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Effects of Processing Mucuna Bean *(Mucuna pruriens L.)* on Protein Quality and Anti-nutrients Content

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Abstract
Mucuna bean is grown in many parts of Kenya as a green manure/cover crop. The bean contains a high protein content but remains a minor food crop due to the presence of anti-nutrient compounds mainly 3,4-dihydroxy-L-phenylalanine (L-Dopa). The potential for utilisation of mucuna bean as an alternative source of protein was evaluated by assessing the effect of various processing methods on its protein quality and anti-nutrient compounds. Mucuna bean was processed to remove L-Dopa and other anti-nutrient compounds by different methods such as soaking, autoclaving, roasting, germination, and alkaline fermentation. Protein quality was determined by amino acid composition, in vitro and in vivo rat balance methodologies. All processing techniques, except roasting, reduced levels of L-dopa by >95% and other anti-nutrient compounds such as total phenols, trypsin inhibitor and phytates. Processing improved in vitro protein digestibility (IVPD) but significantly (P < 0:05) reduced protein content. Soaking in acidic medium (pH 3.2) at 60°C for 48 hrs significantly improved IVPD (80.5 %) and biological value (80.8) of mucuna bean protein. The content of essential amino acids met the recommended FAO/WHO reference requirements for 2–5 yr old except for tryptophan. However, true digestibility for processed bean diet was poor (58 %) and protein digestibility-corrected amino acid score (PDCAAS) low (0.4) compared to that of reference casein (1.0). This was attributed to both low sulphur amino acids content and residual anti-nutrient factors that affect protein hydrolysis such as phenolic compounds. Mucuna protein diet did not support growth of weanling rats indicating amino acids pattern incompatible with the needs of weanling rats. Histological examination of liver and kidney tissues revealed that consumption of processed mucuna bean as the only source of protein caused inflammation of the organs. This suggests possible presence of other toxins in processed bean even though mucuna bean diet contained the recommended safe level of residual L-Dopa (<0.1%). Processing mucuna bean improved the protein quality and reduced the content of anti-nutrient compounds. However, mucuna bean is not recommended as a sole protein in human diet.

Keywords: Anti-nutrients, L-Dopa, mucuna bean, processing, protein, quality

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/cover crop. It is rich in protein (23-35%) and its digestibility is comparable to that of other pulses, like soybean, rice

bean and lima bean (1). Despite its potential, mucuna bean remains a minor food crop. It is poorly adopted in agricultural systems (2) due to the presence of anti-nutritional and toxic compounds that arise from secondary metabolism in plants. Anti-nutritional compounds reduce food intake and nutrient utilization in animals and lower the nutrient value of grain legumes (3). They include protease inhibitors, polyphenols, lectins, tannins and phytates. The major anti-nutritional compound in mucuna bean is a non-protein amino acid, 3,4-dihydroxy-L-phenylalanine (L-Dopa) (4). Increased serum levels of L-Dopa from consumption of mucuna bean leads to high concentration of dopamine in peripheral tissues. It induces antiphysiological effects such as nausea, vomiting, anorexia, paranoid delusions, hallucinations, delirium and unmasking dementia (5). The average L-Dopa content in mucuna bean is 3.1-6.7% (3). 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 (6). The objectives of this study were to process mucuna bean by different methods to remove L-dopa and other anti-nutritional compounds and to evaluate the effects of processing on protein quality in terms of amino acid composition, *in vitro* and *in vivo* digestibility

Material and Methods

Materials

Matured dried seeds of mucuna bean were obtained from Kenya Agricultural Research Institute (KARI), Nairobi, Kenya. Seeds were sorted, cleaned and stored in plastic containers. Beans were dehulled with a hammer mill and ground using a Waring commercial blender to particle size diameter of 1.00 – 1.70 mm.

