Effect of Various Domestic Processing Methods on Antinutrients and in Vitro Protein and Starch Digestibility of Two Indigenous Varieties of Indian Tribal Pulse, *Mucuna pruriens* Var. *utilis*

Perumal Siddhuraju and Klaus Becker*

Department of Animal Nutrition and Aquaculture, Institute for Animal Production in the Tropics and Subtropics, University of Hohenheim (480), D-70593 Stuttgart, Germany

The effect of various domestic processing methods on antinutrients and starch fractions and in vitro protein and starch digestibilities of white and black varieties of *Mucuna pruriens* var. *utilis* was studied. Cooking or autoclaving of both raw seeds and presoaked seeds in different solutions (water, tamarind extract, sodium bicarbonate, and citric acid) significantly ($p < 0.05$) reduced the content of total phenolics, phytic acid, trypsin inhibitor and chymotrypsin inhibitor activities, and L-dopa compared to soaking or dry heating techniques. The germination processes (24 and 48 h) were also effective in the reduction of various antinutrients, although this reduction appeared to be more pronounced in a prolonged period of germination (72 h). Water soaking followed by dehusking was found to be ineffective in the reduction of trypsin and chymotrypsin inhibitor activities in both varieties. All of the treatments were effective in significantly ($p < 0.05$) reducing the resistant starch content in the presently investigated samples. Cooking as well as autoclaving brought about a more significant ($p < 0.05$) improvement in the digestibility of protein and starch compared to germination and dry heat treatment. Moreover, among the different processing techniques, soaking in sodium bicarbonate solution followed by cooking (29.6−34.8%) or autoclaving (33.0−37.2%) seemed to be the best method for improving starch digestibility.

Keywords: *Mucuna pruriens var. utilis; processing; antinutrients; L-dopa; resistant starch; protein and starch digestibilities*

INTRODUCTION

Mucuna bean [*Mucuna pruriens* (L.) DC. var. *utilis* (Wall ex Wight) Baker ex Burck.] is a tropical legume, and the boiled bean is consumed in several parts of India, especially by the northeastern tribals and Kanikkas tribals belonging to Kerala State (*1*, *2*) and as well as by low-income groups in Sri Lanka (*3*). It is eaten by tribals either as such or mixed with other cereals. The seeds were found to contain a high amount of protein (24−30%) with a high proportion of essential amino acids and to be a good source of starch (26−29%) and certain mineral elements (*4*). However, the seeds cause vomiting and diarrhea when large amounts are ingested by humans and other monogastrics (*5*). Raw *Mucuna* seeds in the diet have also been reported to reduce growth rates of broiler chicks and the egg production of laying hens (*6*). Although legumes constitute one of the richest and least expensive sources of protein in human/animal diets, their utilization is limited due to the presence of antinutritional/antiphysiological compounds such as phenolics, tannins, trypsin, chymotrypsin and amylase inhibitors, lectins, phytic acid, alkaloids, hydrogen cyanide, non-protein amino acids, saponins, and oligosaccharides (*7*, *8*).

The mucuna bean is found to contain a high amount (3−6%) of non-protein phenolic amino acid, L-3,4-dihydroxyphenylalanine (L-dopa), trypsin inhibitor, chymotrypsin inhibitor, and phytic acid. However, the pharmacologically active factor, L-dopa (*9*), is potentially toxic (*5*) if ingested in large amounts. It has been reported to cause serious effects of hallucinations (*10*) in addition to gastrointestinal disturbances such as nausea, vomiting and anorexia (*11*), and it has also been proven to be toxic in individuals with glucose-6-phosphate dehydrogenase deficiency in their erythrocytes, thus resulting in inducing favism (*12*). Recently, Takasaki and Kawakishi (*13*) have reported that the oxidation products of L-dopa conjugate with SH compounds (cysteine) of proteins to form protein-bound 5-*S*-cysteinyldopa cross-links, which leads to polymerization of proteins. However, various processing and cooking methods could improve the protein and starch digestibility of legume seeds by decreasing the levels of some antinutrients (*14*−*16*). Moreover, an increase in digestibility after thermal treatment may be attributed to other factors such as disruption of protein structures and cell wall encapsulated starch, starch gelatinization, and physical disintegration of the legume seeds (*17*, *18*). Meanwhile, information on the nutritional characters and various other antinutritional factors in the presently investigated mucuna beans is available (*2*−*4*, *19*). However, information regarding the effect of various indigenous processing methods, particularly those followed by Indian village people, on the removal/reduction of various antinutrients including non-protein amino acid, L-dopa, and improvement of in vitro protein and starch digestibilities in mucuna beans appears to be lacking. Hence, the present investigation has been carried out to determine the extent to which the various antinutrients in two varieties of *M. pruriens* var. *utilis*

* Corresponding author (telephone +49 711 459 3158; fax +49 711 459 3702; e-mail kbecker@uni-hohenheim.de).

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become reduced/eliminated under various processing techniques such as soaking, autoclaving, cooking, dry heating, and germinating and how to improve the protein and starch digestibilities.

MATERIALS AND METHODS

Seed Samples. The mature and dry raw seeds of *M. pruriens* var. *utilis* of white and black varieties were collected from tribal people living in a village near Marthandam, Tamil Nadu, India, in January 1999. Soon after collection, the seeds were dried for 2 days in open sunlight. The dried seeds were cleaned thoroughly, and any broken or immature seeds were removed. The seeds were stored in a plastic container at room temperature (25 °C) until processing.

Processing Methods. *Soaking.* The seeds were soaked in tap water, tamarind extract (tamarind extract was prepared by stirring tamarind pulp in water, 10 g of pulp in 300 mL of water, pH 2.75), sodium bicarbonate solution (0.07% w/v, pH 8.60), and citric acid solution (0.1% w/v, pH 2.60) for 20 h at 25 °C. A seed to solution ratio of 1:10 (w/v) was used. The unimbibed seeds were discarded, and the soaked seeds were rinsed twice in distilled water and then freeze-dried.

Soaking and Dehusking. Mucuna seeds were soaked in distilled water (1:10 w/v) for 20 h at room temperature (25 °C). The seed coats were manually removed, and the cotyledons were cleaned, rinsed with distilled water, and freeze-dried.

Ordinary Cooking. Presoaked seeds were put in round-mouth tall beakers fitted with condensers after they had been rinsed in distilled water. After tap water (in the ratio of seed and water 1:5 w/v) had been added, the samples were cooked at 85−90 °C on a hot plate until they became soft when felt between the fingers (∼90 min). After the cooking water had been drained, the seeds were freeze-dried.

