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*Full Length Research paper*

# Nutritional Composition and Anti-Nutrient Variation in Mucuna Beans: A Study of Processing Techniques

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**Mucuna bean (*Mucuna pruriens*) is an indigenous legume promoted in Kenya as a green manure cover crop. It contains high protein but it is under - utilized due to the presence of 3, 4-dihydroxy- L-phenylalanine (L-Dopa) and other anti nutritional compounds. To improve its nutritional potential as a protein source, mucuna bean was processed and evaluated for nutritional composition. Effects of processing at different pH, temperature and particle size, autoclaving, germination and fermentation on the contents of anti-nutritional compounds and crude protein were investigated. Raw beans contained high crude protein (27.9 g/100$^{-1}$). Contents of ether extract; crude fibre and ash were 3.7, 7.9 and 3.5 g/100$^{-1}$, respectively. Mineral content was comparable to that of common pulses. Raw whole mucuna bean contained high L-Dopa (7.0 g/100$^{-1}$) content. Other anti-nutritional compounds included total phenols 7.1 g/100$^{-1}$, trypsin inhibitor activity (TIA) 5.1 TIU and phytates 0.9 g/100$^{-1}$. All processing techniques, except roasting, reduced levels of L-dopa by > 95% while less than 15% of protein was lost. Soaking dehulled bean in acidic medium (pH 3.2) at 60°C for 48 h reduced L-Dopa content to the recommended safe level of 0.1%. Processing mucuna bean increased its nutritional value and potential to improve food security.**

**Key words:** Mucuna bean, anti-nutritional compounds, L-Dopa, processing methods.

## INTRODUCTION

*Mucuna pruriens (L.) DC* var. *pruriens* is a legume consumed and promoted by smallholder farmers in Africa, South America and South Asia as a green manure or a cover crop (Buckles, 1995; Ezeagu et al., 2003). It is rich in protein (23 - 35%) and its digestibility is comparable to that of other pulses, like soybean, rice bean and lima bean (Bressani, 2002; Gurumoorthi et al., 2003) . Despite its potential, mucuna bean remains a minor food crop. It is poorly adopted in agricultural systems (Eillitta and Carsky, 2003) due to the presence of anti-nutritional and toxic compounds. These arise from secondary metabolism in plants and include trypsin and chymotrypsin inhibitors, polyphenols, nicotine, phytostigmine, serotonin and phytates. Anti-nutritional compounds reduce food intake and nutrient utilization in animals and lower the nutrient value of grain legumes (D'Mello, 1995). Tropical grain legumes such as faba bean, kidney bean, jackbean and grass pea (Aletor et al., 1994; Alonso et al., 2000; D'mello, 1995; Ologhobo et al., 1993) contain several anti- nutritional compounds, some of which have hepatotoxic and neurotoxic effects.. The major anti-nutritional compound in mucuna bean is a non-protein amino acid, 3, 4-dihydroxy-L- phenylalanine (L-Dopa) (Duke, 1981). Increased serum levels of L- Dopa from consumption of mucuna bean leads to high con-centration of dopamine in peripheral tissues. It induces

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antiphysiological effects such as nausea, vomiting, anorexia, paranoid delusions, hallucinations, delirium, severe depression and unmasking dementia (Josephine and Jonardhanan, 1992; Reynolds, 1989). Average L-Dopa content in mucuna bean is 3.1 - 6.7% (D'mello, 1995). To improve nutritional quality and effectively utilize dry legumes to their full potential as food, inactivation or removal of antinutritional factors by adopting economically viable processing techniques is required. Physical and biochemical methods used to process dry legumes include soaking, cooking, selective filtration, irradiation, enzymatic treatments, germination and fermentation. In fermentation, *Bacillus subtilis*, a strongly proteolytic bacterium is mainly used in alkaline fermentation of legumes such as soybean. It causes biochemical changes in beans by hydrolysis of proteins and metabolism of resultant amino acids leading to increase in pH and flavour development (Owens et al., 1997). In legume processing, where a method is not effective in removing antinutritional compounds, a combination of two or more methods is used (Sathe and Salunkhe, 1984). Mucuna seeds contain other anti-nutritional compounds such as protease inhibitors, phenolic compounds and phytates. Methods used to process mucuna bean include heat treatment (boiling and autoclaving), roasting, fermentation and germination. Based on L-Dopa content in cooked broad bean ( *Vicia faba*) of 0.2 to 0.5% that has been safely consumed by a large number of people in many parts of the world, Teixeira et al. (2003) defined a safe target level of residual L-Dopa in the processed mucuna bean as 0.1% (dry weight basis) or at least 99% reduction of the initial L-Dopa content.

Breeding is another approach to anti-nutritional compounds removal in mucuna bean. However, this is a long-term process as anti-nutritional compounds are diverse with respect to chemical and biochemical nature. Also, genetic manipulation requires consideration of agronomical consequences (Sathe and Salunkhe, 1984) such as crop yield, soil tolerance, light and water requirements and resistance towards pests. According to D'Mello (1995), anti-nutritional compounds confer insect and disease resistance to plants. Their removal by genetic means may result in agronomic disadvantages and increased use of pesticides. L-Dopa is associated with insect repulsion by mucuna plant and it also provides symptomatic relief from Parkinson's disease.

The objective of this study was to process mucuna bean by different methods in order to remove L-dopa and other anti- nutritional compounds and assess effects of treatments on nutritional composition.

## MATERIALS AND METHODS

### Seed preparation

Mature and dry seeds of mucuna bean were obtained from Kenya Agricultural Research Institute (KARI), Nairobi, Kenya. Seeds were sorted and damaged seeds discarded. Clean seeds were stored in plastic containers at room temperature before analysis. Beans were dehulled with a hammer mill and ground using a Waring commercial blender, Smart grind, Black and Decker, Towson, MA, USA. A set of standard sieves, American Society for Testing and Materials (ASTM E11, 8 inch) were used to segregate the mixture into four particle size categories with the following particle diameter size range: 0 - 0.36, 0.36 - 0.50, 0.50 - 1.00 and 1.00 - 1.70 mm for processing. Samples of processed bean were ground with a pulverizer (FRITSCH Pulverizer, 02.102, Germany) for analysis.

