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PROTEIN AND COOKING QUALITY AND RESIDUAL CONTENT OF DEHYDROXYPHENYLALANINE AND OF TRYPSIN INHIBITORS OF PROCESSED *MUCUNA* BEANS (*Mucuna* spp)

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SUMMARY

Samples of *Mucuna* beans (white, black and mottled) from Guatemala were used for studies aimed at finding practical conditions for reducing L-Dopa concentration to levels permitting consumption. Initial concentrations of L-Dopa content varied from 4.84 to 7.60%. The quality of the *Mucuna* bean varied depending on the processing. While soaking the beans in water favored partial elimination of L-Dopa, with an increased efficacy in elimination and an increase in water uptake into the bean if the water temperature was at least 66°C, soaking times that allowed for sufficient L-Dopa removal were rather long for practical home preparation of food. However, by soaking the beans at 60°C with changes of water every 12 h, L-Dopa retention decreased 22-30% of the original value in 48 h, which is a retention similar to soaking for 96 h without change of water.

Mucuna bean cooking time to soften the grain is quite long (over 6 h), which is reduced to some extent by pre-soaking the beans in water for 12 h and slightly more by soaking in a 0.45% bicarbonate solution. L-Dopa retention after 5 h of cooking time was higher for the non-soaked bean samples as compared to the water-soaked and bicarbonate-soaked beans. However, the loss of L-Dopa was similar to that previously observed.

To evaluate these food products biologically, 8 young rats (22 d old) were used per treatment. When fed with diets containing raw and soaked raw *Mucuna* beans, the rats did not gain weight in a 16-d experimental period, although organic matter digestibility was 89% and protein digestibility 56%. However, cooking the beans for 3 h at atmospheric pressure after a 48-h soaking allowed rats to gain weight and an NPR of 1.27 (53% that of casein). Increasing cooking time to 6 h at atmospheric pressure decreased protein quality. Pressure cooking for 30, 45 and 60 min did not yield a cooked *Mucuna* bean with the quality achieved through 3-h cooking in atmospheric pressure. These cooking procedures reduced L-Dopa content by 14 to 43% and eliminated all trypsin inhibitor activity. In contrast, roasting the bean did not result in a significant decrease in L-Dopa; however, the trypsin inhibitor activity, another anti-nutritional property found in the beans, did decrease significantly. Roasted *Mucuna* beans had a protein value of about 37% of the casein value, and an increased protein digestibility compared to raw beans. In addition, if the roasted beans were made into flour and cooked in boiling water, the concentration of L-Dopa in the flour was reduced to extremely low levels. This effect was most likely due to the small particle size. Lastly, germination and malting the beans did not significantly reduce L-Dopa levels, and resulted in beans that were similar to the raw beans in terms of nutritional value and animal weight gain.

Key words: *Mucuna* beans, L-Dopa removal, effects of processing methods, protein quality.

INTRODUCTION

In the past two decades, the majority of the research and extension efforts on *Mucuna* beans have focused on its soil-improving attributes. Various studies have confirmed *Mucuna's* high biomass production and its ability to both fix and recycle large amounts of nitrogen, which are among factors explaining its success as a green manure/ cover crop.

Mucuna has also been used as food and feed in a number of countries where it has been introduced. Typically, the beans have been consumed by humans, though in some areas, immature pods and leaves have also been eaten. For animal feed, both beans and foliage have been utilized. As a food crop, it has remained in a minor status, while as a feed, it has been tested and proven on a large scale, especially in the southern U.S. in the first half of the 20th century

(Eilittä and Sollenberger, 2002). Its potential as a food and feed are affected by two opposing forces: on the one hand, by its relatively good seed and forage yield and beneficial composition of nutrients and, on the other hand, by its relatively numerous anti-nutritional and toxic compounds. These compounds include the anti-nutritional factors commonly found in grain legumes, such as enzyme inhibitors and phenolic compounds. *Mucuna* beans also contain variable amounts of L-Dopa, a compound which induces antiphysiological effects that limit its use as a food crop (Josephine and Janardhanan, 1992). In general, there is a great deal of ambiguity surrounding the state of knowledge on toxic compounds present in *Mucuna*, their impact on its potential as a food and feed, and on ways to reduce such compounds through processing.

The present report deals with a series of experiments that evaluated acceptable and practical processing methods to reduce L-Dopa and trypsin inhibitors in *Mucuna* beans, and assessed protein quality of *Mucuna* beans processed by these various techniques. Altogether, five studies were conducted:

1. Effect of temperature on water absorption and L-Dopa retention in *Mucuna* beans
2. Effect of periodic water exchange on water uptake, soluble solids and L-Dopa retention
3. Effect of various cooking times
4. Effect of cooking time of non-soaked, water and bicarbonate soaked *Mucuna* beans on percent cooked, water uptake, hardness and L-Dopa content
5. Effect of processing on the protein quality, L-Dopa, and trypsin inhibitor content

MATERIALS AND METHODS

Mucuna samples and analytical methods

Ten samples of *Mucuna* beans (described in Table 1), were collected from various locations in the southern lowlands of Guatemala; one of the ten samples (100 kg) was from the Instituto de Ciencia y Tecnología Agrícola (ICTA) which had cultivated *Mucuna* as a cover crop in Cuyuta, Escuintla, in 1999. Most samples were grown by individual farmers over fences and the amounts collected were in the order of 4-5 kg. The seed color was either black, mottled or white. In one case, the person who provided the sample was producing the seed for consumption as a roasted flour to make an infusion (i.e., coffee). The average weight varied from 0.64 to 0.97 g per seed, with an average volume of 0.52 to 0.77 mL per seed. Seed coat weight ranged from 11.27 to 14.77% of seed weight. The samples were stored at room temperature in the laboratory until utilized.

L-Dopa analysis was by the method of Brain (1976), while trypsin inhibitors were measured by the method of the AACC (1976). Proximate analyses of the samples were performed using AOAC Official Methods of Analysis (1984). Processing methods are described separately in the following sections. All processing was conducted at the Universidad del Valle de Guatemala, Guatemala City, at an altitude of 1500 m.a.s.l.

Study 1. Effect of temperature on water absorption and L-Dopa retention in *Mucuna* beans

In these studies, 5 of the small samples of *Mucuna* beans collected from various locations in the Guatemalan lowlands were used (Nos. 1, 2, 3, 5, 6). Each test was conducted with 10 grains, which were weighed and placed into perforated plastic bags. Each sample (conducted in triplicate) was placed in 200 mL deionized water at 22, 45 and 66 °C for a time period between from 0 to 96.5 h. At specific intervals, the bags were removed, the bean surfaces dried and the samples weighed. The difference in weight indicated the amount of water absorbed and was expressed as g per 100 g.

Study 2. Effect of periodic water exchange on water uptake, soluble solids and L-Dopa

Two collections of *Mucuna* beans were used: a white (no. 5) and a mottled-colored (no. 1) grain. A total of 10.0 g of each sample was placed in Erlenmeyer flasks with 100 mL of deionized water at 60°C for a period of 12 h. A sample of these beans was tested for moisture and for L-Dopa content. L- Dopa and solids in the soaking water were also determined after which the water replaced with fresh water. The soaking water for similar samples was removed at 24, 36 and 48 h. This treatment was replicated 3 times. The procedure is shown in Table 2.

Table 1. *Mucuna* bean samples used in the study.

