Studies on the Nutritional Composition and Antinutritional Factors of Three Different Germplasm Seed Materials of an Under-Utilized Tropical Legume, *Mucuna pruriens* Var. *Utilis*

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Two different germplasms of a white variety and one germplasm of a black variety of *Mucuna pruriens* var. *utilis* were evaluated for their physicochemical properties as well as their nutritional and antinutritional characteristics. All germplasms had higher grain weight, density, hydration, and swelling capacity than other common legumes. The dehulled samples contained 303.2−335.5 g^{-1} protein and 46.1−53.5 g kg^{-1} lipid, and these values were higher than the respective whole seeds. The levels of macro- and microelements in both whole and dehulled seeds were comparable to those in common pulses. All germplasms had a high dietary fiber content (18−19.5%), made up of mainly insoluble dietary fiber (DF). Seed lipids were high in unsaturated fatty acids (64.7−66.9%), specifically linoleic acid (48−49%). Whole and dehulled seeds of the white variety from Salem were particularly rich in sulfur-containing amino acids with significantly higher levels of in vitro protein digestibility than the other two germplasms. All germplasms had high levels of total phenols and phytate, trypsin, and chymotrypsin inhibitor activities, but were low in tannins, saponins, and α-amylase inhibitor activity. Only weak hemagglutinating activity against cow erythrocytes and no hemagglutinating activity against human erythrocytes (O) was observed in all the samples. Dehulled seeds were higher in total starch, including resistant starch and oligosaccharides (with verbascose as the major fraction) than the respective whole seeds. Both whole and dehulled samples of the white variety of Salem germplasm showed significantly lower concentrations of L-dopa, nonmethylated, and methylated tetrahydroisoquinolines than the respective whole and dehulled samples of other germplasms. In general, dehulling didn't affect the overall nutritional status in any of the presently investigated samples.

Keywords: *Mucuna pruriens var. utilis; proximate composition; amino acids; fatty acids; carbohydrates; dietary fibers; antinutritional factors; tetrahydroisoquinolines*

INTRODUCTION

Large segments of the human population and animals in developing countries suffer from protein malnutrition. About 800 million people are consuming less than 2000 calories a day and are living under conditions of permanent or intermittent hunger so that they are chronically undernourished. Most of the hungry are women and young children (Conway and Toenniessen, 1999). The populations of most developing countries are increasing rapidly: by the year 2020 there will be an additional 1.5 billion mouths to feed, mostly in the developing world. The search for alternative food and feed ingredients for man and livestock continues to attract the attention of researchers all over the world (Grant et al., 1983; Makkar et al., 1997a, 1998; Siddhuraju et al., 1996). The observed increase in the search for alternative or additional food and feed ingredients, especially for the developing countries of the world, is of paramount importance for two main reasons: (1) the low production of oil seeds and grains; and (2) the stiff competition between man and the livestock industry for existing food and feed materials. However, the cheapest food materials are those that are derived from plant sources which, although they occur in abundance in many countries, are underutilized. Exploitation of tropical wild legumes, including the pulses of tribal utility which are generally rich in protein, deserves timely and urgent attention.

Velvet bean (*Mucuna pruriens* (L.) DC. var. *utilis* (Wall. ex Wight) Baker ex. Burck) is a tropical legume. It belongs to the family Fabaceae and is widespread in the Southern and Southeastern Asian regions (Duke, 1981). It is cultivated as green manure or as a cover crop (Buckles, 1995), and it also grows in the wild. This bean is consumed in several parts of India, especially by the Northeastern tribes and Kanikkas tribes belonging to Kerela State (Arora, 1981; Janardhanan and Lakshmanan, 1985), and also by low income groups in Sri Lanka (Ravindran and Ravindran, 1988). It gives a reliable yield under the dry farming and low soil fertility conditions where most other food legumes cannot be grown economically. The seed yield reaches 1.5−2.0 t/ha and the yield of fresh leaves and stems is 20−30 t/ha; thus, it is considered to be one of the most productive

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legumes in the world (Fujii et al., 1991). Velvet bean leaves are used as fodder, and sometimes the ground seed meal is mixed with ground cotton seed meal for use as cattle feed. The seeds are large compared to some of the commonly cultivated legumes and have a long period of viability and a higher germination percentage. The bean is also used in indigenous ayurvedic medicine (Jayaweera, 1981) and the L-dopa extracted from it is used to provide symptomatic relief in Parkinson's disease (Duke, 1981). However, the pharmacologically active factor L-dopa is potentially toxic and antinutrient. One of the side-effects of L-dopa in patients treated for Parkinson's disease is a toxic confusional state (Infante et al., 1990). The seeds cause vomiting and diarrhea in humans and other animals when large amounts are ingested (Duke, 1981; Afolabi et al., 1985). Raw mucuna seeds in the diet have also been reported to reduce growth rates of broiler chicks and egg production of laying hens (Harms et al., 1961).

Although legumes constitute one of the richest and least expensive sources of protein in human/animal diets, their utilization is limited because of the presence of antinutritional compounds such as phenolics; tannins; trypsin, chymotrypsin and amylase inhibitors; lectins; phytic acid; alkaloids; hydrogen cyanide; glucosinolates; nonprotein amino acids; toxic fatty acids; saponins; and oligosaccharides (Grant et al., 1983; Liener, 1994a,b; Makkar and Becker, 1998). Thus, the intoxication associated with overeating of Mucuna beans could be due to the presence of these antinutritional factors. There could be a substantial amount of variation in secondary plant metabolites and antinutritional factors, and also in nutrient levels, which may be caused by genetic differences or by the environment (Makkar et al., 1997; Udedibie and Carlini, 1998; Oluwatosin, 1999). However, there have been no systematic studies on the levels of various antinutritional factors in and the nutritional composition of mucuna seeds specifically when grown in different environments. Hence, the objective of this study was to investigate the nutritive potential and antinutritional constitutents of three different germplasms of the tribal pulse *M. pruriens* var. *utilis* collected from two different environments.

MATERIALS AND METHODS

Samples. The mature and dry raw seeds of *Mucuna pruriens* var. *utilis* white and black varieties (Marthandam germplasms) were purchased from tribal people living in a village near Marthandam, and another germplasm of white variety (Salem germplasm) was collected from Salem, Tamil Nadu, India in the month of January 1999. The dried seeds were cleaned thoroughly, and any broken or immature seeds were removed. The seeds were stored at room temperature (24 °C) until processing, when they were cracked mechanically and the cotyledons were separated. The whole and dehulled seeds (cotyledons) were used for various chemical analyses. All samples were ground to a particle size of less than 0.5 mm.

Physicochemical Properties. Seed samples from the two germplasms (Marthandam and Salem) of white variety and the one germplasm of black variety (Marthandam) of Mucuna beans were analyzed for their physicochemical properties. Seed color was determined subjectively. Proportions of cotyledon and seed coat were determined by taking the average measures of 30 seeds from each germplasm. The density of seeds was calculated by weighing 100 seeds and subsequently measuring their volume by displacement using a cylinder containing 200 mL of deionized water. The seeds were placed in water to sink them and the volume of the displaced water was recorded, which represented the seed volume (mL/100 seeds). The hydration capacity of the seeds was determined in the following manner. The seeds (100) were placed in a 250-mL Erlenmeyer flask, and then 200 mL of distilled water was added. The flask was lightly stoppered and left overnight at room temperature (24 °C). On the next day the seeds were drained, superfluous water was removed with absorbent paper, and the swollen seeds were reweighed. Hydration capacity per seed was determined as the ratio of the weight difference between that of the soaked and unsoaked seeds divided by the number of seeds. The hydration index was then calculated as the ratio of the average hydration capacity per seed and the weight of one seed. To determine swelling capacity, the soaked seeds were transferred to a 250-mL measuring cylinder after reweighing and 200 mL of water was added. Swelling capacity was estimated from the volume difference data of soaked and unsoaked seeds and the number of seeds used. The swelling index was then calculated:

$$\text{swelling index} = \frac{\text{swelling capacity per seed}}{\text{seed volume (mL)}} \qquad (1)$$

per Williams et al., 1983.

Proximate and Mineral Composition. The moisture contents of both whole seeds and cotyledons of all the germplasm varieties of mucuna beans was determined by oven-drying to a constant weight at 105 °C. The crude protein, lipid, crude fiber, and ash were determined in accordance with the standard methods of AOAC (1980). Carbohydrate content was obtained by difference. Gross energy was estimated using a bomb calorimeter. Determination of Mg, Cu, Fe, Mn, and Zn was carried out by using an atomic absorption spectrometer (Model 939, Unicam, U.K), and a flame photometer (Model ELEX 6361, Eppendorf, Germany) was used for the determination of potassium, sodium, and calcium. Phosphorus was spectrophotometrically analyzed (Gericke and Kurmies, 1952) at 436 nm using a UV−visible spectrophotometer (Model U-2000, Hitach, Japan).

Starch Analysis. Total starch (TS) content was determined after dispersion of the starch granules in 2 M KOH (50 mg of sample, 6 mL of KOH) at room temperature (30 min, constant shaking) and hydrolysis of the solubilized starch with 80 μl of amyloglucosidase (EC 3.2.1.3; Cat. No. 102857, Boehringer-Mannheim, Germany) at 60 °C for 45 min (Goni et al., 1997). Glucose was determined using the glucose oxidase/peroxidase reagent (Cat. no. 510-A, Sigma Chemical Co., Deisenhofen, Germany). Total starch was calculated as glucose $\times$ 0.9, after correction of the free glucose content. The glucose content of samples, both free glucose and the glucose moiety of sucrose, was determined in order to correct the total starch values obtained as described above. Samples dispersed in 2 M KOH were treated with invertase (EC 3.2.1.26) during 30 min at 37 °C. After centrifugation, a 1-mL aliquot was precipitated with 2 mL of 96% ethanoland centrifuged again, and glucose was analyzed in the supernatants using the glucose oxidase/peroxidase reagent.

Resistant starch (RS) was analyzed by the following procedure. Samples (100 mg) were treated with 200 mg of pepsin (1 g of pepsin/10 mL of KCl−HCl buffer, pH 1.5; Merck no. 7190, 2000 FIT-U g^{-1}) to remove protein and then incubated for 16 h at 37 °C with 1 mL of pancreatic α-amylase (EC 3.2.1.1, A-3176, Sigma) solution containing 40 mg of α-amylase/mL Tris−maleate buffer, pH 6.9, to remove digestible starch. After centrifugation (15 min, 3000*g*) and removal of the supernatant, the pellet was dispersed with 2 M KOH and hydrolyzed with amyloglucosidase, and the liberated glucose was quantified, all as described above for total starch. RS was calculated as glucose $\times$ 0.9 (Goni et al.,1996). Digestible starch (DS) content was calculated as TS − RS.