### Processing methods

#### *Soaking at different temperature, pH and particle size diameter levels*

Forty grams of bean sample (particle size 0.50 -1.00 mm) were placed in a 1 l capacity glass jar. Distilled, deionized water (800 ml) was added and the mixture stirred for 1 min. The ratio of sample to water was 1:20 (w/v). Samples were placed in an automated temperature control water bath (Scientific engineering, Grant Instruments Ltd, Cambridge. England) set at 20, 40 or 60°C. The pH was adjusted from 6.4 to 7 $\pm$ 0.2 using 1 M NaOH solution. To evaluate pH effect, samples of particle size diameter of 1.00 - 1.70mm were used. Samples were placed in the water bath set at 20°C and pH adjusted from 6.4 to three levels (3, 7 and 9 $\pm$ 0.2) using 18 N acetic acid and 1 M NaOH solutions accordingly. To study effect of particle size on extraction, samples were placed in a water bath set at 20°C and pH was adjusted from 6.4 to 7 $\pm$ 0.2 using 1M NaOH. Three different particle size diameters were used (0.36 - 0.50, 0.50 - 1.00 and 1.00 - 1.70 mm). Samples of 10 g each were taken at the following time intervals: 6, 12, 24, 36 and 48 h. They were frozen overnight at - 21°C and then freeze-dried at - 40 to - 50°C (Freeze Mobile Twin 6, United Scientific, Alcatel vacuum pump M2008A) and (United Scientific, Virtis Bench Top freeze dryer, Gerdiner, NY).

#### *Autoclaving*

Twenty grams of bean sample (particle size 1.0 - 1.7 mm) were placed in a 1 l capacity glass jar. Distilled, deionized water (400 ml) was added and the mixture stirred for 1 min. The ratio of sample to water was 1:20 (w/v). pH was adjusted from 6.4 to 7 $\pm$ 0.2 using 1 M NaOH. Samples were placed in an autoclave and heat treated at 121°C at 1 Kgf/ $cm^2$ pressure for 30 min. They were cooled, frozen at - 21°C then freeze dried.

#### *Alkaline fermentation*

Forty grams of bean sample (particle size 1.0 - 1.7 mm) were placed in a 1 l capacity glass jar. Distilled, deionized water (800 ml) was added and the mixture was stirred for 1 min. The ratio of sample to water was 1:20 (w/v). Samples were autoclaved at 121°C at 1 Kgf/ $cm^2$ pressure for 30 min, cooled, and inoculated with activated *B. subtilis* (Microbiology and Plant Pathology Culture Bank, University of Pretoria) at 5% v/v. Starter culture averaged approximately $10^6$ cfu/ml. Fermentation was carried out at 32°C for 72 h. Samples were frozen at - 21°C and then freeze-dried.

#### *Germination*

One hundred grams of mucuna beans were sterilized by soaking in

ethanol for 1 min. Seeds were soaked in distilled water (1:10, w/v) for 12 h at room temperature (25°C). Water was drained and the seeds were spread between thick layers of wet cotton wool on a tray and allowed to germinate in the dark for 3 days. After 3 days, seeds that had not germinated were discarded. Germinated seeds were removed from the cotton wool, seed coats removed manually and samples placed in plastic bags and frozen at - 21°C for 12 h to stop germination. Seeds were thawed and dried in an oven at 50°C for 24 h. Dried germinated seeds were ground into powder, passed through a 500 μm sieve, frozen at -21°C then freeze-dried.

**Roasting**

Ten grams of bean sample (particle size 1.0 - 1.7 mm) were mixed with approximately fifty grams of sand. The sand had been pre-heated to 80°C for 1 h. Samples were heated in oven set at 100°C for 15, 30, 45 and 60 min. Mixtures were cooled in a desiccator for 2 h, and samples separated from sand by sieving. Samples were then ground into powder, freeze-dried and stored at - 21°C until analysis.

**Analytical methods**

**Proximate composition**

Mucuna bean was ground into fine flour (particle size diameter < 0.5 mm), analyzed for crude protein, moisture, crude fat, ash, crude fibre, tannins, phytate and minerals according to AOAC (Association of Official Analytical Chemists, 1990) methods. Samples were analyzed in triplicate.

***Trypsin inhibitor activity***

Trypsin inhibitor activity (TIA) was determined by the method of Kakade et al. (1974), using benzoyl-DL-arginine-*p*-nitroanilide (BAPNA) as substrate. A 4 g sample of defatted mucuna flour was treated with 40 ml of 0.05 M sodium phosphate buffer, pH 7.5 and 40 ml of distilled water. The sample was shaken for 3 h and centrifuged at 700 g for 30 min at 15°C. Supernatant was diluted in order to obtain inhibition between 40 and 60% of enzyme activity. Incubation mixture consisted of 0.5 ml trypsin solution (5 mg/ml), 2 ml 2% (w/v) Bapna, 1.0 ml sodium phosphate buffer (pH 7.5, 0.1 M), 0.4 ml HCl (0.001 M) and sample extract (0.1 ml). Total volume of incubation mixture was maintained at 4.0 ml. Incubation was carried out in a water bath at 37°C for 20 min after which 6.0 ml of 5% TCA (trichloroacetic acid) solution was added to stop the reaction. Blank sample was treated similarly through the entire determination. Absorbance (A) was read at 410 nm wavelength in a spectrophotometer. Results were expressed as trypsin inhibitor units (TIU). One TIU was defined as an increase of 0.01 in absorbance units under conditions of assay. Trypsin inhibitory activity was defined as the number of TIU.

