phaseolus vulgaris*, *P. limensis*, *Vigna unguiculata*, *Cicer arietinum*, *Pisum sativum* and *Lens culinaris* (Meiners *et al*., 1976a).

Food legumes have been recognized as important sources of several minerals in Indian diets (Gopalan *et al*., 1978). Table 2 shows the mineral composition of the samples. In the present investigation, all the *Vigna* species exhibit higher levels of potassium content than that of other legumes *Phaseolus vulgaris*, *P. lunatus*, *Vigna unguiculata*, *Cicer arietinum*, *Pisum sativum* and *Lens culinaris* (Meiners *et al*., 1976b); *Cicer arietinum* (Alajaji and El-Adawy, 2006; Zia Ul-haq *et al*., 2007) and also register a higher level of potassium when compared with recommended dietary allowance values NRC/NAS (1980). The high content of potassium can be utilized beneficially in the diets of people who take diuretics to control hypertension and suffer from excessive excretion of potassium through the body fluid (Siddhuraju *et al*., 2001). The calcium content of *Vigna trilobata* and *Vigna bourneae* is found to be higher than that of the recommended dietary allowances of calcium (>400mg) for children by the Indian Council of Medical Research (ICMR, 1992), All the investigated *Vigna* species contain higher levels of calcium, magnesium and iron when compared with *Cicer arietinum* ((Meiners *et al*., 1976b) and *Cajanus cajan* (Nwokolo 1987). The phosphorus content is found to be higher than the *Vigna umbellata* var. RBL-40 (Rajaram and Janardhanan, 1990). The ratios of sodium to potassium (Na/K) and calcium to phosphorus (Ca/P) are shown in Tables 2. The Na/K ratio in the body is of great concern for prevention of high blood pressure, Na/K ratio less than one is recommended. Hence, most of the investigated samples would probably reduce high blood pressure disease because they had Na/K less than one. Modern diets which are rich in animal proteins and phosphorus may promote the loss of calcium in the urine (Shills and Young, 1988). This has led to the concept of the Ca/P ratio. If the Ca/P ratio is low (low calcium, high phosphorus intake) more than the normal amount of calcium may be lost in the urine, decreasing the calcium level in bones. Food is considered "good" if the ratio is above one and "poor" if the ratio is less than 0.5 (Nieman *et al*., 1992). The Ca/P ratio in the present study ranged between 1.23 to 2.35 indicating they would serve as good sources of minerals for bone formation.

The presently investigated *Vigna* species exhibits the highest level of niacin content (Table 3). Which is found to be higher than that of an earlier report in *Cajanus cajan*, *Dolichos lablab*, *D. biflorus*, *Mucuna pruriens*, *Phaseolus mungo*, *Vigna catjang* and *Vigna* species (Rajyalakshmi and Geervani, 1994) and *Cicer arietinum* (Alajaji and El-Adawy, 2006). The presently investigated tribal pulses also register higher level of ascorbic acid content than *Vigna radiata* and *V. mungo* (Kakati *et al*., 2010).

The data on fatty acid composition of the total lipids of different species of *Vigna* (Table 4) indicate that palmitic acid, oleic acid, linoleic acid and linolenic acid are the major fatty acids, as in *Glycine max*, *Vigna mungo* and *V. radiata* (Salunkhe *et al*., 1982).

Table 1: Proximate composition of seven species of *Vigna*. *$

Components	*Vigna trilobata*	*V. radiata* var. *sublobata*	*V. aconitifolia*	*V. umbellata*	*V. unguiculata* subsp. *cylindrica*	*V. vexillata*	*V. bourneae*
Moisture	5.24±0.05^{d}	4.80±0.08^{c}	3.84±0.06^{a}	5.56±0.02^{e}	5.44±0.05^{e}	6.94±0.01^{f}	4.24±0.05^{b}
Crude protein (Kjeldahl N × 6.25)	22.10±0.46^{b}	25.64±0.17^{d}	24.40±0.10^{c}	26.12±0.56^{d}	18.24±0.57^{a}	25.84±0.56^{d}	21.40±0.01^{b}
Crude lipid	6.48±0.59^{d}	3.58±0.56^{a}	5.26±0.53^{b}	4.18±0.50^{a}	3.18±0.01^{a}	5.80±0.17^{d}	4.20±0.02^{a}
TDF (Total Dietary Fibre)	7.48±0.34^{d}	6.12±0.58^{c}	6.78±0.71^{d}	4.74±0.01^{b}	3.42±0.55^{a}	4.89±0.05^{b}	5.25±0.05^{b}
Ash	3.12±0.56^{a}	3.48±0.56^{a}	4.12±0.58^{a}	4.04±0.04^{a}	3.10±0.58^{a}	3.94±0.56^{a}	3.38±0.05^{a}
Nitrogen Free Extractives (NFE)	60.82	61.18	59.44	60.92	72.06	59.53	65.77
Calorific value (kJ100g^{-1} DM)	1629.06	1584.87	1598.43	1611.15	1627.90	1644.34	1614.08