Processing methods

Bean samples were processed by extraction at different temperature and pH. 40g samples were placed in glass jars containing distilled, deionized water (800 ml) and stirred for 1 minute. Samples were placed in an automated temperature control water bath set at 20 or 60°C respectively. The pH was adjusted to 3.2 and 9.0 ± 0.2 using 18 N acetic acid and 1M NaOH solutions respectively. Samples of 10g each were taken at the following time intervals: 6, 12, 24, 36 and 48 hrs and stored before analysis. Processing samples by autoclave was done at 121°C at 1 Kgf /cm^2 pressure for 30 min. Roasting was done by mixing samples with preheated sand and heating in oven set at 100°C for 60 min. Samples that were processed by germination were first sterilized in ethanol. They were germinated in the dark for 3 days. Activated *Bacillus subtilis* (Microbiology and Plant Pathology Culture Bank, University of Pretoria) at 5% v/v was used for alkaline fermentation, of autoclaved and cooled seeds. Seeds were fermented at 32°C for 72 hrs. After processing by all methods, bean samples were frozen overnight at ¯21°C then freeze dried at -(40-50)°C and ground with a pulverizer for analysis.

Protein evaluation by biological methods

In vitro protein digestibility (IVPD) was determined by the pH drop multi-enzyme method (7). The multi-enzyme (Sigma-Aldrich Inc, Germany) solution was composed of 1.6 mg trypsin, 3.1 mg chymotrypsin, and 1.3 mg peptidase per ml of distilled water. *In vitro* protein digestibility of sample was calculated using the following equation: % digestibility = $210.464 – 18.103x$
Where x is the pH after the 10-minute incubation

Biological evaluation of protein quality was based on the nitrogen balance method (7). A sample of 30 clinically healthy weanling albino rats of wistar strain at approximately 4-5 weeks of age and weighing 70 ±10 g were obtained from Teaching and Research rat colony of the Department of Biochemistry and Biotechnology, Kenyatta University. They were divided into three groups of ten rats each on the basis of initial weight, sex and litter origin. One group was given the N-free basal diet, and remaining two groups were randomly allocated to the test (processed mucuna bean) and reference diets. The composition of basal diet was as described previously (8). At the end of the study period, rats were anesthetized using diethyl ether and sacrificed. The liver and kidney were harvested, weighed and preserved in 40% formalin for histological examination. Tissues were processed for light microscopy by fixation, dehydration, clearing and embedding in paraffin wax then sectioned. Tissues were stained with haematoxylin and eosin dyes using standard histological protocols. Stained tissues were cover slipped with DPX mountant, dried and examined microscopically for any pathological changes.

Analytical methods

Proximate composition was determined on bean samples. Samples were ground into fine flour (particle size diameter <0.5mm) and analyzed for crude protein, moisture, crude fat, ash, crude fibre, tannins, phytate and minerals according to AOAC (9) methods. Samples were analyzed in triplicate. Trypsin inhibitor activity (TIA) was determined using Benzoyl-DL-arginine-*p*-nitroanilide (BAPNA) as substrate (10). Absorbance (A) was read at 410 nm wavelength. Amino acid profile of mucuna bean protein was determined according to the Pico-Tag Amino Acid Analysis System (Waters Chromatography Div., Millipore Co., Milford, MA, USA) (11) . Samples were acid hydrolyzed, derivatized and subjected to HPLC analysis. L-Dopa was determined after acidic extraction of sample as described by Siddhuraju and Becker, 2005 (12). L-Dopa analysis was on a Pico-Tag C_{-18}, 3.9 x 150mm column under the following conditions: injection volume 20 µl, flow rate: 1.0 ml/min, and column temperature of 37°C.

Statistical analysis

A complete randomized design was used. Mucuna bean was randomized to the treatments (processing methods). Data was analyzed using the Statistical Package for Social Sciences (SPSS) Version 11.5 (SPSS Inc., Chicago, IL USA). Differences between means were compared using paired T-test. Amino acid data was subjected to one way analysis of variance (ANOVA) and post hoc Tukey B test. Differences in means were considered statistically significant at $p < 0.05$. Values expressed as means ± standard deviation (SD).

