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Nutritional and antinutritional composition, in vitro amino acid availability, starch digestibility and predicted glycemic index of differentially processed mucuna beans (*Mucuna pruriens* var. *utilis*): an under-utilised legume

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Abstract

The effects of different solutions, water, 0.07% sodium bicarbonate, 0.1% ascorbic acid and 3% moringa leaf powder in water, and soaking, followed by autoclaving, on the proximate composition and antinutrients, in vitro amino acid digestibility, starch hydrolysis index (HI) and glycemic index (GI) of *Mucuna pruriens* var. *utilis* seeds were evaluated. Even though none of the processing techniques affected the proximate composition on gross energy value, they significantly ($P < 0.05$) reduced the levels of various antinutrients, such as total phenolics, tannins, phytates, saponins, L-dopa, trypsin inhibitor (TI), chymotrypsin inhibitor (CI) and lectin activities of the respective samples. Nonetheless, all the processing methods improved the total protein digestibility and individual amino acid availabilities, particularly those of methionine, tyrosine, lysine and arginine, without affecting the protein quality. When compared to the raw seed sample, all the hydrothermal processing increased the total (TS) and digestible (DS) starch content (351–361 and 303–315 g kg^{-1}, respectively) and decreased the resistant starch (RS) content (46.1–48.1 g kg^{-1}) significantly ($P < 0.05$). The in vitro starch digestion rate was also measured in the freshly processed seed samples, and the values of HI and GI were comparable to that of similarly processed legumes such as moth bean and black gram.

Keywords: *Mucuna pruriens* var. *utilis*; Processing; Antinutrients; L-Dopa; In vitro protein digestibility; Starch digestibility; Glycemic index

1. Introduction

The seeds of *Mucuna pruriens* var. *utilis* are found to be rich in protein (30%) and starch (28%) (Siddhuraju, Becker, & Makkar, 2000). In addition to cultivation as a potential green manure and as a cover crop, the cooked seeds are being consumed by people living in rural areas of India, Africa and Latin America. The crop gives reliable yield under the dry farming and low soil fertility conditions under which most of the other food legumes cannot be grown economically (Buckles, 1995). The bean is also used in indigenous ayurvedic medicine (Prakash & Tewari, 1999) and the L-dopa extracted from it is used to provide symptomatic relief in Parkinson's disease. However, raw mucuna seeds in the diet have been reported to reduce the growth rates of broiler chicks (Del Carmen, Gernat, Myhrman, & Carew, 1999) and also reduce the metabolic growth rate and feed utilisation in common carp (Siddhuraju & Becker, 2001a, 2001b, 2001c). Furthermore, the pharmacologically active factor L-dopa has been found to be a potential antinutrient (Siddhuraju & Becker, 2002).

The digestibility and utilisation of the legume protein is limited by the structure of protein (Deshpande & Damodaran, 1989) and the presence of antimetabolic factors, such as trypsin inhibitor, chymotrypsin

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inhibitor, phytates, phenolics and lectins (Liener, 1994a; Nielsen, 1991). The digestibility of legume starch is also affected by the cell-wall structural features (Tovar, de Francisco, Björck, & Asp, 1991) and antinutrients, such as amylase inhibitors (Lajolo, Filho, & Menezes, 1991), phytates (Urooj & Puttaraj, 1994), and polyphenolics (Thomson & Yoon, 1984; Yadav & Khetarpaul, 1994). Various processing and cooking methods could improve the protein and starch digestibility of legume seeds by decreasing the levels of some antinutrients (Barampama & Simard, 1994; Bishnoi, Khetarpaul, & Yadav, 1994; Chau & Cheung, 1997). Moreover, the improvement on digestibility, after the thermal treatments, might be attributed to some other factors, such as disruption of protein structures and cell wall-encapsulated starch, starch gelatinisation, and physical disintegration of the legume seeds (Björck, Granfeldt, Liljeberg, Tovar, & Asp, 1994; Bravo, Siddhuraju, & Saura-Calixto, 1998; Tovar et al., 1991). Although the hydrothermal treatments were found to be most effective among the different processing methods for the reduction of various antinutrients in mucuna beans (Siddhuraju & Becker, 2001a, 2001b, 2001c) no information is available on the proximate composition, amino acid profiles or their availability, in vitro starch digestion rate, starch hydrolysis index or glycemic response of such processed beans. Therefore, the present study was carried out to compare the effects of various soaking solution, followed by autoclaving, on the antinutrients and in vitro digestibility of the protein and starch in mucuna beans.

2. Materials and methods

2.1. Processing of mucuna seeds

The mature and dry seeds of mucuna beans were obtained from the tribal people living in a village near Marthandam, Tamil Nadu, India during the month of January 1999 (Siddhuraju et al., 2000). A *Moringa oleifera* tree leaf (freeze-dried) sample was received from Mr. Foidl, Nicaragua. Some preweighed raw seed samples were mechanically cracked into two or three parts with a hammer and soaked for 24h at room temperature (24 ± 1 °C) in separate 2 l capacity beakers containing the following solutions: (A) water; (B) 0.07% sodium bicarbonate; (C) 0.1% ascorbic acid; and (D) water containing 3% freeze-dried moringa leaf powder (in order to prevent the non-enzymatic and polyphenoloxidase enzyme mediated oxidation of L-dopa during the processing of mucuna beans, as it was the first time that the dehydrated moringa leaf powder, which contains natural antioxidants, such as ascorbic acid and dietary flavonoids, in water, had been attempted as a soaking medium), in a seed: water/solution ratio of 1:10 (w/v). The solutions were occasionally stirred with a glass rod (2–3 times). After soaking, the solutions were decanted and the soaked seeds were rinsed in distilled water and autoclaved for 20 min at 121 °C at 15 psi (1.05 $kp\,cm^{-2}$), maintaining a seed:water ratio of 1:5 (w/v). After decanting the autoclaved water, the seed samples were freeze-dried. The samples were ground into a fine powder and stored at room temperature until required for further analysis.

