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RESEARCH ARTICLE

Nutrient and Chemical Evaluation of Raw Seeds of Five Varieties of *Lablab purpureus* (L.) Sweet

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ABSTRACT

The proximate and mineral compositions, vitamins (niacin and ascorbic acid), seed protein fractions, amino acid profiles, fatty acids, in vitro protein digestibility and anti-nutritional factors of five varieties of Lablab purpureus were analysed. The major findings of the study were as follows; crude protein ranged from 20.46-25.47%, crude lipid 2.69-4.17%, total dietary fibre 4.98-6.90%, ash 3.97-4.48% and carbohydrates 60.63-66.32%. The energy level of the seed (1524.20-1604.34kJ100^{-1} DM) was comparable with commonly consumed Indian pulses. The seeds were found to be rich source of minerals, such as sodium, potassium, calcium, magnesium, phosphorus and iron. The data on seed protein fractions revealed that the globulins constitute the major bulk of the seed protein as in most legumes. Profiles of amino acids of total seed proteins detected in the present study revealed that they contain relatively higher levels of all essential amino acids except tryptophan and sulphur containing amino acids compared with the FAO/WHO (1991) requirement pattern. The seed lipids contained a large proportion of unsaturated fatty acids. The in vitro protein digestibility of the legumes under study ranged from 64.36-70.30%. The antinutritional factors ranged from; total free phenolics, 0.21-0.32g100g^{-1}, tannin, 0.23-0.40 g100g^{-1}, L-DOPA, 0.21-0.49 g100g^{-}1, phytic acid, 314-421mg 100g^{-1}, hydrogen cyanide 0.22-0.33mg 100g^{-1}, trypsin inhibitor activity, 24.30-34.56 TIU mg^{-1} protein. Stachyose was the principle oligosaccharide of all the varieties of Lablab purpureus. Lower levels of phytohaem-agglutinating activity for human erythrocytes of 'O' blood group than 'A' and 'B' blood groups were found in all the varieties.

***Key Words**: Lablab purpureus, seed protein, amino acid profiles, antinutrients.*

INTRODUCTION

Legumes / pulses are considered to be a very important group of plant food stuffs, particularly in developing world, as a cheap source of protein when animal protein is scarce. In addition to proteins, they supply adequate concentration of minerals, vitamins and carbohydrate [1]. Being a cheap source of dietary protein, legumes are now successfully used in child feeding programmes and food and feed formulations. Cereals and other crops also supply a substantial level of protein and high levels of carbohydrates. However, in some of these foodstuffs, the utilization of available protein and carbohydrates is much less than that calculated from the chemical composition because of the presence of various antinutritional or antiphysiological substances [2, 3]. In this context, a detailed investigation of all plant resources of available world wide, including legumes, is the need of the hour to feed ever increasing population. However, most of the Indian legumes remain uninvestigated biochemically and nutritionally. In the presence study the chemical composition and nutritional potential of the five different varieties of *Lablab purpureus* have been investigated and reported.

MATERIALS AND METHODS

Collection of seed samples

The five different varieties of *Lablab purpureus* were procurred 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 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 [4] and the crude protein content was calculated (N x 6.25). Crude lipid content was determined using Soxhlet apparatus [5].The ash content was determined by heating 2g of the dried sample in a silica dish at 600°C for 6hr [5] Total dietary fibre (TDF) was estimated by the non-enzymatic-gravimetric method [6]. 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.

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

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 [7]. 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 [8].

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) [9]. The phosphorus content in the triple acid digested extract was determined colorimetrically [10].

Ascorbic acid and niacin contents were extracted and estimated as per the method given by Sadasivam and Manickam [11]. 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* [12] 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* [13]. The albumin and globulin fractions of

the seed protein were extracted and separated according to the method of Murray [14]. 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 [13].

Lipid extraction and fatty acid analysis

The total lipid was extracted from the seeds according to the method of Folch *et al* [15] 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* (16). 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.