Dietary Fiber Analysis. Dietary fiber in whole seeds of mucuna beans was analyzed by the enzymatic−gravimetric method of Prosky et al. (1988) modified to prevent some error associated with the ethanol precipitation step (Manas et al., 1994). Samples were treated with heat-stable α-amylase (EC 3.2.1.1; A-3306), protease (P-3910), and amyloglucosidase (EC 3.2.1.3; A-9913) (all from Sigma) to remove digestible starch

and protein. After the treated samples were centrifuged (15 min, 3000*g*) and washed with distilled water, the soluble dietary fiber (SDF) fraction was separated by aspiration and transferred into dialysis tubes (12 000−14 000 MW). These tubes were kept in a dialysis chamber for about 48 h at 25 °C with a constant water flow (5 L h^{-1}). After dialysis, SDF was hydrolyzed with 1M H_2SO_4 for 90 min in a boiling water bath. The residue from the enzymatic treatment was further washed with ethanol and acetone and centrifuged under the same conditions as before, constituting the insoluble dietary fiber (IDF) fractions. IDF residues were subsequently hydrolyzed with 12 M (1 h, 30 °C) and 1 M (1.5 h, 100 °C) H_2SO_4. The residue after the acid hydrolysis was dried at 105 °C to a constant weight and quantified as Klason lignin (KL).

Uronic acid (UA) and neutral sugars (NS) were quantified in the hydrolysates; UA was determined spectrophotometrically by the method of Scott (1979) using galacturonic acid as a standard, and NS was determined by GC as alditol acetates (Englyst and Cummings, 1988) using myoinositol as an internal standard. A Chrompack CP 9001 (Chrompack Inc./Varian USA, Walnut Creek, CA) gas chromatograph fitted with a flame ionization detector (FID) and a CP-9010 autosampler, and connected to a CP 900X MAESTRO data system was used. The column was a DB-225 capillary column (30 m × 0.25 mm i.d.). The operation conditions were column temperature, 140−220 °C; injector temperature, 230 °C; and detector temperature, 230 °C. Carrier gas was nitrogen. IDF content was calculated as (NS + UA) + KL, and SDF was calculated as NS + UA.

Amino Acid Analysis. The amino acid composition of whole and dehulled mucuna seeds was determined using an amino acid analyzer (Bassler and Buchholz, 1993) after hydrolyzing the samples with 6 M HCl at 100 °C. Sulfur-containing amino acids were oxidized using performic acid before the acid hydrolysis. For determination of the tryptophan content of the whole seed and cotyledon proteins, a known amount of sample (100 mg) was dispersed into glass tubes together with 5 mL of papin (30 000 USP−U/mg, Merck) solution (400 mg of papin in 100 mL of 0.1 M sodium acetate buffer, pH 7.0). The tubes were closed tightly and incubated at 65 °C for 16 h. The tryptophan content of the enzyme hydrolysates was determined spectophotometrically by the method of Mertz et al. (1975). The contents of different amino acids recovered were presented as grams per 16 grams (g 16 g^{-1}) of nitrogen. The amino acid content of the reference protein was taken from FAO/WHO (1990). The essential amino acid (EAA) score was calculated:

$$\text{EAA score} = \frac{\text{g of EAA in 16 g N of test sample}}{\text{g of EAA in 16 g N of FAO/WHO ref. pattern}} \times 100 \quad (2)$$

Analysis of Fatty Acid Composition. Total lipids were extracted from the seed flour by using hexane at room temperature and overnight stirring on a magnetic stirrer. Further, this lipid was purified according to the method of Folch et al. (1957) using a chloroform and methanol mixture in the ratio of 2:1 (v/v). Methyl esters were prepared from the total lipids by the method of Lepage and Roy (1986). The fatty acid methyl esters were analyzed by gas chromatography (Perkin-Elmer, Norwalk, CT) using an instrument equipped with a flame ionization detector (FID; 350 grad/min) and a SP2330 Supelco Capillary Column (30m × 0.32 mm i.d.). The column temperature gradient ranged from 70 °C to 225 °C and the carrier gas was nitrogen at a flow rate of 2.0 mL/min. A standard fatty acid and methyl ester mixture was run, and retention times were used in identifying sample peaks. A response factor was calculated to correct GLC response of each fatty acid ester to make a common baseline. The methyl ester of pentadecanoic acid (C15:0) was used as an internal standard. Fatty acid levels were estimated on the basis of peak areas of known concentrations of the standards.

Analysis of Various Antinutritional Components. *Phenolic Substances.* Total phenols, tannins, and condensed tannins were determined by spectrophotometric methods described by Makkar et al. (1998). Total phenols were quantified by Folin−Ciocalteu reagent, and tannins were quantified as the difference of phenolics before and after tannin removal from the extact using insoluble polyvinylpolypyrrolidone. Condensed tannins were measured by butanol−HCl−Fe^{3+} reagent (Porter et al., 1986). Total phenols and tannins were expressed as tannic acid equivalent, and condensed tannins were expressed as leucocyanidin equivalent.

Non-Protein Amino Acid, L-Dopa, and Two Tetrahydroisoquinolines: Extraction and Identification. To 100 mg of finely ground whole seed and cotyledon flour in a glass tube was added 5 mL of 0.1 N HCl, and the samples were stirred for 10 min at room temperature. The mixture was subjected to Ultra-turrax T25 (20 500 min^{-1}) for 30 s in an ice bath, and subsequently it was kept on a magnetic stirrer for 1 h at room temperature. The supernatant was collected by centrifugation (13 000 rpm, 15 min). This extraction procedure was repeated twice, and the supernatants of all three extractions were pooled. Then the solution was filtered through a 0.2-μm glass filter and a 20-μL aliquot was injected into the HPLC (Siddhuraju and Becker, 2000). By using standards, the L-Dopa and two tetrahydroisoquinolines were identified.

HPLC Conditions for the Quantification of L-Dopa and Two Tetrahydroisoquinoline Compounds. The chromatograph consisted of a Merck Hitech Model L-7100 HPLC pump, an L-7450 photodiode array detector, an L-7200 autosampler, a D-7000 interphase module, and an LC organizer. The analytical column was reverse phase C18 (Nucleosil 120, mean particle diameter 5 μm) 250 × 4.6 mm i.d. A guard precolumn was packed with the same material as in the main column. Two solvents were used: (A) water, methanol, and phosphoric acid in the ratio of 975.5:19.5:1 (v/v/v) and (B) 70% methanol. Solvent (A) was prepared from analytical grade stocks, whereas solvent (B) was prepared by using HPLC grade methanol. The gradient used started with 100% A and 0% B up to 12 min; next 5 min solvent B increased from 0% to 100% with 100 to 0% decrease of solvent A; increase A to 100% and decrease B to 0% in the next 5 min; and then the column was washed with solvent A alone in the next 15 min to adjust the column to the starting conditions (A 100% and B 0.00%). Separation was performed at room temperature (22 °C) and the flow rate was 1.2 mL/min. The average elution times for the compounds, CDOTHIQ, MCDOTHIQ, and L-Dopa, were 5.2, 7.1, and 7.6 min, respectively. Absorbance was monitored at 282 nm, and peak heights and areas were determined. By using the authentic standards, the sample results were expressed. The standard for L-Dopa was procured from Sigma, whereas standards for the two tetrahydroisiquinolines were prepared in our laboratory by using the procedures of Bell et al. (1971) and Daxenbichler et al. (1972a and b).

Trypsin, Chymotrypsin, and α-Amylase Inhibitor Analyses. Trypsin inhibitor activity was determined essentially according to Smith et al. (1980) except that the enzyme was added last as suggested by Liu and Markakis (1989). Chymotrypsin inhibitor activity was assayed in a 0.1M borate buffer, pH 7.6, extract of defatted meal by the procedure of Kakade et al. (1970). One chymotrypsin unit is expressed as an increase of 0.01 absorbance unit at 275 nm in 10 min by the reaction mixture of volume 10 mL. Chymotrypsin inhibitor unit was defined in terms of chymotrypsin units inhibited per mg of sample. α-Amylase assay and α-amylase inhibitor activity was determined according to the procedure outlined by Deshpande et al. (1982). One unit of enzyme activity was defined as that which liberates 1 μmol of reducing groups (calculated as maltose)/min at 37 °C and pH 7.0 under the specified conditions from soluble starch. One unit of α-amylase activity inhibited was defined as one α-amylase inhibitor unit.

Phytohemagglutinating Activity. Analysis for lectin content was conducted by hemagglutination assay (as described by Gordon and Marquardt, 1974) in the presence of 10 mM Mn^{2+} in round-bottomed wells of microtiter plates using 2% (v/v) trypsinized cattle blood erythrocytes suspension in saline phosphate buffer, pH 7.0 (Makkar et al., 1997b). The lectin activity against 3% trypsinized type O human erythrocytes was determined as described by Tan et al. (1983). The hemagglu-

tination activity was defined as the minimum amount of the bean material (in mg per ml of the assay medium) which produced aggglutination. The minimum amount was the material per ml of the assay medium in the highest dilution which was positive for agglutination. One hemagglutinating unit (HU) was defined as the least amount of material per mL in the last dilution giving positive agglutination (Grant et al., 1983).

Estimation of Saponins and Phytic Acid. Total saponin (triterpinoid and steroidal) content was determined using a spectophotometric method described by Hiai et al. (1976). To 0.5 g portions of ground meal samples in screw-capped centrifuge tubes was added 10 mL of 80% aqueous methanol. The tubes were tightly capped and the contents were stirred overnight using a magnetic stirrer. The tubes were centrifuged at 3000*g* for 10 min, and the supernatants were collected in 25-mL volumetric flasks. The residues were washed three times with 5 mL of 80% aqueous methanol and centrifuged again, and the supernatants were collected in volumetric flasks. The final volume was made up to 25 mL with 80% aqueous methanol. Aliquot samples from the flasks were used for saponin determination. The results are expressed as diosgenin equivalent from a standard curve of different concentrations of diosgenin in 80% aqueous methanol. Saponins were also determined by hemolytic assay as described in Thilborg et al. (1994).

Phytate content of the sample was determined by a colorimetric procedure described by Vaintraub and Lapteva (1988) and as modified by Alonso et al. (1995). Ground samples (0.5 g each) were stirred in 10 mL of 2.4% HCl for 1 h. The contents were centrifuged at 3500 rpm for 10 min to obtain supernatants. The collected aliquot of the supernatants was diluted with distilled water (3:25). The pH of the diluted extract was adjusted to 6.00 with 1 M NaOH. Afterward, 10 mL of the diluted extract was quantitatively transferred to a column (0.7 $\times$ 15 cm) containing 0.5 g of Dowex 1 (AG 1 $\times$ 8 anion-exchange resin, chloride form, 8% cross-linkage, 100−200 mesh, Dow Corning Corp., Midland, MI). Inorganic phosphate was eluted with 15 mL of 0.1 M NaCl. Finally, phytate was eluted with 15 mL of 0.7 M NaCl and collected. Suitable aliquots of the eluents were diluted with distilled water to make 3 mL and then used for the assay. Results are expressed as percentage phytic acid by using standard phytic acid.