***L-Dopa content***

L-Dopa was determined using HPLC by a modified method of Siddhuraju and Becker (2005). A standard solution of L-Dopa (200 mg/ml) was prepared daily by dissolving appropriate mass of the pure standard in 0.1 M HCl. Prior to analysis; the stock solution was diluted to appropriate concentration with 0.1 M HCl and maintained at a temperature of 2 - 5°C. Finely-ground and defatted processed sample (100 mg) was prepared by placing in a glass tube, adding 5 ml of 0.1 M HCl and mixing for 10 min at room temperature (22°C). The mixture was homogenized at 20, 500 rpm per min (Ultra-turrax T25, 1Ka, Janke and Kunkel, Germany) for 30 s in an ice bath and subsequently kept on a magnetic stirrer for 1 h at room temperature. Supernatant was collected by centrifugation (10,000 g, 15 min). Extraction procedure was repeated and supernatants from extractions pooled and filtered through a 0.2-mm glass filter. Supernatants were then diluted as required with 0.1 M HCl and stored at 2 - 5°C until analyzed. All samples were analyzed within 8 h of preparation. Samples were analyzed for L- dopa on a Pico-Tag $C_{18}$, 3.9 × 150 mm column under the following conditions: injection volume 20 μl, flow rate: 1.0 ml/min, and column temperature: 47°C. Chromatographic condition for L-Dopa analysis was isocratic elution using two solvents. The eluting solution (Solvent A) was made up of water, methanol and phosphoric acid in the ratio of 975.5:19.5:1 (v/v/v), pH 2.0, and the washing solution (Solvent B) comprised of 70% methanol (solvents were stable at 22°C for 1 week). All the aqueous solutions were passed through a 0.20 mm glass filter. Eluents were degassed by filtration or in an ultrasonic bath. Elution conditions were 0% (B) for 12 min, followed by an increase in solvent B to 100% over an interval of 5 min. Thereafter, for another 5 min, solvent B was decreased to 0% and finally the column was washed with solvent 0% B for 15 min to adjust the column to starting conditions.

**Statistical analysis**

Data was exported from excel and analyzed using the Statistical Package for Social Sciences (SPSS) Version 11.5 (SPSS Inc., Chicago, IL USA). Statistical differences between means were compared using paired T-test. Differences in means were considered statistically significant at $p < 0.05$.

## RESULTS AND DISCUSSION

### Chemical and anti-nutritional compounds composition of mucuna bean

The crude protein content of mucuna bean was 27.87% (Table 1). This is higher than that of commonly consumed legumes, such as chick pea (*Cicer arietinum*), green pea (*Pisum sativum*), common bean (*Phaseolus vulgaris*), pigeon pea ( *Cajanus cajan*) and lentil (*Lens culinaris*) with a range from 18.5 to 21.9 % for the raw grains (Costa et al., 2006; Kumar et al., 1991), except for soybean that contains an average of 38% crude protein (Augustin and Klein, 1989). Mucuna bean crude protein content compared well with that of cowpea (*Vigna unguiculata*) at 29.3% and mung bean (*Phaseolus aureus*) at 26.5% (Mwasaru et al., 1999; Sathe and Salunkhe, 1984). Crude protein content in mucuna bean grown in different locations of the tropics and subtropics vary between 21.0 - 30.3% (Pugalenthi et al., 2005). The variation may be attributed to interaction between genetic make up and environment. Mucuna bean contained slightly higher crude fat content (3.65%) than most other legumes. Legumes generally contain low fat contents in the range of 1 - 2% with the exception of chickpea- 6.7% (Costa et al., 2006); soybean- 21% and peanut- 49% (Augustin and Klein, 1989). It was within the range of 2.8
- 4.9% reported for the same variety (Ravindran and Ravindran, 1988; Siddhuraju et al., 2000). Crude fibre content of 7.91% was higher than the 1.6 - 7.9% range

**Table 1.** Proximate composition and antinutrients content in whole, dehulled raw and processed (60˚C, pH 3.2, particle size diameter of 1.0 - 1.7 mm) mucuna bean.

| Parameter | Whole raw seeds g/100g (dwb) | Dehulled raw seeds g/100g (dwb) | Processed dehulled mucuna bean[1] g/100g (dwb) |
|---|---|---|---|
| Crude protein | 27.87 ± 0.48 | 31.9 ± 0.92 | 27.2[b] ± 1.1 |
| Crude fat | 3.65 ± 0.23 | 5.54 ± 0.21 | 5.5 [a] ± 0.1 |
| Crude fibre | 7.91 ± 0.36 | 3.98 ± 0.15 | 1.8 [b] ± 0.2 |
| Ash | 3.53 ± 0.07 | 0.30 ± 0.01 | 0.3 [a] ± 0.01 |
| Invert sugar | 0.16 ± 0.01 | - | |
| L-Dopa | 6.98 ± 0.10 | 5.71 ± 0.26 | 0.1 [b] ± 0.01 |
| Trypsin inhibitor activity[A] | 5.14 ± 0.14 | 9.32 ± 0.19 | No inhibition |
| Phytate[B] | 0.85 ± 0.02 | 1.35 ± 0.01 | 0.4 [b] ± 0.04 |
| Total phenolics[C] | 7.09 ± 0.41 | 5.20 ± 0.20 | 0.1 [b] ± 0.01 |
| Tannins[D] | 2.31 ± 0.04 | ND | 0 |
| Sucrose (mg/100 g) | 1.21 ± 0.02 | - | - |
| Raffinose (mg/100 g) | 0.35 ± 0.01 | - | - |
| Stachyose (mg/100 g) | 0.07 ± 0.01 | - | - |
| Verbascose | ND | ND | - |

Values are means ± SD of triplicate determinations; Values followed by different superscripts in the same row are significantly *(p < 0.05)* different; [A]As TUI / mg sample; [B]As phytic acid; [CD]As tannic acid equivalents. ND not detected; (-) not determined**.** [1]Processing conditions (pH 3.2, 60˚C, particle size diameter (1.0 - 1.7 mm).

reported by Adebowale and Lawale (2003). However, a higher concentration of 5.3 - 11.5% in *Mucana utilis* seeds has been reported (Siddhuraju et al., 2000). Ash content of 3.53% was within the 2.9 - 4.4% range reported for other mucuna varieties (Vijayakumari et al., 1996). It compares well with 3.4 - 4.0% values reported for beans (Augustin and Klein, 1989) but lower than 9.8% reported for chickpea and 10.4% reported for pea, (Costa et al., 2006). Dehulled mucuna seeds contained higher crude protein, crude fat and ash content, but lower crude fibre content than whole bean, implying that the seed coat is comprised mainly of fibre, while protein fat and ash are concentrated in the cotyledon.

