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RESEARCH PAPER

The Biochemical Composition and Nutritional Potential of three Varieties of *Vigna mungo* (L.) Hepper.

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ABSTRACT

Three different varieties of the pulse, Vigna mungo (L.) Hepper *were analysed for their proximate and mineral composition, vitamins (niacin and ascorbic acid), protein fractions, amino acid profile of total seed proteins, fatty acid profile of seed lipids, in vitro protein digestibility and certain antinutritional factors. The major findings of the study were as follows: crude protein content ranged from 24.37 – 26.22%, crude lipid 2.94 – 4.24%, total dietary fibre 4.24 – 5.47%, ash 2.98 –3.33%, carbohydrates 61.24 – 64.43% and calorific value1603.65 – 1691.81kJ100^{-1}g DM. The investigated seed samples contained minerals such as Na, K, Mg and P in abundance. The ratios of Na/K ranged from 0.16 – 0.19% and Ca/P 0.68 – 1.19%. Albumins and globulins seems to be the principle protein of the investigated Vigna mungo varieties. The essential amino acid profile of total seed proteins were found to be high when compared to the FAO/WHO (1991) recommended pattern. The fatty acid profiles of all the three varieties revealed that the seed lipids contained linoleic and linolenic acid in high concentration. The IVPD of the studied samples ranged from 49.76 - 56.21%. The antinutritional factors ranged from: total free phenolics 0.48 – 1.41%, tannins 0.62 – 0.70%, L-DOPA 0.52 – 0.79%, phytic acid 338 – 412 mg100^{-1}, hydrogen cyanide 0.06 – 0.12 mg100^{-1}, trypsin inhibitor activity 32.48 – 41.36 TIUmg^{-1} protein. Raffinose was the principle oligosaccharide of all the three varieties of Vigna mungo. Low levels of phytohaemagglutinating activity for human erythrocytes of 'O' blood group than 'A' and 'B' blood groups were found in all the varieties.*

Key Words: *Vitamins, amino acid profiles, in vitro protein digestibility, antinutritional factors.*

INTRODUCTION

Legumes/pulses are considered to be a very important group of plant food stuffs, particularly in the developing world, as a cheap source of protein when animal protein is scarce. A significant part of human population relies on legumes as staple food for subsistence, particularly in combination with cereals. They are unique foods because of their rich nutrient content including starch, protein dietary fibre, oligosaccharides, phytochemicals and minerals [1]. Their nutritional contents contribute to many health benefits to humans [2, 3].

Most of the research on dry beans has been related to varietal selection. The criteria for selection have always been resistance to diseases or yields but nutritional quality [4]. A study of the composition and nutritive quality of dry beans would therefore be of great interest, because the knowledge provided would help to orient the work of investigators involved in varietal selection and also reduce or eliminate antinutritional factors to make edible and non edible legume seeds more acceptable as an inexpensive source of protein. The data on nutritional /antinutritional content of grain legumes in one region may help to select the specific variety or type of legume to grow on large scale. These kinds of studies would also help to increase the availability of food by processing underutilized varieties into edible forms through research and development [5]. The aim of the present study was to determine the variation in some of the nutrient and antinutrients, which are interesting from the nutritional point of view for varieties of *Vigna mungo* grown in Coimbatore District of Tamil Nadu.

MATERIALS AND METHODS

Collection of seed samples

The three different varieties of *Vigna mungo* were procured from the Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The seeds, after thorough cleaning and removal of broken

seeds, foreign materials and immature seeds were stored in airtight plastic jars at room temperature (25°C).

Proximate composition

The moisture content was determined by drying 50 transversely cut seed in an oven at 80°C for 24 hr and is expressed on a percentage basis. The air-dried samples were powdered separately in a Willy 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 [6] and the crude protein content was calculated (N x 6.25). Crude lipid content was determined using Soxhlet apparatus [7].The ash content was determined by heating 2g of the dried sample in a silica dish at 600°C for 6hr [7]. Total dietary fibre (TDF) was estimated by the non-enzymatic-gravimetric method [8]. To determine the TDF, duplicate 500 mg ground samples were taken in separate 250 ml 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 was added to each beaker and allowed to stand for 1 hr 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 ≥ 2 hr at 105°C and then cooled ≥ 2 hr in a desiccator and weighed. One crucible containing residue was used for ash determination at 525°C for 5 hr. The ash-containing crucible was cooled for ≥ 2hr 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.