Analysis of Oligosaccharides and Total Soluble Sugars. *Preparation of Verbascose.* Oligosaccharides were extracted from dehulled, ground, and petroleum-ether-defatted seeds of green gram according to Amarowicz et al. (1992). A suspension containing 100 g of flour in 2000 mL of 60% methanol was heated at 90 °C for 3 h under a reflex condenser, cooled, and centrifuged at 3800 rpm for 10 min. Methanol was removed from the supernatant by using a rotary vacuum evaporator at a temperature not exceeding 50 °C. This aqueous solution of oligosaccharides was cooled, then purified through extraction in water−*n*-butanol (1:1). By using a separating funnel, the water phase was separated and lyophilised. To find the presence of oligosaccharides in the extract, the sucrose, raffinose, and stachyose standards were also stained on the Silica gel 60G (Merck) TLC plate by using a developing solvent system of *n*-butanol−acetone−water (75:50:50). Oligosaccharides were visualized by spraying the plate with aniline−diphenylamine−phosphoric acid reagents. Column chromatography on silica gel was performed to isolate and purify verbascose from the oligosaccharide mixture. Obtained lyophilisate (3.5 g) was dissolved in 15 mL of 60% methanol and transferred to a column (1 m long and 2.5 cm in diameter) supported with Silica gel 60G (Merck). Oligosaccharides were washed from the column with *n*-butanol−acetone−water (75:50:50). Fractions (30 mL) were collected manually. The presence of sugars was indicated with color reaction (Dubois et al., 1956). The oligosaccharides in each fraction were monitored with the TLC method. Those containing only verbascose were combined and concentrated by using a rotary vacuum evaporator. The purity of this verbascose standard was checked with the high-performance anion exchange (HPAE) chromatographic method.

Extraction of Soluble Sugars. Finely ground whole seed and dehulled samples (100 mg) were extracted in 5 mL of distilled water in screw cap tubes, in a boiling water bath with constant shaking for 1 h. Soluble polymers were precipitated with 96% ethanol (1 h, 4 °C). After centrifugation (3000*g* for 15 min), supernatants were evaporated at reduced pressure with a temperature not exceeding 50 °C. The samples were redissolved in double-distilled water and filtered through 0.22-μm filters, just prior to analysis by HPAE chromatography. The total soluble sugar content in the aqueous sample extracts was determined by the anthrone−sulfuric acid method by using glucose as a standard (Sadasivam and Manickam, 1992).

HPAE Analysis of Oligosaccharides. The chromatographic profiles of oligosaccharides were established using HPAE chromatography combined with pulsed amperometric detection (PAD) in a Dionex Series 300 DX ion chromatograph fitted with a CarboPac PA-1 column (Ernst and Krug, 1998). Flow rate was 1 mL/min at about 1900 psi. Injection volume was 30 μL. Two sodium hydroxide/sodium acetate gradients were used. Carbohydrate elution was effected under alkaline conditions (150 mM NaOH). The high pH (12−13) of the eluant converts hydroxyl groups of the oligosaccharides into oxyanion. The degree of oxyanion interaction with the anion-exchange resin determines carbohydrate retention times. Adding a competing ion such as acetate (0−500 mM NaOAc) to the eluent reduces the retention times. The oligosaccharides (sucrose, raffinose, stachyose, and verbascose) were identified and quantified by comparison with known standards: stachyose and raffinose purchased from Sigma, sucrose purchased from Merck, and verbascose prepared from dehulled green gram.

In-vitro Protein Digestibility. The in vitro protein digestibility of samples was measured according to the multi-enzyme technique (Satterlee et al., 1979). The in vitro protein digestibility of the sample was calculated using the regression equation $Y = 234.84 - 22.56(X)$, where Y= % protein digestibility and X = pH of protein suspension after 20 min digestion with four-enzyme solution.

Statistics. The data were subjected to a one-way analysis of variance (ANOVA) and the significance of difference between means was determined by Duncan's multiple range test ($P<0.05$) using the Excel program (Statistica for Windows, 5.1, 97 edition).

RESULTS AND DISCUSSION

Physicochemical properties of three germplasms of *Mucuna* beans are presented in Table 1. The seed density of all the germplasms (1.23−1.24 g mL^{-1}) is comparable to that of chick pea (1.29 g mL^{-1}) but higher than that of faba bean (0.75−0.78 g mL^{-1}) (Williams et al., 1983); and hydration capacity, hydration index, and swelling index are higher than those of chick peas (Williams et al., 1983). Mean grain weight, hydration capacity, hydration index, and swelling capacity of Marthandam germplasm (white variety) are significantly higher than those of Salem germplasm of white variety and Marthandam germplasm of black variety. The higher swelling and hydration capacity of mucuna seeds compared to other beans and peas suggests that they could be preferred to others in respect to these characteristics which are determining factors for food processes such as cooking and processing and, hence, would give scope for further product development.

The proximate composition of whole and dehulled seeds of the three germplasms in mucuna beans are presented in Table 2. Among the samples, Marthandam germplasm contained a significantly higher amount of crude protein (298.3 g kg^{-1}), followed by Salem germplasm (262.5 g kg^{-1}) of white variety, and Marthandam germplasm of black variety (243.1 g kg^{-1}), which seems to be comparable to an earlier report on *M. pruriens*

Table 1. Physicochemical Properties of Three Different Germplasm Seed Samples of *Mucuna pruriens* var. *Utilis*[a]

parameter	white variety: Marthandam germplasm	white variety: Salem germplasm	black variety: Marthandam germplasm
color of seed	white	white	black
seed weight (g 100 seeds^{-1})	$109.25^b \pm 2.51$	$78.79^c \pm 1.80$	$73.52^c \pm 3.88$
cotyledon weight (g 100 g^{-1} seeds)	$90.30^b \pm 2.74$	$88.18^b \pm 0.76$	$88.10^b \pm 0.42$
seed coat weight (g 100 g^{-1} seeds)	$9.70^b \pm 0.27$	$11.82^c \pm 0.34$	$11.90^c \pm 0.67$
seed density (g mL^{-1})	$1.24^b \pm 0.03$	$1.23^b \pm 0.03$	$1.23^b \pm 0.07$
seed volume (mL 100 seeds^{-1})	$88.00^b \pm 2.01$	$64.00^c \pm 1.47$	$60.00^c \pm 3.17$
hydration capacity (g seed^{-1})	$0.72^b \pm 0.02$	$0.60^c \pm 0.02$	$0.55^d \pm 0.03$
hydration index	$0.66^c \pm 0.02$	$0.76^b \pm 0.02$	$0.75^b \pm 0.04$
swelling capacity (mL seed^{-1})	$0.80^b \pm 0.02$	$0.64^c \pm 0.02$	$0.58^d \pm 0.03$
swelling index	$0.91^c \pm 0.02$	$1.00^b \pm 0.02$	$0.97^{b,c} \pm 0.05$

[a] Values are mean of three determinations ± standard deviation. [b–d] Values with the same superscript in each row do not differ significantly from each other ($p \leq 0.05$).

Table 2. Proximate Composition of Velvet Bean (*Mucuna pruriens* var. *utilis*) White (Two Germplasms) and Black (One Germplasm) Varieties of Whole and Dehulled Seed Samples (g kg^{-1} DM)[a]

component	whole seeds, white variety: Marthandam germplasm	whole seeds, white variety: Salem germplasm	whole seeds, black variety: Marthandam germplasm	dehulled seeds, white variety: Marthandam germplasm	dehulled seeds, white variety: Salem germplasm	dehulled seeds, black variety: Marthandam germplasm
dry matter	$898.3^c \pm 3.76$	$919.7^c \pm 9.46$	$903.7^c \pm 7.61$	$898.1^c \pm 9.00$	$893.1^c \pm 8.59$	$908.6^c \pm 10.21$
crude protein	$298.3^c \pm 11.45$	$262.5^d \pm 3.17$	$243.1^c \pm 4.09$	$335.5^c \pm 7.77$	$307.2^d \pm 9.62$	$303.2^d \pm 12.20$
lipid	$44.7^c \pm 3.68$	$46.8^c \pm 3.02$	$49.4^c \pm 1.40$	$46.1^d \pm 3.18$	$49.6^{c,d} \pm 3.40$	$53.5^c \pm 2.51$
ash	$34.4^d \pm 2.78$	$45.5^c \pm 3.42$	$38.6^d \pm 2.26$	$45.1^c \pm 2.92$	$47.9^c \pm 2.72$	$44.2^c \pm 3.17$
crude fiber	$87.6^c \pm 1.77$	$79.1^d \pm 1.78$	$90.4^c \pm 1.83$	$61.0^{c,d} \pm 2.64$	$58.7^d \pm 5.67$	$68.7^c \pm 4.51$
NFE[b]	535.1	566.1	578.5	512.3	536.6	530.4
gross energy value (MJ kg^{-1}DM[b])	$19.43^c \pm 0.02$	$19.15^c \pm 0.03$	$19.62^c \pm 0.04$	$19.63^c \pm 0.03$	$19.91^c \pm 0.03$	$19.69^c \pm 0.02$

[a] Values are mean of triplicate determinations ± standard deviation. [b] NFE, nitrogen free extractive; DM, dry matter basis. [c,d] Values with same superscript in each row of whole or dehulled samples do not differ significantly from each other ($p \leq 0.05$).

Table 3. Macro and Micro Mineral Composition of Whole and Dehulled Seed Samples of White (Two Germplasms) and Black Varieties (One Germplasm) of *Mucuna pruriens* var. *utilis*[a]

minerals	whole seeds, white variety: Marthandam germplasm	whole seeds, white variety: Salem germplasm	whole seeds, black variety: Marthandam germplasm	dehulled seeds, white variety: Marthandam germplasm	dehulled seeds, white variety: Salem germplasm	dehulled seeds, black variety: Marthandam germplasm
macro elements (mg 100 g^{-1} DM[b])						
phosphorus	443.0 ± 12.17	498.9 ± 8.12	376.5 ± 6.43	678.4 ± 11.20	620.7 ± 8.76	720.8 ± 17.13
potassium	1417.5 ± 21.65	1574.6 ± 20.61	1343.0 ± 31.41	1468.1 ± 15.87	1490.7 ± 26.52	1694.1 ± 28.11
calcium	101.7 ± 7.20	87.8 ± 5.42	104.5 ± 4.67	44.5 ± 2.10	49.0 ± 1.78	46.3 ± 3.28
magnesium	109.8 ± 3.81	119.7 ± 2.54	109.0 ± 4.01	81.9 ± 3.10	100.3 ± 1.72	97.8 ± 1.24
sodium	14.1 ± 0.62	12.7 ± 1.03	25.7 ± 0.43	18.3 ± 1.29	23.2 ± 0.91	14.9 ± 0.23
micro elements (mg 100 g^{-1} DM[b])						
copper	1.38 ± 0.05	2.42 ± 0.03	1.65 ± 0.07	1.30 ± 0.04	1.67 ± 0.12	2.59 ± 0.06
zinc	9.88 ± 0.37	5.26 ± 0.34	12.20 ± 1.02	6.35 ± 0.04	8.74 ± 0.03	7.62 ± 0.11
iron	7.07 ± 0.32	5.79 ± 0.26	7.47 ± 0.07	6.13 ± 0.08	7.44 ± 0.35	6.45 ± 0.42
manganese	2.09 ± 0.05	1.49 ± 0.08	2.41 ± 0.04	1.70 ± 0.16	1.74 ± 0.23	1.17 ± 0.05

[a] Values are mean of duplicate determinations ± standard deviation. [b] DM, dry matter basis.