Sequential Cooking. Raw beans were cooked for ∼130 min using a long-neck round-bottom glass beaker fitted with a condenser. After every 40 min, the cooking water was drained and boiling water was added, maintaining seed to water ratio at 1:5 (w/v). Finally, at 130 min, the cooking water was removed, and then the samples were freeze-dried.

Autoclaving. The presoaked seeds were autoclaved at 15 lb of pressure (121 °C) in distilled water (1:5 w/v) for 30 min, whereas a seed to water ratio of 1:7 (w/v) was used in the case of raw seeds for the same process. Excess water was drained, and the autoclaved samples were freeze-dried.

Dry Heating. Mucuna beans were put in an iron pot and mixed with clean fine sand to prevent burning of the seed coat and to ensure uniform heat distribution. The materials were roasted for ∼30 min at 120−130 °C. The sand was then separated from the beans using a sieve, and the beans were allowed to cool at room temperature (25 °C).

Germination. The seeds soaked in distilled water for 20 h were germinated in sterile Petri dishes lined with wet filter paper for 24, 48, and 72 h at 30 °C in the dark and frequently watered. The sprouts were then rinsed with distilled water and freeze-dried.

The processed and unprocessed seed samples were ground to fine powder (particle size < 0.5 mm) and then stored in airtight plastic bottles at room temperature (25 °C) prior to analysis. All analyses were carried out in triplicate and reported on a dry matter basis. Residual moisture was determined by oven-drying to a constant weight at 105 °C.

Chemical Analysis. Total Phenolics. Total phenolics were determined by spectrophotometric methods described by Makkar et al. (*20*). Total phenolics were quantified by Folin−Ciocalteu reagent and were expressed as tannic acid equivalents.

Trypsin and Chymotrypsin Inhibitor Analyses. Trypsin inhibitor activity was essentially determined according to the method of Smith et al. (*21*) except that the enzyme was added last as suggested by Liu and Markakis (*22*). Chymotrypsin inhibitor activity was assayed in a 0.1 M borate buffer, pH 7.6, extract of defatted meal according to the procedure of Kakade et al. (*23*). One chymotrypsin unit is expressed as the increase of 0.01 absorbance unit at 275 nm in 10 min by the reaction mixture of volume of 10 mL. A chymotrypsin inhibitor unit was defined in terms of chymotrypsin units inhibited per milligram of sample.

Estimation of Phytic Acid. The phytate content of the sample was determined by a colorimetric procedure described by Vaintraub and Lapteva (*24*) and as modified by Alonso et al. (*25*). Ground samples (0.5 g each) were stirred in 10 mL of 2.4% HCl for 1 h. The contents were centrifuged at 3000*g* at 4 °C 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 × 15 cm) containing 0.5 g of Dowex 1 (AG 1X8 anion-exchange resin, chloride form, 8% cross-linkage, 100−200 mesh, Dow Corning Corp.). 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 of phytic acid by using standard phytic acid.

Extraction of 3,4-Dihydroxyphenylalanine (L-Dopa). Five milliliters of 0.1 N HCl was added to 50−100 mg of finely ground raw and processed seed flour taken in a glass tube, and the samples were stirred for 10 min at room temperature. The mixture was initially subjected to an Ultra-turrax T25 (20500 min^{-1}) for 30 s in an ice bath and subsequently kept on a magnetic stirrer for 1 h at room temperature. The supernatant was collected by centrifugation (10000*g*, 4 °C, 15 min). This extraction procedure was repeated twice, and the supernatant of all three extractions were pooled and made up to 50−100 mL by using the same extracting medium. Then the solution was filtered through a 0.2 μm glass filter, and 20 μL aliquots were injected into the HPLC (*26*).

HPLC Conditions for the Quantification of L-Dopa. The chromatograph consisted of a Merck Hitachi model L-7100 HPLC pump, an L-7450 photodiode array detector, an L-7200 autosampler with injector valve containing a 100-μL sample loop, a D-7000 interphase module, and an LC organizer (Hitachi Instruments Inc., San Jose, CA). The analytical column was reversed phase C_{18} (Nucleosil 120, mean particle diameter = 5 μm, 250 × 4.6 mm i.d., Macherey-Nagel GmbH & Co.). 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 was as follows: Starting with 100% solvent A and 0% solvent B up to 12 min, for the next 5 min solvent B was increased from 0 to 100% with a 100 to 0% decrease of solvent A; solvent A was increased to 100% with a decrease of solvent 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 (100% solvent A and 0% solvent B). Separation was performed at room temperature (23 ± 2 °C), and the flow rate was 1.2 mL/min. The average elution time for L-dopa was 7.6 min. Absorbance was monitored at 282 nm, and peak heights and areas were determined. By using the authentic standard, the sample results were expressed. The standard, L-dopa, was procured from Sigma (St. Louis, MO).

In Vitro Protein Digestibility (IVPD). The IVPD of raw and processed samples, except those samples soaked in various solutions, was measured according to a multienzyme technique (*27*). Samples containing 62.5 mg of protein were suspended in 10 mL of distilled water, and the pH was adjusted to 8.0 with 0.1 N HCl and/or NaOH while they were stirred in a 37 °C water bath for 15 min. The multienzyme solution, consisting of 1.6 mg of trypsin, 3.1 mg of chymotrypsin, and 1.3 mg of peptidase/mL, was maintained in an ice bath and adjusted to pH 8.0 with 0.1 N HCl and/or NaOH. One milliliter of the above multienzyme solution was added to the protein suspension with stirring at a constant temperature of 37 °C. After exactly 10 min from the time of the addition of the three