To prevent the possible retragradation of the starch, another set of samples was analysed for in vitro starch digestibility rate and resistant starch content immediately after autoclaving. For the above-mentioned analysis, a portion of cracked seed (~120 mg), with similar seed coat and cotyledon proportions, was soaked in the respective solutions and autoclaved, in triplicate, in separate centrifuge tubes and the autoclaving liquid was discarded.

2.2. Proximate composition

The moisture content of the raw and processed seed samples was determined by oven-drying to a constant weight at 105 °C. Crude protein, lipid, crude fibre and ash were determined in accordance with the standard methods of AOAC (1990). Carbohydrates (nitrogen-free extractives) were obtained by difference. Gross energy was estimated by an adiabatic bomb calorimeter (IKA C7000), using benzoic acid as a standard.

2.3. Analysis of total phenolics and tannins

Total phenolics and tannins were extracted and determined by spectrophotometric methods described by Makkar, Becker, Abel, and Pawelzik, 1997. Total phenolics were quantified by the Folin–Ciocalteu reagent, and tannins were quantified as the difference between phenolics before and after tannin removal from the extract using insoluble polyvinylpolypyrrolidone (PVPP). Results of total phenolics and tannins were expressed as tannic acid (T-0125; Sigma Chemical Co. St. Louis, MO) equivalents.

2.4. Estimation of phytic acid and total saponin contents

The phytic acid content of the sample was determined by a colorimetric procedure described by Vaintraub and Lapteva (1988), as modified by Alonso et al. (1995). A suitable aliquot was diluted with distilled water to make 3 ml and was then used for the assay. The results were expressed as $g\,kg^{-1}$ of dry matter by using standard phytic acid (P-8810; Sigma, St. Loius, MO). Total saponin content was determined by a spectrophotometric method, as described by Hiai, Oura, and Nakajima (1976). The results were expressed as diosgenin equiva-

lent from a standard curve of different concentrations of diosgenin (Art. 7044; Carl Roth GmbH, Karlsruhe) in 80% aqueous methanol.

2.5. Extraction and estimation of L-dopa (L-3,4-dihydroxyphenylalanine)

Five millilitres of 0.1 N HCl were added to 80–100 mg of finely ground raw and processed seed flour taken in a glass tube, and the samples were stirred for 20 min at room temperature. The mixture was initially subjected to an Ultra-turrax T25 (20,500 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 (10,000*g*, 4 °C, 15 min). The extraction procedure was repeated twice, and the supernatants from all the 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 (Siddhuraju & Becker, 2001a, 2001b, 2001c). By using the authentic standard, the sample results were expressed as g kg^{-1} dry matter. The standard, L-dopa, was purchased from Sigma (St, Louis, MO).

2.6. Trypsin and chymotrypsin inhibitor activity determination

The trypsin inhibitor activity was essentially determined according to Smith, Van Megen, Twaalfhoven, and Hitchcook (1980) except that the enzyme was added last, as suggested by Liu and Markakis (1989). The chymotrypsin inhibitor activity of defatted seed samples was assayed in a 0.1 M borate buffer, pH 7.6, by the procedure of Kakade, Swenson, and Liener (1970). 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 10 ml. A chymotrypsin inhibitor unit (CIU) was defined in terms of chymotrypsin units inhibited per mg of sample.

2.7. Lectin activity assay

Analysis of the lectin content was conducted by hemagglutination assay (Gordon & Marquardt, 1974) in the presence of 10 mM Mn^{2+} in round-bottomed wells of microtitre plates using 2% (v/v) trypsinised cattle blood erythrocytes suspension in saline phosphate buffer, pH 7.0 (Makkar & Becker, 1997). The hemagglutination activity was defined as the minimum amount of the bean material (in mg per ml of the assay medium), which produced agglutination. 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, More, McKenzie, Stewart, & Pusztai, 1983).

2.8. Amino acid analysis

The amino acid compositions of the raw and the differentially processed mucuna seeds were determined using an automated amino acid analyser after hydrolysing the samples with 6 M HCl at 110 °C for 24 h (Bassler & Buchholz, 1993). The sulphur-containing amino acids were oxidised using performic acid before the acid hydrolysis. The tryptophan contents of the above-mentioned samples were determined spectrophotometrically by the method of Pintèr-Szakàcs and Molnár-Perl (1990). The contents of different amino acids recovered were presented as g 16 g^{-1} of nitrogen. The amino acids content of the mucuna samples were compared with the FAO/WHO (1990) reference pattern and soybean (Vasconcelos et al., 1997).

2.9. In vitro protein digestibility evaluation by dialysis cell method

Each sample containing 250 mg (40 mg of nitrogen) of protein was suspended in 16 ml of 0.01 N HCl, pH was adjusted to 1.9 at 37 °C and 1 mg pepsin 1:60,000 (3500 units/mg protein; porcine stomach mucosa, Sigma Co. St. Louis, MO) was added. The enzymatic hydrolysis was carried out for 30 min at 37 °C and the digestion was stopped by raising the pH to 7.5 with 1N NaOH. The digestate thus obtained was transferred into the dialysis bag Spectra/Por 6 tubing (Spectrum Medical Industries, Los Angeles, CA) that had a specific molecular exclusion rating of 1000 Da and then 10 mg pancreatin (5 × USP, hog pancreas, ICN Pharmaceuticals, Cleveland, OH) were added to the mixture. The digestion products, which dialysed to the outer compartment of the cell for 6 h at 37 °C against circulating 0.01 M sodium phosphate buffer at pH 7.5, were collected at a rate of 1.6 ml/min by means of a peristaltic pump (Gauthier, Vachon, & Savoie, 1986; Savoie & Gauthier, 1986).