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

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

The nitrogen free extract (NFE) was obtained by difference [9]. 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 [10].

Minerals and vitamins analysis

Five hundred milligrams of the ground legume seed was digested with a mixture of 10ml concentrated nitric acid, 4ml of 60% perchloric acid and 1ml of concentrated sulphuric acid. After cooling, the digest was diluted with 50ml of deionised distilled water, filtered with Whatman No. 42 filter paper and the filtrates were made up to 100ml in a glass volumetric flask with deionised distilled water. All the minerals except phosphorus were analysed from a triple acid-digested sample by an atomic absorption spectrophotometer – ECIL (Electronic Corporation of India Ltd., India) [11]. The phosphorus content in the triple acid digested extract was determined colorimetrically [12].

Ascorbic acid and niacin contents were extracted and estimated as per the method given by Sadasivam and Manickam [13]. For the extraction of ascorbic acid, 3g air-dried powdered sample was ground with 25ml of 4% oxalic acid and filtered. Bromine water was added drop by drop to 10ml 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 25ml with 4% oxalic acid and used for ascorbic acid estimation. Two millilitres of the extract was made up to 3ml with distilled H_2O in a test tube. One millilitre 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, 7ml of 80% H_2SO_4 was 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 30ml concentrated H_2SO_4 for 30min. After cooling, this suspension was made up to 50ml with distilled H_2O and filtered. Five millilitres of 60% basic lead acetate was added to 25ml of the filtrate. The pH was adjusted to 9.5 and centrifuged to collect the supernatant. Two millilitres of concentrated H_2SO_4 was added to the supernatant. The mixture was allowed to stand for 1hr and centrifuged. The 5ml of 40% $ZnSO_4$ was

added to the supernatant. The pH was adjusted to 8.4 and centrifuged again. Then the pH of the collected supernatant was adjusted to 7 and used as the niacin extract. For estimation, 1ml extract was made up to 6ml with distilled water in a test tube, 3ml cyanogen bromide was added and shaken well, followed by addition of 1ml 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 100grams of powdered samples.

Extraction and estimation of total proteins and protein fraction

The total (true) protein was extracted by the method of Basha *et al* [14] with slight modification (ethanol treatment was omitted to save prolamin fraction). The extracted proteins were purified by precipitation with cold 20% trichloroacetic acid (TCA) and estimated by the method of Lowry *et al* [15]. The albumin and globulin fractions of the seed protein were extracted and separated according to the method of Murray [16]. The prolamin fraction was extracted from the residual pellet by treating the pellet with 80% ethanol (1:10w/v) overnight. After centrifugation (20,000g for 20 min at room temperature) the supernatant containing prolamins was air-dried and dissolved in 0.1N NaOH. The resulting pellet was extracted with 0.4N NaOH (1:10w/v) overnight and centrifuged as above. The supernatant was designated as glutelins. All four fractions so obtained were precipitated and washed with cold 10% TCA. All samples were redissolved in 0.2M NaOH and protein content was determined by the Lowry *et al* method [15].

Amino acid analysis

The total seed protein was extracted by a modified method of Basha *et al* [14]. 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 de-ionized water. Dilution was effected by means of citrate buffer pH 2.2 to such an extent that the solution contained 0.5 mg protein ml^{-1}. The solution was passed through a millipore filter (0.45µM) and derivitized with O-phthaldialdehyde by using an automated pre-column (OPA). Aminoacids 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 Liddelle and Saville [17] 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 tryptophan contents of the alkaline hydrolysates were determined colorimetrically using the method of Spies and Chambers [18] as modified by Rama Rao *et al* [19]. The contents of the different amino acids were expressed as g100g-1 proteins and were compared with FAO/WHO (1991) reference pattern [20]. The essential amino acid score was calculated as follows:

$$\text{Essential amino acid score} = \frac{\text{grams essential amino acid in 100g of total protein}}{\text{grams of essential amino acid in 100g of FAO/WHO (1991) reference pattern}} \times 100$$

Lipid extraction and fatty acid analysis

The total lipid was extracted from the seeds according to the method of Folch *et al* [21] 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* [22]. 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 (2mX3mm) 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 30ml/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.