(Siddhuraju et al., 1996) and higher than some of the commonly cultivated legumes (Bravo et al., 1998). The similar trend of germplasm and varietal influence on protein content has also been observed in *Vicia faba* (Makkar et al., 1997b). When compared to whole seeds, dehulled seeds appear to be relatively higher in crude protein, crude lipid, and ash contents and lower in crude fiber and NFE. Both whole and dehulled seeds have a high energy value (19.2–19.9 MJ kg^{-1}) due to the presence of a relatively high amount of lipid, and this value is comparable to the value for soybean (19.4 MJ kg^{-1}) (Makkar et al., 1997b).

Food legumes are a good source of minerals such as calcium, iron, copper, zinc, potassium, and magnesium (Salunkhe et al., 1985). The data on mineral composition (Table 3) indicated that all the germplasms (both whole and dehulled seeds) of this underexploited legume are found to be rich sources of potassium and have high contents of phosphorus, magnesium, zinc, and iron, comparable to common legumes such as *Vigna unguiculata*, *Cicer arietinum,* and *Pisum sativum* (Meiners et al., 1976). 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 body fluid.

The amino acid compositions and essential amino acid scores of the total seed proteins of whole seeds and dehulled seeds are given in Tables 4 and 5, respectively, along with those for FAO/WHO (1990) reference pattern and soyabean protein (Bau et al., 1994). The contents of essential amino acids such as valine, isoleucine, tyrosine, and phenylalanine in all the samples, and

Table 4. Amino Acid Composition and Essential Amino Acid Score of Whole Seed Samples of White (Two Germplasms) and Black (One Germplasm) Varieties of *Mucuna pruriens* var. *utilis*[a], FAO Reference Protein[b], and Soyabean

	amino acid composition (g 16 g^{-1} N)							
	white variety				black variety			
amino acids	Marthandam germplasm	essential amino acid score	Salem germplasm	essential amino acid score	Marthandam germplasm	essential amino acid score	reference[b]	soyabean[c]
aspartic acid	10.23		11.14		9.75			11.30
threonine	2.84	83.53	3.11	91.47	3.29	96.77	3.40	3.76
serine	3.55		3.44		3.60			5.67
glutamic acid	10.15		10.89		9.92			16.90
glycine	3.96		4.72		4.48			4.01
alanine	2.91		3.23		3.03			4.23
cystine	1.12	77.60	1.45	107.60	1.05	89.60	2.50	1.70
methionine	0.82		1.24		1.19			1.22
valine	4.18	119.43	4.80	137.14	4.74	135.43	3.50	4.59
isoleucine	4.07	145.36	4.68	167.14	4.52	161.43	2.80	4.62
leucine	5.86	88.79	6.38	96.67	6.32	95.76	6.60	7.72
tyrosine	3.96	129.21	3.35	124.60	5.18	169.40	6.30	3.39
phenylalanine	4.18		4.55		5.49			4.84
histidine	3.06	161.05	2.94	154.74	3.86	203.16	1.90	2.50
lysine	5.67	97.76	6.25	107.76	5.53	95.34	5.80	6.08
arginine	5.30		5.71		5.22			7.13
proline	4.40		4.80		5.09			4.86
tryptophan	0.84	76.36	0.77	70.00	0.94	85.46	1.10	1.24

[a] Values are of single determination. [b] Data from FAO/WHO, 1990. [c] Bau et al., 1994.

Table 5. Amino Acid Composition and Essential Amino Acid Score of Dehulled Seed Samples of White (Two Germplasms) and Black (One Germplasm) Varieties of *Mucuna pruriens* var. *utilis*[a], FAO Reference Protein, and Soyabean

	amino acid composition (g 16 g^{-1} N)							
	white variety				black variety			
amino acids	Marthandam germplasm	essential amino acid score	Salem germplasm	essential amino acid score	Marthandam germplasm	essential amino acid score	FAO/WHO reference values[b]	soyabean[c]
aspartic acid	9.92		9.66		10.01			11.30
threonine	2.72	80.00	2.95	86.76	3.01	88.53	3.40	3.76
serine	3.58		3.43		3.52			5.67
glutamic acid	9.89		9.55		9.29			16.90
glycine	3.65		3.97		4.03			4.01
alanine	2.75		2.84		2.87			4.23
cystine	1.10	82.40	1.42	99.20	1.52	98.40	2.50	1.70
methionine	0.96		1.06		0.94			1.22
valine	3.55	101.43	3.83	109.43	3.96	113.14	3.50	4.59
isoleucine	3.72	132.86	3.83	136.79	3.77	134.64	2.80	4.62
leucine	5.61	85.00	5.61	85.00	5.70	86.36	6.60	7.72
tyrosine	3.25	110.63	3.35	113.33	3.74	123.33	6.30	3.39
phenylalanine	3.72		3.79		4.03			4.84
histidine	3.15	165.79	2.70	142.11	3.05	160.53	1.90	2.50
lysine	5.28	91.03	5.29	91.21	5.66	97.59	5.80	6.08
arginine	5.48		4.92		4.97			7.13
proline	4.71		4.81		5.33			4.86
tryptophan	0.91	82.73	1.06	96.36	0.98	89.09	1.10	1.24

[a] Values are of single determination. [b] Data from FAO/WHO, 1990. [c] Bau et al., 1994.

sulfur amino acids in Salem germplasm (whole seed) of white variety, were found to be higher than those of the FAO/WHO (1990) recommended pattern. The other essential amino acids, leucine and lysine, were at levels comparable to the reference pattern. The contents of tryptophan and threonine in all the samples, and particularly sulfur amino acids in Marthandam germplasms of white and black variety, seem to be the limiting amino acids. However, except for sulfur amino acids and tryptophan, levels of all other essential amino acids are more or less comparable to soyabean protein (Bau et al., 1994). No wider variations between the amino acid profiles of whole and dehulled seed proteins of respective samples have been observed.

Table 6 shows the total, digestible, and resistant starch (TS, DS, and RS, respectively) contents of different germplasms of whole and dehulled mucuna beans. Average values of total, digestible, and resistant starch of all germplasms of whole seeds are 27.8%, 17.6%, and 10.15%, respectively and no significant variation has been observed between the germplasms. However, when compared to whole seeds, dehulled samples contain higher total (34.3%), digestible (20.5%), and resistant (14.2%) starch contents. Among the different germplasms of dehulled samples, digestible starch content in Marthandam germplasm of black variety and resistant starch content in Salem germplasm of white variety seem to be significantly higher. The total and digestible starch values in dehulled samples of all the germplasms seem to be quite similar to those of chick pea and horse gram (Bravo et al., 1999). In general, mucuna beans contain apparently low DS contents due

Table 6. Composition of the Starch Fractions of Three Different Germplasms of *Mucuna pruriens* var. *utilis* in Whole and Dehulled Seeds (g 100 g^{-1} DM)[a]

	total starch	digestible starch[b]	resistant starch
	whole seeds		
	white variety		
Marthanda germplasm	26.90^{d} ± 0.82	17.13^{d} ± 0.41 (63.67)[c]	9.77^{d} ± 0.64
Salem germplasm	28.09^{d} ± 0.71	17.92^{d} ± 1.13 (63.80)	10.17^{d} ± 0.45
	black variety		
Marthandam germplasm	28.30^{d} ± 0.78	17.78^{d} ± 1.14 (62.83)	10.52^{d} ± 0.70
	dehulled seeds		
	white variety		
Marthanda germplasm	33.10^{d} ± 0.57	19.67^{e} ± 0.51 (59.49)	13.40^{e} ± 0.82
Salem germplasm	35.02^{d} ± 0.49	19.86^{e} ± 0.77 (56.71)	15.16^{d} ± 0.46
	black variety		
Marthandam germplasm	36.04^{d} ± 0.66	21.96^{d} ± 0.73 (60.94)	14.10^{e} ± 0.24

[b] Calculated by difference, as TS − RS (total starch − resistant starch). [a] Results are mean of three determinations ± standard deviation. DM, dry matter basis. [d,e] Values with same superscript in each column of whole or dehulled samples do not differ significantly from each other ($p \leq 0.05$). [c] Values in parentheses indicate the percent of total starch.

to the presence of a very high amount of RS (mainly RS_2): about 40% of the TS. These results correspond with the previous report on black gram (Bravo et al., 1999). Starch in raw foods is contained within granules that are poorly affected by hydrolytic enzymes, and degree of crystallinity or amylose/amylopectin ratio of the starch granules are major factors known to affect starch digestibility (Englyst et al., 1992). This accounts for the high RS content of raw legumes. However, during hydrothermal processing the starch granules are gelatinized and partially solubilized, thereby becoming more available to digestive enzymes and reducing the level of RS.

The fatty acid compositions of the total seed lipids of three germplasms of mucuna beans are given in Table 7. The data reveal that all the seed lipids are rich in unsaturated fatty acids (64.7−66.9%), and have very high contents of linoleic acid (48−49%) and low levels of saturated fatty acids. These values are nutritionally desirable and also comparable to those of certain common legume seeds (Salunkhe et al., 1985). The samples also contain more palmitic acid (19.6−21.8%) and stearic acid (7.0−7.5%) than the other legume seeds, and they have a fair amount of behenic acid (3.36−3.76%). Similar results were reported by Achinewhu (1982) in *Mucuna uriens* seed fat. With regard to the fatty acid profiles, no variations have been observed among the germplasms of white and black variety.