*All values are means of triplicate determinations expressed on a dry weight basis ± denotes standard error.

$ Mean values in the following row sharing a common letter are not statistically significant according to DMRT.

Table 2. Mineral composition of seven species of *Vigna* (mg 100 g^{-1} seed flour) *$

Components	*Vigna trilobata*	*V. radiata* var. *sublobata*	*V. aconitifolia*	*V. umbellata*	*V. unguiculata* subsp. *cylindrica*	*V. vexillata*	*V. bourneae*
Sodium	34.12±0.01^{a}	32.10±0.03^{a}	24.78±0.58^{c}	22.18±0.02^{d}	18.78±0.06^{e}	26.48±0.57^{b}	27.34±0.58^{b}
Potassium	1696.10±5.2^{e}	1948.40±0.01^{c}	2452.78±0.60^{a}	2618.16±2.39^{a}	1848.10±0.57^{d}	2012.62±1.08^{b}	2142.30±0.57^{b}
Calcium	418.46±0.01^{a}	284.20±1.72^{b}	264.12±1.53^{c}	274.14±0.38^{b}	258.54±0.56^{c}	201.18±0.01^{d}	438.14±0.01^{a}
Magnesium	242.00±0.57^{a}	158.24±0.56^{c}	144.10±0.58^{d}	156.10±0.58^{d}	161.20±0.33^{c}	178.10±0.01^{a}	168.20±1.20^{b}
Phosphorus	178.18±0.02^{c}	212.12±0.57^{b}	141.28±0.02^{d}	126.20±0.58^{e}	210.10±0.56^{b}	248.08±0.58^{a}	231.16±0.31^{a}
Iron	9.48±0.11^{c}	8.40±0.01^{d}	6.48±0.01^{f}	6.12±0.57^{f}	11.40±0.01^{a}	10.40±0.58^{b}	7.54±0.01^{e}
Zinc	2.56±0.01^{a}	1.74±0.01^{b}	1.21±0.01^{d}	0.78±0.01^{e}	2.40±0.01^{a}	1.58±0.57^{c}	2.10±0.01^{a}
Copper	1.22±0.05^{a}	0.84±0.01^{c}	0.48±0.02^{f}	0.54±0.01^{f}	0.58±0.01^{e}	1.10±0.01^{b}	0.72±0.01^{d}
Manganese	2.34±0.03^{b}	1.24±0.01^{d}	0.94±0.01^{f}	1.10±0.01^{e}	1.68±0.02^{c}	2.50±0.01^{b}	2.66±0.01^{a}
Na/K	0.02	0.02	0.01	0.01	0.01	0.01	0.01
Ca/P	2.35	1.34	1.87	2.17	1.23	0.81	1.90

*All values are of means of triplicate determination expressed on dry weight basis ± denotes Standard error.

$ Mean values in the following row sharing a common letter are not statistically significant according to DMRT.

The linolenic acid content of *Vigna aconitifolia, V. umbellata, V. vexillata* and *V. bourneae* is more than that of *Vigna unguiculata* (Thangadurai, 2005), where as the palmitic acid and oleic acid contents of *Vigna aconitifolia, V. unguiculata* subsp. *cylindrica, V. vexillata* and *V. bourneae* seems to be equal to that of *Cajanus cajan* (Salunkhe *et al*., 1982). Similarly the linoleic acid content of all the investigated *Vigna* species is relatively high when compared with *Vigna unguiculata* and *Phaseolus vulgaris* (Omogbai, 1990).