No.	Identification	Seed wt (g)	Grain volume (mL)	Density (g mL^{-1})	Seed coat (%)
1	Mottled LM-00	0.89 ± 0.02	0.75 ± 0.00	1.30 ± 0.04	11.71 ± 0.06
2	Black SFR – 99	0.64 ± 0.03	0.52 ± 0.04	1.27 ± 0.11	14.77 ± 0.90
3	White TC – 01	0.80 ± 0.03	0.62 ± 0.02	1.31 ± 0.01	12.74 ± 0.43
4	Mottled SFR - 99	0.88 ± 0.02	0.76 ± 0.15	1.18 ± 0.18	12.38 ± 0.23
5	White JEP – 01	0.88 ± 0.02	0.70 ± 0.01	1.30 ± 0.01	12.33 ± 0.09
6	White SFR – 99	0.86 ± 0.04	0.69 ± 0.04	1.31 ± 0.01	11.27 ± 0.15
7	Black EP - 01	0.79 ± 0.01	0.65 ± 0.02	1.31 ± 0.02	13.31 ± 0.32
8	White EP - 01	0.92 ± 0.01	0.71 ± 0.03	1.30 ± 0.00	11.54 ± 0.14
9	Mottled EP - 01	0.97 ± 0.01	0.77 ± 0.01	1.31 ± 0.01	11.38 ± 0.17
10	Black ICTA-99	0.74±0.03	0.61± 0.01	1.30 ± 0.02	15.72 ± 1.12

Note: % seed coat: weight of seed coat/total seed weight.

Table 2. Activities and analyses conducted of samples taken at different times during the Study 2.

Time (h)	Activity	Analyses conducted
0	Sample preparation	Beans: % moisture, L-Dopa
12	Soaking water removed A sample of soaked beans removed Addition of fresh water	Water: L-Dopa and solids Beans: % moisture, L-Dopa
24, 36	Soaking water removed A sample of soaked beans removed Addition of fresh water	Water: L-Dopa and solids Beans: % moisture, L-Dopa
48	Soaking water removed A sample of soaked beans removal	Water: L-Dopa and solids Beans: % moisture, L-Dopa

Study 3. Effect of various cooking times

In regions where beans are an important component of diets, cooking time is one of the most important acceptability characteristics (El-Tabey Shehata, 1992; Shellie-Dessert and Hosfield, 1990; Bressani, 1993). The hard-to-cook condition does not yield beans that are chewed easily and if excess heat is applied, nutritional properties decrease and fuel costs increase. In previous studies it was noticed that not all *Mucuna* bean kernels absorbed water equally; in fact, some of the collections tested showed very low rates of water uptake. Therefore, the following cooking time studies were conducted. First, cooking time without previous soaking was determined. The procedure involved dropping into 300 mL boiling deionized water 100 grains of each of the 5 collections (Nos. 1,2,3,5,6). This was zero time. The level of water was kept constant by adding boiling water to 300 mL. Samples of the beans (10 grains) were withdrawn at periodic intervals and pressed between the fingers. The percentage cooked was calculated based on those beans which could be pressed and had a pasty consistency. Both beans that could and could not be pressed were placed in petri dishes and dried to constant weight with hot air at 60°C to establish moisture content. Second, the impact of soaking prior to cooking on cooking time was determined. In these experiments the bean samples (5 collections, each 100 grains) were soaked in 250 mL deionized water or in 0.45% sodium bicarbonate water solution for 13 h before the cooking study following the above-mentioned procedure was started.

Study 4. Effect of cooking time of non- soaked, water- and bicarbonate- soaked *Mucuna* beans on percent cooked, water uptake, hardness and L-Dopa content

In these studies 150 g of two *Mucuna* bean samples, a white (B-EP, no. 5) and a mottled (M-LM; no. 1), were processed by a) non-soaking, b) soaking in water for 12 h and c) soaking in 0.45% bicarbonate solution for 12 h at room temperature. The samples were placed in beakers and cooked until soft. The volume of water was 200 mL. Subsamples were withdrawn at hourly intervals for measurements of cooking percentage, water content, seed hardness and L-Dopa content. Hardness was measured by a penetrometer through a puncture test.

Study 5. Effect of processing on the protein quality, L-Dopa, and trypsin inhibitor content of *Mucuna*

The beans used for these studies were obtained from ICTA. Three biological trials using the Net Protein Ratio method (NPR) were conducted. These three trials utilized different processing methods for *Mucuna*: 1. pressure and atmospheric cooking, 2. roasting, and 3. germination.

In the first trial, the effect of two types of cooking was studied in beans that had been soaked in plain water for 48 h. Cooking consisted of pressure (15 psi) cooking (for 30, 45 and 60 min) and atmospheric cooking (for 3 and 6 h). After processing the beans were separated from the cooking liquor and dried to a low moisture content. They were then ground into a flour and analyzed for protein, L-Dopa and trypsin inhibitors (TI).

In the second biological trial, the *Mucuna* beans were subjected to roasting (105-120 °C) in a coffee roaster using gas as the energy source for 10, 20 and 30 min. The beans were cooled, dehulled with a disc dehuller, cleaned, ground into flour, and then analyzed as indicated above.

The third biological assay was conducted with *Mucuna* beans that had been germinated for 0, 3 and 6 d. In this experiment a second sample germinated for 3 d was heat-treated, simulating malting, in a microwave oven for 7 min at 30% power level. For germination, all samples were surface sterilized and soaked for 12 h before initiation of germination. Germination was carried out in a temperature and relative humidity controlled cabinet.

Once processed, all the bean samples were dried with hot air at 60 °C to constant weight and ground into flour. The resulting flour was analyzed for protein, L-Dopa and TI as indicated above.

The four types of processed *Mucuna* bean flour samples (i.e., cooked with pressure and at atmospheric pressure, roasted, and germinated) were each incorporated into a basal diet for rats to provide 10% protein. Each diet also contained 5% vegetable oil, 4% mineral mixture, 1% cod liver oil, starch to adjust to 100 g and 5 ml of a complete B-vitamin solution. Two additional diets were prepared: one, in which casein, a reference protein, was selected to provide 10% protein and the second, an NFD (nitrogen free diet) to measure endogenous nitrogen metabolism. The experiments were conducted for 16 d using 8 young rats (22 d old) per treatment (total of 168) with an initial average weight of 44 g. Food was provided *ad libitum* and food consumption and weight were measured every 2 d. During the last 5 d of the 16-d experimental period, feces were collected for determination of organic matter digestibility, protein digestibility and L-Dopa absorption.

RESULTS AND DISCUSSION

Study 1. Effect of temperature on water absorption and L-Dopa retention in *Mucuna* beans

The results are summarized in Table 3. Water uptake by the beans was different between samples particularly at low temperature (22 °C); however, these differences diminished as the temperature of the water increased to 45 and 66 °C. After 96.5 h, at 22 °C, the average water uptake for the 5 samples was 51.8%. The low water absorption is a characteristic of many species of grain legumes and is associated with the seed coat, the hilum, the drying of the seed in the pod and with the hard-to-cook problem. It is overcome in many cases by increasing water temperature, as is the case in this study. Water absorption increased to 83.5 ± 5.6 % when water temperature was 45 °C and to 88.4 ± 5.1% at 66 °C. Only small increases in water uptake took place between 45 and 66 °C at 19 h and longer soaking periods.

Table 3. Water uptake and change in L-Dopa content of *Mucuna* beans soaked for different times at 3 water temperatures. Averages of 5 bean samples and 3 replications at each soaking time are given.

Soaking time (h)	Water uptake (%) 22 °C	45 °C	66 °C
0	0	0	0
3	2.46 ± 1.58	30.24 ± 10.46	42.76 ± 5.74
19	22.16 ± 25.27	84.74 ± 7.55	84.22 ± 3.78
24.25	26.60 ± 30.30	84.66 ± 7.45	84.18 ± 3.64
46	36.40 ± 39.33	86.04 ± 8.90	86.18 ± 5.49
96.5	51.76 ± 49.42	83.50 ± 5.62	88.42 ± 5.09
		L-Dopa (%)	
0	5.69 ± 0.53	5.69 ± 0.53	5.69 ± 0.53
96.5	4.00 ± 0.86	2.91 ± 0.38	1.56 ± 0.34
Retention (%)	70.3	51.1	27.4

Table 3 also summarizes the changes in L-Dopa upon soaking at different water temperatures. Soaking for 96.5 h at 22 °C resulted in a 70% L-Dopa retention (i.e., 30% loss). Retention decreased to 51% at 45 °C and to 27% at 66 °C after 96.5 h of soaking. Although water uptake did not increase significantly with prolonged soaking time or with higher temperatures, L-Dopa dropped significantly as water temperature increased. Note that there was no change in water during the complete soaking period at the higher temperatures. It seems that water temperature therefore plays a significant role in L-Dopa removal from the beans. Figure 1 shows the mathematical expression of this relationship as a linear equation. Projection to 0% L-Dopa content would indicate that a temperature of 95 °C for 96.5 h would be required, assuming the solubility of L-Dopa will increase with temperature. However, soaking time for 96.5 h is not attractive for standard food preparations. The soaking water was of a dark color for black samples and creamy white for the white and mottled beans.