Results and Discussion

Chemical and anti-nutrients composition of mucuna bean

Composition of mucuna bean is shown in Table 1. Crude protein content of mucuna bean (27.87%) was higher than that of commonly consumed legumes, such as chick pea (*Cicer arietinum*), green pea (*Pisum sativum*) and common bean (*Phaseolus vulgaris*) with a range from 18.5 to 21.9 % for the raw grains (13), but lower compared to soybean that contains an average of 38% crude protein (14). 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%) (13); soybean (21%) and peanut (49%) (14). Ash content of 3.53% was within the range of 3.4-4.0% reported for beans (14) but lower than 9.8% reported for chickpea and 10.4% reported for pea, (13). 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 (6.98%) was within range (6.7-7%) reported for black accessions in India but higher than 5.9% reported for white seed coat (15). Wide variations in L-Dopa content in mucuna species have been attributed to genetic make up

and on growing locations (16). 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 of mucuna bean were higher than 3.82% reported for cowpea (17). A higher phytate content of 1.35% of dehulled mucuna seeds could be attributed to their location which is mainly in the cotyledons. Trypsin inhibitor activity (TIA) of 9.32 TIU/mg was higher than that previously reported of 4.7-6.9 TIU/mg for the same variety (18).

Effect of processing on the content of L-Dopa and other anti-nutritional compounds

Effects of processing mucuna bean are shown in Table 2. All processing methods except roasting resulted in significant ($P<0.05$) reduction in L-Dopa content. According to the Merck Index (19), L-Dopa has only limited solubility in water (66mg in 40 ml at 20˚C) but it is readily soluble in dilute solutions of hydrochloric acid. Extraction at high pH (9.0) reduced L-Dopa by 99.65% to 0.02%. However, the extract and the bean sample turned blackish, implying chemical conversion of L-Dopa to melanin, rather than actual extraction into water (20). Fermentation and germination reduced L-Dopa levels by 73% and 22%, respectively. Phenolic compounds interfere with digestion and absorption of dietary protein and carbohydrates and availability of vitamins and minerals. They lower the activity of digestive enzymes such as α–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} (21). Reduction in phenolic content during soaking, followed by thermal processing has been observed in other legumes such as *Lablab Parpareus var. vulgaris* and *Vigna radiate* (21). Phytate binds divalent cations, such as, iron, zinc, magnesium and calcium and forms insoluble complexes, making the minerals unavailable for absorption (22) Protease inhibitors in diet lead to formation of irreversible trypsin enzyme-trypsin inhibitor complex, causing a trypsin drop in the intestine and subsequent slower animal growth (23). 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.

Table 1 Proximate composition and antinutrients content of raw and processed 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^{b} ± 0.92	27.2^{a} ± 1.1
Crude fat	3.65 ± 0.23	5.54^{a} ± 0.21	5.50^{a} ± 0.1
Crude fibre	7.91 ± 0.36	3.98^{b} ± 0.15	1.80^{a} ± 0.2
Ash	3.53 ± 0.07	0.30^{a} ± 0.01	0.30^{a} ± 0.01
L-Dopa	6.98 ± 0.10	5.71^{b} ± 0.26	0.10^{a} ± 0.01
Trypsin inhibitor activity[A]	5.14 ± 0.14	9.32^{b} ± 0.19	0.0^{a}
Phytate[B]	0.85 ± 0.02	1.35^{b} ± 0.01	0.40^{a} ± 0.04
Total phenolics[C]	7.09 ± 0.41	5.20^{b} ± 0.20	0.10^{a} ± 0.01
Tannins[D]	2.31 ± 0.04	0	0

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. [1]Processing conditions (pH 3.2, 60˚C)

Crude protein decreased significantly ($P<0.05$) during processing of mucuna bean except for germination and fermentation. The two processing methods significantly ($P<0.05$) increased the crude protein to 32.9% and 37.6%, respectively. During germination, extensive breakdown of seed-storage compounds and synthesis of structural proteins and other cell components take place. High amino acid biosynthetic activity in the seedling result in high content of free amino acids (24). Leaching of protein in processed mucuna bean has been reported (20). Processing mucuna bean at low pH (3.2), moderate temperature (60˚C) for 48 hrs reduced L-Dopa content to 0.07% (recommended level of ≤0.1%) and crude protein to 27.2%. Protein loss (14.7%) was

significantly high but residual crude protein was higher than in most legumes. This processing method for L-Dopa reduction in mucuna bean could be used for household application.

Table 2 Effect of processing mucuna bean on the content of L-Dopa, other anti-nutritional compounds, crude protein (%) and *in vitro* protein digestibility (%).