Amino acid analysis

The total seed protein was extracted by a modified method of Basha *et al* [12]. 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:

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

Analysis of antinutritional compounds

The antinutritional compounds, total free phenolics [21], tannins [22], the non-protein amino acid, L-DOPA (3, 4-dihydroxyphenylalanine) [23], phytic acid [24] and hydrogen cyanide [25] were quantified. Trypsin inhibitor activity was determined by the enzyme assay of Kakade *et al* [26] 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 [27]. Five grams each of all the five 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 [28]. 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* [28]. 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* [29]. 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* [13] 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* [30]. 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 [31]. 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 5C for 1h. The samples containing protein and enzymes were all adjusted to pH 8.0 at 37C. The IVPD was determined by the sequential digestion of the samples containing protein with a multi-enzyme mixture (trypsin, -chymotrypsin and peptidase) at 37C followed by protease at 55C. 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 $Y = 234.84\ \ 22.56X$, where Y is the % digestibility and X the pH drop.

RESULTS AND DISCUSSION

The proximate composition of seeds of five different varieties of *Lablab purpureus* was shown in Table1. Crude protein content ranged from 20.46-25.47%. Among the five varieties, Co1 was found to be higher (25.47%) amount of crude protein when compared to certain legumes such as *Cicer arietinium* (20.7%), *V*igna *mungo* (23.6) and *V.radiata* (24.5%) as reported by Bravo *et al* [32]. The crude lipid content of *Lablab purpureus* var.Co11 was higher when compared with *Cicer arietinum* [33]. The total dietary fibre content of five different varieties ranged from 4.98-6.90%. The ash content range of this legume (3.97 - 4.48%) would be important to the extent that it contains the nutritionally important mineral elements shown in Table 2. From the data the different varieties of *Lablab purpureus* had a higher range of carbohydrate (60.63-66.32%), than peanut and soyabeans [34]. The high NFE contents of *Lablab purpureus* enable this legume to act as a good source of calories which would be antimarasmus, especially in infant nutrition [35]. The range in calorific values exceeds energy values of cowpea, green gram, horse gram, moth bean and peas [36], which are in the range of 1318-1394 kJ $100g^{-1}$DM.Food legumes

Table 1: Proximate composition of seeds of five different varieties of *Lablab purpureus*[a] (g 100 g^{-1} seed flour)

Components	Co_1	Co_2	Co_9	Co_{11}	Co_{12}
Moisture	7.25 ± 0.02	8.04 ± 0.01	8.42 ± 0.05	7.80 ± 0.09	8.05 ± 0.02
Crude protein (Kjeldhal N × 6.25)	25.47 ± 0.45	24.98 ± 0.14	23.41 ± 0.28	23.02 ± 0.14	20.46 ± 0.28
Crude lipid	2.69 ± 0.05	3.87 ± 0.02	2.28 ± 0.02	4.17 ± 0.02	3.76 ± 0.02
Total dietary fibre(TDF)	6.90 ± 0.02	6.32 ± 0.03	5.25 ± 0.02	5.80 ± 0.05	4.98 ± 0.04
Ash	4.31 ± 0.03	3.97 ± 0.03	4.43 ± 0.03	4.28 ± 0.02	4.48 ± 0.06
Nitrogen Free Extractives (NFE)	60.63	60.86	64.63	62.73	66.32
Calorific value (kJ100g^{-1} DM)	1524.20	1579.43	1556.22	1589.23	1604.34

[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 five different varieties of *Lablab purpureus*[a] (mg 100 g^{-1} seed flour)

Components	Co_1	Co_2	Co_9	Co_{11}	Co_{12}
Sodium	129.38 ± 0.05	108.74 ± 1.06	152.87 ± 0.09	108.47 ± 0.18	87.00 ± 0.12
Potassium	1725.07 ± 0.18	1674.64 ± 0.98	1768.95 ± 1.06	1692.07 ± 1.60	1653.07 ± 1.52
Calcium	575.03 ± 2.49	435.00 ± 2.01	363.67 ± 1.98	434.00 ± 0.58	478.67 ± 2.51
Magnesium	225.00 ± 2.80	261.00 ± 1.98	262.00 ± 1.60	156.00 ± 0.96	391.50 ± 1.74
Phosphorus	733.00 ± 5.68	637.96 ±5.92	567.81 ± 0.01	513.43 ± 2.29	500.27 ± 2.25
Zinc	2.69 ± 0.02	2.72 ± 0.02	2.73 ± 0.06	2.71 ± 0.01	2.72 ± 0.01
Copper	1.62 ± 0.01	1.44 ± 0.03	4.91 ± 0.02	4.34 ± 0.02	4.89 ± 0.03
Iron	10.24 ± 0.09	9.79 ± 0.06	6.55 ± 0.03	8.68 ± 0.06	10.33 ± 0.09
Manganese	7.01 ± 0.08	5.44 ± 0.04	7.09 ± 0.05	4.88 ± 0.02	5.44 ± 0.06
Vitamins					
Niacin	18.33 ± 0.11	21.36 ± 0.21	24.04 ± 0.09	19.38 ± 0.06	16.32 ± 0.14
Ascorbic acid	28.14 ± 0.09	31.34 ± 0.14	25.06 ± 0.07	36.23 ± 0.17	39.06 ± 0.17