Study 2. Effect of periodic water exchange on water uptake, soluble solids and L-Dopa

The results of the study are summarized in Table 4. L-Dopa levels of the white and mottled *Mucuna* samples that were soaked with periodically changed 60°C water were reduced to 22-30% of the initial value in 48 h, a level similar to that obtained when the beans were soaked for 96 h at 66 °C in the first study. Thus, L-Dopa loss was estimated to be 70% for the white collection and 78% for the mottled collection. The table shows that the levels of L-Dopa in the soaking water decreased with soaking time, obviously due to the lower concentration. L-Dopa recovery was close to 100% for both samples at each soaking time. Water uptake was shown to increase with soaking time from around 54.5 to 61.0 % in 48 h for both samples. Solids lost during soaking varied from 0.96 to 1.1 g from the 10 g bean sample soaked at each period. As expected, losses of solids decreased with soaking time.

Figure 2 shows the mathematical expression of L-Dopa changes with respect to the soaking time for the two *Mucuna* collections (at 60°C, with periodic water changes). The best fit was an exponential equation, which indicates that L-Dopa removal slowed as soaking time increased. By this time and under the conditions used, starch probably started to gelatinize and protein denature, making it more difficult for L-Dopa to solubilize in the water media. Factors other than L-Dopa solubility in water play a role in L-Dopa removal from *Mucuna* beans. To speed up L- Dopa removal, reducing particle size of the seed would probably be necessary.

Based on the first two studies it was concluded that for best L-Dopa removal, soaking water should be at a high temperature and should be changed often.

Study 3. Cooking time studies

The results of the cooking studies without previous soaking are summarized in Table 5. For 100% cooked beans, two samples required 6.1 h (370 min) cooking time and the other three required around 5.8 h at 1500 m.a.s.l. (i.e., the altitude of Guatemala City where the study was conducted). Cooking time was established by pressing individual beans between the fingers. The percent cooked was calculated from the number which gave a pasty consistency. From the data on cooking time and water content, polynomial regressions were calculated (Table 6). These equations permit one to establish cooking time at fixed water content levels, or to study the behavior of water content with cooking time. The low coefficient of x^2 (1.21) was indicative of longer cooking time while the high coefficient (1.95) suggested beans with lower cooking times.

Table 4. Changes in water uptake, solids in soaking water, and L-Dopa content in beans and soaking water in two *Mucuna* beans collections. The results are averages of 3 replications per soaking time.

Soaking time (h)	L-Dopa in beans (g %)	L-Dopa in soaking water (g %)	Water uptake (g %)	Solids in soaking water (Brix Units)
	WHITE-SFR			
0	7.04 ± 1.10	0	0	0
12	3.61 ± 0.17	2.46 ± 0.10	54.1 ± 2.6	3.1 ± 0.1
24	2.67 ± 0.10	1.33 ± 0.12	57.8 ± 1.6	2.1 ± 0.1
36	2.28 ± 0.26	0.65 ± 0.06	58.4 ± 1.5	2.0 ± 0
48	2.14 ± 0.66	0.38 ± 0.15	59.5 ± 0.3	2.0 ± 0
	MOTTLED-LM			
0	7.34 ± 1.05	0	0	0
12	4.28 ± 0.02	2.48 ± 1.58	55.3 ± 3.3	3.1 ± 0.1
24	2.92 ± 0.35	1.63 ± 0.09	58.5 ± 1.7	2.3 ± 0.1
36	2.56 ± 0.71	0.77 ± 0.17	60.1 ± 1.3	2.0 ± 0
48	1.63 ± 0.26	0.42 ± 0.13	61.8 ± 0.6	2.0 ± 0

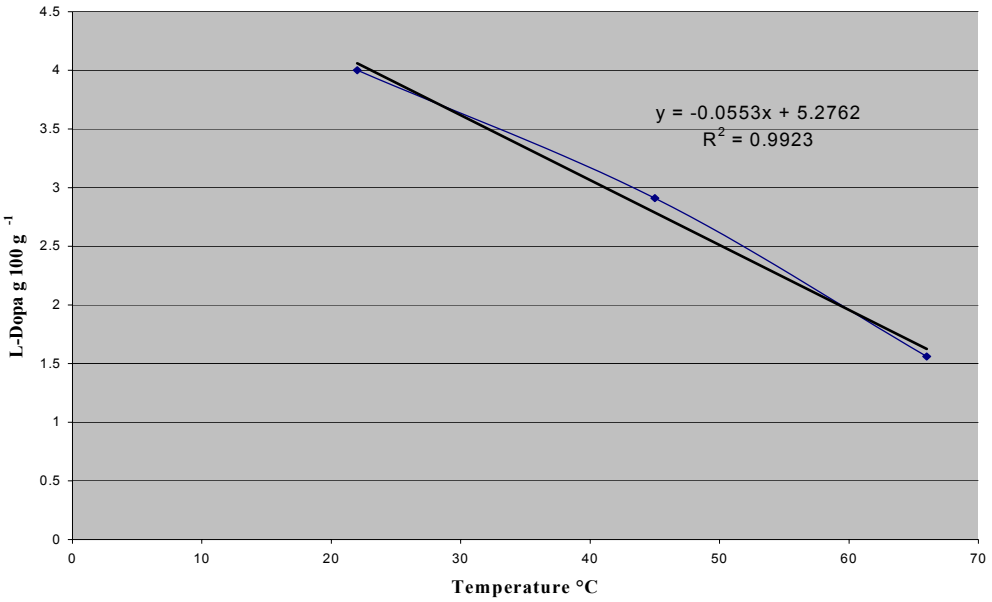

Figure 1. Effect of water temperature on L-Dopa content of two collections of *Mucuna* beans during a 96.5-h soaking time.

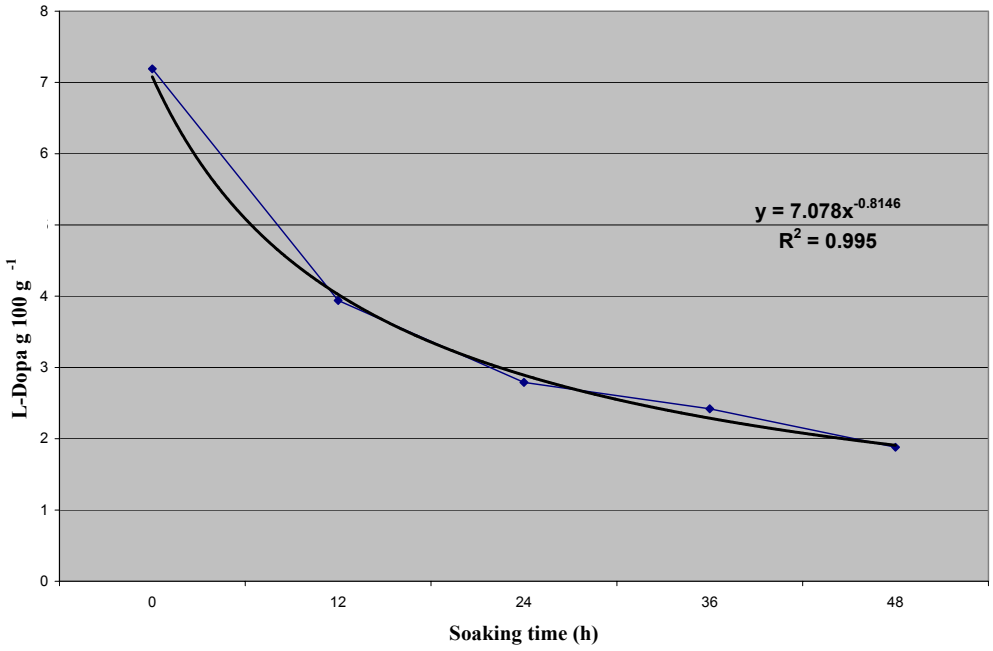

Figure 2. Effect of periodic water exchange on L-Dopa in two collections of *Mucuna* beans. The water temperature was 60°C.