Analysis of antinutritional compounds

The antinutritional compounds, total free phenolics [23], tannins [24], the non-protein amino acid, L-DOPA (3, 4-dihydroxyphenylalanine) [25], phytic acid [26] and hydrogen cyanide [27] were

quantified. Trypsin inhibitor activity was determined by the enzyme assay of Kakade *et al* [28] 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 [29]. Five grams each of all the three varieties of seed flours were extracted 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 25 ml 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 double-distilled water.

Separation of oligosaccharides was done by TLC. Thirty g 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. Five 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 water (6:1:3), and dried [30]. The plates were sprayed with α –naphthol reagent (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 verbascose (procured from sigma chemical co., St. Louis, USA). Separated sugars that appeared were verbascose, stachyose and raffinose. The sugar spots were scrapped, eluted in 2 ml of distilled water kept overnight 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* [30]. 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 phytohaemagglutinating (lectin) activity

Lectin activity was determined by the method of Almedia *et al* [31]. One g of air-dried seed flour was stirred with 10ml of 0.15N sodium chloride solution for 2hours and the pH was adjusted to 4.0. The contents were centrifuged at 10,000 X g for 20min. and the supernatants were collected separately. The protein content was estimated by the Lowry *et al* [15] 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. Supernatants were removed with Pasteur pipettes. The washing procedure was repeated three times. The washed cells were diluted by one drop of cells with 24 drops of phosphate – buffered saline.

The determination of lectin was done by the method of Tan *et al* [32]. Clear supernatant (50µl) was poured into the depression (pit) on a micro-titration 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 3 hours at room temperature. After the incubation period, the titre values were recorded. One haemagglutinating unit (HU) is defined as the least amount of haemagglutinin that will produce positive evidence of agglutination of 25µl of a blood group erythrocyte after 3hr incubation at room temperature. The phytohaemagglutinating activity was expressed as haemagglutinating units (HU) / mg protein.

Determination of in vitro protein digestibility (IVPD)

This was determined using the multi-enzyme technique [33]. 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 1h. 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, ∝-chymotrypsin and peptidase) at 37°C followed by protease 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.

RESULTS AND DISCUSSIONS

The proximate composition of seeds of three different varieties of *Vigna mungo* was shown in Table 1. Crude protein content ranged from 24.37 – 26.22%. Among the three varieties, Co_5 was found to be higher (26.22%) amount of crude protein when compared to certain legumes such as *Cicer arietinum* (20.7%), *Vigna mungo* (23.6%) and *Vigna radiata* (24.5%) as reported by Bravo *et al*, [34]. The crude lipid content of *Vigna mungo var* TMV – 1 was higher when compared with *Vigna capensis* and *Vigna sinensis* [35]. The total dietary fibre content of three different varieties ranged from 4.24 – 5.47%. The ash content range of this legume (2.98- 3.33%) would be important to the extent that it contains the nutritionally important mineral elements shown in Table 2. From the data the diffeent varieties of *Vigna mungo* had a higher range of carbohydrate (NFE) (61.24 – 64.43%), than peanut and soybeans [36]. The high NFE contents of *Vigna mungo* enable this legume to act as a good source of calories which would be antimarasmus, especially infant nutrition [37]. The range in calorific values exceeds energy values of cowpea, green gram, horse gram, moth beans and peas [36] which are in the range of 1318–1394 KJ 100 g^{-1} DM.