The data on dietary fiber (DF) composition of the three different germplasms of mucuna beans (whole seeds) are given in Table 8. Among the different germplasms, Marthandam germplasm of black variety registers relatively more content of total dietary fiber (TDF, 19.5%) than the content of the other two germplasms, namely, Marthandam (18.0%) and Salem (18.8%) of white variety. In all the samples TDF is mainly composed of insoluble dietary fiber (about 92−95%), with soluble dietary fiber (SDF) accounting for only between 5 and 8%. The neutral sugars such as arabinose (3.54−3.75%), xylose (2.05−2.70%), glucose (2.0−2.53%), and uronic acid (1.65−1.87%) are found to be the dominating insoluble dietary fiber components in all the investigated germplasms of mucuna beans. Even though the pectic substances, both neutral (arabinogalactans) and acidic (galacturonates), are the main soluble nonstarch polysaccharides in the SDF, their concentrations are very low when compared to the respective constituents of IDF. The uronic acid content of IDF, which is higher than that in SDF, suggests the presence of pectic substances linked to other cell wall polysaccharides. Low amounts of rhamnose and traces of fucose and ribose are also present in both IDF and SDF, and similar results in common legumes have also been reported by Bravo et al. (1999). Because of the presence of high concentrations of phenolics, all the samples seem to contain more Klason lignin (KL, 6.64−7.68%). Some polyphenolics may be retained along with resistant protein and lignin in the residue quantified as KL (Sauro-Calixto et al., 1991).

The contents of total soluble sugars and oligosaccharides in the three germplasms of mucuna (whole and dehulled seeds) are shown in Table 9. The oligosaccharides, such as sucrose, raffinose, stachyose, and verbascose, were detected in all the samples. The total content of soluble sugars measured by the anthrone method, expressed as glucose, range from 9.2 to 10.5% in whole seeds and from 10.1 to 11.5% in dehulled seeds. These values are similar to those of common legumes such as *Cajanus cajan* (3.5−10.2%), *Cicer arietinum* (3.5−9.0%), and *Vigna unguiculata* (6.0−13.0%) (Reddy et al., 1984). The α-galactosides of total sugars in all the germplasms of mucuna amount to 49.7−54.8% in whole seeds and 51.3−56.0% in dehulled samples. These results show the content of α-galactosides represents a large proportion of the total soluble carbohydrate content, compounds which are related to the production of flatulence. Within the oligosaccharides, verbascose is present in higher amounts in both whole (2.45−2.87%) and dehulled (2.79−3.32%) seeds as in the case of faba bean (3.32%) (Ruperez, 1998). Stachyose and verbascose (but not raffinose) contents in both whole and dehulled samples of mucuna beans seem to be higher when compared to those in earlier reports of Revilleza et al. (1990) and Vijayakumari et al. (1996) in *M. pruriens.* Among the three germplasms of both whole and dehulled seeds, Marthandam germplasm of white variety appears to contain a high level of oligosaccharides. These variations are mainly influenced by the environmental factors with seed maturity conditions rather than genetic characteristics.

The data on various antinutritional factors are presented in Tables 10 and 11. Among the different germplasms of whole seeds, the contents of total phenols (TP) and tannins (T) are significantly higher in the black variety (6.1 and 0.6%, respectively) than in the two germplasms of the white variety, Marthandam and Salem (5.54 and 0.37%; 5.24 and 0.28%, respectively). The TP values appear to be similar to those of earlier reports in *M. pruriens* (Siddhuraju et al.,1996) and higher than those of different cultivars of faba bean and soyabean (Makkar et al., 1997b). All the germplasms of whole seeds contain negligible amounts of tannins. The dehulled samples of the respective germplasms contain moderately low levels of TP (10−30%) and quite lower levels of T (60−80%) than whole seeds. With regard to the content of TP and T, levels in the

Table 7. Fatty Acid Composition of Three Different Germplasm (Whole) Seed Materials of *Mucuna pruriens var. utilis* Lipids[a] (%)

	white variety		black variety
fatty acid	Marthandam germplasm	Salem germplasm	Marthandam germplasm
C12:0 (lauric)	0.06	0.03	ND
C14:0 (myristic)	0.17	0.16	0.17
C14:1 (myristoleic)	ND	0.02	ND
C16:0 (palmitic)	19.61	20.05	21.81
C16:1 (palmitoleic)	0.31	0.29	0.30
C17:1 (*cis*-10-heptadecenic)	0.07	0.06	0.07
C18:0 (stearic)	7.53	7.06	7.43
C18:1n9t (elaidic)	0.03	0.03	0.03
C18:1n9c (oleic)	7.99	8.33	6.95
C18:2n6t (linolelaidic)	1.67	2.66	1.50
C18:2n6c (linoleic)	49.15	48.75	47.98
C18:3n3 (linolenic)	6.83	6.52	7.67
C20:0 (arachidic)	1.51	1.40	1.46
C20:1n9 (*cis*-11-eicosenoic)	0.11	0.12	0.09
C21:0 (henicosanoic)	0.05	0.05	0.05
C20:2 (*cis*-11,14-eicosadienoic)	0.03	0.05	0.05
C20.3n6 (*cis*-8,11,14-eicosatrienoic)	0.04	0.04	0.02
C22:0 (behenic)	3.76	3.43	3.36
C22:5n3 (*cis*-7,10,13,16,19-docosapentaenoic)	ND	0.04	0.04
C23:0 (tricosanoic)	0.10	ND	0.09
C24:0 (lignoceric)	0.96	0.91	0.87
C26:0 (cerotic)	ND	ND	0.05
total unsaturated fatty acids	66.23	66.91	64.70
total saturated fatty acids	33.75	33.09	35.29
essential fatty acids	55.98	55.27	55.65

[a] Average values of two independent determinations. ND, not detected.

Table 8. Composition of the Dietary Fiber Fractions of Three Germplasm (Whole) Seed Samples of *Mucuna pruriens* var. *utilis* (g 100 g^{-1} DM)[a]

	white variety						black variety		
	Marthandam germplasm			Salem germplasm			Marthandam germplasm		
	IDF	SDF	TDF	IDF	SDF	TDF	IDF	SDF	TDF
rhamnose	0.162	0.030	0.192	0.188	0.025	0.213	0.120	0.033	0.153
fucose	0.035	0.015	0.050	0.024	0.022	0.046	0.060	0.019	0.079
ribose	0.096	0.033	0.129	0.116	0.043	0.159	0.090	0.047	0.137
arabinose	3.570	0.175	3.745	3.756	0.105	3.861	3.545	0.112	3.657
xylose	2.052	0.038	2.090	2.708	0.063	2.771	2.702	0.078	2.780
mannose	0.087	0.068	0.155	0.041	0.079	0.120	0.074	0.093	0.167
galactose	0.320	0.068	0.388	0.339	0.075	0.414	0.345	0.069	0.414
glucose	2.469	0.058	2.527	2.157	0.074	2.231	2.004	0.058	2.062
total neutral sugars	8.790	0.485	9.275	9.327	0.486	9.813	8.939	0.509	9.448
uronic acids	1.645	0.408	2.053	1.778	0.474	2.252	1.871	0.546	2.417
klason lignin	6.641		6.641	6.784		6.784	7.684		7.684
total dietary fiber	17.076	0.893	17.969	17.889	0.960	18.849	18.493	1.055	19.548

[a] Average values of two independent determinations. IDF, insoluble dietary fiber; SDF, soluble dietary fiber; TDF, total dietary fiber; DM, dry matter basis.

Marthandam germplasm of black variety have been found to be significantly higher than levels in the two germplasms of white variety as in the case of faba bean cultivars (Makkar et al., 1997b). Only trace amounts of condensed tannins have been detected in all the germplasms. 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 (Vijayakumari et al., 1996).

Phytic acid has an antinutritional property because of its ability to lower the bioavilability of essential minerals, and to form a complex with proteins, thereby inhibiting the enzymatic digestion of ingested protein (Nolan and Duffin, 1987). The phytate content of all germplasms of dehulled mucuna seeds (1.05−1.22%) seems to be higher than that of whole seeds (0.86−1.10%) because phytate (P) is known to be the primary storage form of P in mature legume seeds and their values are lower than those of some of the pea cultivars (Alonso et al., 1998). Regarding the content of phytate, among whole seeds the Salem germplasm of white variety has been found to contain significantly higher amounts than the other two germplasms, namely Marthandam of white and black variety. However, no significant variation has been observed between the different germplasms of dehulled samples. It is worthwhile to note that the phytate content in mucuna beans could be substantially eliminated by processing methods such as soaking and cooking (Vijayakumary et al., 1996).

Among the different samples of mucuna beans, the Salem germplasm of white variety has been found to contain significantly higher amounts of saponins (1.32 and 1.19% in whole and dehulled seeds, respectively) than the other two Marthandam germplasms of white

Table 9. Total Soluble Sugars and Oligosaccharide Contents in Whole and Dehulled Seeds of Three Different Germplasms of *Mucuna pruriens* var. *utilis* (g 100 g^{-1} DM)[a]

	sucrose	raffinose	stachyose	verbascose	total oligosaccharides	total α-galactosides	total soluble sugars[b]
whole seeds							
white variety							
Marthandam germplasm	2.26 ± 0.03	1.192 ± 0.02	1.46 ± 0.04	2.87 ± 0.15	7.79	5.53 (54.82)	10.08 ± 0.51
Salem germplasm	2.45 ± 0.07	0.90 ± 0.04	1.22 ± 0.06	2.66 ± 0.08	7.22	4.78 (49.65)	9.62 ± 0.61
black variety							
Marthandam germplasm	2.32 ± 0.10	0.91 ± 0.05	1.27 ± 0.04	2.45 ± 0.06	6.94	4.62 (50.34)	9.19 ± 0.13
dehulled seeds							
white variety							
Marthandam germplasm	2.49 ± 0.07	1.29 ± 0.04	1.82 ± 0.03	3.32 ± 0.08	8.92	6.43 (55.98)	11.48 ± 0.26
Salem germplasm	2.49 ± 0.12	1.08 ± 0.05	1.40 ± 0.02	3.00 ± 0.06	7.96	5.48 (51.32)	10.67 ± 0.57
black variety							
Marthandam germplasm	2.75 ± 0.16	1.17 ± 0.05	1.55 ± 0.09	2.79 ± 0.20	8.26	5.51 (54.85)	10.05 ± 0.34

[a] Values are mean of duplicate determinations ± standard deviation. Data in parentheses are percentage of total soluble sugars. DM, dry matter basis. [b] Estimated by the anthrone method.