The L-Dopa content of raw whole mucuna bean of 6.98% was within 6.7 - 7% range reported for black accessions in India but higher than 5.9% reported for white seed coat (Vadivel and Janardhanan, 2000). Wide variations in L- Dopa content in mucuna species have been attributed to genetic make up and on growing locations (Eillita et al., 2002). St Laurent et al. (2002) reported that degree of maturity of mucuna bean determined L-Dopa content with half-mature seeds having a higher L-Dopa content than mature dry seeds. Dehulled raw mucuna beans contained a lower L-Dopa content (5.71%) than whole raw mucuna bean (6.98%), indicating the presence of L-Dopa in seed coat.

Total phenolic content (7.09%) of mucuna bean was higher than 3.82% reported for cowpea (Preet and Punia, 2005), but lower than 10.6% reported for pigeon pea (Singh, 1988). Siddhuraju et al. (2000) reported a lower content of 5.2 - 5.5% total phenols and 0.27 - 0.37% tannin concentration among white germplasms of *M. pruriens.* Phenolic compounds decrease digestibility of carbohydrates and availability of vitamins and minerals. They lower the activity of the digestive enzymes such as -amylase, trypsin, chymotrypsin, and lipase and may cause damage to mucosa of the digestive tract and reduce absorption of nutrients such as vitamin $B_{12}$ (Liener, 1994) . Phenolics also interfere with digestion and absorption of proteins. Decrease in total phenolics and tannin contents in dehulled mucuna bean compared to the whole bean could be attributed to high concentration of tannins in the seed coat. Low or negligible amounts of tannins are located in the cotyledons (Alonso et al., 2000). In processed mucuna bean, tannins are therefore of little nutritional significance.

The 0.85% phytate content of mucuna bean compared well with 0.9 and 0.86%, reported for white and black mucuna varieties, respectively (Siddhuraju and Becker, 2001), and to other conventional legumes such as 0.97% for chickpea (Rehman and Shah, 2005) and 0.81% for pigeon pea (Oloyo, 2004,). It was however, lower than 0.6 - 1.5% reported for soybean (Ologhobo and Fetuga, 1984); 1.25% for lentils; 1.10%, for black grams (Rehman and Shah, 2005) and 0.5 - 3.0% for dry beans (Deshpande and Cheryan, 1984). A higher phytate content of 1.35% of dehulled mucuna seeds could be attributed to their location, which is mainly in the cotyledons. The physical removal of testa by dehulling is associated with increased phytate content depending on the proportion of the hull in relation to whole seed. Phytates in legumes bind multivalent cations lowering bioavailability of minerals. They also form complexes with proteins and starch, inhibiting enzymic digestion of the

**Table 2.** Mineral content of raw mucuna bean.

| Mineral | Concentration in mg 100 g $^{-1}$ (dwb) |
|---|---|
| **Macro elements** | |
| Potassium | 807.5 ± 24.9 |
| Sodium | 149.0 ± 11.4 |
| Calcium | 38.0 ± 2.8 |
| Magnesium | 8.8 ± 4.2 |
| Phosphorus | 88.1 ± 3.2 |
| **Micro elements** | |
| Copper | 1.9 ± 0.1 |
| Zinc | 4.1 ± 0.2 |
| Iron | 7.9 ± 0.3 |
| Manganese | 2.6 ± 0.1 |

Values are means ± SD of triplicate determinations.

same (Oatway et al., 2001). Presence of phytates in mucuna bean lowers its nutritional quality.

Trypsin inhibitor activity (TIA) observed for mucuna bean of 9.32 TIU/mg was less than the range of 13.7 - 14.2 TIU/mg reported by Siddhuraju and Becker (2001) but higher than that of 4.7 - 6.9 TIU/mg reported for the same variety (Del Carmen et al., 2002). However, the TIA in mucuna bean was lower than 15.4 TIU/mg reported for pigeon pea (Oloyo, 2004). Protease inhibitors, such as trypsin inhibitor in diets lead to formation of irreversible trypsin enzyme-trypsin inhibitor complexes. This causes a decrease in trypsin in the intestine and subsequently in digestibility of dietary protein, thus leading to slower animal growth (Pugalenthi et al., 2005). Increase in TIA after dehulling is attributed either to their presence in cotyledon fraction of bean or to husk, contributing a substantial portion of whole seed weight (Deshpande et al., 1986).

Raffinose and stachyose contents of 0.35% and 0.01% in mucuna bean were lower than that reported by Siddhuraju et al. (2000) for three germplasms of *M. pruriens* as: 0.90 - 1.19, 1.22 - 1.46 and 2.45 - 2.87% for raffinose, stachyose, and verbascose, respectively. Janardhanan et al. (2003) also reported higher concentration of raffinose (0.95 - 1.10%), stachyose (1.05 - 1.22) and verbascose (3.95 - 4.34%) in mucuna bean and attributed variability among germplasms to environmental factors and seed maturity. Oligo-saccharides content in mucuna bean was low, compared to that of field bean (0.2% raffinose and 1.2% stachyose) but similar to that in soybean (0.8 and 5.4%, respectively) (Arora, 1983). Oligosaccharides such as raffinose and stachyose are not desirable in diet as they are associated with flatulence, following consumption of legumes (Janardhanan et al., 2003). This is attributed to the presence of microorganisms in the large intestine that utilize sugars, leading to production of flatus gases (hydrogen, carbon dioxide and small amounts of methane), abdominal rumbling, diarrhea, and discomfort (Liener, 1994).