Table 5. Percentage of cooked beans and their moisture content at different cooking times of five collections of *Mucuna* beans which had not been soaked prior to cooking. The results are averages of 10 beans withdrawn at periodic intervals from the cooking water.

Time (min)	Black-SFR N-SFR Cooked	Black-SFR N-SFR Moist.	White-SFR B-SFR Cooked	White-SFR B-SFR Moist.	White EP B-EP Cooked	White EP B-EP Moist.	White TC B-TC Cooked	White TC B-TC Moist.	Mottled LM P-LM Cooked	Mottled LM P-LM Moist.
	%									
0	0	9.7	0	9.5	0	11.6	0	12.1	0	11.1
60	0	33.7	0	33.5	0	35.6	0	34.0	0	32.4
120	0	43.6	0	42.0	0	44.1	0	35.2	0	43.3
180	0	54.1	0	55.8	0	52.3	0	53.8	0	48.7
240	10	56.9	0	52.5	10	55.5	0	57.8	10	57.0
270	20	56.2	0	62.9	20	59.2	20	61.4	50	59.4
290	-	-	-	-	-		-	-	40	58.5
300	30	60.5	20	51.3	40	63.5	-	63.2	50	60.4
310	-	-	-	-	-	-	40	-	-	-
320	50	64.8	30	56.5	60	63.9	-	65.0	80	67.6
325	-	-	-	-	-	-	60	-	80	61.3
330	60	64.1	-	-	70	64.6	80	64.6	100	64.8
335	-	-	-	-	-	-	-	-	-	-
340	70	66.2	70	60.5	90	64.5	90	66.1	-	-
350	70	66.7	60	61.6	-	-	-	-	-	-
360	80	64.7	60	61.8	-	-	-	-	-	-
366	100	65.6	70	62.9	-	-	-	-	-	-
370	-	-	90	60.9	-	-	-	-	-	-

Table 6. Regression equation for cooking time (x) and water uptake by the beans (y) for five *Mucuna* accessions which had not been soaked prior to cooking.

Sample	Equation	r^2
N-SFR	$Y= -1.40x^2 \pm 16.93x \pm 13.60$	0.97
B-SFR	$Y= -1.59x^2 \pm 17.32x \pm 13.36$	0.93
B-EP	$Y= -1.46x^2 \pm 16.86x \pm 15.08$	0.98
B-TC	$Y= -1.21x^2 \pm 16.00x \pm 13.84$	0.97
P-LM	$Y= -1.95x^2 \pm 16.73x \pm 13.69$	0.98

The results on cooking time with previous soaking in water and in $NaHCO_3$ are summarized in Table 7. Even though the beans were soaked, cooking time did not decrease significantly. When soaked in bicarbonate, cooking time decreased only by 10-20 minutes. Cooking time was shorter when bean moisture content was high when soaking was initiated. For example, the Black SFR had an initial moisture of 40.2% and was cooked in 305 min while the Mottled LM cooked in 350 min and had an initial moisture content of 12.4%. The relationship between cooking time and moisture level is shown in Table 8 for the three bean samples.

The equations show that soaking in water or in bicarbonate increases bean water content at all times, with water being more effective than the bicarbonate. It was observed that at about 3 h of cooking the seed coats were easily removed when the beans were cooked without soaking. This happened in about 2 h when the beans were soaked and even sooner if soaked with bicarbonate. Seed coat removal is an advantage to the nutritional quality of the beans, since seed coats are very high in dietary fiber and in phenolic compounds. In *P. vulgaris* the protein quality of processed cotyledons is higher than the protein quality of processed whole beans. This was interpreted to be due to phenolic compounds (which interfere with protein digestibility) and to dietary fiber (constituting almost 80%) of the seed coats (Acevedo *et al*., 1994; Bressani *et al*., 1988; Bressani, 1993). It was also observed that *Mucuna* beans do not cook as *Phaseolus* beans do. *Mucuna* beans break apart when pressed and the pieces are not as pasty as those of *Phaseolus*.

Table 7. Percentage of cooked beans and their moisture content at different cooking times of three collections of *Mucuna* beans subjected to cooking after soaking in water or in Na HCO_3. The results are averages of 10 beans withdrawn at periodic intervals from the cooking water.

Time	Black SFR				White EP				Mottled LM			
(min)	H_2O		NaH CO_3		H_2O		NaH CO_3		H_2O		NaH CO_3	
	Cooked	Moist.	Cooked	Moist.	Cooked	Moist.	Cooked	Moist.	Cooked	Moist.	Cooked	Moist.
	%											
0	0	40.2	0	32.3	0	26.0	0	20.0	0	12.4	0	12.9
60	0	39.1	0	41.3	0	34.4	0	36.7	0	37.0	0	33.5
120	0	51.5	0	50.8	0	49.5	0	46.4	0	43.5	0	43.9
180	0	54.5	0	59.6	0	51.3	0	54.3	0	49.8	0	50.8
210	0	61.4	30	60.0	20	56.6	10	57.0	0	55.5	0	55.4
230	-	-	30	59.3	-	-	-	-	-	-	-	-
240	30	62.0	-	-	20	58.0	20	57.4	0	56.5	10	56.9
250	-	-	50	61.1	-	-	-	-	-	-	-	-
260	40	62.5	50	64.1	-	-	-	-	-	-	-	-
270	40	65.0	80	64.5	30	61.8	40	61.6	10	59.2	20	59.7
280	60	67.2	80	63.0	-	-	-	-	-	-	-	-
285	-	-	70	63.9	-	-	-	-	-	-	-	-
290	60	65.0	100	63.7	-	-	-	-	-	-	-	-
295	80	65.9	-	-	-	-	-	-	-	-	-	-
300	-	-	-	-	50	62.8	70	64.1	30	61.5	40	61.4
305	100	69.7	-	-	-	-	90	63.2	-	-	-	-
310	-	-	-	-	80	64.3	100	64.0	-	-	-	-
315	-	-	-	-	80	57.9	-	-	-	-	-	-
320	-	-	-	-	100	63.4	-	-	40	60.8	60	63.3
330	-	-	-	-	-	-	-	-	-	-	80	65.9
335	-	-	-	-	-	-	-	-	60	64.8	90	65.0
340	-	-	-	-	-	-	-	-	70	64.2	100	61.2
345	-	-	-	-	-	-	-	-	80	64.1	-	-
350	-	-	-	-	-	-	-	-	100	65.5	-	-

Table 8. Polynomial regressions between cooking time (x) and moisture content (y) of three *Mucuna* bean accessions cooked after no soaking, after soaking in water and after soaking in bicarbonate.