Table 10. Levels of Various Heat-Stable Antinutritonal Factors in Different Germplasms of Whole and Dehulled Seed Samples, *Mucuna pruriens* var. *utilis* (Values are on Dry Matter Basis)[a]

	total phenols[b] (g 100 g^{-1})	tannins[c] (g 100 g^{-1})	condensed tannins[c] (g 100 g^{-1})	phytic acid (g 100 g^{-1})	saponins[d] (g 100 g^{-1})	L-DOPA (g 100 g^{-1})	CDTHIQ (mg 100 g^{-1})	MCDTHIQ (mg 100 g^{-1})
whole seeds								
white variety								
Marthandam germplasm	$5.539^{f} \pm 0.05$	$0.365^{f} \pm 0.03$	trace	$0.904^{f} \pm 0.02$	$1.150^{f} \pm 0.03$	$4.700^{e} \pm 0.01$	$131.5^{e} \pm 1.50$	$115.00^{e} \pm 4.00$
Salem germplasm	$5.237^{g} \pm 0.13$	$0.289^{g} \pm 0.02$	trace	$1.103^{e} \pm 0.12$	$1.319^{e} \pm 0.05$	$3.625^{g} \pm 0.07$	$85.75^{g} \pm 0.95$	$62.40^{g} \pm 3.04$
black variety								
Marthandam germplasm	$6.142^{e} \pm 0.16$	$0.552^{e} \pm 0.03$	trace	$0.856^{f} \pm 0.02$	$1.172^{f} \pm 0.05$	$4.387^{f} \pm 0.03$	$117.6^{f} \pm 2.29$	$92.50^{f} \pm 1.60$
dehulled seeds								
white variety								
Marthandam germplasm	$5.202^{e} \pm 0.08$	$0.120^{e} \pm 0.02$	trace	$1.052^{e} \pm 0.06$	$0.926^{f} \pm 0.07$	$5.054^{e} \pm 0.10$	$138.42^{e} \pm 1.93$	$123.66^{e} \pm 1.04$
Salem germplasm	$5.048^{e} \pm 0.14$	$0.107^{e} \pm 0.01$	trace	$1.155^{e} \pm 0.16$	$1.186^{e} \pm 0.10$	$4.073^{f} \pm 0.09$	$96.35^{g} \pm 1.90$	$70.11^{g} \pm 0.73$
black variety								
Marthandam germplasm	$5.121^{e} \pm 0.26$	$0.135^{e} \pm 0.01$	trace	$1.224^{e} \pm 0.07$	$1.098^{e} \pm 0.07$	$4.874^{e} \pm 0.14$	$130.67^{f} \pm 1.56$	$102.78^{f} \pm 1.94$

[a] Values are mean of triplicate determinations ± standard deviation. Trace, less than 0.005% level. L-DOPA, L-3,4-dihydroxyphenylalanine. CDTHIQ, L-3-carboxy-6,7-dihydroxy-1,2,3,4,-tetrahydroisoquinoline. MCDTHIQ, 1-methyl-3-carboxy-6,7-dihydroxy-1,2,3,4,-tetrahydroisoquinoline. [b] As tannic acid equivalent. [c] As leucocyanidin equivalent. [d] As diosgenin equivalent. [e−g] Means followed by same superscript in a column of whole or dehulled seeds are not statistically significant ($p \leq 0.05$).

and black variety (1.15 and 1.17; 0.93 and 1.10%), respectively. However the values of saponins in dehulled seeds of all the germplasms seems to be relatively lower (10−20%) than the respective whole seeds. With respect to both whole and dehulled seeds, no significant variation has been observed between the germplasm of white and black variety collected from Marthandam. Saponins comprise a large family of structurally related compounds containing a steroid or triterpinoid aglycon (sapogenin) linked to one or more oligosaccharide moieties; therefore, not all the saponins have the same pathogenicity. Even though saponins from some plants in general produce adverse effects on the growth of animals (especially nonruminents), some plant saponins have been shown to have positive effects (Makkar et al., 1997b; Liener, 1994a). When compared to soyabean saponin content (4.94%, Makkar et al., 1997b), in all the germplasms of the presently investigated mucuna beans, both whole and dehulled, contain low levels (about 70%) of saponins, and none of the germplasms appear to have a positive hemolytic effect against cow erythrocytes as does soyabean (Makkar et al., 1997b).

Among the different germplasms of whole seeds, Marthandam germplasm of white variety contained the highest levels of L-dopa (4.7%), nonmethylated tetrahydroisiquinoline (0.132%), and methylated tetrahydroisoquonoline (0.115%); the lowest amount of L-dopa (3.63%), and nonmethylated (0.086%) and methylated (0.062%) tetrahydroisoquinolines were found in the Salem germplasm of white variety. When compared to whole seeds, respective germplasms of dehulled seeds seemed to contain a relatively (10−15%) higher amount of L-dopa, non-methylated and methylated tetraisoquinoline compounds, because more than 90% of these

Table 11. Various Heat-Labile Antinutritional Components and in Vitro Protein Digestibility of Three Different Germplasms of *Mucuna pruriens var. utilis* Whole and Dehulled Seed Samples (Values are on Dry Matter Basis[a]

	trypsin inhibitor activity[b]	chymotrypsin inhibitor activity (CIU mg^{-1} sample)	α-amylase inhibitor activity (AIU g^{-1} sample)	hemagglutinating activity (mg ml^{-1}) cattle erythrocytes[c]	human blood group O[d]	in vitro protein digestibility (%)
			whole seeds			
			white variety			
Marthandam germplasm	$13.78^e \pm 0.16$	$10.97^e \pm 0.81$	$1.38^e \pm 0.23$	51.00 (0.19)	Nil	$68.12^f \pm 0.23$
Salem germplasm	$15.89^d \pm 0.41$	$12.50^d \pm 0.56$	$1.27^e \pm 0.20$	52.02 (0.19)	Nil	$70.27^d \pm 0.39$
			black variety			
Marthandam germplasm	$13.84^e \pm 0.23$	$10.81^e \pm 0.63$	$4.07^d \pm 0.21$	51.42 (0.19)	Nil	$69.36^e \pm 0.23$
			dehulled seeds			
			white variety			
Marthandam germplasm	$14.40^f \pm 0.45$	$13.80^e \pm 0.12$	$1.75^e \pm 0.16$	50.66 (0.20)	Nil	$68.91^e \pm 0.49$
Salem germplasm	$16.39^e \pm 0.42$	$14.57^e \pm 0.86$	$1.57^e \pm 0.29$	50.26 (0.20)	Nil	$70.26^d \pm 0.38$
			black variety			
Marthandam germplasm	$19.25^d \pm 0.62$	$15.96^d \pm 0.56$	$4.95^d \pm 0.06$	51.12 (0.20)	Nil	$67.45^f \pm 0.24$

[a] Values are mean of triplicate determinations ± standard deviation. [b] Units are mg pure trypsin inhibited g^{-1} sample; CIU, chymotrypsin inhibitor unit; AIU, amylase inhibitor unit. [c] Values in parentheses indicate the hemagglutinating unit (HU mg^{-1} sample). [d] Nil, no hemagglutination. [e–g] Means followed by same superscript in a column of whole or dehulled seeds are not statistically significant ($p \leq 0.05$).

compounds are concentrated in cotyledons as in the case of other *Mucuna* spp.(Daxenbichler et al., 1972b). These quinoline compounds are similar to other tetrahydroisiquinolines used in reducing the blood pressure (Amarasekara and Jansz, 1980). The levels of L-dopa content in all the germplasms of whole seeds have been found to be lower than those in previous studies in *M. pruriens* (Siddhuraju et al.,1996) and similar to those of other *Mucuna* spp.(Lorenzetti et al., 1998). However, the pharmacologically active factor, L-dopa (Pieris et al., 1980), is potentially toxic (Duke, 1981; Afolabi et al., 1985) if ingested in large amounts. L-dopa, a compound chiefly used in the treatment of Parkinson's disease, has been reported to have the serious side effect of causing hallucinations, in addition to causing gastrointestinal disturbances such as nausea, vomiting, and anorexia (Reynolds, 1989). It has also been shown to be toxic in individuals with glucose-6-phosphate dehydrogenase deficiency in their erythrocytes, and as a result, to induce favism (Nechama and Edward, 1967). Recently, Takasaki and Kawakishi (1997) have reported that the oxidation products of L-dopa conjugate with SH compounds (cysteine) of proteins to form a protein-bound 5-*S*-cysteinyldopa cross link and it leads to polymerization of proteins and/or other protein, although the amount of formation is low. These compounds are readily oxidizable in alkaline pH and high temperatures with more moisture and form a dark colored compound (personal observations, unpublished). This might also be one of the factors which could be responsible for lowering of protein and starch digestibilities. However, L-dopa and the other two quinoline compounds are found to be soluble in water, and it could be possible for consumers to remove or reduce them by adopting simple household processing methods such as water-soaking with boiling.

The mean values for the trypsin, chymotrypsin, and amylase inhibitor activities, hemagglutinating activity, and in vitro protein digestibility of the different germplasms of mucuna beans (whole and dehulled seeds) are presented in Table 11. In whole seeds, trypsin (15.89 mg/g) and chymotrypsin (12.50 CIU/mg) inhibition activity of Salem germplasm of white variety was significantly higher than that in the other two germplasms, namely Marthandam of white and black varieties (13.78 and 13.84 mg/g; 10.97 and 10.81 CIU/mg, respectively), and these values have been found to be relatively higher (about 10–30%) in the respective decorticated samples. The trypsin inhibitor activities of all the studied samples (whole seeds) were higher than that of cow pea (2.54 mg/g) *Phaseolus lunatus,* and *Dolichos lablab* (2.11 mg/g) (Aletor and Aladetimi, 1989), and different cultivars of *Vicia faba* (1.72–3.35 mg/g) (Makkar et al., 1997b), and they also seem to be lower than that of soyabeans (29.1–30.2 mg/g) (Smith et al., 1980). The chymotrypsin inhibitor activity of all the germplasms in both whole and dehulled samples has been found to be higher than that of pigeon pea raw sample (0.2 CIU/mg) (Mulimani and Paramjothi, 1995). Protease inhibitors induce pancreatic hypertrophy and hyperplasia which lead to depression of growth in animals (Liener, 1994b). Even though the mucuna beans contain high levels of protease inhibitors, they have been found to be inactivated partially (Siddhuraju et al., 1996) and completely (Udedibi and Carlini, 1998) by cooking processes. Such processed mucuna beans could be a valuable addition to monogastric diets when supplemented with cereal protein, specifically wheat flour (de la Vega and Sotelo, 1981). The levels of α-amylase inhibitor activity in whole and dehulled seeds of all the germplasms range between 1.27 and 4.07 AIU/g and 1.57 and 4.95 AIU/g, respectively. AIU activity in the Marthandam germplasm of black variety seems to be significantly higher than that in the two germplasms of white variety. When compared to different varieties of chick pea (Mulimani et al., 1994) and *M. pruriens* (Siddhuraju et al., 1996), the presently investigated germplasms show a negligible level of AIU activity. This could be inactivated when subjected to hydrothermal processing techniques.