Ash is important in legumes as it contains nutritionally important mineral elements. Mucuna bean was observed to contain fairly good amounts of minerals such as potassium, copper, iron and zinc (Table 2). Micro elements content: iron, copper and zinc for mucuna bean compared well with other mucuna varieties (Siddhuraju et al., 2000) and to legumes such as pigeon pea and green gram (Rajyalakshmi and Geervani, 1994). The iron content (7.85 mg 100g $^{-1}$) in mucuna seeds was noteworthy as diets in many developing countries are deficient in iron (Bressani, 2002). Compared to field beans, mucuna bean had a higher content of iron, zinc and manganese (Arulmozhi and Janardhanan, 1992) but compared well with soybean in iron and zinc levels (8.66, 5.43 mg 100g $^{-1}$, respectively). The content of potassium, calcium, magnesium and phosphorus in mucuna bean were lower compared to soybean whose levels were reported as: 1820, 223, 284 and 477 mg 100g$^{-1}$, respectively (Augustine and Klein, 1989). Unlike most legumes, the concentration of sodium in mucuna bean observed in this study (150 mg 100 g $^{-1}$) was higher than 7 mg 100 g$^{-1}$ reported for soybean (Augustine and Klein, 1989). Potassium was the predominant mineral in mucuna seeds (807.54 mg 100g $^{-1}$). Notable variation in mineral content in four mucuna accessions was attributed to genetic origin, geographical source and levels of soil fertility (Vadivel and Janardhanan, 2000). Ash content is significant in foods as an index for quality of feeding materials used for poultry and cattle. The ash content in mucuna bean could contribute to its use in compounding of animal feed (Pugalenthi et al., 2005). It is noteworthy that phytate phosphorus, the predominant phosphorus in legumes is biologically unavailable to man. Dehulling of seeds has been reported to cause loss of minerals through leaching during soaking and cooking of legumes (Augustin and Klein, 1989).

**Effect of processing on the content of L-Dopa, other anti-nutritional compounds and crude protein**

The content of anti-nutritional compounds and crude protein of processed mucuna bean is shown in Table 3. Processing mucuna bean at 20 and 60°C temperature levels resulted in significant ($p < 0.05$) reduction in L-Dopa content to 0.31 and 0.12%, respectively from 5.71% in raw dehulled seed. Reduction of L-Dopa achieved after 48 h extraction period at 20°C was 94.55% while at 60°C it was 97.88%. Autoclaving for 30 min significantly ($p < 0.05$) reduced level of L-Dopa to 0.43%. Increasing temperature significantly ($p<0.05$) reduced trypsin inhibitor activity and total phenolic compounds but had no effect on phytate content. L-Dopa content was significantly ($p < 0.05$) reduced at the three pH levels studied and the residual L-Dopa attained was, 0.09% in

**Table 3.** Effect of processing mucuna bean at different temperature, pH and particle size diameter (p/s) on the content of L-Dopa, other anti-nutritional compounds and crude protein (%).

| Treatment | L-Dopa | Trypsin inhibitor activity[A] | Phytates[B] | Total phenolics[C] | Tannins[D] | Crude protein | Protein/residual L-Dopa |
|---|---|---|---|---|---|---|---|
| Raw mucuna bean | 5.71$^{b}$ ± 0.05 | 9.32$^{d}$ ± 0.05 | 1.35$^{c}$ ± 0.01 | 5.20$^{b}$ ± 0.08 | 0.0$^{a}$ | 31.9 $^{d}$ ± 0.30 | 5.59 |
| Autoclaving | 0.43$^{a}$± 0.05 (92.54) | 0.0$^{a}$ | 0.50$^{a}$±0.01 (62.93) | 0.44$^{a}$±0.13 (91.51) | 0.0$^{a}$ | 29.7$^{c}$± 0.28 (- 6.90) | 69.07 |
| Extraction at pH 7.0, 60°C , p/s 0.50 - 1.0 mm | 0.12$^{a}$± 0.01 (97.88) | 1.73$^{b}$ ± 0.07 (81.43) | 2.59$^{d}$ ± 0.01 | 0.0$^{a}$ (100) | 0.0$^{a}$ | 27.8$^{a}$±0.14(- 12.85) | 231.67 |
| Extraction at pH 7.0, 20°C, p/s 0.50 - 1.0 mm | 0.31$^{a}$± 0.06 (94.55) | 6.34$^{c}$ ± 0.07 (31.97) | 0.81$^{b}$±0.01 (40.38) | 0.37$^{a}$±0.09 (92.97) | 0.19$^{b}$± 0.01 | 28.9$^{b}$ ±0.14 (- 9.40) | 93.22 |
| Extraction at 20°C, pH 3.2, p/s 1.0 - 1.7 mm | 0.09$^{a}$± 0.02 (98.46) | 0.0 $^{a}$ | 0.53$^{a}$±0.02 (60.94) | 0.35$^{a}$±0.08 (93.31) | 0.0$^{a}$ | 26.9$^{b}$ ± 0.00 (15.67) | 298.89 |
| Extraction at 20°C , pH 9.0, p/s 1.0 - 1.7mm | 0.02$^{a}$± 0.01 (99.65) | 2.87$^{b}$ ± 0.08 (69.21) | 0.95$^{c}$±0.01 (29.72) | 0.38$^{a}$±0.06 (92.64) | 0.0$^{a}$ | 23.1$^{a}$ ± 0.42 (27.59) | 1155.0 |
| Small p/s (0.355- 0.5 mm) 20°C , pH 7.0 | 0.34$^{b}$± 0.02 (97.41) | 2.40$^{a}$ ± 0.07 (74.25) | 0.49$^{a}$±0.01 (63.42) | 0.23$^{a}$±0.03 (95.60) | 0.0$^{a}$ | 22.0$^{a}$ ± 0.14 (31.03) | 64.71 |
| Big p/size (1.0- 1.7 mm), 20°C (pH 7.0) | 0.28$^{a}$± 0.02 (96.83) | 6.56$^{b}$ ± 0.03 (29.61) | 1.01$^{c}$±0.01 (25.61) | 9.50$^{c}$±0.70 (82.66) | 1.66$^{c}$ | 27.8$^{b}$ ± 0.14 (12.85) | 99.29 |

Values are means ± SD of triplicate determinations; Values followed by different superscripts in the same column are significantly *(p < 0.05)* different; Values in brackets indicate % reduction; $^{A}$As TIU / mg sample; $^{B}$As phytic acid; $^{CD}$As tannic acid equivalents.