Amino acid contents of the hydrolysates were determined according to the procedure mentioned earlier. The nitrogen content of the enzymatic digests was determined by the micro-Kjeldahl method. The in vitro digestibility of proteins was evaluated on the basis of the nitrogen content of the dialysate as follows:

$$\text{Nitrogen digestibility (\%)} = \frac{\text{Protein nitrogen dialysed out}}{\text{Native protein nitrogen}} \times 100$$

$$\text{Amino acid digestibility (\%)} = \frac{\text{Amino acid in dialysate}}{\text{Amino acid in the native protein}} \times 100.$$

2.10. Total, digestible and resistant starch analyses

The total starch (TS) content was determined after dispersion of the starch granules in 2 M KOH (50 mg sample, 6 ml KOH) at room temperature (30 min, constant shaking) and hydrolysis of the solubilized starch with 80 μl amyloglucosidase (14 U mg^{-1}; EC 3.2.1.3; Cat. No. 102857, Boehringer–Mannheim, Germany) at 60 °C for 45 min (Goni, Garcia-Alonso, & Saura-Calixto, 1997). The glucose content was determined by using the glucose oxidase/peroxidase reagent (Cat. No. 510-A, Sigma Chemical Co., Deisenhofen, Germany). Total starch was calculated as glucose × 0.9, after correction for the free glucose content. The glucose content of the samples, both free glucose and the glucose moiety of sucrose, was determined in order to correct the total starch values obtained. The samples dispersed in 2 M KOH were treated with 0.2 ml of (10 mg in 1 ml of 0.4 M sodium acetate buffer, pH 5.0) invertase (EC 3.2.1.26; 300 U mg^{-1}) for 30 min at 37 °C. After centrifugation, a 1 ml aliquot was mixed with 2 ml of 96% ethanol to precipitate proteins and macromolecular carbohydrates, centrifuged again and the glucose was analysed in the supernatants, using the glucose oxidase/peroxidase reagent.

The resistant starch (RS) was analysed as follows: sample (100 mg) was treated with 20 mg pepsin (1 g pepsin per 10 ml 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 1ml pancreatic α-amylase (EC 3.2.1.1, A-3176, Sigma) solution, containing 40 mg α-amylase per ml Tris maleate buffer, pH 6.9, to remove digestible starch. After centrifugation (3000*g*, 15 min) and removal of the supernatant, the pellet was dispersed with 2 M KOH, hydrolysed with amyloglucosidase and the liberated glucose quantified, as described above, for total starch. RS was calculated as glucose × 0.9 (Goni, Garcia-Diz, Manas, & Saura-Calixto, 1996). The digestible starch (DS) content was calculated as the difference between TS and RS.

2.11. In vitro starch digestion rate, HI and GI determination

Fresh soaking in various solutions, followed by pressure-cooking (autoclaving) of samples were used to assess the kinetics of starch digestion. Soon after draining the autoclaved water, the samples were homogenised through a tissue homogeniser (Ultra-Turrax T25, Janke & Kunkel), in HCl–KCl buffer of pH 1.5 at constant speed (13,500 rpm min^{-1}) during 1.5 min. The protein was hydrolysed by using the procedure mentioned in the total starch determination section. The pH was then adjusted to 6.9 after the addition of Tris maleate buffer, and 5 ml of α-amylase solution, containing 2.6 IU of α-amylase in Tris maleate buffer, were added. The samples were then incubated at 37 °C in a shaking water bath. At 30 min intervals, 1 ml samples of the supernatants were taken (without disturbing the pellet), placed in separate tubes and incubated at 100 °C for 5 min with vigorous shaking to inactivate the enzyme. The digested starch was completely hydrolysed using amyloglucosidase and glucose was determined as described above by calculating the digested starch as glucose × 0.9. The rate of starch digestion was expressed as the percentage of TS hydrolysed at different times. The area under the hydrolysis curve (AUC) was calculated using the first-order equation described by Goni et al. (1997)

$$\mathrm{AUC} = C_{\infty}(t_f - t_0) - (C_{\infty}/k)[1 - \exp[-k(t_f - t_0)]],$$

where C_{∞} corresponds to the concentration at equilibrium (t_{180}), t_f is the final time (180 min), t_0 is the initial time (0 min), and k is the kinetic constant. A hydrolysis index (HI) was calculated by comparison with the AUC of a reference food (fresh white bread) as described previously (Goni et al., 1997). By using the in vitro starch HI values, the GI was estimated by the following equation established by Goni et al. (1996)

$$\mathrm{GI} = 39.71 + (0.549 \times \mathrm{HI}).$$

2.12. Statistical analyses

The data were subjected to a one way analysis of variance (ANOVA) and the significance of the difference between means was determined by Duncan's multiple range test ($P < 0.05$) using the Statistica for Windows H'97, Version 5.1 (Statsoft Inc., Tulsa, USA). Values expressed are means ± SD.