Lectins combine with the cells that line the intestinal mucosa and cause a nonspecific interference with the absorption of available nutrients, and also reduce feed intake (Liener, 1994b). Phytohemagglutinating activity (HU/mg sample) of both whole and dehulled samples of all the germplasms against cow erythrocytes shows weak agglutination of 0.19−0.20; these values have been observed to be statistically similar. When compared to some of the cultivars of faba bean and soybean (Makkar et al., 1997b), the lectin activity of all the germplasms of both whole and dehulled seeds of white and black varieties of *Mucuna pruriens* has been found to be much lower. However, all the germplasms of both whole and dehulled seeds failed to agglutinate O-type human erythrocytes. Similarly, Udedibie and Carlini (1998), in their study of Brazilian *M. pruriens* seeds, have reported the lack of hemagglutinating activity against the erythrocytes of the three human blood groupings. However, the presence of phytohemagglutinating activity in *M. pruriens* seeds against the different human blood erythrocytes (namely A, B, and O systems) (Siddhuraju et al., 1996), and specific to the B system in *M. flagellipes* (Mbadiwe and Agogbua, 1978), have also been reported. Hence, the above-reported findings reveal that the concentrations of toxic and antinutritional factors are known to be greatly influenced by climatic and ecological conditions also, rather than being based on genetic characteristics alone. In general, germplasms of the white variety were found to contain a relatively higher amount of certain antinutrients than the germplasm of black variety.

The in vitro protein digestibility (IVDP) of raw mucuna bean (whole and dehulled) flour (67.45−70.27%) is similar to that of raw chick pea (Attia et al., 1994) and soyabean and lower than that of raw kidney beans (Ekfenyong and Borchers, 1979). Similarly, in *M. pruriens* a low level of protein digestibility (48%) in vivo has also been reported (Siddhuraju et al., 1996). Among the different germplasms of whole seeds, the Marthandam germplasm of white variety seems to have a significantly lower level of IVDP; whereas among the dehulled samples, the Marthandam germplasm of black variety has been found to contain a significantly lower level of IVDP. Such a low level of IVDP in the mucuna samples might be due to the presence of high levels of protease inhibitors and phytic acid along with a high concentration of the phenolic nonprotein amino acid L-dopa and a substantial amount of tetrahydroisoquinoline compounds.

CONCLUSIONS

From the preceding discussion it appears that all the germplasms of *Mucuna pruriens* var. *utilis* are rich in crude protein, essential fatty acids, energy value, dietary fiber, starch content, most of the macro and micro elements, and certain essential amino acids, and have favorable physicochemical properties such as high levels of hydration and swelling capacity. However, substantial quantities of various antinutritional factors, such as protease inhibitors, total phenolics, flatulence-producing factors (raffinose, stachyose, and verbascose), L-dopa, and methylated and nonmethylated tetrahydroisoquinolines are present. After inactivation or removal of such antinutrients by adopting economically viable and indigenous processing techniques (after conducting suitable animal feeding experiments), the mucuna bean can serve as a cheap and alternate protein source not only for the teeming population of the tropics but also for animals in those regions. Further, it could also be a valuable addition to monogastric diets when supplemented with cereal proteins. Further research studies on toxicological effects, inactivation of detected antinutrients, and protein and starch quality evaluation through in vitro and in vivo systems are in progress.

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LITERATURE CITED

Achinewhu, S. C. Composition and food potential of African oil bean (*Pentaclethra macrophylla*) and velvet bean (*Mucuna uriens*). *J. Food Sci.* **1982**, *47*, 1736−1737.

Afolabi, O. A.; Osshuntogun, B. A.; Adewusi, S. R.; Fapojuwo, O. O.; Ayorinde, F. O.; Grissom, F. E.; Oke, O. L. Preliminary nutritional and chemical evaluation of raw seeds from *Mucuna solanei*: an underutilized food source. *J. Agric. Food Chem.* **1985**, *33*, 122−124.

Aletor, V. A.; Aladetimi, O. O. Compositional evaluation of some cowpea varieties and some under-utilised edible legumes in Nigeria. *Nahrung* **1989**, *33*, 999−1007.

Alonso, R.; Fruhbeck, G.; Aguirre, A.; Castiella, V.; Marzo, F.; Santidrian, S. C. Colorimetric determination of total phytate in raw seeds of *Vicia faba* L. after anion-exchange chromatography. In: *2nd European Conference on Grain Legumes-Improving Production and Utilisation of Grain Legumes*; AEP (European Association for Grain Legume Research) Ed.; July 9−15, Copenhagen, Denmark, 1995; p 377.

Alonso, R.; Orue, E.; Marzo, F. Effect of extrusion and conventional processing methods on protein and antinutritional factor contents in pea seeds. *Food Chem.* **1998**, *63*, 505−512.

Amarasekara, A. E.; Jansz, E. R. Studies on *Mucuna* species of Sri Lanka. II. Determination of Tetrahydroisoquinoline content of seeds. *J. Natl. Sci. Found. Sri Lanka* **1980**, *8*, 99−103.

Amarowicz, R.; Ciska, E.; Kozlowska, H. Method for obtaining verbascose standard. *Pol. J. Food Nutr. Sci.* **1992**, *1/42*, 125−128.

Arora, R. K. Native food plants of the Northeastern tribals. In *Glimpses of Indian Ethnobotany*; Jain, S. K. Ed.; Oxford and IBH Publishing Co.: New Delhi, India, 1981; pp 91−106.

AOAC. *Official Methods of Analysis*, 13th ed.; Association of Official Analytical Chemists: Washington, DC, 1980.

Attia, R. S.; Shehata, M. E. T.; Aman, A. E.; Hamza, M. A. Effects of ripening and parching on the quality characteristics, the chemical composition and the nutritive value of chickpea (*Cicer arietinum* L). *J. Sci. Food Agric.* **1994**, *65*, 385−390.

Bassler, R.; Buchholz, H. Amino acid analysis. In *Methodenbuch, Die Chemische Untersuchung von Futtermitteln;* VD-LUFA-Verlag: Darmstadt, Germany, 1993; vol. III, section 4.11.1, pp 1−5.

Bau, H. M.; Villaume, C.; Lin, C. F.; Evard, F.; Quemener, B.; Nicolas, J. P.; Mejean, L. Effect of solid-state fermentation using *Rhizophus oligosporus* sp. T-3 on elimination of antinutritional substances and modification of biochemical constituents of defatted rapeseed meal. *J. Sci. Food Agric.* **1994**, *65*, 315−322.

Bell, E. A.; Janzen, D. H. Medical and ecological considerations of L-Dopa and 5-HTP in seeds. *Nature* **1971**, *229*, 136−137.

Bell, E. A.; Nulu, J. R.; Cone, C. L-Dopa and L-3-carboxyl-6,7-dihydroxy- 1,2,3,4-tetrahydroisoquinoline, a new amino acid,

from seeds of *Mucuna mutisiana. Phytochem.* **1971**, *10*, 2191−2194.

Bravo, L.; Sidddhuraju, P.; Saura-Calixto, F. Effect of various processing methods on the *in vitro* starch digestibility and resistant starch content of Indian pulses. *J. Agric. Food Chem.* **1998**, *46*, 4667−4674.

Bravo, L.; Sidddhuraju, P.; Saura-Calixto, F. Composition of underexploited Indian pulses. Comparison with common legumes. *Food Chem*. **1999**, *64*, 185−192.

Buckles, D. Velvetbean: a 'new' plant with a history. *Econ. Bot*. **1995**, *49*, 13−25.

Conway, G.; Toenniessen, G. 1999. Feeding the world in the twenty-first century. *Nature* **1999**, *402* (*Suppl.*), C55−C68.

Daxenbichler, M. E.; Kleiman, R.; Weisleder, D.; VanEtten, C. H.; Carlson, K. D. A new amino acid, (−)-1-methyl-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, from velvet bean. *Tetrahedron Lett.* **1972a**, *18*, 1801−1802.

Daxenbichler, M. E.; VanEtten, C. H.; Earle, F. R.; Tallent, W. H. L-Dopa recovery from *mucuna* seed. *J. Agric. Food Chem.* **1972b**, *20*, 1046−1048.

Desphande, S. S.; Sathe, S. K.; Salunkhe, D. K.; Cornforth, T. Effect of dehulling on phytic acid, polyphenols and enzyme inhibitors of dry beans (*Phaseolus vulgaris* L.). *J. Food Sci.* **1982**, *47*, 1846−1850.

Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorometric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350−356.

Duke, J. A. *Handbook of Legumes of World Economic Importance*; Duke, J. A., Ed., Plenum Press: New York, 1981; pp 170−173.

Ekfenyong, T. E.; Brochers, R. L. Digestibility of proteins of winged bean seed. *J. Food Sci. Technol.* **1979**, *16*, 92−95.

Englyst, H. N.; Cummings, J. H. Improved method for measurement of dietary fibre as nonstarch polysaccharides in plant foods. *J. Assoc. Offic. Anal. Chem.* **1988**, *71*, 808−814.

Englyst, H. N.; Kingman, S. M.; Cummings, J. H. Classification and measurement of nutritionally important starch fractions. *Eur. J. Clin. Nutr*. **1992**, *46* (*Suppl. 2*), S33−S50.

Ernst, M.; Krug, H. Seasonal growth and development of Asparagus (*Asparagus officinalis* L). III. The effect of temperature and water stress on carbohydrate content in storage roots and rhizome buds. *Gartenbauwissenschaft* **1998**, *63*, S, 202−208.

FAO/WHO. Protein quality evaluation. In *Report of Joint FAO/WHO Expert Consultation;* Food and Agriculture Organization of the United Nations: Rome, 1990; p 23.

Folch, J.; Lees, M.; Solane-Stanley, G. H. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497−509.

Fujii, Y.; Shibuya, T.; Yasuda, T. L-3,4-Dihydroxyphenylalanine as an allelochemical candidate from *Mucuna pruriens* (L.) DC. Var. *utilis*. *Agric. Biol. Chem.* **1991**, *5*, 617−618.

Gericke, S. V.; Kurmies, B. Die kolorimetrische phosphorsäurebestimmung mit ammonium-vanadat-molybdat und ihre Anwendung in der Pflanzenanalyse. *Z. Pflanzenernaehr. Bodenkd.* **1952**, *59*, 235−247.

Goni, I.; Garcia-Diz, L.; Manas, E.; Saura-Calixto, F. A. Analysis of resistant starch: a method for foods and food products. *Food Chem.* **1996**, *56*, 445−449.

Goni, I.; Garcia-Alonso, A.; Saura-Calixto, F. A. A starch hydrolysis procedure to estimate glycemic index. *Nutr. Res.* **1997**, *17*, 427−437.

Gordon, J. A.; Marquardt, M. D. Factors affecting haemagglutination by concanavalin A and soybean agglutinin. *Biochim. Biophys. Acta* **1974**, *332*, 136−144.

Grant, G.; More, L. J.; McKenzie, N. H.; Stewart, J. C.; Pusztai, A. A survey of the nutritional and haemagglutination properties of legume seeds generally available in the UK. *British J. Nutr.* **1983**, *50*, 207−214.

Harms, R. H.; Simpson, C. F.; Waldroup, P. W. Influence of feeding various levels of velvet beans to chicks and laying hens. *J. Nutr.* **1961**, *75*, 127−134.

Hiai, S.; Oura, H.; Nakajima, T. Color reaction of some sapogenins and saponins with vanillin sulfuric acid. *Planta Med.* **1976**, *29*, 116−122.