**Table 4.** Effect of roasting, germination and fermentation of mucuna bean on the content of anti-nutritional compounds and crude protein (%).

| Treatment | L-Dopa | Trypsin inhibitor activity[A] | Phytates[B] | Total phenolics[C] | Tannins[D] | Crude protein | Protein/ residual L-Dopa |
|---|---|---|---|---|---|---|---|
| Raw mucuna bean | 5.71$^{b}$ ± 0.05 | 9.32$^{d}$ ± 0.05 | 1.35$^{c}$ ± 0.01 | 5.20$^{b}$ ± 0.08 | 0.0$^{a}$ | 31.9 $^{d}$ ± 0.30 | 5.59 |
| Roasting at 100°C | 6.00$^{c}$ ± 0.18 4.96 | 5.70$^{c}$ ± 0.04 (38.84) | 0.42$^{a}$ ± 0.02 (69.09) | 0.38$^{a}$ ± 0.05 (92.57) | 0.0$^{a}$ | 31.8$^{a}$ ± 0.14 (0.31) | 5.30 |
| Germination | 4.43$^{b}$±0.10(22.32) | 5.24$^{b}$ ± 0.07 (43.78) | 1.26 $^{b}$ ± 0.01 (6.77) | 3.11 $^{b}$ ± 0.03 (40.24) | 0 | 32.9$^{b}$ ± 0.14 3.13 | 7.43 |
| Fermentation | 1.49$^{a}$±0.08(73.96) | 0.00 $^{a}$ (100) | 2.47 $^{c}$ ± 0.01 82.97 | 2.76 $^{b}$ ± 0.03 (47.05) | 2.00 $^{b}$ | 37.6$^{c}$ ± 0.14 17.87 | 25.23 |

Values are means ± SD of triplicate determinations; Values followed by different superscripts in the same column are significantly *(p < 0.05)* different; Values in brackets indicate % reduction; $^{A}$As TIU / mg sample; $^{B}$As phytic acid; $^{CD}$As tannic acid equivalents.

in acidic media (pH 3.2), 0.31% at pH of 7.0 and 0.02% in alkaline medium (pH 9.0). Processing mucuna bean at all pH levels significantly ($p < 0.05$) reduced TIA and the contents of phytates, and phenols. At low pH (pH 3.2), there was no residual TIA in the processed bean. At fixed temperature (20°C) and pH (7.0) extraction conditions, reducing particle size diameter significantly ($p < 0.05$) decreased L-Dopa content of mucuna bean. For all three different particle size diameters (0.355 -0.5, 0.5 -1.0, 1.0-1.7 mm) used, residual L-Dopa content in processed bean were 0.34, 0.31 and 0.28%, respectively. The treatment also decreased TIA and phytate content but had varying effects on reduction of phenols and tannins.

Effects of roasting, germination and fermentation of mucuna bean are presented in Table 4. There was no significant change in residual L-Dopa during roasting of mucuna seeds at 100°C for 60 min. However, TIA, phenolic and phytate content were significantly ($p < 0.05$) decreased. Germination and alkaline fermentation of mucuna bean for three days reduced L - Dopa content

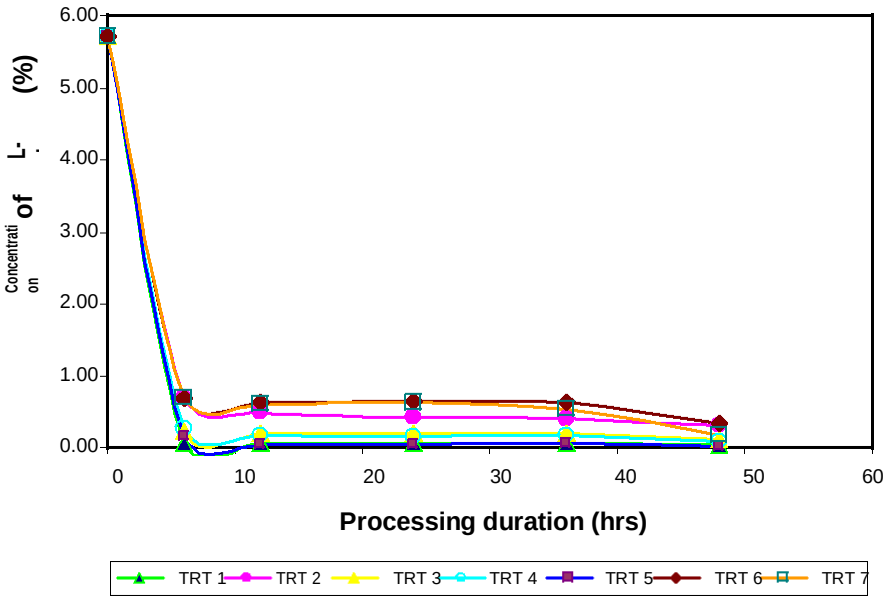


**Figure 1.** Effect of processing duration on the L-Dopa content (%) of mucuna bean. TRT 1 is extraction at pH 3.2, 60°C; TRT 2 extraction at 20°C, pH 7.0; TRT 3 extraction at 60°C, pH 7.0; TRT 4 pH 3.2, 20 ˚C; TRT 5 pH 9.0, 20 ˚C; while TRT 6 and TRT 7 represent extraction at pH 7.0, 20°C and particle size diameter of 0.3 - 0.5 and 0.5 - 1.0 mm respectively.

significantly ($p < 0.05$) to 4.43 and 1.49%, respectively. In addition, germination reduced TIA and phytate content of mucuna bean while alkaline fermentation completely inactivated trypsin inhibitor but increased the phytate content.