Table 3. Vitamins (niacin and ascorbic acid) content of seven species *Vigna* (mg $100g^{-1}$ seed flour) *$

Species	Niacin	Ascorbic acid
Vigna trilobata	26.20 ± 0.02^{e}	52.04 ± 0.58^{f}
V. radiata var. *sublobata*	32.10 ± 0.56^{b}	62.14 ± 0.58^{d}
V. aconitifolia	38.48 ± 0.58^{a}	79.40 ± 0.56^{b}
V. umbellata	22.30 ± 0.57^{f}	58.10 ± 0.57^{e}
V. unguiculata subsp. *cylindrica*	18.36 ± 0.01^{g}	98.14 ± 0.57^{a}
V. vexillata	30.40 ± 0.57^{c}	66.42 ± 0.57^{c}
V. bourneae	28.34 ± 0.59^{d}	48.36 ± 0.63^{g}

*All values are of means of triplicate determination expressed on dry weight basis ± denotes standard error.

$ Mean values in the following row sharing a common letter are not statistically significant according to DMRT.

The essential amino acid profiles of total seed proteins are compared favourably with the FAO/WHO (1991) requirement pattern, except that there were deficiencies of sulphur containing amino acids in all the *Vigna* species and also in threonine content of *Vigna trilobata*, leucine content of *V. aconitifolia*; lysine content of *V. trilobata*, and *V. umbellata* (Table 5). Among the seven species of *Vigna*, the *Vigna radiata* var. *sublobata*; *V. unguiculata* subsp. *cylindrica* and *V. vexillata* register higher amounts of IVPD than the other four species of *Vigna* presently investigated (Table 6) and their protein digestibility is found to be higher than that of *Cajanus cajan* (Singh and Eggum, 1984) and *Cicer arietinum* (Attia *et al*., 1994).

The problem of plant protein digestibility has been suggested to be because of the interplay of several factors such as protease inhibitors, phytates, oxalates, lectins, goitrogens and other antinutritional factors. In societies where legumes are consumed rather than much more expensive animal foods, there is bound to be great concern over the level of antinutrients in the diets. For this reason, in the present investigation an attempt has been made to detect the presence of certain antinutritional factors in different species of *Vigna* (Table 6). The content of total free phenolics in the samples investigated appears to be lower when compared with *Vigna sesquipedalis* (Rajaram and Janardhanan, 1990) and *Phaseolus lunatus* (Egbe and Akinyele, 1990) and comparable to that of *Vigna umbellata* and *V. sinensis* (Rajaram and Janardhanan, 1990). The tannin content of different species of *Vigna* is relatively lower than the domesticated legumes like *Vigna mungo*, *Cicer arietinum*, *Vigna unguiculata* and *V. radiata* (Khan *et al*., 1979; Rao and Deosthale, 1982). The content of the non-protein amino acid, L-DOPA, is relatively lower when compared with other tribal pulse reported earlier *M. pruriens* var. *utilis* (Mohan and Janardhanan, 1995; Vadivel and Janardhanan, 2000; Vadivel and Pugalenthi, 2008). It has been demonstrated that in *Mucuna pruriens*, the level of L-DOPA is significantly eliminated by dry heat treatment (Siddhuraju *et al*., 1996), cooking and autoclaving (Vijayakumari *et al*., 1996). Phytic acid content of different species of *Vigna* is found to be low when compared with that of some commonly consumed grain legumes like *Vigna mungo* (Kataria *et al*., 1988), *V. radiata*, (Kataria *et al*., 1989) tribal pulses, *Dolichos lablab* var. *vulgaris* (Vijayakumari *et al*., 1995) and *Mucuna pruriens* var. *utilis* (Janardhanan *et al*., 2003). Hydrogen cyanide is known to cause acute or chronic toxicity. The content of HCN level in the presently investigated tribal pulses is far below the lethal level i.e. 36mg/100g (Oke, 1969) and comparable with those of *Vigna sinensis* and *Pisum sativum* (Montgomery, 1980); *Dolichos lablab* var. *vulgaris*, *Bauhinia purpurea* (Vijayakumari *et al*., 1995, 1997); *Entada phaseoloides* (Siddhuraju *et al*., 2001). Though the trypsin inhibitor activity has been studied in a number of pulses, the results obtained in the present investigation cannot be compared because the expression of trypsin inhibitor activity, nature and concentration of the substrate, etc. are different. However, based on investigations that have been reported and where the same experimental conditions were used, the trypsin inhibitor activities in cultivated legumes like *Cajanus cajan* (67.1 – 71.3 $TIUmg^{-1}$ protein) (Singh and Eggum, 1984) is higher than that of different *Vigna* species. Trypsin inhibitor activity has the greatest impact on the IVPD of the legumes when the former is known to be heat labile. Stachyose seems to be the principle oligosaccharide of all the *Vigna* species. It is in conformity with the earlier reports in cow pea (Onigbinde and Akinyele, 1983); *Canavalia ensiformis*, *Phaseolus lunatus* and *Canavalia gladiata* (Ravilleza *et al*., 1990) and *Dolichos lablab* var. *vulgaris* (Vijayakumari *et al*., 1995).