Table 1. Effect of Processing on the Levels of Total Phenols, Phytic Acid, and Trypsin and Chymotrypsin Inhibitor Activities in Two Varieties of *M. pruriens* Var. *utilis* Seeds (Dry Matter Basis)[a]

	white variety				black variety			
treatment[b]	total phenols[c] (g 100 g^{-1})	phytic acid (g 100 g^{-1})	TIA[d]	CIA[e] (CIU mg^{-1} of sample)	total phenols[c] (g 100 g^{-1})	phytic acid (g 100 g^{-1})	TIA[d]	CIA[e] (CIU mg^{-1} of sample)
raw seed	5.539^{a}	0.904^{a}	13.78^{a}	10.97^{a}	6.142^{a}	0.856^{a}	13.84^{b}	10.84^{b}
RA	3.200^{ef}	0.553^{h}	0.546^{gh}	0.759^{gh}	2.369^{hij}	0.563^{fg}	0.473^{i}	0.943^{hi}
RSC	2.818^{g}	0.660^{g}	0.562^{gh}	1.083^{g}	2.576^{hi}	0.540^{fgh}	0.512^{i}	1.278^{h}
WSA	2.257^{h}	0.481^{i}	0.549^{gh}	ND[f]	1.799^{l}	0.440^{i}	0.500^{i}	0.149^{jk}
WSC	2.273^{h}	0.655^{g}	0.608^{gh}	0.615^{hi}	1.882^{kl}	0.501^{ghi}	0.585^{i}	0.553^{ijk}
TSA	2.622^{g}	0.435^{j}	0.486^{gh}	ND	2.155^{jk}	0.479^{hi}	0.569^{i}	0.110^{jk}
TSC	3.236^{e}	0.644^{g}	0.270^{h}	ND	2.269^{ij}	0.500^{ghi}	0.304^{i}	ND
ASA	2.055^{h}	0.493^{i}	1.065^{g}	ND	2.241^{j}	0.528^{fgh}	0.459^{i}	ND
ASC	2.307^{h}	0.513^{i}	0.554^{gh}	0.456^{hi}	2.058^{jkl}	0.528^{fgh}	0.494^{i}	0.617^{ij}
CSA	2.262^{h}	0.556^{h}	0.270^{gh}	0.168^{i}	1.739^{l}	0.496^{hi}	0.162^{i}	ND
CSC	2.916^{fg}	0.645^{g}	0.184^{h}	1.128^{g}	2.639^{h}	0.586^{ef}	0.138^{i}	2.266^{g}
TS	4.982^{bc}	0.860^{bc}	8.062^{d}	7.319^{e}	4.584^{ef}	0.715^{d}	10.89^{e}	8.058^{d}
AS	4.447^{d}	0.805^{de}	6.792^{e}	6.904^{e}	4.253^{g}	0.705^{d}	7.837^{g}	6.843^{e}
CS	5.042^{bc}	0.824^{cd}	8.412^{d}	8.560^{d}	4.427^{fg}	0.778^{bc}	10.68^{ef}	9.045^{c}
WSD	4.819^{bc}	0.870^{ab}	14.06^{a}	11.01^{a}	4.622^{ef}	0.787^{b}	15.72^{a}	13.08^{a}
DH	5.125^{b}	0.874^{ab}	3.493^{ef}	4.827^{f}	5.602^{b}	0.781^{bc}	3.435^{h}	3.991^{f}
G24	4.976^{bc}	0.778^{ef}	13.30^{a}	10.37^{b}	5.418^{bc}	0.789^{b}	12.91^{c}	10.43^{b}
G48	4.737^{cd}	0.757^{f}	12.51^{b}	9.858^{c}	5.154^{cd}	0.724^{cd}	11.84^{d}	9.187^{c}
G72	4.505^{d}	0.592^{g}	10.09^{c}	8.502^{d}	4.876^{de}	0.644^{e}	10.05^{f}	8.116^{d}

[a] Values are the mean of triplicate determinations. Values in the same column with different roman superscripts are significantly different ($p < 0.05$). [b] RA, raw seeds autoclaved; RSC, raw seeds sequentially cooked; WSA, water soaked and autoclaved; WSC, water soaked and cooked; TSA, tamarind extract soaked and autoclaved; TSC, tamarind extract soaked and cooked; ASA, alkaline soaked and autoclaved; ASC, alkaline soaked and cooked; CSA, citric acid soaked and autoclaved; CSC, citric acid soaked and cooked; TS, tamarind extract soaked; AS, alkaline soaked; CS, citric acid soaked; WSD, water soaked and dehusked; DH, dry heated; G, geminated for 24, 48, and 72 h. [c] As tannic acid equivalent. [d] Milligrams of pure trypsin inhibited per gram of sample. [e] CIU, chymotrypsin inhibitor unit. [f] ND, not detected.

enzyme solutions (still stirring), 1 mL of bacterial protease (type XIV *Streptomyces griseus*) solution (7.95 mg of enzyme/mL of H_2O) was added to the sample. Immediately the solution was transferred to a 55 °C water bath. Nine minutes after the bacterial protease solution had been added to the sample, the sample was transferred to a 37 °C water bath. Ten minutes after the addition of the bacterial protease (1 min back in the 37 °C water bath), the pH of the enzyme hydrolysate was recorded and the in vitro protein digestibility of the sample was calculated by using the following regression equation: $Y = 234.84 - 22.56X$, where Y is the percent protein digestibility and X is the pH of the protein suspension after 20 min of digestion with the four-enzyme solution.

Total, Digestible, and Resistant Starch Analyses. Total starch (TS) content was determined, except for the samples soaked in various solutions, 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 amyloglucosidase (EC 3.2.1.3; catalog no. 102857, Boehringer-Mannheim, Mannheim, Germany) at 60 °C for 45 min (*28*). Glucose was determined using the glucose oxidase/peroxidase reagent (catalog no. 510-A, Sigma Chemical Co., Deisenhofen, Germany). Total starch was calculated as glucose × 0.9, after correction of the free glucose content. The glucose content of the samples, both free glucose and the glucose moiety of sucrose, was determined to correct the total starch values obtained before. Samples dispersed in 2 M KOH were treated with invertase (EC 3.2.1.26) for 30 min at 37 °C. After centrifugation, a 1 mL aliquot was precipitated with 2 mL of 96% ethanol and centrifuged again, and the glucose was analyzed in the supernatants using the glucose oxidase/peroxidase reagent (*18*).

Resistant starch (RS) was analyzed as follows: Samples, except those soaked in various solutions (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, 3000g) and the 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 × 0.9 (*29*). The digestible starch (DS) content was calculated as the difference between TS and RS.

In Vitro Starch Digestibility (IVSD). Raw and processed samples, except the samples soaked in various solutions (50 mg), were prepared as explained above. Ten milliliters of HCl−KCl buffer with a pH of 1.5 was added. Then 0.2 mL of a solution containing 1 g of pepsin in 10 mL of HCl−KCl buffer was added to each sample, and the samples were incubated at 40 °C for 1 h in a shaking water bath. Volume was completed to 25 mL with Tris−maleate buffer, pH 6.9. Five milliliters of α-amylase solution in Tris−maleate buffer containing 2.6 UI was added to each sample. Samples were then incubated at 37 °C in a shaking water bath for 3 h. From this, a 1 mL aliquot was taken and placed into a tube that was shaken vigorously at 100 °C for 5 min to inactivate the enzyme. Then 3 mL of 0.4 M sodium acetate buffer, pH 4.75, was added to the aliquot, and 60 μL of amyloglucosidase was used to hydrolyze the digested starch into glucose for 45 min at 60 °C in a shaking water bath. Volume was adjusted to 10−100 mL with distilled water. Triplicate aliquots of 0.5 mL were incubated with a glucose oxidase/peroxidase reagent (catalog no. 510-A, Sigma Chemical Co.). The glucose was converted into starch by multipyling by 0.9 (*28*). Percentage of starch digestibility was calculated as percent starch hydrolyzed from the total starch content of the sample.