Effects of processing duration is shown in Figure 1. For all the different extraction conditions, there was a significant ($p < 0.05$) L-Dopa reduction after 6 h extraction. Increasing processing time from 6 to 48 h did not significantly ($p < 0.05$) reduce further the L-Dopa content for mucuna bean processed at a temperature of 60°C, pH 7.0 and particle size diameter of 1.0 - 1.7 mm. Residual L-Dopa after processing for 6 and 48 h was 0.07 and 0.06%, respectively. In solvent extraction, concentration of solute in solvent increases as extraction proceeds in a closed batch process, thus reducing the concentration gradient (driving force), and the rate of extraction decreases over time. Temperature increase during solvent extraction processes gives a higher extraction rate due to increased solute solubility (Teixeira and Rich, 2003). During mucuna bean processing, there was no significant difference in residual L-Dopa content between extraction at 60 and 20°C; the residual L-Dopa levels after 48 h were 0.12 and 0.31%, respectively. Particle size also influences the rate of solute extraction in that, the smaller particle size has greater interfacial surface area at the solid/ liquid interface and higher rate of transfer of solute at the surface. Smaller particle size also reduces distance traveled by solute molecules within the interior of particle as they migrate towards the surface (diffusion). However, for very small particle size diameter such as powders, circulation of solvent around particles can be impeded, compromising the benefit of greater surface area. This explains the significantly ($p < 0.05$) higher residual L- Dopa in mucuna bean (0.34%) for the smallest particle size diameter (0.355 - 0.5 mm) compared to that for the bigger particle size diameter (1.0 - 1.7 mm) which was 0.28%. There was no significant ($p < 0.05$) difference in residue L-Dopa content of the seed after 48 h processing between the two smaller particle size diameter ranges (0.3 - 0.5 and 0.5 - 1.0 mm).

According to the Merck Index (Merck, 1983), L-Dopa has only limited solubility in water (66 mg in 40 ml at 20°C) but it is readily soluble in dilute solutions of hydrochloric acid. Processing mucuna bean at different pH levels did not significantly affect the residual L-Dopa content. Extraction at high pH (9.0) reduced L-Dopa by 99.65 to 0.02% and this is highly below the recommended residual level of 0.1% dry solids. However, the extract and the bean sample turned blackish, implying chemical conversion of L-Dopa to melanin, rather than actual extraction into water. Similar observations were made by Teixeira and Rich (2003). Extraction at high and low pH, and autoclaving reduced L-Dopa content by more than 90%. However, no significant interactions were noted between the effects of temperature and pH treatments during processing of mucuna bean for L-Dopa extraction. Fermentation and germination reduced L-Dopa levels by 73 and 22%, respectively. However, Prakash and Tewari (1999) reported a higher (31%) reduction of L-Dopa after 3 days germination. Roasting at 100°C for 60 min did not

significantly affect the L-Dopa content of mucuna bean. This could be explained by resistance of L-Dopa to thermal processing (Bressani et al., 2003). In addition to L-Dopa, other anti-nutritional compounds such as trypsin inhibitors, phenols, phytates and tannins were also reduced during mucuna bean processing. However, high content of total phenolics is undesirable for human consumption because they interfere with digestion and absorption of dietary protein. Polyphenols also react with proteins and enzymes and act as trypsin inhibitors and amylase inhibitors (Deshpande et al., 1986). Reduction in phenolic content during soaking, followed by thermal processing has been observed in commonly- consumed legumes such as *Lablab Parpareus var. vulgaris* and *Vigna radiate* (Kataria et al., 1989). Polyphenols are generally water- soluble and their loss during soaking at various temperatures may be due to leaching out of phenolics from the seeds under the influence of absorbed solution (Vijayakumari et al., 1998).

All extraction treatments reduced the polyphenols by more than 90% except for the bigger particle size treatment where the reduction was 82.7%. The latter could be attributed to reduction in surface area. Change in pH did not affect reduction of polyphenols while germination resulted to 40.2% decrease. Reduction of polyphenols during roasting could be attributed to thermal degradation and denaturation, changes in chemical reactivity or to formation of insoluble complexes during heating (Kataria et al., 1989; Siddhuraju and Becker, 2001). Tannins cause a decrease in the digestibility of proteins and carbohydrates due to the formation of insoluble enzyme-resistant complexes. An enzymatic hydrolysis by polyphenolase causes loss of tannins in grains during germination (Reddy et al., (1985). Reduction in tannin content may be due to effects of soaking and germination of seeds (Sangronis and Machado, 2007). High phytate content (0.85%) in mucuna bean is undesirable in the diet as phytate binds essential, nutritionally- important divalent cations, such as, iron, zinc, magnesium and calcium and forms insoluble complexes, making the minerals unavailable for absorption (Deshpande and Cheryan, 1984). Zinc binds to phytate in the physiological pH range more tightly than other minerals. Decrease in phytic acid has been attributed to leaching-out effect during hydration (Beleia et al., 1993). Increase in ionic concentration of the soaking medium enhances loss of phytate (Deshpande and Cheryan, 1984). The apparent decrease in phytate during hydrothermal processing of legume seeds is attributed to formation of insoluble complexes between phytate and other components such as phytate-protein and phytate-protein-mineral complexes or to hydrolysis of inositol hexaphosphate to penta- and tetra phosphates. Removal of phytate from legumes during germination is attributed to enzymatic (phytase) hydrolysis of phytate followed, by diffusion (Bau et al., 1997).