Table 3: Data on total (true) protein and protein fractions of seed flour of five different varieties of *Lablab purpureus*[a]

Components	Co_1		Co_2		Co_9		Co_{11}		Co_{12}	
	g 100g^{-1} seed flour	g 100g^{-1} seed protein	g 100g^{-1} seed flour	g 100g^{-1} seed protein	g 100g^{-1} Seed flour	g 100g^{-1} seed protein	g 100g^{-1} seed flour	g 100g^{-1} seed protein	g 100g^{-1} Seed flour	g 100g^{-1} seed protein
Total (true) protein	22.90 ± 0.06	100.00	21.69 ± 0.05	100.00	20.28 ± 0.06	100.00	19.62 ± 0.07	100.00	17.71 ± 0.05	100.00
Albumins	6.33 ± 0.44	31.44	7.36 ± 0.03	33.93	6.33 ± 0.03	31.21	46 ± 0.03	27.83	5.78 ± 0.02	32.63
Globulins	14.50 ± 0.03	63.32	12.87 ± 0.04	59.34	12.44 ± 0.08	61.34	12.95 ± 0.06	66.00	10.93 ± 0.05	61.72
Prolamins	0.57 ± 0.02	2.49	0.39 ± 0.01	1.80	0.62 ± 0.01	3.06	0.59 ± 0.03	3.01	0.49 ± 0.01	2.77
Glutelins	0.63 ± 0.01	2.75	1.07 ± 0.02	6.41	0.89 ± 0.02	4.39	0.62 ± 0.01	3.16	0.51 ± 0.01	2.88

Table 4: Amino acid profiles of acid-hydrolysed, purified total seed proteins of five different varieties of *Lablab purpureus*[a] ($g100g^{-1}$)

Amino acid	Co_2	EAAS	Co_2	EAAS	Co_9	EAAS	Co_{11}	EAAS	Co_{12}	EAAS	FAO/WHO 1991) requirement pattern
Glutamic acid	15.94		16.05		15.73		15.61		15.72		
Aspartic acid	14.03		13.91		13.52		13.04		12.86		
Serine	5.62		5.24		5.11		5.04		5.24		
Threonine	5.24	154.12	5.20	152.94	5.62	165.29	4.91	144.41	5.02	147.64	3.4
Proline	4.52		3.81		4.12		4.06		4.21		
Alanine	4.41		4.02		3.89		4.22		4.03		
Glycine	4.66		4.34		4.06		4.11		3.95		
Valine	5.32	152.0	5.42	154.86	5.21	148.86	5.12	146.29	5.12	146.29	3.5
Cystine	Trace	43.2	Trace	38.4	Trace	36.4	Trace	34.4	Trace	33.6	2.5
Methionine	1.08		0.96		0.91		0.86		0.84		
Isoleucine	4.41	157.5	4.24	151.43	4.32	154.29	4.50	160.71	4.65	166.07	2.8
Leucine	8.41	127.42	8.71	131.97	8.61	130.45	8.52	129.09	8.62	130.60	6.6
Tyrosine	3.90	142.86	3.62	133.81	3.82	149.84	4.07	142.69	3.81	143.33	6.3
Phenylalanine	5.10		4.81		5.62		4.92		5.22		
Lysine	7.24	124.83	7.14	123.10	7.60	131.03	6.84	117.93	7.04	121.37	5.8
Histidine	3.42	180.0	3.06	161.05	3.32	174.74	2.92	153.68	2.91	153.16	1.9
Tryptophan	0.62	-	0.84	-	0.41	-	0.48	-	0.74	-	1.1
Arginine	6.08	-	5.81	-	5.64	-	5.92	-	5.63	-	-