Treatment	L-Dopa	TIA[A]	Phytates[B]	Total phenolics[C]	Tannins[D]	Crude protein	IVPD
Raw bean	5.71[b] ±0.05	9.32[d] ±0.05	1.35[c] ±0.01	5.20[c] ±0.08	0.0[a]	31.9[d] ±0.30	67.21[a] ±0.05
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)	78.68[e] ±0.05
Extraction at 60˚C, pH 3.2,	0.06[a] ±0.01 (90.02)	0.0 [a]	0.39[a] ±0.01 (70.80)	0.06[a] ±0.04 (98.77)	0.0[a]	27.2[b] ±0.01 (14.73)	80.54[f] ±0.01
Extraction at 20˚C, pH 9.0	0.02[a] ±0.01 (99.65)	2.87[b] ±0.08 (69.21)	0.95[b] ±0.01 (29.72)	0.38[a] ±0.06 (92.64)	0.0[a]	23.1[a] ±0.42 (27.59)	75.48[d] ±0.01
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[d] ±0.14 (0.31)	67.15[a] ±0.18
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)	0.0	32.9[e] ±0.14 3.13	69.14[b] ±0.10
Fermentation	1.49[a] ±0.08 (73.96)	0.0[a] (100)	2.47 [c] ±0.01 82.97	2.76[b]±0.03 (47.05)	2.00 [b]	37.6[d] ±0.14 17.87	70.83[c] ±0.08

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.

Effect of processing mucuna bean on protein quality

The IVPD for raw mucuna bean was 67.21%. All processing methods except roasting significantly (P<0.05) improved IVPD (Table 2). Mucuna bean processed at pH of 3.2, 60˚C showed high IVPD value of 80.54%. This was higher compared to the range reported for cowpea (75.5-78.3%) (26). Based on the composition of processed mucuna bean, this processing method was selected and used for *in vivo* digestibility study. Amino acid composition of raw and mucuna bean processed by this method is shown in Table 3. Processing increased concentration of essential amino acids (EAAs) in mucuna beans. Except for methionine and tryptophan, the content of other EAAs in raw and processed mucuna bean protein met the recommended reference requirements for 2-5 yr old (27).

Table 3: Essential amino acid (EAA) composition of raw and processed (soaked in acidic medium at pH 3.2 and at 60˚C for 48 hrs) mucuna bean (mg/g)

Amino acid	Raw mucuna bean	Processed mucuna bean	EAA requirement for children[1] (2-5 yrs)
Histidine	20.1	25.0	19.0
Isoleucine	39.2	49.3	28.0
Leucine	66.8	82.7	66.0
Lysine	58.0	69.5	58.0
Methionine	8.2	11.8	25.0
Phenylalanine and tryrosine	87.2	101.1	63.0
Tryptophan	7.2	8.0	11.0
Threonine	39.2	41.5	34.0
Valine	44.8	51.8	35.0

Adapted from: [1]FAO/WHO (1991); Values are means of duplicate determinations.

Table 4: Protein quality of casein and mucuna bean fed to rats

Assay	Casein	Mucuna bean diet
TD (%)	$93.6^{a} \pm 3.14$	$58.0^{b} \pm 4.40$
BV (%)	$94.2^{a} \pm 2.35$	$80.8^{b} \pm 2.62$
AAS[1]	1.24^{a}	0.63^{b}
PDCAAS	$1.16^{a} \pm 0.05$	$0.37^{b} \pm 0.05$

Values are means ± SD of ten replicate determinations; Values followed by different superscripts in the same row are significantly *(P<0.05)* different, [1]Values are means of duplicate determinations.

Histological examination liver specimens obtained from rats fed on casein diet (Figure 1) was normal compared to that from rats fed processed mucuna bean diet revealed liver infiltrates, vacuolar degeneration, venous congestion, perivascular cuffing with lymphocytes and necrosis of liver cells (Figure 2) indicated liver function abnormality leading to toxic injury (27). Examination of kidney specimens showed normal histology (Figure 3) for rats fed casein diet and loss of kidney function characterized by interstitial infiltrates, fibrosis and inflammatory atrophy associated with the mucuna diet (Figure 4). However, report on studies on raw and roasted mucuna bean do not indicate presence of mutagenic or substances that can be converted into mutagens by metabolism in the liver (28). This implies that though mucuna diet contained recommended safe level of residual L-Dopa (<0.1%) there could be other toxins in the processed mucuna bean that were toxic to rats.