Table 4: Fatty acid profiles of seed lipids of seven species of *Vigna* [a].

Fatty acid (%)	*Vigna trilobata*	*V. radiata* var. *sublobata*	*V. aconitifolia*	*V. umbellata*	*V. unguiculata* subsp. *cylindrica*	*V. vexillata*	*V. bourneae*
Palmitic acid [C16:0]	24.78	16.10	19.28	26.48	20.70	20.40	21.28
Stearic acid [C18:0]	10.50	5.42	9.40	7.10	12.44	7.32	9.68
Oleic acid [C18:1]	18.48	21.40	18.76	11.42	19.24	10.48	13.14
Linoleic acid [C18:2]	28.40	20.12	28.32	29.10	25.10	34.60	29.02
Linolenic acid [C18:3]	16.20	32.00	23.10	24.78	21.04	24.10	25.14
Others [unidentified]	1.64	4.96	1.14	1.12	1.48	3.10	1.74

[a] All values are of two determination.

Table 5. Amino acid profiles of acid-hydrolysed, purified total seed proteins of seven species of *Vigna* ($g100g^{-1}$) [a].

Amino acid	*Vigna trilobata*	EAAS	*V. radiata* var. *sublobata*	EAAS	*V. aconitifolia*	EAAS	*V. umbellata*	EAAS	FAO/WHO (1991) requirement pattern
Glutamic acid	12.46		14.78		12.78		13.20		
Aspartic acid	9.78		13.46		10.37		12.46		
Serine	4.13		4.76		3.01		4.21		
Threonine	3.10	91.17	3.68	108.23	3.60	105.88	4.08	120.00	3.4
Proline	3.46		3.50		4.31		5.42		
Alanine	5.60		4.24		4.72		3.86		
Glycine	4.10		4.10		3.46		3.26		
Valine	5.09	145.42	5.25	150.00	4.94	141.14	5.12	146.28	3.5
Cystine	0.53	}55.6	0.43	}65.60	0.23	}68.40	0.12	}60.00	}2.5
Methionine	0.86		1.21		1.48		1.38		
Isoleucine	3.13	111.76	5.78	206.42	4.78	170.71	4.31	153.92	2.8
Leucine	6.61	100.15	6.93	105.00	5.96	90.30	6.80	103.03	6.6
Tyrosine	3.11	}114.12	3.46	}137.14	3.38	}122.06	2.76	}119.68	}6.3
Phenylalanine	4.08		5.18		4.31		4.78		
Lysine	5.18	89.31	6.31	108.79	5.96	102.75	5.30	91.37	5.8
Histidine	3.16	166.31	3.20	168.42	2.96	155.78	2.36	124.21	1.9
Tryptophan	0.56	50.90	0.66	60.00	0.73	66.36	0.78	70.90	1.1
Arginine	5.10		5.34		4.36		4.46		

Table 5. Continues

Amino acid	*V. unguiculata* subsp. *cylindrica*	EAAS	*V. vexillata*	EAAS	*V. bourneae*	EAAS	FAO/WHO (1991) requirement pattern
Glutamic acid	14.16		15.70		11.30		
Aspartic acid	9.48		11.24		12.48		
Serine	3.78		4.12		4.36		
Threonine	3.54	104.11	4.56	134.11	4.30	126.47	3.4
Proline	5.68		3.46		3.74		
Alanine	5.10		4.17		5.24		
Glycine	3.28		3.33		3.48		
Valine	5.48	156.57	4.98	142.28	5.30	151.42	3.5
Cystine	0.63	}80.40	0.33	}59.20	0.78	}64.40	}2.5
Methionine	1.38		1.15		0.83		
Isoleucine	5.18	185.00	3.36	120.00	4.76	170.00	2.8
Leucine	6.43	97.42	7.04	106.66	6.74	102.12	6.6
Tyrosine	3.17	}142.06	2.86	}136.03	3.14	}132.53	}6.3
Phenylalanine	5.78		5.71		5.12		
Lysine	6.30	108.62	6.06	104.48	5.90	101.72	5.8
Histidine	5.14	270.52	1.86	97.89	4.18	220.00	1.9
Tryptophan	0.94	85.45	0.74	67.27	0.86	78.18	1.1
Arginine	5.30		6.16		5.36		