EAAS: Essential amino acid score

Table- 5: Fatty acid profile of the seed lipids of five different varieties of *Lablab purpureus*

Fatty acid (%)	Co_1	Co_2	Co_9	Co_{11}	Co_{12}
Palmitic acid [C16:0]	29.00	28.28	28.80	28.10	28.62
Stearic acid [C18:0]	1.62	1.52	1.50	1.48	1.60
Oleic acid [C18:1]	13.00	13.60	13.30	13.27	13.10
Linoleic acid [C18:2]	43.78	43.25	43.40	44.04	42.96
Linolenic acid [C18:3]	12.60	13.35	13.00	13.11	13.72

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

Table 6: Data on IVPD and anti nutritional factors of seeds of five different varieties of *Lablab purpureus*

Component	Co1			Co2			Co9			Co11			Co12		
In-vitro protein digestibility[a]	64.36			66.58			70.30			67.32			65.31		
Total free phenolics (%)[a]	0.21 ± 0.02			0.26 ± 0.02			0.24 ± 0.01			0.27 ± 0.01			0.32 ± 0.01		
Tannins (%)[a]	0.35 ± 0.01			0.53 ± 0.01			0.66 ± 0.02			0.40 ± 0.00			0.37 ± 0.02		
L-Dopa(%)[a]	0.30 ± 0.01			0.49 ± 0.01			0.45 ± 0.01			0.21 ± 0.02			0.47 ± 0.07		
Phytic acid[b] ($mg100g^{-1}$)	421 ± 1.26			344 ± 1.24			314 ± 2.21			332 ± 0.78			396 ± 0.57		
Hydrogen cyanide[b] ($mg100g^{-1}$)	0.32 ± 0.11			0.26 ± 0.09			0.14 ± 0.07			0.02 ± 0.03			0.33 ± 0.19		
Trypsin inhibitor activity (TIU mg^{-1} protein)[a]	31.36			34.56			24.36			29.10			33.54		
Oligosacchride[b] (g $100g^{-1}$)	Raff	Stac	Verb	Raff	Stac	Verb	Raff	Stac	Verb	Raff	Stac	Verb	Raff	Stac	Verb
	0.34 ±0.01	1.68 ±0.03	1.17 ±0.01	0.48 ±0.03	1.76 ±0.01	1.01 ±0.01	0.58 ±0.01	1.58 ±0.07	0.96 ±0.01	0.36 ±0.03	1.96 ±0.09	1.21 ±0.07	0.68 ±0.05	1.92 ±0.07	1.11 ±0.09
Phytohaemagglutinating activity [Hu mg^{-1} protein[b]]	A group	B group	O group	A group	B group	O group	A group	B group	O group	A group	B group	O group	A group	B group	O group
	36	154	17	52	161	24	42	142	19	39	156	29	54	138	21

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

are a good source of minerals such as calcium, iron, copper, zinc, potassium and magnesium [37]. In the present investigation, all the varieties of *Lablab purpureus* registered a higher level of potassium (Table. 2) when compared with recommended dietary value (RDA) for infants and children (<1550mg) [38]. 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 [39]. The seeds of five different varieties of *Lablab purpureus* contained higher level of sodium, potassium, calcium magnesium, phosphorus and iron when compared with other legumes *Phaseolus vulgaris*, *P. limeneis*, *V.unguiculata*, *Cicer arietinum*, *Pisum sativum* and *lens culinaris* [40]. The manganese content of *Lablab purpureus* was found to be higher than that of Estimated Safe and Adequate Daily Dietary Intake of minerals (ESADDI) [41].

The presently investigated seeds of *Lablab purpureus* exhibited the highest level of niacin content (Table2) which was 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* sp [42]; *Vigna unguiculata* supsp. *unguiculata* and *Vigna unguiculata* supsp. *cylindrica* [43,44]. The presently investigated seeds of five different varieties of *Lablab purpureus* also registered higher level of ascorbic acid content than *Atylosia scarbaeoides* and *Teramnus labialis* [43, 44].