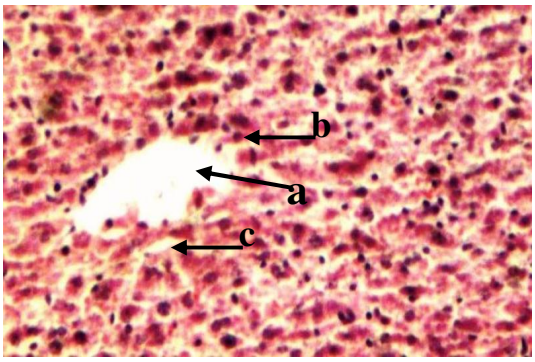

Figure 1: Histological section of liver of a rat fed the casein diet showing normal liver histology: a) central vein, b) hepatic cord and c) liver sinusoid. Magnification: X 100

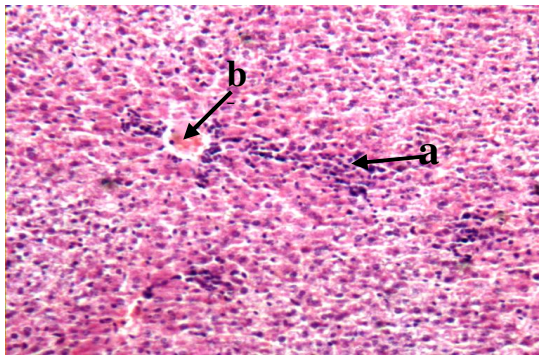

Figure 2: Histological section of liver of a rat fed mucuna bean diet showing a) infiltration and b) venous congestion. Magnification: X 100

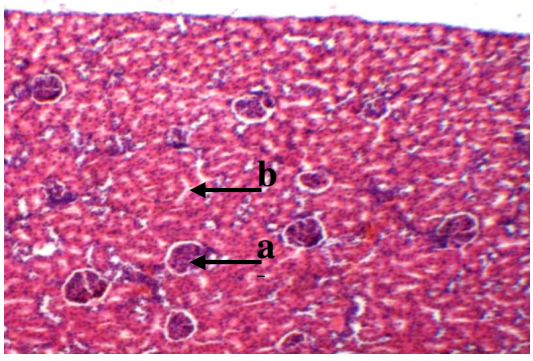

Figure 3: Histological section of kidney of a rat fed casein diet showing normal kidney histology: a) glomerulus and b) tubules. Magnification: X 100

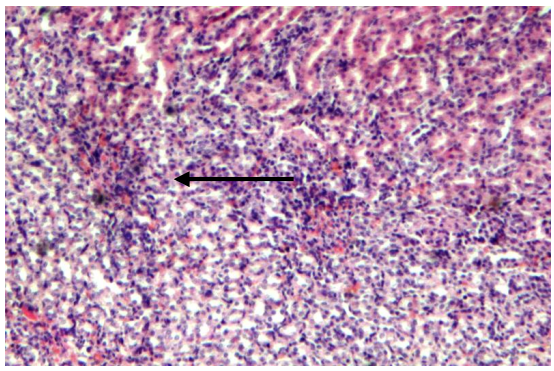

Figure 4: Histological section of kidney of a rat fed mucuna bean diet showing medulla tubular atrophy. Magnification: X 100

Conclusions and Outlook

Proximate composition of mucuna bean compared favorably with that of conventional edible legumes. All processing methods except roasting significantly reduced L-Dopa content in mucuna bean. Simultaneously, other anti-nutritional compounds such as trypsin inhibitor, phytates and phenolic compounds were significantly reduced. Specifically, processing at low pH (3.2) and at 60˚C reduced the L-Dopa content to recommended safe level of 0.1% and improved BV of protein. However, a reduction of crude protein (by 15%) was observed. Residual protein level (27.1%) was high in processed mucuna bean compared to that in commonly- consumed legumes. All processing methods (except roasting) improved protein quality (IVPD) of mucuna bean. However, the processed bean did not support growth of weanling rats when fed as sole protein. Low PDCAAS for mucuna bean protein could be attributed to very low content of sulphur amino acids and possible presence of factors that hinder protein hydrolysis thereby reducing nutritional value. Consumption of processed mucuna bean by weanling rats caused inflammation of liver and kidney suggesting presence of toxins other than L-Dopa. Hence, mucuna bean may not be used as the sole protein in the human diet.

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