Sample	Without soaking	Soaked in water	Soaked in NaH CO_3
W-SFR	$Y=-1.59x^2 \pm 17.32x \pm 13.36$	$Y=0.079x^2 \pm 5.57x \pm 38.08$	$Y=-1.05x^2 \pm 11.72x \pm 31.73$
W-EP	$Y=-1.46x^2 \pm 16.86x \pm 15.08$	$Y=-1.05x^2 \pm 12.57x \pm 25.32$	$Y=-1.31x^2 \pm 14.91x \pm 21.35$
M-LM	$Y=-1.95x^2 \pm 16.73x \pm 13.69$	$Y=-1.26x^2 \pm 15.39x \pm 16.73$	$Y=-1.40x^2 \pm 16.40x \pm 15.24$

Study 4. Effect of cooking time of non-soaked, water-, and bicarbonate-soaked *Mucuna* beans on percent cooked, water uptake, hardness and L-Dopa content

The data shown in Table 9 suggests that bicarbonate-soaked beans cooked faster than water-soaked or non-soaked beans. Non-soaked beans cooked in 6 h while soaked beans (in either water or bicarbonate) cooked in 5 h. Water uptake at 5 h was higher for water-soaked and non-soaked beans, and significantly lower for bicarbonate-soaked beans. This is not easily explained; however, it is possible that the HCO_3 ion or Na reacted with the cellular wall, thus reducing water uptake. Studies with potassium bicarbonate should therefore be conducted. Seed hardness decreased with cooking time. The appearance of cooked *Mucuna* is strikingly different than that of cooked *Phaseolus*. The first splits into small pieces and then gives a paste. *Phaseolus* gives a paste when cooked. The changes found in water uptake, cooking time and hardness are similar to those previously reported.

Table 10 summarizes the changes in L-Dopa upon soaking and cooking. In all instances, L-Dopa decreased as cooking time increased from 0 to 6 h even though the initial value for the samples was quite variable. The change in L-Dopa content of the non-soaked bean at 6 h of cooking time was 50.8 and 28.0% for the W-EP and M-LM samples, respectively.

In the beans soaked in water, L-Dopa decreased by 55.1 and 52.5% during a 6-h cooking for W-EP and M-LM *Mucuna* samples, respectively. The loss was higher in soaked beans compared to non-soaked beans. Finally, the losses of L-Dopa for the samples soaked in bicarbonate were 58.9% (W-EP) and 55.8% (M-LM), and were higher when compared to the other treatments.

In a second study using the same samples, L-Dopa loss for the non-soaked W-EP sample was 60.8%; this increased to 67.6% with 12-h soaking. For M-LM sample, the L-Dopa loss for the non-soaked sample was 36.9%, which increased to 50.1% with soaking.

Figure 3 shows the mathematical expression of the results of sample W-EP (Table 10) indicating a decreasing rate of L-Dopa removal as cooking time increased. The same tendency was also evident for *Mucuna* sample M-LM (Figure 4). These equations are similar to those shown in Figure 2. They suggest that L-Dopa will only decrease very slowly as cooking time increases unless the seed structure is changed drastically by e.g., cracking or chopping the bean.

Based on the above results, soaking is an attractive procedure to use for *Mucuna* preparation since it, particularly if bicarbonate solution is used, reduces cooking time. The process also reduces L-Dopa content by about 55-58%. It would have been interesting to have a treatment with soaking in bicarbonate at higher water temperatures, as the previous studies suggested higher temperatures are more effective in reducing L-Dopa levels. Likewise, the effect of the pH should be studied.

Study 5. Effect of processing on the protein quality, L-Dopa, and trypsin inhibitor content

Changes in moisture, protein and L-Dopa content of the samples as a result of atmospheric and pressure cooking are shown in Table 11.

Processing resulted in a higher protein content, possibly because of the removal of the seed coat. These processes did not result in a consistent decrease in L-Dopa content. To verify this, the products were analyzed a number of times. This finding was interpreted to mean that L-Dopa is quite heat-stable.

The results on protein quality of *Mucuna* beans cooked by atmospheric and pressure-cooking are presented in Table 12. All rat diets were calculated to contain 10% protein from *Mucuna* beans; casein at the same level served as a control. The 10% protein level of *Mucuna* protein was equivalent to 35 g of *Mucuna* flour.

Table 9. Effect of cooking time on percentage of cooked beans, on water uptake, and on bean hardness in two non-soaked, water-soaked, and bicarbonate-soaked *Mucuna* samples. The values are averages of three replications at each cooking time.

Time (h)	Sample W-EP								
	Bicarbonate Soaked			Water Soaked			Non Soaked		
	Cooked (%)	Water Uptake (g %)	Hardness (mm)	Cooked (%)	Water Uptake (g %)	Hardness (mm)	Cooked (%)	Water Uptake (g %)	Hardness (mm)
0	0	16.5±1.9	0.11	0	24.7±0.8	0.38	0	3.8±0.3	0
1	0	30.1±0.1	1.06	0	31.9±3.2	0.29	0	28.7±0.3	0.27
2	0	40.8±1.1	1.73	0	41.5±0.1	0.92	0	40.4±2.2	1.06
3	0	41.7±1.7	2.15	5	52.6±1.8	2.55	0	46.1±0.3	1.81
4	55	47.7±5.6	2.81	45	60.5±1.7	2.61	5	55.2±1.1	2.34
5	100	41.6±3.6	Split	100	72.5±1.5	Split	60	60.6±1.7	3.76
6	-	-	-	-	-	-	100	71.4±1.7	Split
					Sample M-LM				
0	0	21.8±0.2	0.09	0	30.8±0.3	0.30	0	3.9±0.1	0
1	0	35.5±0.1	0.97	0	37.5±0.9	0.39	0	28.4±0.3	0.26
2	0	40.5±4.8	2.18	0	44.6±2.0	0.67	0	41.5±0.2	0.87
3	15	37.7±0.4	2.75	0	55.5±1.6	2.44	0	48.0± 0.7	1.47
4	75	49.0±1.8	3.39	55	62.9±0.6	2.54	5	52.5±1.3	2.16
5	100	35.2±3.5	Split	100	72.9±0.9	Split	65	60.3±0.9	3.16
6	-	-	-	-	-	-	100	66.8±2.0	3.06

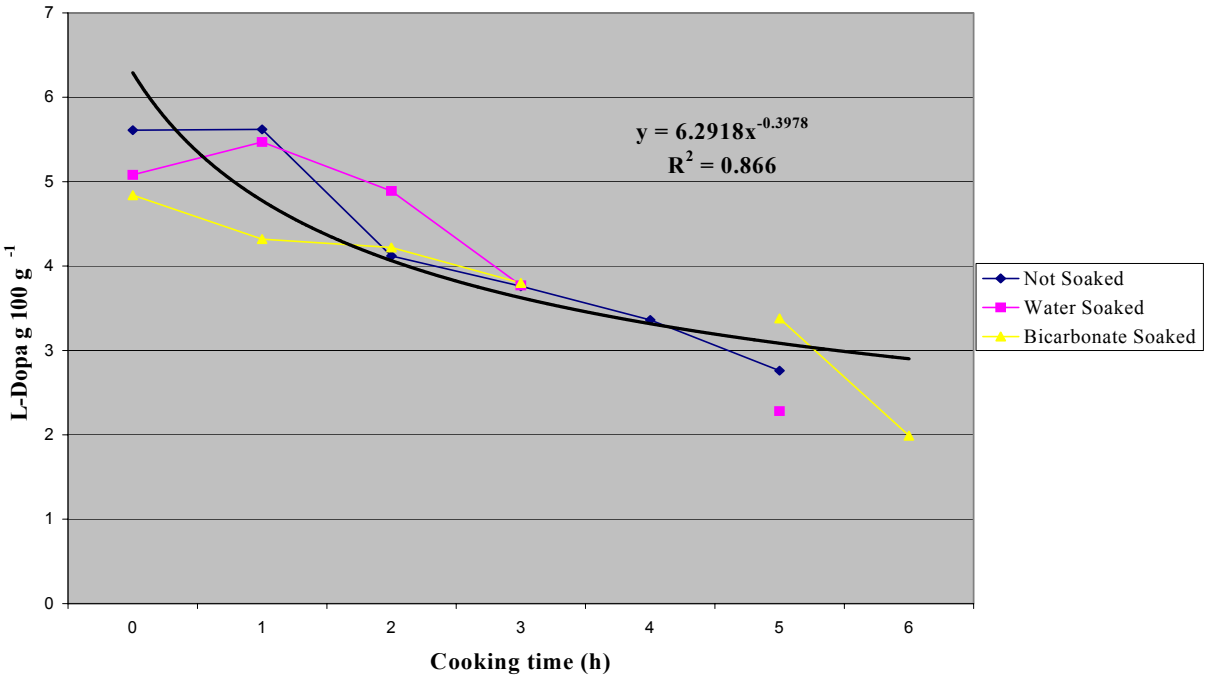

Figure 3. Impact of various cooking times and soaking in water or bicarbonate on L-Dopa content. Sample B-EP was used in the study.