Statistical Analysis. Results were expressed as mean values ± standard deviations of three separate determinations. Data were statistically analyzed using the Statistica program, version 5.1. The significant difference between means was calculated by one-way analysis of variance (ANOVA) using Duncan's multiple-range test at $p < 0.05$.

RESULTS AND DISCUSSION

Total Phenolics. The higher content of total free phenolics is undesirable for human consumption because they interfere with the digestion and absorption of dietary protein (*15*). The polyphenolic contents of the white and black varieties of mucuna beans were 5.5 and

6.1 g 100 g^{-1}, respectively (Table 1). These values are comparable to values reported in velvet bean (*30*) and higher than that of cow pea (*31*) and different varieties of peas (*32*). A more significant reduction in the content of total free phenolics has been observed under soaking in different solutions followed by autoclaving or cooking of both white (41.6−59%) and black varieties (57−71%). Similarly, a significant reduction of total phenolic contents during soaking followed by thermal processing has been observed in commonly consumed legumes such *Dolichos lablab* var. *vulgaris*, *Lathyrus sativus*, and *Vigna radiata* (*33−35*). This might be due to either the increased leaching out of phenolic substances or poor extractability of phenolics from the seeds under the influence of absorbed solution concentrations by thermal processing. When the seeds were soaked in alkaline, tamarind solution, or citric acid or soaked in water and dehusked, the decrease in polyphenolic contents observed was greater in the black variety than in the white variety. When the water-soaked seeds were subsequently germinated for 24, 48, and 72 h, the decrease in polyphenolic content was 7−21%, which appears to be lower than the values reported for different lines of *Lathyrus sativus* (*35*).

Phytic Acid. The phytate molecule is negatively charged at the physiological pH and is reported to bind essential, nutritionally important essential divalent cations, such as iron, zinc, magnesium, and calcium. This forms insoluble complexes, thereby making minerals unavailable for absorption (*36*). The seeds of both the white (0.9%) and black (0.86%) varieties of mucuna beans (Table 1) are known to contain higher levels of phytic acid than various conventional legumes such as *Phaseolus angularis* and *Phaseolus calcaratus* (*16*) as well as chick pea and pigeon pea (*37*). However, this value appears to be lower than in dry beans (0.5−3.0%) (*38*) and soybeans (0.6−1.5%) (*39*). During soaking in different solutions and water soaking followed by dehusking, only a 4−8% reduction in the content of phytic acid has been noticed in both varieties of mucuna beans. These results are in good agreement with the previous findings of Srivastava and Khokhar (*35*) in different lines of *Lathyrus sativus* seeds. These losses are mainly due to leaching and are particularly favored when the compounds possess low molecular weight and ionic character. Deshpande and Cheryan (*40*) reported that when the ionic concentration of the soaking medium was increased, losses of phytate were enhanced. On the other hand, the removal of phytate in legumes by water soaking followed by germination has been attributed to the enzymatic (phytase) hydrolysis of phytate followed by diffusion (*41*). The action of phytase during the germination of mucuna beans (72 h) led to a significant reduction in the phytate content (24−34%) as compared with the reductions found after 24 and 48 h of germination. However, such lesser reduction of phytate content observed at 24 and 48 h germination might be related to slower germination of seeds coupled with lower phytase activity. Autoclaving or cooking of presoaked mucuna seeds in different solutions was significantly ($p < 0.05$) effective in reducing the phytate (27−34 and 38−51%, respectively) content. A similar loss of phytic acid during hydrothermal processing of presoaked seeds has also been reported in *L. sativus* (*35*). The apparent decrease in the phytic acid content of legume seeds during cooking or autoclaving may be partly due either to the formation of insoluble complexes between phytate and other components such as phytate−protein and phytate−protein−mineral complexes or to the inositol hexaphosphate hydrolyzed to penta- and tetraphosphates.

Trypsin and Chymotrypsin Inhibitors. The presence of protease inhibitors in the diet leads to the formation of the irreversible trypsin enzyme−trypsin inhibitor complex, causing a trypsin drop in the intestine and a decrease in the diet protein digestibility, leading to slower animal growth. Under this situation, the organism increases the secretory activity of the pancreas, which could cause pancreatic hypertrophy and hyperplasia (*7*, *8*). The trypsin inhibitor and chymotrypsin inhibitor activities of the white and black varieties of mucuna beans [13.78 and 13.84 mg g^{-1} (10.97 and 10.84 CIU mg^{-1}), respectively] are shown in Table 1. The trypsin inhibitor activity assayed in mucuna beans is lower than that of soybean (29.1−30.2 mg g^{-1}) (*21*) and higher than the values (1.72−3.55 mg g^{-1}) reported in certain cultivars of faba bean (*42*). The estimated value of the chymotrypsin inhibitor activity in both mucuna bean varieties seems to be much higher than the results found in *Cajanus cajan* (*43*). A major beneficial effect of cooking and autoclaving of legume grains is the destruction of protease inhibitors, which interfere in protein digestibility. Significant reductions of trypsin inhibitor (92−99%) and chymotrypsin inhibitor (90−100%) activities have been noticed after cooking/autoclaving of both raw and presoaked seed samples. Similarly, a significant reduction in trypsin inhibitor activity (76%) during the hydrothermal processing has been observed in different lines of *L. sativus* (*35*) as well as in certain other common legumes. Even total inactivation of the trypsin inhibitor from Brazilian velvet bean has been reported by Udedibie and Carlini (*19*) after ordinary cooking or pressure cooking of presoaked samples. Even though dry heat treatment showed a greater effect in the reduction of trypsin inhibitor (75−79%) and chymotrypsin inhibitor (56−63%) activities, these values seem to be significantly lower when compared to the other hydrothermal processing methods that were employed in mucuna beans. Similarly, Sathe and Salunkhe (*44*) observed that the trypsin inhibitor activity (TIA) of beans, cowpea, and black gram was resistant to dry heat. Nonetheless, only a 69% reduction of TIA was achieved in faba bean when it was subjected to dry-heating treatment (*45*).