3. Results and discussion

3.1. Proximate composition

The proximate composition of raw and processed mucuna beans is shown in Table 1. The crude protein and crude lipid content of various processed seeds (317–332 and 49.3–50.2 g kg^{-1}, respectively) were relatively higher than the raw seeds (301 and 43.4 g kg^{-1}, respectively). The substantial reduction of ash content in the processed seeds (20.6–24.2 g kg^{-1}) might be due to the leaching of both micro and macro elements into the different soaking solutions and subsequent autoclaving medium through the mechanically broken and enhanced permeability of seed coats when compared to the unprocessed seed sample (35.2 g kg^{-1}). Similar results have also been reported in soya bean (*Glycine max*) and lima bean (*Phaseolus lunatus*), when samples were subjected to the autoclaving process (Aletor & Ojo, 1989). However, these processing techniques did not affect the levels of crude fibre, nitrogen-free extractives and gross energy values.

Table 1
Proximate composition and antimetabolic factors in raw mucuna seeds and those soaked in different solutions followed by autoclaving (g kg^{-1} dry matter)

Component	Raw mucuna seeds	Treated mucuna seeds			
		A	B	C	D
Crude protein	301	332	328	317	322
Crude lipid	43.4	50.2	48.7	49.3	50.1
Ash	35.2	20.6	23.4	22.9	24.2
Crude fibre	73.5	80.1	72.3	78.6	72.4
NFE	546.9	517.3	528.0	532.2	531.1
Gross energy (MJ kg^{-1})	19.6	20.0	19.6	20.1	20.0
L-Dopa	$48.1^{a} \pm 0.9$	$13.6^{c} \pm 0.8$	$12.2^{d} \pm 0.4$	$15.1^{b} \pm 0.6$	$14.2b^{c} \pm 0.4$
Total phenolics[A]	$56.5^{a} \pm 2.4$	$23.2^{c} \pm 0.6$	$20.1^{d} \pm 0.6$	$24.8^{c} \pm 1.2$	$28.5^{b} \pm 0.9$
Tannins[A]	$3.0^{a} \pm 0.3$	$1.0^{c} \pm 0.3$	$0.7^{cd} \pm 0.1$	$0.5^{d} \pm 0.2$	$1.5^{b} \pm 0.2$
Phytic acid	$9.5^{a} \pm 0.5$	$4.0^{c} \pm 0.2$	$3.3^{d} \pm 0.3$	$5.1^{b} \pm 0.4$	$4.7^{b} \pm 0.3$
Saponins[B]	$12.1^{a} \pm 1.0$	$2.3^{c} \pm 0.4$	$2.8^{bc} \pm 0.3$	$3.1^{bc} \pm 0.2$	$3.6^{b} \pm 0.3$
Trypsin inhibitor activity[C]	$14.0^{a} \pm 0.23$	$0.51^{b} \pm 0.03$	$0.41^{b} \pm 0.05$	$0.53^{b} \pm 0.02$	$0.44^{b} \pm 0.04$
Chymotrypsin inhibitor activity[D]	9.8 ± 0.3	ND	ND	ND	ND
Lectin activity (HU)[E]	Trace	ND	ND	ND	ND

NFE, nitrogen-free extract = 1000−(crude protein + lipid + ash + crude fibre).
Values are means of triplicate determinations.
Values followed by different superscripts in same row are significantly ($P < 0.05$) different.
Soaking in (A) water, (B) 0.07% sodium bicarbonate solution, (C) 0.1% ascorbic acid solution, and (D) water containing 3% freeze-dried moringa leaf power, followed by autoclaving. ND, not detected.
[A] As tannic acid equivalents.
[B] As diosgenin equivalent. L-Dopa, 3,4-dihydroxyphenylalanine.
[C] mg pure trypsin inhibited g^{-1} sample.
[D] CIU, chymotrypsin inhibitor unit mg^{-1} sample.
[E] Phytohaemagglutinating unit (HU mg^{-1} sample).

3.2. Effects of processing on antinutrients

Table 1 shows that the various processing methods, such as cracked seed soaking in water, sodium bicarbonate, ascorbic acid and water containing 3% moringa leaf powder, followed by autoclaving, significantly reduced ($P < 0.05$) the levels of the total phenolics, tannins, phytates, saponins, L-dopa, TIA, CIA and lectin of *Mucuna pruriens* var. *utilis*. Similarly, cooking/pressure-cooking of raw and presoaked seeds in different solutions, of various legumes, such as *Vigna aconitifolia*, mung bean, faba bean, *Lathyrus sativus*, *Canavalia ensiformis*, *C. braziliensis*, *Phaseolus angularis* and *P. calcaratus* significantly decreased the various antinutrients (Carlini & Udedibie, 1997; Chau & Cheung, 1997; Kataria, Chauhan, & Punia, 1989; Khalil & Mansour, 1995; Khokhar & Chauhan, 1986; Srivastava & Khokhar, 1996). Among the various treatments, there were no significant differences in the levels of TIA, CIU and lectin activity, but soaking in sodium bicarbonate solution, followed by autoclaving, brought about significantly ($P < 0.05$) lower total phenolics and phytate contents than all other processing methods. Although the various hydrothermal processing techniques have been found to be more effective than other processing methods for the reduction of non-protein amino acid L-dopa in mucuna beans (Siddhuraju & Becker, 2001a, 2001b, 2001c), the remaining concentration seems to be still higher. However, in the present study, soaking of cracked seeds in the different solutions, particularly in 0.07% sodium bicarbonate, followed by autoclaving, significantly decreased the L-dopa content (12.2 g kg^{-1}) when compared to whole seeds soaked in various solutions, followed by cooking or autoclaving (16.2–27.8 g kg^{-1}) (Siddhuraju & Becker, 2001a, 2001b, 2001c). Similar effects for cracked mucuna seeds after soaking them overnight in water, followed by boiling, on reduction of L-dopa have also been reported by (Lorenzetti, MacIsaac, Arnason, Awang, & Buckles, 1998). Such a greater reduction of L-dopa in the present study during soaking in different solutions, followed by autoclaving, could be explained by the exposure of a larger surface area of cotyledons to the soaking medium under different ionic strengths, which further increased permeability of seed coats, along with non-dependent seed coat permeability effects. Moreover, the remaining concentrations, of L-dopa levels in all the processed samples seem to be close to the value, 1.5 g/person/day, which is considered to be safe for consumption (Lorenzetti et al., 1998). In general, the decrease in the levels of these antinutrients, particularly L-dopa, during heat treatment might also be due to thermal degradation and denaturation, as well as the formation of insoluble complexes (Kataria et al., 1989; Siddhuraju & Becker, 2001a, 2001b, 2001c).