Table 1: Proximate composition of seeds of three different varieties of *Vigna mungo*[a]

Component	$g100^{-1}g$ seed flour		
	Co_5	**TMV-1**	**Vamban-1**
Moisture	8.20 ± 0.05	8.54 ± 0.05	8.43 ± 0.07
Crude protein (Kjeldahl x 6.25)	26.22 ± 0.27	26.07 ± 0.14	24.37 ± 0.27
Crude lipid	2.94 ± 0.03	4.24 ± 0.06	3.63 ± 0.09
Total Dietary Fibre	4.55 ± 0.03	4.24 ± 0.06	3.63 ± 0.09
Ash	3.12 ± 0.04	2.98 ± 0.04	3.33 ± 0.08
NFE(Nitrogen Free Extractives)	63.17	61.24	64.43
Calorific value ($kJ100g^{-1}$ DM)	1603.65	1617.93	1691.81

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

Table 2: Mineral composition and vitamins (niacin and ascorbic acid) of seed of three different varieties of *Vigna mungo*[a] (mg 100 g^{-1} seed flour)

Name of the mineral	mg $100g^{-1}$ seed flour		
	Co_5	**TMV-1**	**Vamban-1**
Sodium	239.65 ± 0.65	328.01 ± 0.62	283.94 ± 0.52
Potassium	1681.48 ± 0.88	1601.78 ± 0.90	1625.20 ± 0.60
Calcium	493.83 ± 0.72	495.65 ± 0.80	334.89 ± 0.89
Magnesium	209.00 ± 0.16	297.33 ± 0.42	297.33 ± 0.42
Phosphorus	421.21 ± 1.69	415.48 ± 1.12	495.07 ± 1.43
Zinc	2.72 ± 0.02	2.19 ± 0.03	2.18 ± 0.02
Copper	5.45 ± 0.03	4.92 ± 0.02	4.64 ± 0.01
Iron	6.54 ± 0.07	5.41 ± 0.03	9.83 ± 0.01
Manganese	4.90 ± 0.05	4.92 ± 0.06	6.01 ± 0.04
Na/K	0.16	0.24	0.19
Ca/P	1.17	1.19	0.68
Niacin	3.30 ± 0.04	2.94 ± 0.01	4.27 ± 0.04
Ascorbic acid	7.14 ± 0.11	5.20 ± 0.07	6.36 ± 0.05

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

Food legumes are good source of minerals such as calcium, iron, copper, zinc, potassium and magnesium [38]. In the present investigation, all the varieties of *Vigna mungo* registered a higher level of potassium (Table-2) when compard with recommended dietary value (RDA) for infants and children (<1550mg) [39]. 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 [40]. The seeds of three different varieties of *Vigna mungo* contained higher level sodium, potassium, calcium, magnesium and phosphorus when compared with *Phaseolus vulgaris, P.limensis, V. unguiculata, Cicer arietinum, Pisum sativum and Lens culinaris* [41]. The manganese content of *Vigna mungo* was found to be higher than that of Estimated Safe and Adequate Daily Dietary Intake of minerals (ESADDI) [42].

The ratios of sodium to potassium (Na/K) and calcium to phosphorus (Ca/P) are also shown in Table 2. 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, in the present study, all the three varieties of *Vigna mungo* 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 [43]. This had 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 loss in the urine, decreasing the calcium level in bones. Food is considered “good” if the ratio is a above one and “poor” if the ratio is less than 0.5 [44]. The Ca/P ratio in the present study ranged between 0.68 to 1.19 indicating they would serve as good sources of minerals for bone formation.

The presently investigated *Vigna mungo* varieties exhibits the highest level of niacin content (Table 2) which was found to be higher than that of earlier report in *Cajanus cajan, Dolichos lablab, D.biflorus,Phaseolus mungo, Vigna catajang* and *Vigna* species [45]; *Phaseolus aureus* and *Cicer arietinum* [46] and *Vigna unguiculata* subsp. *cylindrica* [47]. The presently investigated *V. mungo* varieties also registered higher level of ascorbic acid content than *Cicer arietinum* [48]; *Vigna radiata* and *Vigna mungo* [49].

In the samples investigated, albumins and globulins (4.88 – 5.98% and 11.36 – 13.58% respectively) constitute the major bulk of the proteins (Table 3) as in most legumes and their *Vigna* species reported earlier [50, 51, 35].