In addition to heat stable protease inhibitors (*7*), the existing residual TIA and chymotrypsin inhibitor activity (CIA) might be due to the presence of non-protein TIA and CIA inhibitors such as browning substances (resulting from Maillard effect), phytate, phenolics, and a relative concentration of fiber which could also give rise to the residual inhibitor activity after thermal processing (*46*, *47*). The non-protein TIA comprised 21.2−55.8 and 3.37−14.2% of the total TIA in soybean and winged bean, respectively (*46*). In our present study, the presence of a relatively higher amount of phytate and phenolics including 3,4-dihydroxyphenylalanine in heated mucuna beans might partially contribute to residual TIA and CIA. Soaking of mucuna beans in different solutions resulted in 39−51% TIA and 16−37% CIA reductions, respectively. However, when compared to acidic solutions, alkaline solution soaking appears to be the more effective one. Some authors have reported that soaking hardly decreases TIA in kidney bean (*40*) and faba bean (*48*). Our results show that only when

Table 2. Effect of Autoclaving, Cooking, and Dry Heating Processess on Non-protein Amino Acid, L-dopa, in Mucuna Beans (*M. pruriens* Var. *utilis*)[a]

treatment[b]	white variety (g 100 g^{-1} of DM[c])	% loss from raw seed	black variety (g 100 g^{-1} of DM)	% loss from raw seed
raw seed	4.70 ± 0.12^{a}		4.28 ± 0.20^{a}	
RA	2.28 ± 0.04^{e}	51.5	1.74 ± 0.05^{e}	59.4
RSC	1.88 ± 0.02^{g}	60.0	1.87 ± 0.03^{d}	56.4
WSA	1.85 ± 0.02^{g}	60.7	1.56 ± 0.03^{f}	63.6
WSC	2.06 ± 0.04^{f}	56.2	1.59 ± 0.05^{f}	62.9
TSA	2.04 ± 0.03^{f}	56.6	1.68 ± 0.03^{ef}	60.8
TSC	2.78 ± 0.14^{c}	40.9	1.86 ± 0.04^{d}	56.6
ASA	1.62 ± 0.05^{h}	65.5	1.66 ± 0.04^{def}	61.2
ASC	1.86 ± 0.04^{g}	60.4	1.60 ± 0.05^{f}	62.6
CSA	1.87 ± 0.06^{g}	60.2	1.27 ± 0.04^{g}	70.3
CSC	2.58 ± 0.03^{d}	45.1	2.20 ± 0.03^{c}	48.6
DH	4.23 ± 0.04^{b}	10.0	3.16 ± 0.04^{b}	26.2

[a] Values are the mean of triplicate determinations ± standard deviation. Values in the same column with different roman superscripts are significantly different ($p < 0.05$). [b] RA, raw seeds autoclaved; RSC, raw seeds sequentially cooked; WSA, water soaked and autoclaved; WSC, water soaked and cooked; TSA, tamarind extract soaked and autoclaved; TSC, tamarind extract soaked and cooked; ASA, alkaline soaked and autoclaved; ASC, alkaline soaked and cooked; CSA, citric acid soaked and autoclaved; CSC, citric acid soaked and cooked; DH, dry heated. [c] DM, dry matter basis.

mucuna beans were soaked in tamarind and citric acid solutions was less reduction of protease inhibitor levels found. This might be due to the relative hardness of the seed coat caused by soaking at acidic pH involving less diffusion of some compounds such as those with protease inhibitor activity into the soaking liquid (*49*). Conversely, substantial amounts of trypsin and chymotrypsin inhibitors have been reported to leach out of *C. cajan* seeds soaked in salt solutions (*43*) and great northern beans soaked in acidic and alkaline solutions (*50*). Prolonging germination (72 h) considerably lowered but could not completely remove TIA (27%) and CIA (22−25%) levels from mucuna beans. The reduction may be attributed to the mobilization and enzymatic degradation of proteins including protease inhibitors of seeds during germination. A similar trend of lower reduction of protease inhibitors has also been reported in moth bean (*51*) and *P. calcaratus* (*16*). The increase in the relative contents of TIA and CIA after dehusking may be attributed to at least two factors. First, these antinutritional factors may be characteristically present in the cotyledon fractions of the beans. Second, the seed coat contributes a substantial portion of the whole seed weight (*52*).

L-Dopa. The effect of various processing methods on the level of L-dopa in mucuna beans is given in Tables 2 and 3. Raw seed samples of the white and black varieties of mucuna beans contain 4.70 and 4.28% L-dopa, respectively. These values appear to be lower than that of the earlier reports in *M. pruriens* (*30*). However, when compared to the conventional legume, faba bean (*Vicia faba*) (*53*), the L-dopa values in the presently investigated samples were found to be much higher. Recently, Prakash and Tewari (*54*) reported that three Indian *Mucuna* spp. contained levels of L-dopa in the range between 3.6 and 4.2%, and these values are comparable to our present findings. Such wide variations in the L-dopa content in *Mucuna* spp. not only is due to genetic makeup but also depends on the growing locations. Lorenzetti et al. (*55*) observed that the plants cultivated within 10° of the equator contained significantly higher levels of L-dopa than plants cultivated far away from the equator regions. Even the half-mature seeds of mucuna beans have been reported to have a higher L-dopa content than the matured dry seeds (*54*), and therefore it could be determined that the maturity as well as harvesting period of the seeds influences the L-dopa content. Similar results have also been observed in different stages of pod development in the two cultivars of faba bean seeds (*53*).

Table 3. Effect of Soaking, Dehusking, and Germination on Non-protein Amino Acid, L-dopa, in Mucuna Beans (*M. pruriens* Var. *utilis*)[a]

treatment	white variety (g 100 g^{-1} of DM[c])	% loss from raw seed	black variety (g 100 g^{-1} of DM)	% loss from raw seeds
raw seed	4.70 ± 0.12^{a}		4.28 ± 0.20^{a}	
TS	4.29 ± 0.02^{b}	8.72	3.54 ± 0.03^{c}	17.3
AS	4.00 ± 0.15^{cd}	14.9	3.60 ± 0.12^{c}	15.9
CS	4.38 ± 0.11^{b}	6.81	4.06 ± 0.08^{b}	5.14
WS	4.29 ± 0.09^{b}	8.72	4.02 ± 0.05^{b}	6.10
WSD	4.61 ± 0.07^{a}	1.91	4.15 ± 0.06^{ab}	3.04
G24	4.07 ± 0.12^{c}	13.4	3.58 ± 0.19^{c}	16.4
G48	3.89 ± 0.09^{de}	17.2	3.46 ± 0.03^{cd}	19.2
G72	3.76 ± 0.04^{e}	20.0	3.30 ± 0.05^{d}	22.9

[a] Values are the means of triplicate determinations ± standard deviation. Values in the same column with different roman superscripts are significantly different ($p < 0.05$). [b] TS, tamarind extract soaked; CS, citric acid soaked; AS, alkaline soaked; WS, water soaked; WSD, water soaked and dehusked; G, germination for 24, 48, and 72 h. [c] DM, dry matter basis.