Infante, M. E.; Perez, A. M.; Simao, M. R.; Manda, F.; Baquete, E. F.; Fernandes, A. M.; Cliff, J. L. Outbreak of acute toxic psychosis attributed to *Mucuna pruriens. Lancet* **1990**, *336*, 1129.

Janardhanan, K.; Lakshmanan, K. K. Studies on the tribal pulse, *Mucuna utilis*: Chemical composition and antinutritional factors. *J. Food Sci. Technol*. **1985**, *22*, 369−371.

Jayaweera, D. M. A. *Medicinal Plants Used in Ceylon*; National Science Council of Sri Lanka, Colombo, 1981.

Kakade, M. L.; Swenson, D. H.; Liener, I. E. Note on the determination of chymotrypsin and chymotrypsin inhibitor activity using casein. *Anal. Biochem*. **1970**, *33*, 255−258.

Lepage, G.; Roy, C. C. Direct transesterification of all classes of lipids in a one step reaction. *J. Lipid Res.* **1986**, *27*, 114−120.

Liener, I. E. Implications of antinutritional components in soybean foods. *Crit. Rev. Food Sci. Nutr*. **1994a**, *34*, 31−67.

Liener, I. E. Antinutritional factors related to proteins and amino acids. In *Foodborne Disease Handbook*; Hui, Y. H., Gorham, J. R., Murrell, K. D., Cliver, D. O., Eds.; Marcel Dekker: New York, 1994b; Vol. 3, pp 261−309.

Liu, K.; Markakis, P. Trypsin inhibition assay as related to limited hydrolysis of inhibitors. *Anal. Biochem*. **1989**, *178*, 159−165.

Lorenzetti, F.; MacIsaac, S.; Arnason, J. T.; Awang, D. V. C.; Buckler, D. The phytochemistry, toxicology, and food potential of velvet bean (*Mucuna* Adans. Spp., Fabaceae). In *Cover Crops in West Africa Contributing to Sustainable Agriculture;* Buckles, D., Eteka, A., Osiname, O., Galiba, M., Galiano, N., Eds.; IDRC, Ottawa, Canada and IITA, Ibadan, Nigeria, 1998.

Makkar, H. P. S.; Becker, K. Plant Toxins and detoxification methods to improve feed quality of tropical seeds. Review. Pre-Conference Symposium II on Management of Feed Resources and Animal Waste for Sustainable Animal Production in Asia − Pacific Region Beyond 2000; *8th World Conference on Animal Production*, June 28, 1998; Seoul National University: Seoul, Korea, 1998; pp 467−480.

Makkar, H. P. S.; Becker, K.; Sporer, F.; Wink, M. Studies on nutritive potential and toxic constituents of different provenances of *Jatropha curcas. J. Agric. Food Chem.* **1997a**, *45*, 3152−3157.

Makkar, H. P. S.; Becker, K.; Abel, H.; Pawelzik, E. Nutrient contents, rumen protein degradability and antinutritional factors in some colour- and white- flowering cultivars of *Vicia faba* beans. *J. Sci. Food Agric*. **1997b**, *75*, 511−520.

Makkar, H. P. S.; Aderibige, A. O.; Becker, K. Comparative evaluation of nontoxic and toxic varieties of *Jatropha curcas* for chemical composition, digestibility, protein degradability and toxic factors. *Food Chem*. **1998**, *62*, 207−215.

Manas, E.; Bravo, L.; Saura-Calixto, F. Sources of error in dietary fibre analysis. *Food Chem*. **1994**, *50*, 331−342.

Mbadiwe, E. I.; Agogbua, S. I. O. An anti-B specific haemagglutinin from the seeds of *Mucuna flagellipes*. *Phytochem*. **1978**, *17*, 1057−1058.

Meiners, C. R.; Derise, N. L.; Lau, H. C.; Crews, M. G.; Ritchey, S. J.; Murphy, E. W. The content of nine mineral elements in raw and cooked mature dry legumes. *J. Agric. Food Chem.* **1976**, *24*, 1126−1133.

Mertz, E. T.; Jambunathan, R.; Misra, P. S. Protein Quality, In *Agricultural Research Station Bulletin No 7*, Purdue University: West Lafayette, IN, 1975; p 9.

Mulimani, V. H.; Paramjyothi, S. Changes in trypsin and chymotrypsin inhibitory activity on soaking of redgram *(Cajanus cajan* L.). *Plant Foods Hum. Nutr.* **1995**, *47*, 185−190.

Mulimani, V. H.; Rudrappa, G.; Supriya, D. Alpha-amylase inhibitors in chick pea (*Cicer arietinum* L.). *J. Sci. Food Agric.* **1994**, *64*, 413−415.

Nechama, S. I.; Edward, R. K. Does 3,4-dihydroxyphenylalanine play a part in favism? *Nature* **1967**, *215*, 285−286.

Nolan, K. B.; Duffin, P. A. Effect of phytate on mineral bioavailability. *In vitro* studies on Mg^{2+}, Ca^{2+}, Fe^{2+}, Cu^{2+} and Zn^{2+} solubilities in the presence of phytate. *J. Sci. Food Agric*. **1987**, *40*, 79−83.

Oluwatosin, O. B. Genotype x environment influence on cowpea (*Vigna unguiculata* (L) Walp) antinutritional factors: 1-trypsin inhibitors, tannins, phytic acid and haemagglutinin. *J. Sci. Food Agric.* **1999**, *79*, 265−272.

Pieris, N.; Jansz, E. R.; Dharmadasa, H. M. Studies on Mucuna species of Sri Lanka. I. The L-DOPA content of seeds. *J. Natl. Sci. Found. Sri Lanka* **1980**, *8*, 35−40.

Porter, L. J.; Hrstich, L. N.; Chan, B. C. The conversion of procyanidines and prodelphinidins to cyanidin and delphinidin. *Phytochemistry* **1986**, *25*, 223−230.

Prosky, L.; Asp, N. G.; Schweizer, T. F.; de Vries, J. W.; Furda, Y. Determination of insoluble, soluble and total dietary fibre in foods and food products: interlaboratory study. *J. Assoc. Offic. Anal. Chem.* **1988**, *71*, 1017−1023.

Ravindran, V.; Ravindran, G. Nutritional and anti-nutritional characteristics of mucuna (*Mucuna utilis*) bean seeds. *J. Sci. Food Agric*. **1988**, *46*, 71−79.

Reddy, N. R.; Pierson, M. D.; Sathe, S. K.; Salunkhe, D. K. Chemical, nutritional and physiological aspects of dry bean carbohydrates: A review. *Food Chem*. **1984**, *13*, 25−68.

Revilleza, M. J. R.; Mendoza, E. M. T.; Raymundo, L. C. Oligosaccharides in several Philippine indigenous food legumes: determination, localization and removal. *Plant Foods Hum. Nutr*. **1990**, *40*, 83−93.

Reynolds, J. E. F. *Martindale; The Extra Pharmacopoeia*. The Pharmaceutical Press: London, 1993; pp 1015−1020.

Ruperez, P. Oligosaccharides in raw and processed legumes. *Z. Lebensm.- Unters.-Forsch. A* **1998**, *206*, 130−133.

Sadasivam, S.; Manickam, A. Determination of total carbohydrate by anthrone method. In *Biochemical Methods for Agricultural Sciences;* Wiley Eastern Limited: New Delhi, 1992a, pp 8−9.

Salunkhe, D. K.; Kadam, S. S.; Chavan, J. K. Chemical composition. In *Postharvest Biotechnology of Food Legumes*; Salunkhe, D. K., Kadam, S. S., Chavan, J. K., Eds.; CRC Press Inc.: Boca Raton, FL, 1985; pp 29−52.

Satterlee, L. D.; Marshall, H. F.; Tennyson, J. M. Measuring protein quality. *J. Am. Oil Chem. Soc.* **1979**, *56*, 103−109.

Saura-Calixto, F.; Goni, I.; Manas, E.; Abia, R. Klason lignin, condensed tannins and resistant protein as dietary fiber constituents: determination in grape pomaces. *Food Chem.* **1991**, *39*, 299−309.

Scott, R. W. Colorimetric determinations of hexauronic acids in plant materials. *Anal. Chem.* **1979**, *51*, 936−941.

Siddhuraju, P.; Becker, K. Rapid reversed-phase high performance liquid chromatographic method for the quantification of L-dopa, nonmethylated and methylated tetrahydroisoquinoline compounds from Mucuna beans. *Food Chem.* **2000**, submitted for publication.

Siddhuraju, P.; Vijayakumari, K.; Janardhanan, K. Chemical composition and protein quality of the little-known legume, velvet bean (*Mucuna pruriens* (L.) DC.). *J. Agric. Food Chem.* **1996**, *44*, 2636−2641.

Smith, C.; Van Megen, W.; Twaalfhoven, L.; Hitchcook, C. The determinations of trypsin inhibitor levels in foodstuffs. *J. Sci. Food Agric.* **1980**, *31*, 341−350.

Takasaki, S.; Kawakishi, S. Formation of protein-bound 3,4-dihydroxyphenylalanine and 5-*S*-Cysteinyl-3,4-dihydroxyphenylalanine as new cross-linkers in gluten. *J. Agric. Food Chem*. **1997**, *45*, 3472−3475.

Tan, N. G.; Rahim, Z. H. A.; Khor, H. T.; Wong, K. C. Winged bean (*Psophocarpus tetragonolobus*) tannin level, phytate content, and hemagglutinating activity. *J. Agric. Food Chem.* **1983**, *31*, 916−917.

Thilborg, S. T.; Christensen, S. B.; Cornett, C.; Olsen, C. E.; Lemmich, E. Molluscicidal saponins from a Zimbabwean strain of *Phytolacca dodecandra*. *Phytochemistry* **1994**, *36*, 753−759.

Udedibe, A. B. I.; Carlini, C. R. Brazilian *Mucuna pruriens* seeds (velvet bean) lack hemagglutinating activity. *J. Agric. Food Chem.* **1998**, *46*, 1450−1452.

Vaintraub, I. A.; Lapteva, N. A. Colorometric determination of phytate in unpurified extracts of seeds and the products of their processing. *Anal. Biochem.* **1988**, *175*, 227−230.

Vega, A.; Girla, F.; Sotelo, A. Nutritional evaluation of the velvet bean (*Stizolobium cinerium*) alone and supplemented with methionine or wheat flour. *Nutr. Rep. Int.* **1981**, *24*, 817−823.

Vijayakumari, K.; Siddhuraju, P.; Janardhanan, K. Effect of different post-harvest treatments on antinutritional factors in seeds of the tribal pulse, *Mucuna pruriens* (L.) DC. *Int. J. Food Sci. Nutr.* **1996**, *47*, 263−272.

Williams, P. C.; Nakoul, H.; Singh, K. B. Relationship between cooking time and some physical characteristics in chickpeas (*Cicer arietinum* L.). *J. Sci. Food Agric*. **1983**, *34*, 492−496.

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