Table 3: Data on total (true) protein and protein fractions of seed flour of three different varieties of *Vigna mungo* [a]

Protein fraction	$C0_5$		TMV-1		Vamban-1	
	g 100 g^{-1} seed flour	g 100 g^{-1} seed protein	g 100 g^{-1} seed flour	g 100 g^{-1} seed protein	g 100 g^{-1} seed flour	g 100 g^{-1} seed protein
Total (true) protein	22.91±0.03	100.00	20.88±0.04	100.00	20.44±0.07	100.00
Albumins	5.98±0.03	26.10	4.88±0.05	23.37	5.20±0.03	25.44
Globulins	13.58±0.05	59.28	12.95±0.08	62.02	11.36±0.06	55.58
Prolamins	1.46±0.09	6.37	1.14±0.01	5.46	1.89±0.03	9.25
Glutelins	1.89±0.01	8.25	1.91±0.03	9.15	1.99±0.02	9.73

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

The amino acid profiles of the purified seed proteins and the essential amino acid score were presented in Table 4. The content of essential amino acids in all the varieties of *Vigna mungo* were found to be higher compared to the FAO / WHO [20] requirement pattern. Among the three varieties of seed materials of *Vigna mungo* Co_5 registered highest level of *in vitro* protein digestibility (56.21%) and higher than that of an earlier investigation in the seeds of *Phaseolus mungo* [52].

Fatty acid composition of the total seed lipids of three different varieties of *Vigna mungo* were given in Table 5. The data revealed that all the seed lipids were rich in unsaturated fatty acids (76.00 – 76.13%) and had very high contents of linolenic acid (46.25- 47.65%) and low level of saturated fatty acids (23.07- 24.00%). These values are nutritionally desirable and also comparable with already investigated *Vigna mungo* [53]. The fatty acid composition and high amounts of unsaturated fatty

acids make *Vigna mungo* a special legume. Linoleic and Linolenic acids are the most important essential fatty acids required for growth, physiological functions and maintenance [54]. The presence of high levels of unsaturated fatty acids, in all the presently studied varieties, is nutritionally desirable.

Table 4: Amino acid profiles of acid hydrolysed, purified total seed proteins of three different varieties of *Vigna mungo*

Amino acid	Co_5		TMV-1		Vamban-1		FAO/WHO (1991) recommended pattern g 100 g^{-1} protein
	g100g^1 protein	EAAS	g100g^1 protein	EAAS	g100g^1 protein	EAAS	
Glutamic acid	20.50	-	20.00	-	20.30	-	-
Aspartic acid	11.80	-	11.90	-	12.20	-	-
Serine	4.60	-	4.30	-	4.00	-	-
Threonine	4.50	132.35	4.10	120.59	4.30	126.70	3.40
Proline	3.90	-	4.00	-	3.80	-	-
Alanine	4.00	-	3.90	-	3.80	-	-
Glycine	3.80	-	3.80	-	3.90	-	-
Valine	5.60	160.00	5.80	165.71	5.70	162.60	3.50
Cystine	0.70	100.00	0.80	100.00	0.60	96.00	2.50
Methionine	1.80		1.70		1.80		
Isoleucine	5.80	207.14	5.50	196.43	5.30	189.90	2.80
Leucine	7.90	119.69	8.30	125.76	8.00	121.10	6.60
Tyrosine	2.90	134.92	3.30	144.44	3.00	138.90	6.30
Phenylalanine	5.60		5.80		5.70		
Lysine	7.50	129.31	7.60	131.03	7.90	136.10	5.80
Histidine	2.50	131.58	2.40	126.32	2.60	136.40	1.90
Tryptophan	1.12		1.10		1.24		1.1
Arginine	5.30		5.20		5.30		

EAAS: Essential amino acid score

Table 5: Fatty acid composition of the seed lipids of three different varieties of *Vigna mungo*

Fatty acid	Co_5 percentage	TMV-1 percentage	Vamban-1 percentage
Palmitic acid (C16:0)	17.7O	17.92	18.00
Stearic acid (C18:0)	5.93	5.15	6.00
Oleic acid (C18:1)	17.57	18.00	17.90
Linoleic acid (C18:2)	11.15	12.60	11.85
Linolenic acid (C18:3)	47.65	46.33	46.25

[a]All values are of means of triplicate determination expressed on dry weight basis