3.3. Protein quality and amino acid availability

The amino acid compositions of raw and differentially treated mucuna beans, along with the amino acid profiles of soybean and the essential amino acid requirements pattern suggested by FAO/WHO (1990), are shown in Table 2. The amino acid profiles of mucuna beans revealed that the proteins of this seeds contained adequate levels of essential amino acid as compared with the FAO/WHO (1990) and soybean (Vasconcelos et al., 1997), except for the sulphur-containing amino acids, cystine and methionine, which are considered to be the most limiting amino acids in legumes. Soaking in different solutions, followed by autoclaving for 20 min, caused substantial reduction of most of the EAA except methionine and isoleucine in water-soaking, followed by autoclaving, cystine in ascorbic acid soaking followed by autoclaving, methionine in water (containing 3% moringa leaf) powder soaking, followed by autoclaving and tryptophan in all the treated seeds. The reduction in methionine and cystine during prolonged cooking has been reported by Ziena, Youssef, and El-Mahdy (1991) and Khalil and Mansour (1995) in faba beans. In general, both sodium bicarbonate and ascorbic acid solution soaking, followed by autoclaving, were found to cause more pronounced effects on the relative destruction of most of the essential amino acids, particularly threonine, leucine, phenylalanine, histidine and lysine (17% and 16.2%; 21.3% and 17.9%; 14.9% and 16.8%; 17.3% and 18.4%; 11.5% and 15.2%, respectively), than other treatments. Chau, Cheung, and Wong (1997) reported that cooking for 30 min caused significant reduction of most of the EAA, except leucine and phenylalanine, for *Phaseolus angularis* and except phenylalanine, for *P. calcaratus*. Moreover, the same authors have also reported that cooking the seeds for 60 min significantly reduced the contents of all of the EAAs, except leucine, histidine, lysine and threonine in *Dolichos lablab*. Even though the various domestic preparations reduced the EAA levels, the total EAA retention in the seeds of mucuna beans were still found to be adequate or higher than the FAO/WHO (1990) requirement pattern.

Digestibilities and relative digestibilities of amino acids of the raw and treated seed samples are given in Table 3. After a 6 h it in vitro digestion, among the

Table 2
Amino acid composition of differentially processed mucuna seeds, FAO/WHO (1990) recommended allowances and soybean

Amino acid	Raw mucuna seeds	Amino acid composition (g 16 g^{-1} N)								Soybean[a]	FAO/WHO (1990) values[b]
		Differentially processed mucuna seeds[e]									
		A	β	B	β	C	β	D	β		
Aspartic acid	11.4	10.6	6.8	10.2	10.4	10.4	8.2	10.4	8.3	11.3	
Threonine	3.58	3.40	5.0	2.97	17.0	3.00	16.2	3.23	9.8	3.76	3.40
Serine	4.37	4.16	4.8	3.87	11.4	3.91	10.5	4.26	2.5	5.67	
Glutamic acid	12.3	11.9	3.3	11.0	10.3	11.2	8.4	11.9	3.0	16.9	
Glycine	4.30	4.13	4.0	3.87	10.0	3.91	9.1	4.05	5.8	4.01	
Alanine	3.22	3.05	5.3	3.10	3.7	2.86	11.2	3.02	6.2	4.23	
Cystine	1.01	0.94	6.9	0.90	10.9	1.02	−1.0	1.00	1.0	1.70	2.50[c]
Methionine	0.72	0.76	−5.6	0.70	2.8	0.71	1.4	0.76	−5.6	1.22	
Valine	4.23	4.23	0.0	4.00	5.4	3.95	6.6	4.05	4.3	4.59	3.50
Isoleucine	4.16	4.27	−2.6	3.83	7.9	3.78	9.1	3.88	6.7	4.62	2.80
Leucine	7.88	7.53	4.4	6.20	21.3	6.47	17.9	6.53	17.1	7.72	6.60
Tyrosine	4.45	4.06	8.8	4.00	10.1	3.68	17.3	3.78	15.1	3.39	6.30[d]
Phenylalanine	4.70	4.44	5.5	4.00	14.9	3.91	16.8	4.16	11.5	4.84	
Histidine	3.47	3.09	11.0	2.87	17.3	2.83	18.4	2.89	16.7	2.50	1.90
Lysine	6.18	5.83	5.7	5.47	11.5	5.24	15.2	5.64	8.7	6.08	5.80
Arginine	5.28	5.13	2.8	5.07	4.0	5.00	5.3	5.33	−0.9	7.13	
Proline	5.06	4.51	10.9	4.83	4.5	4.59	9.3	4.78	5.5	4.86	
Tryptophan	1.22	1.30	−6.6	1.31	−7.4	1.23	−0.8	1.30	−6.6	1.24	1.10
Limiting amino acid											
I	cys + met	cys + met		cys + met		cys + met		cys + met			
II				thr		thr		thr			

β, Percent loss of amino acid from raw mucuna beans. Values are means of two independent analyses.

[a] Vasconcelos et al. (1997).