Trypsin inhibitor activity observed in mucuna bean is lower than that of soybean (29.1 - 30.2 mg/g) (Smith et al., 1980) and higher than levels of 1.7 - 3.6 mg/g reported for faba bean (Makkar et al., 1997). Enzyme inhibition may lead to under-utilization of corresponding substrate in a diet. Protease inhibitors in diet lead to formation of irreversible trypsin enzyme- trypsin inhibitor complex, causing a trypsin drop in the intestine. This decreases digestibility of diet protein, resulting to slower animal growth. The organism in this situation increases the secretory activity of the pancrease, which could cause pancreatic hyperplasia (Liener, 1994). Trypsin inhibitors in legumes are significantly decreased by heat treatments, a major beneficial effect of cooking legume grains (Armour et al., 1998). In this study, processing mucuna bean at low pH 3.2, autoclaving, alkaline fermentation, and processing at low pH (3.2), moderate temperature (60°C) reduced TIA completely. However, residual TIA was observed after roasting, extraction at low temperature (20°C) and at high pH (9.0), and germination. Complete inactivation of trypsin inhibitor in mucuna bean grown in Brazil was reported by Udedibie and Carlini (1998) after ordinary cooking of pre-soaked seeds. Sathe and Salunke (1984) reported that trypsin inhibitor in beans, cowpea, and black gram was resistant to dry heat. Heat resistance of trypsin inhibitor has also been reported for bean flour processed at 97°C for 30 min (Carvalho and Sgarbieri, 1997). In addition to heat stable protease inhibitors, existing residual TIA is attributed to the presence of non-protein components such as browning substances (resulting from Maillard re-actions), phytate, phenolics, and a relative concentration of fibre which could give rise to residual inhibitor activity after thermal processing. Polyphenolic compounds constitute majority of residual TIA in heated winged beans (Deshpande, 1992). Presence of phytate, phenolics including L-Dopa in heated mucuna beans might partially contribute to residual TIA. Leaching of pro-tease inhibitors was reported in pigeon pea seeds soaked in salt solutions (Mulimani and Puramjyothi, 1995) and from great Northern beans soaked in acidic and alkaline solutions (Eicher and Satterlee, 1988). Germination is mainly a catabolic process as the reserved substances present in the cotyledon are used for the development and growth of the embryo (Sangronis and Machado, 2007). Siddhuraju and Becker (2001) reported that germination for 3 days reduced TIA level in mucuna bean by 44% and attributed it to mobilization and enzymatic degradation of proteins including protease inhibitors in seeds during germination. Total phenolic and phytate content were also decreased.

Crude protein decreased significantly ($p < 0.05$) during processing of mucuna bean except by germination and fermentation. Germination and fermentation significantly ($p < 0.05$) increased the crude protein to 32.9 and 37.6%, respectively. This is due to extensive breakdown of seed-storage compounds and synthesis of structural proteins and other cell components that take place during

germination. The seedlings are sites of high amino acid biosynthetic activity, resulting in high contents of free protein amino acids supporting the synthesis of proteins and the development of the plant (Kuo et al., 2004). Increase in protein could also be attributed to loss of dry matter through respiration and microbial spoilage (Mbithi-Mwikya et al., 2000).

Leaching of protein occurs especially at high temperature and pH. Myhrman (2002) reported a 50% loss of protein from mucuna bean flour after several successive soakings in tap water at room temperature but less than 10% loss for larger bean fragments (4 to 5 parts per bean) even after boiling in water. A loss of 11% was reported by Teixeira and Rich (2003), using mucuna bean particle size diameter of less than 1 mm and soaking in tap water for 44 h. Increased surface area due to small particle size diameter (0.35 - 0.50 mm) is attributed to the high protein loss (31.03%). Crude fat and ash were not significantly affected by processing while crude fibre decreased significantly. Reduction in ash content during processing may be due to the leaching of both micro and macro elements into the extracting medium.

This study shows that the processing method used reduced the anti nutritional constituents of mucuna bean but resulted in protein loss. The “processing effectiveness” of a method was defined based on the two most important parameters determining the quality of processed mucuna bean and these are the protein content and residual L-dopa. As shown in Table 3, the protein to L-dopa ratio was used to select effectiveness of a processing method. Processing at high pH (9.0) gave the highest ratio (1155) but resulted to melanin formation.

At low pH (3.2) and moderate temperature (60°C), the processing effectiveness was 298.9 and 231.1 respectively. To increase processing effectiveness, the two parameters were selected and mucuna bean was processed by extraction at low pH (3.2), moderate temperature (60˚C), particle size diameter (1.0 - 1.7 mm) and adequate quantity of water (X20 w/v) for a period of 6 h. The residual L-Dopa content in processed bean was 0.07% and crude protein of 27.2% (Table 1). The processing effectiveness value was improved to 388.6. The residual L-dopa content in processed bean was within the recommended level of 0.1% but protein loss (14.7%) was relatively high. This processing method for L-Dopa reduction in mucuna bean could be used for household application. Under these processing conditions, there was no significant difference in the residual L-Dopa content of processed bean among different processing durations. Increasing processing period from 6 to 48 h did not significantly reduce further the L-Dopa content of mucuna bean.

Processing for the two intervals resulted in 98.8 and 99.0% reduction in L-Dopa, respectively and residual L-Dopa content in the bean of 0.07 and 0.06%, respectively.

## Conclusion

Proximate composition and mineral contents of mucuna bean compared favorably with that of conventional edible legumes. Mucuna bean is notably rich in protein and micronutrients. All processing methods except roasting significantly reduced the L-Dopa content in mucuna bean. Specifically, processing at low pH (3.2) and at 60°C reduced the L-Dopa content to recommended safe level of 0.1%. Simultaneously, other anti-nutritional compounds such as trypsin inhibitor, phytates and phenolic compounds were significantly reduced. During processing, some reduction of crude protein (by 15%) was observed. However, residual protein level (27.1%) was high in processed mucuna bean compared to that in commonly-consumed legumes. Processed mucuna bean has good potential as a cheap and alternate source of protein in the diet and it could be utilized to improve food security.

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