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 antinutritional or toxic compounds associated with the large content of protein in their seeds [55]. For this reason, in the present investigation an attempt has been made to detect the presence of certain antinutritional factors in different varieties of *Vigna mungo*. The content of total free phenolics in the samples investigated appeared to be lower when compared with earlier reported *Vigna mungo* [52]. The tannin content of the investigated samples were relatively lower than the other domesticated legumes like black gram, chick pea, cow pea and green gram [56; 57] red gram, Bengal gram, lentil [5] and different chick pea cultivars [58]. Phenolics and tannins are known to inhibit activities of digestive enzymes and hence, the presence of even low levels of tannins and phenolics is not desirable from a nutritional point of view. However, in legumes the soaking and cooking process is known to reduce phenolics and tannins significantly [59]. Recently plant phenolics are increasingly gaining importance in relation to human health as wellness since they exhibit anticarcinogenic, antioxidant, antiviral, antimicrobial, anti-inflammatory and hypotensive

properties [60]. The content of non-protein amino acid, L-DOPA is relatively low when compared with other pulses reported earlier from our laboratory [61; 47].

Table 6 :Data on IVPD and antinutritional factors of three different varieties of *Vigna mungo* [a]

Components	CO_5	Tmv-1	Vamban-1
In vitro protein digestibility(%)[a]	56.21	49.76	55.36
Total free phenolics (%)[a]	0.41± 0.06	0.48 ± 0.03	0.68 ± 0.12
Tannins (%)[a]	0.30 ± 0.01	0.38 ± 0.03	0.32 ± 0.03
L-DOPA (%)[a]	0.12 ± 0.01	0.22 ± 0.06	0.19 ± 0.08
Phytic acid[a] mg $100g^{-1}$	338 ± 1.26	412 ± 1.74	398 ± 0.78
Hydrogen cyanide mg $100g^{-1}$	0.12 ± 0.01	0.06 ± 0.01	0.09 ± 0.03
Trypsin inhibitor[b] TIU mg^{-1} protein	38.74	41.36	32.48
Oligosaccharide[a] $g100g^{-1}$			
Raffinose	1.12 ± 0.04	1.22 ± 0.04	1.18 ± 0.03
Stachyose	0.96 ± 0.01	1.02 ± 0.08	0.94 ± 0.01
Verbascose	0.82 ± 0.07	0.92 ± 0.06	0.86 ± 0.01
Phytohaemagglutinating activity (Hu mg^{-1} protein[b]) A group	48	38	33
B group	98	87	92
O group	18	22	14

Raff: Raffinose; Stac: Stachyose; Verb: Verbascose
[a]All values are of means of triplicate determination expressed on dry weight basis
[b]Values are two independent experiments

The phytate molecule is negatively charged at physiological pH and is reported to bind with essential, nutritionally important divalent cations such a Fe, Zn, Mg and Ca etc., and forms insoluble complexes, thereby making minerals unavailable for absorption [62]. It also formed complexes with proteins and starch and inhibits their digestion [63]. The phytic acid content of investigated seed samples were found to be low when compared with that of some commonly consumed legumes *Vigna mungo* [64]; *Dolichos lablab* var. *vulgaris* [65]; tribal pulses *Mucuna pruriens* var, *utilis* [66] and *Mucuna atropurpurea* [67]. Hydrogen cyanide is known to cause acute or chronic toxicity. The content of HCN level in the presently investigated legume was far below the lethal level ie., 36mg/100g [68] and comparable with those of *Vigna sinensis* and *Pisum sativum* [69].

Raffinose seems to be the principle oligosaccharide of investigated *Vigna mungo* varieties. It was in conformity with earlier reports in black gram [70]; soya bean [71] and *Mucuna monosperma* [72]. The lectin (phytohaemagglutinating activity) of seed samples exhibit a high level of agglutination activity specifically in 'B' group compared other two blood group 'A' and 'O'(Table 6). This is in good agreement with earlier reports in the tribal pulse *Dolichos lablab* var. *vulgaris* [65].

On the basis of the above findings, it is concluded that *Vigna mungo* varieties investigated seem to be a good source of protein, essential amino acids, essential fatty acids, minerals and vitamins like niacin and ascorbic acid. 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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