[b] Data from FAO/WHO (1990) reference pattern of essential amino acid requirement for pre-school children (2–5 years old).

[c] Cystine + methionine.

[d] Tyrosine + phenylalanine.

[e] Soaking in (A) water, (B) 0.07% sodium bicarbonate solution, (C) 0.1% ascorbic acid solution, and (D) water containing 3% freeze-dried moringa leaf power, followed by autoclaving.

Table 3
Amino acid availability of raw and differentially processed mucuna beans (g 16 g^{-1} N)

Amino acid	Raw mucuna seeds		Differentially processed mucuna seeds[a]							
			A		B		C		D	
	α	β	α	β	α	β	α	β	α	β
Aspartic acid	2.19	19.3	3.70	34.9	4.23	41.6	4.42	42.4	3.89	37.4
Threonine	0.72	20.1	1.34	39.3	1.31	44.2	1.27	42.2	1.19	36.7
Serine	0.99	22.6	1.74	41.9	1.88	48.6	1.86	47.5	1.73	40.7
Glutamic acid	2.34	19.1	3.96	33.4	4.32	39.3	4.43	39.4	3.84	32.3
Glycine	0.70	16.3	1.50	36.3	1.65	42.7	1.68	43.0	1.51	37.4
Alanine	0.88	27.4	1.03	33.7	1.27	40.9	1.30	45.5	1.15	38.2
Cystine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Methionine	0.29	39.7	0.42	55.8	0.42	59.8	0.34	47.2	0.43	57.1
Valine	1.06	25.0	1.72	40.6	1.90	47.6	2.00	50.6	1.76	43.5
Isoleucine	0.83	20.0	1.47	34.5	1.67	43.7	1.71	45.2	1.57	40.3
Leucine	1.52	19.3	2.92	38.7	3.26	52.5	3.44	53.2	3.13	47.9
Tyrosine	1.58	35.4	2.46	60.6	3.28	82.1	2.98	81.0	3.19	84.3
Phenylalanine	1.44	30.5	2.03	45.6	2.75	68.8	2.67	68.4	2.54	61.0
Histidine	0.81	23.3	1.13	36.6	1.33	46.5	1.41	49.8	1.12	38.9
Lysine	1.72	27.9	3.30	56.7	3.43	62.8	3.81	72.6	3.33	59.1
Arginine	1.89	35.8	3.11	60.7	3.55	69.9	3.64	72.7	3.48	65.3
Proline	2.02	40.0	1.66	36.9	1.93	39.9	1.79	39.0	2.31	48.3
Tryptophan	0.36	29.5	0.52	40.0	0.61	46.6	0.49	39.8	0.52	40.0
Protein Digestibility(%)[b]		30.4		40.4		43.3		39.6		39.8

α, Amino acids liberated (g 16 g^{-1} N) after 6 h enzymatic digestion.
β, Amino acids availability (%) after 6 h enzymatic digestion.
Values are means of two determinations. ND, not detected.
[a] Soaking in (A) water, (B) 0.07% sodium bicarbonate solution, (C) 0.1% ascorbic acid solution, and (D) water containing 3% freeze-dried moringa leaf power, followed by autoclaving.
[b] Protein digestibility% = [Sample protein ($N \times 6.25$) dialysed out/native sample protein ($N \times 6.25$)] × 100.

various processed samples, soaking in sodium bicarbonate solution, followed by autoclaving, exhibited the highest nitrogen digestibility (43.3%). This value is well comparable to those of pinto bean, kidney bean, lentils, chickpea, freeze-dried soybean protein and heat-dried soybean protein (Rozan et al., 1997; Savoie, Charbonneau, & Parent, 1989). However, the lowest (30%) nitrogen digestibility level was observed in raw mucuna beans. The contribution of each amino acid to the total nitrogen release was variable. It was obvious that some amino acids, such as tyrosine, phenylalanine, lysine, arginine and methionine more readily released by enzymes. On the other hand, amino acids, such as aspartic acid, glutamic acid, threonine, isoleucine, leucine and histidine, were generally were more difficult to release in raw samples. However, when samples subjected to various soaking solutions, followed by the hydrothermal processing relatively increased the availability rate of both essential and non-essential amino acids, except proline. Nonetheless, among the different treatments, ascorbic acid-treated samples were found to show greater amino acid release rate. However, the presence of phytate and protease inhibitors could have had inhibitory action on the protein digestibility in the raw seed samples (Liener, 1994a, 1994b). On the other hand, this little known legume has a high concentration of total phenolics with a major proportion of the non-protein phenolic amino acid, 3,4-dihydroxyphenylalanine, and oxidation of such *o*-diphenolics forms a *o*-quinones and such generated, unstable, highly reactive molecules, subsequently react with other *o*-quinones, amino acids or proteins, evolving into brown, black or red heterogeneous polymers which might also partially have been responsible for the reduction of proteolytic digestion of proteins (Rohn, Rawel, Pietruschiski, & Kroll, 2001). Further the primary, secondary, tertiary structures and amino acid sequences of the protein could also influence the overall digestion of the protein (total nitrogen) as well as the individual amino acid availability. Apart from this, the non-starch polysaccharide constituent (NSPs) should also have decreased the digestibility of seed proteins through the interaction with the enzymes or proteins rather than by changing the apparent viscosity of the digestive mixtures (Kossori et al., 2000). However, the significant reduction of various detected antinutrients, along with denaturation of protein structure during the processing of mucuna seeds, might have influenced the higher amino acid availability. Similarly, soaking, followed by thermal processing, of legumes, such as *Vigna umbellata*, *Phaseolus angularis* and *Phaseolus calcaratus* also significantly improved the in vitro protein digestibility (Chau & Cheung, 1997;

Kaur & Kapoor, 1990). Although the alkali-treated plant proteins were found to be relatively less digested under in vitro enzymatic digestion conditions (Vachon, Gauthier, Jones, & Savoie, 1983), in the present study the mucuna seeds treated with low concentration of sodium bicarbonate (0.07%) did not show any drastic reduction of amino acid availability when compared to the other treated samples.