The L-dopa content in the white and black varieties was reduced to 8.72 and 17.3%, 14.9 and 15.9%, 6.81 and 5.14%, and 8.72 and 6.10% when the samples were soaked in tamarind pulp extract, sodium bicarbonate, citric acid, and water solutions, respectively. Similarly, Vijayakumari et al. (*56*) reported that reductions of L-dopa of only 16 and 18% were observed when *M. pruriens* seeds were soaked for 9 h in distilled water and sodium bicarbonate solution, respectively. The results of our present research findings are in good agreement with the above quoted results. However, both soaking in tamarind pulp extract and sodium bicarbonate solutions improved the reduction of L-dopa content ($p < 0.05$) significantly compared to the raw, citric acid solution and water soaking treatments in both the varieties of mucuna beans. Srivastava and Khokhar (*35*) have also observed the significant effect of tamarind pulp extract and sodium bicarbonate solution soaking on the reduction of non-protein amino acid, β-ODAP, in *L. sativus.* D'Mello and Walker (*57*) also reported that for whole seeds of *Canavalia ensiformis*, when subjected to dilute $KHCO_3$ solution at 80 °C, the concentration of canavanine declined to negligible levels within 48 h. However, such a low and variable reduction of L-dopa in the present study during the soaking in different solutions could be explained by two factors: first, the permeability of the seed coat along with the diffusion rate of L-dopa in different ionic strength solutions and, second, by the presence of L-dopa in the intact cell compartments of the cotyledons rather than in the seed coat. The second factor might be more pronounced on the least leaching of L-dopa during various soaking processes, although it is a water/other solutions soluble compound. Further, the above-mentioned second factor has been proven by quantification of more concentration of L-dopa retained in the soaking followed by dehusked seed samples of both varieties. When the raw or presoaked seeds were cooked or pressure cooked in distilled water, the losses in L-dopa contents reached 45−66 and 49−70% in the white and black varieties,

Table 4. Total Starch (TS), Resistant Starch (RS), and Digestible Starch (DS) Contents of Raw and Processed *M. pruriens* Var. *utilis* Seeds (Grams per 100 Grams of Dry Matter)[a]

	white variety			black variety		
treatment[b]	TS	DS[c]	RS	TS	DS	RS
raw seed	27.19 ± 0.29^{f}	17.42 ± 0.07^{g}	9.77 ± 0.22^{a}	28.27 ± 0.60^{h}	17.75 ± 0.30^{i}	10.51 ± 0.30^{a}
RA	33.40 ± 0.54^{b}	29.14 ± 0.61^{bc}	4.26 ± 0.08^{hi}	31.36 ± 0.46^{f}	25.89 ± 0.45^{f}	5.47 ± 0.38^{d}
WSA	36.01 ± 0.90^{a}	32.07 ± 0.76^{a}	3.94 ± 0.15^{hij}	35.33 ± 0.18^{b}	31.81 ± 0.21^{b}	3.52 ± 0.20^{ij}
TSA	30.76 ± 0.41^{c}	25.59 ± 0.31^{d}	5.16 ± 0.13^{ef}	30.44 ± 0.76^{g}	26.29 ± 0.76^{f}	4.16 ± 0.09^{g}
ASA	33.14 ± 0.21^{b}	29.92 ± 0.19^{b}	3.22 ± 0.05^{k}	34.73 ± 0.12^{bc}	31.16 ± 0.25^{b}	3.57 ± 0.14^{hij}
CSA	29.45 ± 0.26^{d}	25.06 ± 0.09^{d}	4.39 ± 0.18^{gh}	33.37 ± 0.22^{e}	29.61 ± 0.30^{d}	3.76 ± 0.14^{ghij}
DH	26.38 ± 0.50^{f}	19.88 ± 0.73^{f}	6.50 ± 0.29^{c}	28.21 ± 0.11^{h}	21.87 ± 0.27^{g}	6.33 ± 0.21^{c}
RSC	28.83 ± 0.24^{de}	24.02 ± 0.31^{e}	4.81 ± 0.22^{fg}	33.78 ± 0.64^{de}	27.90 ± 0.41^{e}	5.88 ± 0.24^{cd}
WSC	32.61 ± 0.47^{b}	28.80 ± 0.47^{c}	3.81 ± 0.09^{ij}	34.22 ± 0.08^{cd}	30.37 ± 0.16^{c}	3.86 ± 0.11^{ghi}
TSC	28.64 ± 0.69^{de}	23.23 ± 0.81^{e}	5.41 ± 0.19^{e}	31.45 ± 0.32^{f}	26.46 ± 0.30^{f}	4.99 ± 0.16^{e}
ASC	32.85 ± 0.44^{b}	29.32 ± 0.37^{bc}	3.54 ± 0.08^{jk}	36.23 ± 0.17^{a}	32.91 ± 0.36^{a}	3.33 ± 0.26^{j}
CSC	28.31 ± 0.52^{e}	23.53 ± 0.50^{e}	4.78 ± 0.02^{fg}	33.22 ± 0.57^{e}	29.20 ± 0.63^{d}	4.02 ± 0.09^{gh}
G24	25.39 ± 1.04^{g}	17.74 ± 1.04^{g}	7.65 ± 0.47^{b}	26.32 ± 0.41^{i}	19.04 ± 0.58^{h}	7.28 ± 0.17^{b}
G48	21.89 ± 0.49^{h}	15.89 ± 0.46^{h}	6.01 ± 0.57^{d}	24.01 ± 0.30^{j}	18.08 ± 0.43^{i}	5.93 ± 0.72^{cd}
G72	17.45 ± 0.57^{i}	12.65 ± 0.41^{i}	4.80 ± 0.59^{fg}	18.93 ± 0.42^{k}	14.38 ± 0.43^{j}	4.55 ± 0.07^{ef}

[a] Values are the mean of triplicate deteminations ± standard deviation. Values in the same column with different roman superscripts are significantly different ($p < 0.05$). [b] RA, raw seeds autoclaved; WSA, water soaked and autoclaved; TSA, tamarind extract soaked and autoclaved; ASA, alkaline soaked and autoclaved; CSA, citric acid soaked and autoclaved; DH, dry heated; RSC, raw seeds sequentially cooked; WSC, water soaked and cooked; TSC, tamarind extract soaked and cooked; ASC, alkaline soaked and cooked; CSC, citric acid soaked and cooked; G, geminated for 24, 48, and 72 h. [c] DS, calculated by difference as TS − RS (total starch − resistant starch).