EAAS: Essential amino acid score, [a] Values are single determination.

Table 6: Data on *in vitro* protein digestibility (IVPD) and antinutritional factors of seven species of *Vigna.* $

Components	*Vigna trilobata*	*V. radiata* var. *sublobata*	*V. aconitifolia*	*V. umbellata*	*V. unguiculata* subsp. *cylindrica*	*V. vexillata*	*V. bourneae*
IVPD (%)[g]	74.30	79.12	70.38	72.40	77.30	76.21	72.68
Total free phenolics[h] g100g^{-1}	0.78±0.01^{b}	1.07±0.01^{f}	1.02±8.82^{e}	0.58±0.01^{a}	0.83±0.02^{c}	0.98±0.01^{d}	1.21±0.01^{f}
Tannins[h] g 100g^{-1}	0.23±0.01^{a}	0.31±0.01^{c}	0.48±0.01^{e}	0.24±0.01^{b}	0.27±0.03^{b}	0.19±0.01^{a}	0.36±0.01^{d}
L-DOPA[h]g 100g^{-1}	0.78±0.02^{c}	0.44±0.06^{a}	0.56±0.01^{b}	0.36±0.01^{a}	1.12±0.03^{d}	0.74±0.01^{c}	1.04±0.01^{d}
Phytic acid [h] mg100g^{-1}	312±1.76^{a}	394±3.48^{d}	376±0.33^{c}	336±0.57^{b}	406±1.45^{e}	414±0.01^{f}	436±0.88^{f}
Hydrogen cyanide[h] mg100g^{-1}	0.18±0.01^{c}	0.24±0.17^{d}	0.26±0.01^{d}	0.09±0.01^{a}	0.27±0.01^{e}	0.12±0.01^{b}	0.14±0.02^{b}
Trypsin inhibitor activity[g] (TIU mg^{-1} protein)	23.19	24.48	31.36	34.30	26.40	33.20	24.60
Oligosaccharide[h] g 100g^{-1}							
Raffinose	0.41±0.01^{b}	0.38±0.01^{c}	0.58±0.01^{a}	0.78±0.01^{d}	0.56±0.01^{d}	0.66±0.01^{b}	1.01±0.01^{c}
Stachyose	1.78±0.01^{b}	1.58±0.02^{d}	1.98±0.01^{c}	2.01±0.01^{e}	1.76±0.01^{a}	2.06±0.01^{b}	2.12±0.01^{d}
Verbascose	1.17±0.01^{b}	0.98±0.01^{c}	1.36±0.01^{e}	1.76±0.01^{d}	1.01±0.01^{a}	1.74±0.01^{b}	1.84±0.02^{c}
Phytohaemagglutinating activity Hu mg^{-1} protein[g]							
A group	34	64	45	58	46	42	54
B group	166	150	148	138	112	124	134
O group	14	24	29	34	13	28	26

[g]All values of two independent experiments, [h]All values are of means of triplicate determination expressed on dry weight basis ± standard error,
$ Mean values in the following row sharing a common letter are not statistically significant according to DMRT.

The lectins of different species of *Vigna* exhibit a high level of agglutination activity specifically in 'B' group compared to other two blood groups 'A' and 'O'. This is in good agreement with earlier reports in the tribal pulse *Dolichos lablab* var. *vulgaris* (Vijayakumari *et al*., 1995). However, dry heat and autoclaved are known to inactivate completely that trypsin inhibitors and phytohaemagglutins in *Mucuna* beans (Siddhuraju *et al*., 1996).

CONCLUSIONS

On the basis of the above findings, it is concluded that the tribal pulses investigated seem to be good source of protein, 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 cooking process, since they are heat labile.

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