3.4. Effect of processing on starch fractions, in vitro digestion rate and glycemic response

The enzymatically assessed total, digestible and resistant starch contents of the raw and differentially processed mucuna beans are given in Table 4. The raw mucuna seeds contained 271 g kg^{-1} of total starch with a high amount of resistant starch, 88.3 g kg^{-1}, mainly RS_2, which contributed to the low starch digestibilities and digestible starch contents. However, when compared to conventional legumes, such as Bengal gram, cow pea, green gram and black gram (Bravo et al., 1998; Urooj & Puttaraj, 1994), the total starch content of raw mucuna beans was found to be low. On the other hand, the indigestible starch (RS; percent of total starch) levels in mucuna beans were also found to be much lower than those in white beans, pinto beans and chickpeas (Bravo, 1999). During the processing with different soaking solutions, followed by autoclaving, TS and DS were significantly increased (351–361 and 303–314 g kg^{-1}, respectively) and RS content was decreased (46.1–48.1 g kg^{-1}) without registering any significant differences ($P < 0.05$) among them when compared to the unprocessed seed sample. This might be due to the partial loss of soluble components, such as oligosaccharides and phenolic substances, including L-dopa. Similar results have also been reported in cowpea, green gram, chickpeas and white beans during pressure-cooking/boiling (Bravo, 1999; Urooj & Puttaraj, 1994). The starch in raw samples is contained within the granules that are poorly affected by hydrolytic enzymes and it is therefore mostly indigestible (Colonna, Leloup, & Buleon, 1992) and this accounts for the higher RS content of raw legumes (RS_2). The variation in the starch digestibility is being influenced by factors, such as degree of crystallinity or amylose/amylopectin ratio of the starch granules (Englyst, Kingman, & Cummings, 1992). During soaking in different solutions, followed by pressure-cooking, starch granules are gelatinised and partly solubilized, becoming available to digestive enzymes. This explains the great improvement of starch digestibility attained after different solution soakings, followed by hydrothermal processing, with a significant decrease in the RS values. However, there is still a portion of starch (12.8–13.5% of total starch) not digested by the amylolytic enzymes in the processed seeds. Multiple factors are involved in the reduced bioavailability of legume starches. The presence of intact cell/tissue structure, enclosing starch granules, hinders the swelling and solubilization of starch and the formation of retrograded starch (RS_3), resulting in a reduced digestion rate in vitro (Tovar, Björck, & Asp, 1990; Würsch, Del Vedovo, & Koellreuter, 1986), and incomplete digestion in vivo (Tovar, Björck, & Asp, 1992). Other factors affecting the legume starch digestibility are the high contents of viscous of soluble dietary fibre constituents as well as the relatively high amylose/amylopectin ratios and the presence of various antinutrients, such as polyphenols, phytic acid and other antinutrients (Deshpande & Cheryan, 1984; Thomson & Yoon, 1984). However, starch digestibility improves during solutions soaking, followed by autoclaving, possibly due to the decreasing levels of these antinutritional factors in the seed. The in vitro starch hydrolysis rate of raw mucuna beans at different time intervals was significantly lower than all the processed samples (Table 5 and Fig. 1). Although the starch hydrolysis rate were found to be similar among the processed samples with respect to time intervals, starch hydrolysis rates, at 60 and 150 min, of moringa leaf powder-treated seed samples were significantly lower than the sodium bicarbonate-treated samples. The observed HI and estimated GI of the raw and processed mucuna beans are given in Table 6. The highest HI (40.5) and GI (62.0) were observed in sodium bicarbonate treated samples and these values were

Table 4
Total, resistant and digestible starch contents of raw and differentially processed mucuna seeds (g kg^{-1} dry matter)[A]

Component	Raw mucuna seeds	Differentially processed mucuna seeds			
		A	B	C	D
Total starch	$271^{b} \pm 13.6$	$351^{a} \pm 5.6$	$351^{a} \pm 19.6$	$356^{a} \pm 13.4$	$361^{a} \pm 12.0$
Resistant starch	$88.3^{b} \pm 3.6$	$48.1^{a} \pm 1.6$	$46.3^{a} \pm 2.3$	$46.1^{a} \pm 0.8$	$46.4^{a} \pm 0.8$
Digestible starch[B]	$183^{b} \pm 13.3$	$303^{a} \pm 4.0$	$310^{a} \pm 21.1$	$309.7^{a} \pm 13.1$	$315^{a} \pm 11.9$
(DS% of TS)	67.4	86.3	86.8	87.0	87.2

Values followed by different superscript in a row are statistically significant ($P < 0.05$).

Soaking in (A) water, (B) 0.07% sodium bicarbonate solution, (C) 0.1% ascorbic acid solution, and (D) water containing 3% freeze-dried moringa leaf power, followed by autoclaving.

[A] Values are means of triplicate determinations ± SD.

[B] Calculated by difference as total starch–resistant starch (TS–RS); DS, digestible starch.