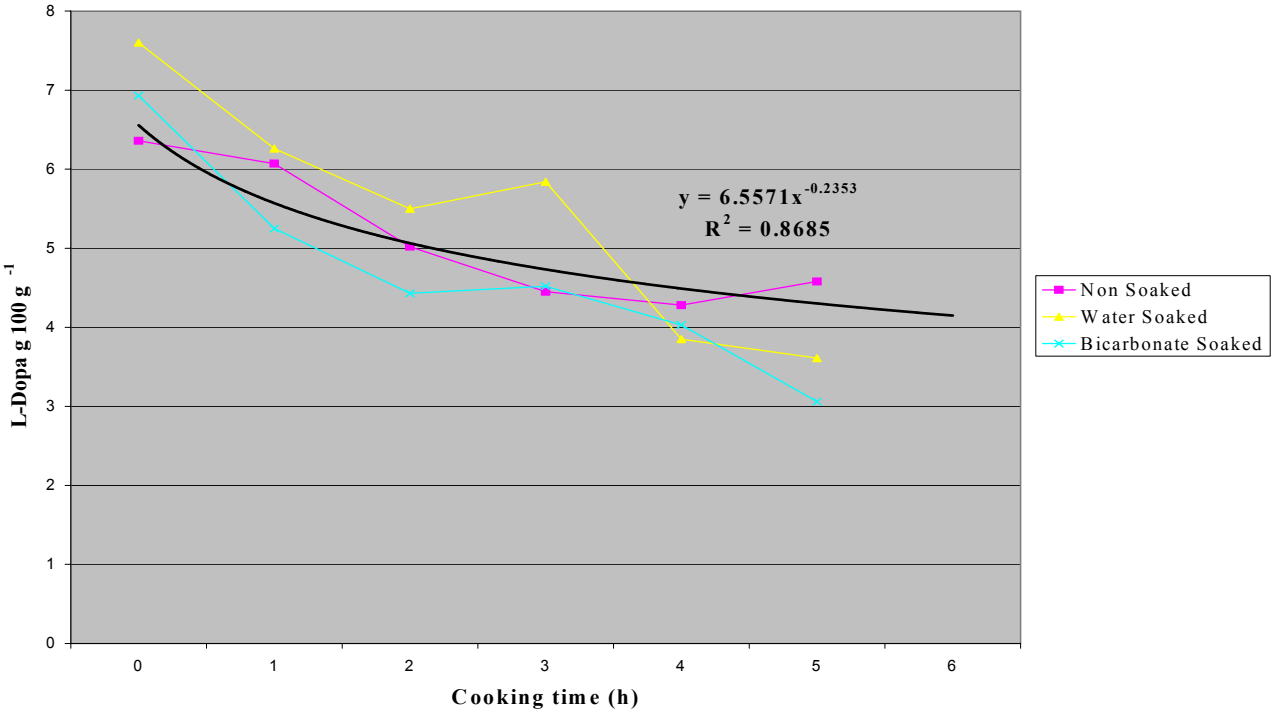

Figure 4. Impact of various cooking times and soaking (in water or bicarbonate) on L-Dopa content. Sample P-LM was used in the study.

Table 10. Effect of cooking time on L-Dopa content in non-soaked and soaked *Mucuna* beans. Values are averages of four replications per cooking time.

Time (h)	Sample W-EP			Sample M-LM		
	Non-Soaked	Water Soaked	Bicarbonate Soaked	Non-Soaked	Water Soaked	Bicarbonate Soaked
	- % -					
0	5.61 ± 0.37	5.08 ± 0.03	4.84 ± 0.20	6.36 ± 0	7.60 ± 0	6.93 ± 0.61
1	---	5.47 ± 0.16	4.32 ± 0.44	n.d.	6.26 ± 0.47	5.25 ± 0.94
2	5.62 ± 0.01	4.89 ± 0.05	4.22 ± 0.12	6.07 ± 0.08	5.50 ± 0.12	4.43 ± 0.37
3	4.12 ± 0.63	3.77 ± 0.41	3.80 ± 0.37	5.02 ± 0.33	5.84 ± 0.19	4.52 ± 0.18
4	3.76 ± 0.04	n.d.	3.38 ± 0.08	4.45 ± 0.24	3.85 ± 0.35	4.03 ± 0.05
5	3.36 ± 0.04	2.28 ± 0.83	1.99 ± 0.61	4.28 ± 0.08	3.61 ± 0.04	3.06 ± 0.47
6	2.76 ± 0.22	n.d.	n.d.	4.58 ± 0.14	n.d.	n.d.
Loss	50.8	55.1	58.9	28.0	52.5	55.8

Table 11. Moisture, protein and L-Dopa content of *Mucuna* beans cooked by atmospheric and pressure cooking after soaking. The results are averages of two replications.

Flour	Moisture (%)	Protein (%)	L-Dopa (g 100 g^{-1})
Raw	3.3	25.6	5.15
48-h soaking	5.6	26.4	6.87
48-h soaking, 3-h cooking at atm. pressure	2.4	31.4	3.92
48-h soaking, 6-h cooking at atm. pressure	2.6	29.5	4.93
48-h soaking, 30 min. cooking at 15 psi	5.7	28.9	5.89
48-h soaking, 45 min. cooking at 15 psi	4.9	29.3	5.78
48-h soaking, 60 min. cooking at 15 psi	4.4	30.2	5.51

Table 12. Protein quality of *Mucuna* beans cooked by atmospheric pressure and by pressure cooking. The values are averages of eight beans per treatment.

Treatment	Wt. gain (g)*	NPR	Digestibility		True Protein
			OM	Apparent Protein	
			- - - - - - - - - - % - - - - - - - - -		
Raw	- 14 ± 2.4	-	89.2 ± 2.4	54.3 ± 7.7	58.7 ± 7.5
48-h soaking	- 15 ± 1.8	-	89.2 ± 2.8	53.3 ± 9.8	57.1 ± 9.6
48-h soaking, 3-h cooking at atm. pressure	18 ± 6.6	1.27 ± 0.39	95.3 ± 1.6	86.9 ± 3.6	89.1 ± 3.4
48-h soaking, 6-h cooking at atm. pressure	12 ± 4.6	0.95 ± 0.33	95.3 ± 0.9	85.3 ± 1.4	87.9 ± 1.3
48-h soaking, 30-min. cooking at 15 psi	12 ± 6.0	0.91 ± 0.38	95.8 ± 0.9	86.1 ± 2.2	88.6 ± 2.2
48-h soaking, 45-min. cooking at 15 psi	10 ± 4.9	0.78 ± 0.31	94.5 ± 1.0	80.1 ± 2.4	82.5 ± 2.4
48-h soaking, 60-min. cooking at 15 psi	8 ± 5.7	0.68 ± 0.42	95.2 ± 1.4	80.1 ± 2.0	83.6 ± 2.0
Casein	50 ± 10.5	2.39 ± 0.34	--	94.8 ± 0.9	96.1 ± 0.9

*Initial Average Wt. 44 g ; NPR = net protein ratio; OM = organic matter.