Table 5. Effect of Various Processings on the in Vitro Digestibility of Protein (IVPD) and Starch (IVSD) of *M. pruriens* Var. *utilis* Seeds (Dry Matter Basis)[a]

	IVPD (%)		IVSD (% of total starch)	
treatment[b]	white variety[c]	black variety[c]	white variety[d]	black variety[d]
raw seed	68.25^{h}	69.37^{j}	6.74 ± 0.77^{i}	5.62 ± 1.06^{j}
RA	$80.58\ (15.30)^{bc}$	$79.57\ (12.82)^{e}$	23.46 ± 2.30^{ef}	17.33 ± 2.44^{g}
WSA	$83.86\ (18.61)^{a}$	$82.01\ (15.41)^{c}$	31.34 ± 1.72^{b}	25.86 ± 1.08^{cd}
TSA	$79.65\ (14.30)^{cd}$	$78.44\ (11.56)^{f}$	25.92 ± 0.72^{de}	20.21 ± 0.99^{f}
ASA	$83.74\ (18.50)^{a}$	$80.74\ (14.08)^{d}$	37.17 ± 1.01^{a}	32.86 ± 1.20^{a}
CSA	$81.30\ (16.05)^{b}$	$83.01\ (16.43)^{b}$	29.74 ± 1.44^{bc}	23.43 ± 0.82^{de}
DH	$74.96\ (9.83)^{f}$	$73.08\ (5.34)^{h}$	11.13 ± 1.89^{g}	7.22 ± 1.23^{ij}
RSC	$80.68\ (15.40)^{bc}$	$83.31\ (16.73)^{b}$	22.92 ± 1.94^{ef}	18.26 ± 1.31^{fg}
WSC	$82.88\ (17.64)^{a}$	$84.54\ (17.94)^{a}$	27.30 ± 2.90^{cd}	27.97 ± 1.60^{bc}
TSC	$78.48\ (13.03)^{de}$	$77.34\ (10.31)^{g}$	25.08 ± 1.89^{def}	23.04 ± 2.20^{e}
ASC	$83.61\ (18.36)^{a}$	$82.53\ (15.95)^{bc}$	34.80 ± 1.21^{a}	29.58 ± 2.19^{b}
CSC	$80.31\ (15.02)^{bc}$	$83.16\ (16.58)^{b}$	22.55 ± 2.57^{f}	25.50 ± 1.66^{cde}
G24	$69.40\ (1.64)^{h}$	$70.65\ (1.84)^{i}$	8.20 ± 0.21^{hi}	7.39 ± 0.47^{ij}
G48	$71.14\ (4.23)^{g}$	$72.43\ (4.41)^{h}$	10.03 ± 0.41^{gh}	9.01 ± 0.47^{i}
G72	$78.09\ (12.60)^{e}$	$76.73\ (9.56)^{g}$	11.50 ± 1.19^{g}	12.54 ± 1.00^{h}

[a] Values in the same column with different roman superscripts are significantly different ($p < 0.05$). [b] RA, raw seeds autoclaved; WSA, water soaked and autoclaved; TSA, tamarind extract soaked and autoclaved; ASA, alkaline soaked and autoclaved; CSA, citric acid soaked and autoclaved; DH, dry heated; RSC, raw seeds sequentially cooked; WSC, water soaked and cooked; TSC, tamarind extract soaked and cooked; ASC, alkaline soaked and cooked; CSC, citric acid soaked and cooked; G, germinated for 24, 48, and 72 h. [c] Figures in parentheses indicate the percent increase over the control values. [d] Values are the mean of triplicate determinations ± standard deviation.

respectively. However, among the various hydrothermal treatments, soaking in alkaline solutions followed by autoclaving in the white variety and soaking in citric acid solution followed by autoclaving in the black variety appear to be more effective for the removal of L-dopa, and the remaining contents of L-dopa closely resemble those values (1.5 g/person/day), which are considered to be safe for the consumption of mucuna beans (*55*). However, in some experiments the L-dopa content was reduced by thoroughly cracking the seeds, soaking them overnight, boiling them for 20 min, and soaking them again overnight (*55*). This technique could reduce the substantial quantity of potentially available nutrients, particularly, minerals. The relative reduction of (10−26%) of L-dopa in mucuna seeds during the dry heat treatment might be due to either partial oxidation or racemization. Germination of presoaked seeds for 24, 48, and 72 h was also more effective for the reduction of the L-dopa content in 13.4−16.4, 17.2−19.2, and 20.0−23.0%, respectively. Prakash and Tewari (*54*) have also observed a 31% reduction of L-dopa content in 3 days after germinating *Mucuna pruriens* seeds.

In Vitro Protein Digestibility. The IVPD of raw and processed seeds of mucuna beans is given in Table 5 . Raw seeds of the white and black varieties of mucuna beans exhibited 68 and 69% IVPD, respectively. Similar percentages of IVPD have been observed recently in Chinese indigenous legumes such as *P. angularis* and *P. calcaratus* (*16*). The relatively low protein digestibility of legumes may be due to their structural characteristics. Numerous studies have indicated that the globulin fraction of these legumes, which represents the major storage protein and comprises 50−75% of the total protein of the dry seed, is quite resistant to attack by proteolytic enzymes (*47*). The IVPD of the two varieties of mucuna beans was increased significantly ($p < 0.05$) by processing methods such as soaking, cooking, autoclaving, dry heating, and germination. Cooking or autoclaving treatments result in more (13.0−18.6 and 10.3−18% improvement in the white and black varieties, respectively) protein digestibility ($p < 0.05$) than dry heating and germination. A similar improvement of IVPD by hydrothermal treatment has also been reported in several legumes such as rice bean, faba

bean, *P. calcaratus*, and *P. angularis* (*14*, *16*, *48*). Compared with the control group, a longer time of germination (72 h) resulted in a significantly higher value of IVPD for both varieties of mucuna beans. Generally, many previous studies indicated that the decrease in the levels of antinutrients during soaking, heat treatment, and germination might be partly responsible for the improved IVPD (*14*, *16*, *58*). The improvement in the IVPD during the above processes might also be partly attributed to the changes in the activities of endogenous enzymes or the alternation of the storage protein structures including structural disintegration of enzyme inhibitors (*59*, *60*).