Table 5
Kinetics of in vitro starch digestion of raw and differentially processed mucuna beans (percent TS hydrolysed at different time intervals)

Treatment	30 min	60 min	90 min	120 min	150 min	180 min
R	6.3 ± 0.9^{b}	7.3 ± 0.3^{c}	$7.9 \pm 0.\ 1^{b}$	8.2 ± 0.1^{b}	8.4 ± 0.1^{c}	8.6 ± 0.1^{b}
A	17.0 ± 1.6^{a}	22.4 ± 1.6^{ab}	26.6 ± 3.6^{a}	28.2 ± 2.2^{a}	31.5 ± 0.9^{ab}	32.2 ± 2.8^{a}
B	18.8 ± 2.4^{a}	25.5 ± 0.7^{a}	28.7 ± 2.0^{a}	31.5 ± 2.2^{a}	32.8 ± 1.1^{a}	34.0 ± 3.1^{a}
C	16.8 ± 2.3^{a}	23.4 ± 2.7^{ab}	26.9 ± 2.1^{a}	30.4 ± 1.8^{a}	31.8 ± 1.2^{ab}	33.2 ± 1.9^{a}
D	17.3 ± 2.5^{a}	22.0 ± 2.0^{b}	26.3 ± 2.5^{a}	28.8 ± 1.4^{a}	30.9 ± 0.6^{b}	32.1 ± 3.0^{a}

R, raw sample. Soaking in (A) water, (B) 0.07% sodium bicarbonate solution, (C) 0.1% ascorbic acid solution, and (D) water containing 3% freeze-dried moringa leaf power, followed by autoclaving.

Values followed by different superscript in each column denote statistically significant differences among the treatments ($P < 0.05$).

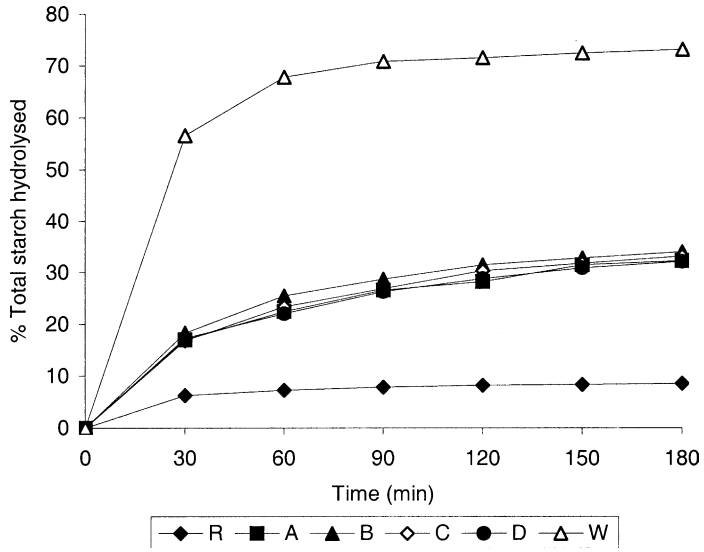

Fig. 1. In vitro starch hydrolysis rate of fresh white bread and differentially processed mucuna seeds: W, white bread; R, raw seeds; A, water-soaking; B, sodium bicarbonate (0.07%) soaking; C, ascorbic acid (0.1%) soaking; D, soaking in water containing 3% freeze-dried moringa leaf powder and followed by autoclaving.

similar in all the other treated samples (37.1–38.4 and 60.1–60.8, respectively). Similar trends, with comparable values of HI and GI, have also been reported in cooked moth bean and black gram after soaking in water and sodium bicarbonate solution, respectively (Bravo et al., 1998). Because of this poor starch digestibility, legumes promote slow and moderate postprandial glucose and insulin response (Jenkins, Wolever, Taylor, Barker, & Fielden, 1980). Such a "lente carbohydrate" property of legumes has been suggested to have beneficial effects in the management of diabetes and hyperlipidemia (Jenkins et al., 1994, 1988). From these results it seems revealed that the presently investigated legume also maintain the lente carbohydrate quality, which is common to all conventional pulses.

Table 6
Hydrolysis index (HI) and predicted Glycemic index (GI) of raw and processed mucuna beans

Sample	HI	GI
Raw	12.1	46.3
A	37.3	60.2
B	40.5	62.0
C	38.3	60.8
D	37.1	60.1
Moth bean[a]	42.8	63.2
Moth bean[b]	51.4	68.0
Black gram[a]	38.7	60.9
Black gram[b]	40.2	61.8

Soaking in (A) water, (B) 0.07% sodium bicarbonate solution, (C) 0.1% ascorbic acid solution, and (D) water containing 3% freeze-dried moringa leaf power, followed by autoclaving.

Soaked in water[a] and soaked in sodium bicarbonate solution[b], followed by cooking, respectively (Bravo et al., 1998).

4. Conclusions

The present study indicated that, compared to the other conventional pulses, mucuna bean contained considerably smaller amounts of certain antinutrients, except for the non-protein amino acid, L-dopa. Although the various domestic preparations did not influence the overall nutritional composition of beans, the reductions of antinutrients, such as total phenolics, tannins, trypsin inhibitor and chymotrypsin inhibitor

activities, phytates, saponins and L-dopa of mucuna beans were acceptable. Interestingly, all the processing methods improved the in vitro protein digestibility, amino acid availability and starch digestibility. Moreover, through the predicted glycemic index values, the bean has been proved to be a good slow releasing carbohydrate food material. Thus, the cracking of raw seeds and then soaking in any one of the specified solutions and subsequent pressure-cooking may be considered as a potential and cost-effective processing device to inactivate/eliminate the above antinutrients. After conducting a thorough investigation on nutrient utilisation and the effects of residual antimetabolic factors of processed seeds through suitable in vivo experiments, the beans can be advocated as an alternative/supplemental food source not only for humans but also for animals, where plant-based food sources are found to be inadequate and scarce, particularly in the remote villages and hilly regions of the developing world.

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