In the samples investigated, albumins and globulins (5.46-7.33% and 10.93-14.50% respectively) constitute the major bulk of the proteins (Table3) as in most legumes and other V*igna* species reported earlier [45, 46, 47]. The amino acid profiles of the purified seed proteins and the essential amino acid score were presented in Table4. The content of sulphur containing amino acid and tryptophan seem to be deficient in all the varieties of *Lablab purpureus*; where as threonine, valine, isoleucine, leucine, tyrosine, phenylalanine, lysine and histidine in all the varieties were found to be higher compared to the [1] requirement pattern. Among the five varieties of seed materials of *Lablab purpureus* Co9 registered highest level of *in vitro* protein digestibility (70.30%) and higher than that of an earlier investigation in the seeds of *Dolichos lablab* var. *vulgaris* [48]. The fatty acid composition of the total seed lipids of five different varieties of *Lablab purpureus* were given in Table5. The data revealed that all the seed lipids were rich in unsaturated fatty acids (69.38-70.42%) and had very high contents of linoleic acid (42.96-44.04%) and low levels of saturated fatty acids. These values are nutritionally desirable and also comparable to those of certain common legume seeds [37]. The samples also contained more palmitic acid (28.10-29.00%) than the other legumes such as *Vigna unguiculata* and *Phaseous vulgaris* [49].

Food legumes are important sources of dietary protein in the developing countries, but acceptability and utilization has been limited due to the presence of high concentration of certain antinutritional factors [50]. For this reason, in the present investigation an attempt has been made to detect the presence of certain antinutritional factors in different varieties of *Lablab purpureus* (Table6). The content of total free phenolics in the samples investigated appeared to be lower when compared with *Neonotonia wightii* var. *coimbatorensis*, *Vigna trilobata*, *Vigna unguiculata* subsp. *unguiculata*, *Vigna radiata* var.*sublobata* and *Vigna unguiculata* subsp. *cylindrica* [43, 44]. The tannin content of the investigated samples was relatively lower than the domesticated legumes like black gram, chickpea, cowpea and green gram [51, 36] and *Teramnus labialis* [44]. Tannins are known to inhibit the activities of digestive enzymes [52] and hence the presence of even a low level of tannin is not desirable from nutritional point of view. 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 properities [53]. The content of the non-protein amino acid, L-DOPA is relatively lower when compared with other pulses reported earlier from our laboratory [43, 44]. Phytic acid has an antinutritional property because of its ability to lower the bio availability of essential minerals, and to form a complex with protein, thereby inhibiting the enzymatic digestion of ingested protein [54].Phytic acid content of five different varieties of *Lablab purpureus* was found to be low when compared with that of some commonly consumed legumes, *Vigna mungo* [55], *V. radiata* [56]; *Dolichos lablab var. vulgaris* [48] and *Mucuna pruriens* var. *utilis* [57]. Hydrogen cyanide is known to cause acute or chronic toxicity. The content HCN level in the presently investigated legume was far below the lethal level i.e., 36mg/100g [58] and comparable with those of *Vigna sinensis* and *Pisum sativun* [59] and certain tribal pulses [43,44].The trypsin inhibitor activities of all the the studied samples were higher than that of cow pea (2.54mg/g), *Phaseolus lunatus* and *Dolichos lablab* (2.11mg/g) [60] and different cultivars of *Vicia faba* (1.72-3.35mg/g) [61] and they

seem to be lower than of Pigeon pea (67.1-71.3mg/g) [62]. Stachyose seems to be the principle oligosaccharide of all varieties of *Lablab purpureus*. It was in conformity with the earlier reports in cow pea [63]; jack bean, lima and sword bean [64] and *Dolichos lablab* var. *vulgaris* [48]. The lectin (Phytohaemagglutinating activity) of five different varieties of *Lablab purpureus* exhibit a high level of agglutination activity specifically in 'B' group compared to other two blood groups 'A and 'O'. This is good agreement with earlier reports in the tribal pulse *Dolichos lablab* var. *vulgaris* [48]. All the antinutritional factors reported except L-Dopa are heat labile. Hence they can be removed by wet or dry thermal treatment [65]. In an earlier study, it has been demonstrated that the L-DOPA contents can also be significantly reduced by repeated soaking and boiling of the seeds in water under optimum heat condition to realize the maximum nutritional advantages [66].

From the chemical investigations it is concluded that the presently investigated legume can be used as protein sources to curtail with the problem of protein deficiency in most of the developing countries which may result in many child killer diseases. The presence of antinutritional factors identified in the current report should not pose a problem for humans if the beans are properly processed.

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