Starch Fractions and in Vitro Starch Digestibility. TS, DS, and RS contents of raw and processed white and black varieties of mucuna beans are given in Table 4. Both raw samples of the white and black varieties had similar amounts of TS (~28%) and very high contents of RS (mainly RS_2), 36 and 38% of the TS, respectively, resulting in low apparent starch digestibilities and low DS contents.

Hydrothermal processing resulted in an apparent increase of TS in both legumes. The TS content of the presoaked and then autoclaved or cooked samples was significantly higher than in the raw ones. Of all the treatments assayed, germination at 72 h resulted in the lowest TS values of 17.5 and 18.9% in the white and black varieties, respectively. DS, calculated as the difference between TS and RS, was higher in heat-treated samples, except dry heat treatment, than in the raw ones. About 82−90% of the starch in cooked or autoclaved seeds was digestible as compared with 64 and 62% in raw samples of white and black varieties of mucuna beans, respectively. Cooked or autoclaved samples contained a significant amount of RS (mainly RS_3), although processing greatly reduced the RS in all samples, because native granules, responsible for the high RS content of raw samples, are gelatinized during cooking. However, among the hydrothermal processing methods (cooking or autoclaving), water-soaked samples or samples soaked in sodium bicarbonate solution followed by cooking or autoclaving appeared to be more effective to reduce the RS content. On the other hand, germination (24−72 h) has relatively decreased the content of both TS (26.3−17.5%) and RS (7.3−4.6%) along with DS (19.0−12.7%).

The effects of different processing treatments on the in vitro starch digestion of mucuna beans are shown in Table 5. The results of starch digestion are shown as a percentage of the TS hydrolyzed at 3 h. Raw/soaking followed by either autoclaving or cooking greatly increased the starch digestibility in both legumes (17.3−37.2%). Among the various treatments, dry heating and germination registered the lowest in vitro starch digestibilities in both seed samples. However, substantial differences have been observed between autoclaving and cooking of the various solution-soaked samples. Among the various hydrothermal processing methods, soaking in sodium bicarbonate ($NaHCO_3$) solution/water followed by autoclaving or cooking appears to be more effective ($p < 0.05$) in improving the starch digestibility of both samples.

The apparent increase of the TS content observed in the processed legumes may be due to the partial loss of soluble materials during soaking and cooking. Soluble sugars, oligosaccharides, soluble polyphenols, or soluble dietary fiber components are some of the constituents that can be lost during processing. An increase of the TS content in cooked samples compared to the respective raw seed samples was also reported by Bravo et al. (*18*) in moth bean, horse gram, and black gram and by Periago et al. (*61*) in peas. On the other hand, starch in legume seeds is known to be hydrolyzed to oligosaccharides and ultimately to monosaccharides during germination; monosaccharides are then utilized to produce the energy required for various metabolic processes taking place during germination (*14*, *62*). This amylolysis, catalyzed by phosphorylase and amylases, may be responsible for the decreased TS content of sprouted legumes in comparison with the other processed samples. Similarly, during the germination of chickpea, cowpea, and green gram the increase in starch digestibility and decrease in total starch content have been observed by Urooj and Puttaraj (*63*). Starch in raw samples is contained within granules that are poorly affected by hydrolytic enzymes and it is, therefore, mostly indigestible (*64*). This accounts for the high RS content of raw legumes (RS_2). The variations in starch digestibility among the studied samples might be due to differences in the degree of crystallinity or amylose/amylopectin ratio of the starch granules, which are factors also known to affect starch digestibility (*65*). During cooking or autoclaving, starch granules are gelatinized and partly solubilized, becoming available to digestive enzymes. This explains the great improvement of starch digestibility attained after hydrothermal processing, with a significant decrease in the RS values. However, there is still a fraction of starch (10−17% of total starch) not digested by the amylolytic enzymes in the processed seeds. Several factors are involved in the reduced bioavailability of legume starches. The presence of intact tissue/cell structures enclosing starch granules hinders the swelling and solubilization of starch, the formation of retrograded starch (RS_3), resulting in a reduced digestion rate in vitro (*66*, *67*) and incomplete digestion in vivo (*68*). Other factors affecting the legume starch digestibility are the high contents of viscous, soluble dietary fiber components as well as the relatively high amylose/amylopectin ratios of legume starches and the presence of various antinutrients such as polyphenols, phytic acid, and other antinutrients (*38*, *69*). However, starch digestibility improves during soaking and hydrothermal processing, possibly because of decreasing levels of these antinutritional factors in the seed. As a consequence of this poor starch digestibility, pulses promote slow and moderate postprandial glucose and insulin responses (*70*). Such a "lente carbohydrate" (slower rate of digestion and absorption of carbohydrates) property of legumes has been suggested to have beneficial effects in the management of diabetes and hyperlipidimia (*71*, *72*). However, when the white and black varieties of mucuna beans were dry heated, decreases in starch digestibility (7.2 and 11.1%) were observed as compared to the other processed forms. The high temperature coupled with a low moisture level might have also resulted in rendering the starch resistant to enzyme attack. Similar observations have been reported by other workers (*63*, *73*).

Conclusions. In the present study, all of the investigated domestic processing methods appeared to be effective in reducing the levels of various detected antinutrients and improving the in vitro protein and starch digestibilities of both varieties of *M. pruriens* var. *utilis*. However, to obtain best results for the reduction

of antinutrients and for the improvement of starch and protein digestibilities in mucuna beans, seeds should be soaked in water or in sodium bicarbonate solution and then cooked or pressure cooked. Particularly, intoxication associated with the overeating of mucuna beans (L-dopa) can be eliminated up to a level of safer consumption by adapting the above-mentioned cost-effective methods. Consumption of such processed mucuna beans may serve as an additional dietary protein and carbohydrate source for the alleviation of malnutrition, which is prevalent among the tribal and rural people living in the developing world. To ensure the nutritional quality of processed samples and to compare the protein digestibility values, further investigations on chemical analyses and in vivo studies are in progress.

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