Rats fed raw *Mucuna* beans and beans soaked for 48 h did not gain weight. On the contrary, they lost more weight (± 5 g) than rats fed with the nitrogen-free diet. Cooked at atmospheric pressure, the 48-h soaked beans gave better rat weight gain when cooked 3 h than when cooked 6 h. Pressure cooking improved animal performance when cooking time was 30 min; however, weight gain and protein quality decreased when the beans were cooked for 60 min. Atmospheric cooking resulted in better weight gain and protein

quality than pressure-cooking. The highest NPR value of 1.27, achieved through a 3-h cooking in atmospheric pressure, was 53-54% of that of casein. The organic matter digestibility of the processed *Mucuna* beans varied between 94.5 and 95.8%, while the raw beans showed an organic matter digestibility of 89%. Protein digestibility was higher for processed as compared to raw beans. Higher protein digestibilities were observed for samples that gave higher NPR values. The intake of L-Dopa varied from 1.2 to 2.3 g during the experimental period (16 d) and were 1.2 (raw), 1.7 (soaked), 1.7 (3-h atm. cooking), 1.8 (6-h atm. cooking), 2.3 (30 min in 15 psi), 2.3 (45 min in 15 psi) and 1.7 (60 min in 15 psi).

There was only a small decrease in L-Dopa due to roasting which apparently was larger for the 0-20 min than for the 0-30 min interval. These results cannot be explained, unless the lower moisture level of 2.3% in the sample contributed to a higher L-Dopa content. In any case the results show the high stability of L-Dopa to temperature. On the other hand, trypsin inhibitor activity decreased significantly upon roasting.

Table 13 summarizes the changes in chemical constituents of *Mucuna* beans with respect to roasting time. As expected, increasing roasting time resulted in a decrease in moisture content, which was responsible for the apparent increase in protein and fat content.

The protein quality of the roasted bean samples is shown in Table 14. Average weight gain of rats improved as roasting time increased from 0 to 30 min; however, weight gain was very low as compared to the rats fed on casein. Intake of 10-, 20-, and 30-min roasted samples was similar and protein quality increased with roasting time from 13 to 37% that of casein. A mortality of 37.5% was observed in the group fed the raw beans. L-Dopa intake was 0.42, 0.91, 1.05 and 1.15 g in the experimental period for the group consuming flour produced by roasting for 0, 10, 20 and 30 min, respectively. It is therefore difficult to explain the results on weight gain, intake, and mortality with the intake of L-Dopa alone. The high level of TI in raw beans is probably responsible for the high mortality in group 1.

Table 15 presents the apparent and true protein digestibility of the roasted *Mucuna* beans. The raw *Mucuna* beans had a low digestibility, both apparent and true, possibly due to the presence of trypsin inhibitors. Roasting induced an increase at 10 min and then a non-significant decrease which was expected, since the roasting process denatures protein and induces non-enzymatic browning. The digestibility of these samples was similar to that of the cooked samples.

Table 13. Moisture, protein and fat content of *Mucuna* beans roasted for different periods. The values are averages of three replications per roasting time.

Roasting time (min)	Moisture	Protein	Fat	L-Dopa	Trypsin inhibitors
	(%)				(TUI mg^{-1})
0	8.0 ± 0.11a	22.1 ± 0.17a	3.8 ± 0.08a	5.31 ± 1.23a	18.90 ± 0.29 a
10	4.5 ± 0.02b	25.8 ± 1.13a	4.2 ± 0.11a	3.79 ± 0.11a	4.70 ± 1.71 b
20	4.2 ± 0.37b	27.4 ± 0.45a	4.1 ± 0.41ab	3.74 ± 0.08a	2.61 ± 1.14 b
30	2.3 ± 0.96c	27.5 ± 0.83b	4.3 ± 0.11b	4.64 ± 0.40a	1.58 ± 1.14 b

Averages followed by a different letter are statistically different (P<0.05).

Table 14. Average weight gain, food intake, net protein ratio (NPR), and mortality of rats fed with *Mucuna* beans roasted for different periods. The values are averages of 8 replications per roasting time.

Roasting time (min)	Weight gain (g/16 d)	Intake (g/16 d)	NPR	Mortality (%)
0	- 10.76 ± 4.20	54.25 ± 20.87	0.57 ± 0.78 c	37.5%
10	- 1.11 ± 6.58	75.13 ± 13.89	0.89 ± 0.64 b	0
20	4.44 ± 9.04	82.70 ± 17.24	1.43 ± 0.99 b	0
30	4.01 ± 2.85	77.60 ± 5.03	1.60 ± 0.38 b	0
Casein	59.43 ± 9.05	154.5 ± 14.86	4.39 ± 0.45 a	0

Averages followed by a different letter are statistically different (P<0.05).

Table 15. Protein digestibility of *Mucuna* beans roasted for different periods. The values are averages of 8 replications per roasting time.

Roasting time (min)	Protein Digestibility (%)	
	Apparent	True
0	61.0 ± 13.0 b	64.6 ± 12.7 c
10	85.8 ± 2.7 a	89.3 ± 3.1 ab
20	84.2 ± 5.1 a	87.2 ± 5.8 b
30	84.9 ± 4.9 a	88.1 ± 5.1 ab
Casein	96.5 ± 0.7 a	100.1 ± 0.77 a

Averages followed by a different letter are statistically different (P<0.05).

In the experiments on germination and malting, the degree of germination of the individual grains was not the same possibly due to different rates of water absorption. Therefore, the samples used for the analysis were not completely homogeneous. Table 16 shows the moisture, protein and fat contents of samples germinated up to six days. The moisture content was similar from 0 to 6 days since all samples were subjected to drying after the germination treatment. There was a slight apparent increase in protein from 27.44 to 28.92% but the difference was not statistically significant. Fat content increased with germination time from 2.89% to 5.83% at 6 days. The germinated samples were then malted as indicated above. The data on chemical analyses are shown in Table 17. There was a slight reduction in moisture content and an apparent small, but non-significant increase in protein content. Fat content was higher in the malted samples.

The changes in L-Dopa and TI upon germination are shown in Table 16. There was a small decrease in L-Dopa content, from 5.11 ± 1.23 to 4.37 ± 0.47%, but it was not significant. These data suggest that enzymes are not developed upon germination to metabolize L-Dopa as is the case for phytase on phytic acid. Trypsin inhibitors significantly decreased from 15.19 ± 2.63 to 2.09 ± 0.06 TUI mg^{-1}.

Table 17 shows the L-Dopa and TI content in malted *Mucuna* beans. The L-Dopa content of the malted samples was no different than that of samples that had been only germinated. On the other hand, TI decreased to the same extent due to malting. There was a small increase in protein and fat content with germination and malting. These two processes decreased TI activity significantly, but induced only a small, non-significant reduction of L-Dopa content.

The 3- and 6-d germinated seed and the 3-d germinated and malted seed were tested for protein quality using the NPR method (Table 18). Animals fed germinated *Mucuna* beans performed very poorly, although some improvement in weight change was observed with germinated beans. The protein quality of the 6-d germinated sample was 14% of the casein value while the NPR of the 3-day germinated and malted sample showed a value that was 17% that of casein; these are both very low. On the other hand, digestibility tended to improve with processing. These studies showed little beneficial effect of germination in improving the protein quality of *Mucuna* beans even though TI decreased (but not L-Dopa). It is important to note that germination does not necessarily improve the protein quality of germinated products. The L-Dopa intake varied from 1.12 g with raw beans to 0.89 (3-d germination), 1.02 (6-d germination) and 1.09 g (3-d germination with malting) for the 16-d experimental period.

Taking advantage of the collection of fecal matter to study protein digestibility, the feces were analyzed for L-Dopa content and compared with L-Dopa intake. The results on roasted and germinated *Mucuna* samples are presented in Table 19.

Table 16. Moisture, protein, fat, L-Dopa and trypsin inhibitor content of *Mucuna* beans germinated for 0, 2, 4, and 6 days. The values are averages of three replications.

Germination time (d)	Moisture	Protein	Fat	L-Dopa	Trypsin inhibitors
	- - - - - - - - - - - - - - - - - % - - - - - - - - - - - - - - - - -				(TUI mg^{-1})
Raw	-	-	-	5.11±1.23a	15.19±2.63a
0	6.27± 0.92a	27.44±0.41a	2.84±1.83b	4.76±0.49a	12.11±1.15b
2	5.08±0.61a	28.42±1.24a	3.66±0.76b	4.57±0.16a	4.90±0.50c
4	6.61± 2.53a	28.11±2.72a	3.91±1.52ab	4.42±0.33a	4.39±0.26c
6	6.45±0 .84a	28.92±2.45a	5.83±0.49a	4.37±0.47a	2.09±0.06d

Note: Raw samples refer to unprocessed samples. Samples at "0 germination time" were surface sterilized and then soaked for 12 h.
Averages followed by a different letter are statistically different (P<0.05).

Table 17. Moisture, protein, fat, L-Dopa and trypsin inhibitor contents of *Mucuna* beans germinated and malted for 2, 4, and 6 days. The values are averages of three replications.

Germination time (d)	Moisture	Protein	Fat	L-Dopa	Trypsin inhibitors (TUI mg^{-1})
	- % -				
2	4.64 ± 0.37 a	27.07 ± 3.71 a	4.82 ± 0.18 ab	4.66±0.45a	1.88±0.38a
4	4.02 ± 0.98 a	27.59 ± 0.62 a	5.27 ± 0.27 ab	4.44±0.42a	1.74±0.33a
6	4.95 ± 0.61 a	28.57 ± 2.74 a	5.68 ± 0.59 a	4.41±0.80a	0.82±0.14b

Averages followed by a different letter are statistically different (P<0.05).

Table 18. Weight gain, intake, net protein ratio, mortality, and protein digestibility of germinated and malted *Mucuna* beans. The values are averages of 8 replications.

Sample	Weight change (g)	Intake (g)	NPR	Mortality (%)	Protein Digestibility (%)	
					Apparent	True
Raw	- 11 ± 4.16b	68.4 ± 9.5ab	-	37.5	61.0 ± 13.0 b	64.6 ± 12.6 b
3-d germinated	- 9 ± 33b	61.1 ± 16.8b	-	12.5	65.5 ± 4.9 ab	69.3 ± 4.4 ab
6-d germinated	- 4 ± 1.5a	73.3 ± 5.1ab	0.61 ± 0.22a	0	65.9 ± 3.4 ab	69.3 ± 3.2 ab
3-d germinated and malted	- 2 ± 3.8a	77.0 ± 6.5a	0.76 ± 0.96a	0	72.0 ± 3.0 a	75.2 ± 2.9 a
Casein	59.4 ± 9.0	154.5 ± 14.9	4.39 ± 0.45	0	96.5 ± 0.7	100.1 ± 0.77 a

Note: weight gain and intake are for the full experimental period (16 days).
Averages followed by a different letter are statistically different (P<0.05).

Table 19. L-Dopa ingested by rats, found in feces, and absorbed. The values are averages of 8 replications per processing method.

Processing method	Ingested (g)	In feces (g)	Absorbed (%)
Roasted 0 min.	0.76 ± 0.17	0.006 ± 0.001	98.7
Roasted 10 min.	0.56 ± 0.15	0.010 ± 0.008	98.2
Roasted 20 min.	0.47 ± 0.12	0.013 ± 0.005	97.9
Roasted 30 min.	0.63 ± 0.08	0.014 ± 0.004	98.4
Raw	1.41 ± 0.28	0.03 ± 0.36	97.9
Germinated 3 d	1.59 ± 0.67	0.04 ± 0.61	97.5
Germinated 6 d	2.04 ± 0.18	0.06 ± 0.56	97.0
Germinated 3 d and malted	2.57 ± 0.19	0.09 ± 0.37	96.5

The results from both experiments with roasted grain and with germinated grain showed very small amounts of L-Dopa in rat feces which indicates that it is absorbed by the animal. This is also implied by the relatively high protein digestibility found for *Mucuna* protein.

As is well known, *Mucuna* beans are consumed as an infusion made from roasted grain. The grain that has been roasted for up to 30 min was used to make an infusion by adding boiling water to the roasted flour. Both the infusion and the flour were analyzed for L-Dopa and it was found that L-Dopa could be extracted almost completely from the roasted flour as shown in Table 20.

These data show that the infusions of roasted *Mucuna* beans contain practically all the L-Dopa, and is therefore not a recommended drink for healthy people. On the other hand roasting could be an interesting approach to eliminating L-Dopa from *Mucuna* beans. However, boiling of raw *Mucuna* beans as flour may also results in a flour with no L-Dopa content.

Table 20. L-Dopa concentration in infusion made from roasted *Mucuna* flour. The values are averages of three replications per roasting time.

Roasting time (min)	L-Dopa in 100 ml infusion (mg)	L-Dopa in g of flour (mg)	Extraction (%)
0	32 ± 5.4	31.3 ± 5.9	102.2
10	41 ± 9.0	39.8 ± 1.6	103.0
20	44 ± 7.0	41.9 ± 5.4	105.0
30	49 ± 2.2	52.0 ± 3.8	94.2

The three biological trials discussed above were conducted with a total of 168 weanling, 22-d old white rats, with an average initial weight of 44 g. On the basis of diet consumed, on the amount of *Mucuna* flour incorporated in the diet, and the L-Dopa content of the bean flour, L-Dopa intake was calculated. This was then expressed as mg of L-Dopa ingested daily and also expressed on a per kg body weight of the rats. In the first trial, the daily L-Dopa average intake was 121 mg (range 75-166 mg) or 2450 mg kg^{-1} body wt (2083-3667 mg kg^{-1}); in the second trial with roasted *Mucuna* bean flour, daily L-Dopa intake averaged 74 mg (69-82 mg) and total average intake was 1941 mg kg^{-1} (range 1605-2242 mg kg^{-1}). In the third study with germinated beans daily L-Dopa intake averaged 119 mg (88-161 mg) or 3126 mg kg^{-1} (2667-3833 mg kg^{-1}). No relationship between weight and L-Dopa intake was suggested by the data and all values of intake fell below the 50% lethal dose of 4000 mg kg^{-1} body wt indicated by Budavaris *et al.* (1989). The daily intakes were also below initial doses given to humans with Parkinson's disease of 250 mg day^{-1}. Rat mortality was associated with a high trypsin inhibitor activity rather than with L-Dopa content, which was not affected by the various processing methods applied. On the other hand, weight change differences for the other treatments were probably more related to changes in amino acid availability, mainly lysine, which is known to be reduced by excessive moist or dry heat processing.

CONCLUSIONS

The eating quality of *Mucuna* beans consists of a number of factors, including rapid water absorption, shorter cooking time and better texture and low levels of L-Dopa. This study showed that soaking *Mucuna* beans at a high temperature with repeated removal of the soaking water is a processing method that is of benefit to its eating quality, especially if the soaking water contains bicarbonate (which also reduces cooking time). Cooking of soaked beans for different times at atmospheric and under pressure also improved its protein quality, particularly when cooking was done at atmospheric pressure for 3 h. The content of trypsin inhibitors, but not L-Dopa, decreased at longer times or under pressure. Roasting for up to 30 minutes also improved bean protein quality but not as much as the improvement due to atmospheric pressure cooking for 3 h. This process also destroyed almost all trypsin inhibitors but little change was observed in L-Dopa content. Germination and malting were processes not effective in improving the nutritional quality of *Mucuna* beans, even though some decrease in trypsin inhibitors took place; again, no major change occurred with L-Dopa. Germination did not induce the enzymatic system to metabolize L-Dopa. Organic matter digestibility of the raw beans was low, but it improved with processing. L-Dopa is quite resistant to thermal processing conditions; it is not protein-bound and its removal can be accomplished by hot water extraction with a reduced particle size. The mortality of the rats and their performance with diets of processed *Mucuna* beans flour was more associated with trypsin inhibitor activity and processing effects than with L-Dopa content.

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