Brazilian *Mucuna pruriens* Seeds (Velvet Bean) Lack Hemagglutinating Activity

Adive B. I. Udedibie* and Celia R. Carlini

Department of Biochemistry-ICB, Federal University of Rio de Janeiro, CEP 21, 941-590 Rio de Janeiro, Brazil

The mature dry seeds of *Mucuna pruriens* (velvet bean) from Brazil were analyzed for two major antinutritional factors, antitryptic and hemagglunating activities, to determine their potential as human food or animal feed. The raw seeds contained 321.5 g kg^{-1} crude proteins and 11864.8 trypsin inhibitor units (TIU) (g of seed)$^{-1}$, but the protein extract failed to agglutinate any of the three types of human erythrocytes (A, B, and O) or rabbit or pig erythrocytes. Ordinary cooking (boiling) of the seeds for an hour at 96 °C, pressure cooking for 20 min, or ordinary cooking for 30 min after soaking in water for 48 h completely inactivated the antitryptic activities.

Keywords: *Brazilian Mucuna pruriens seeds; hemagglutinating and antitryptic activities*

INTRODUCTION

Most developing tropical countries have depended on soybean and peanut meals as key conventional protein concentrates for feeding livestock. The heavy demand for these items has given rise to a disproportionate increase in their prices and, consequently, in the costs of livestock feeds. This invariably has escalated the prices of animal products out of reach of the common man. There is need therefore for identification and exploitation of other novel legumes which fortunately are in abundance in the region.

Mucuna pruriens (L) DC. var. *utilis* (Wight) Burck, commonly known as velvet bean, is a highly productive black-seeded tropical legume that is little known and utilized as human food or animal feed. In Brazil, it is valuable only as a green manure/cover crop. The seeds of mature, unripe, or young pods of *M. pruriens* are, however, soaked in water and boiled/roasted and eaten as such or mixed with salt and eaten by the poor Northeast Indian tribes (Arora, 1981). Some Indian tribes also consume the seeds for increased potency, and the hairs of the pods are used as vermifuge (Vasudeva and Shanpru, 1981).

The use of legume grains as human food or animal feed is limited by their relatively high concentrations of antinutritional factors (Liener, 1994). *M. pruriens* from India has been reported to contain trypsin inhibitors, phytates, cyanogenic glycosides, tannins, and L-3,4 dehydroxyphenylalanine (L-DOPA) (Ravindran and Ravindran, 1988; Josephine and Janardhanan, 1992; Vijayakumari, 1994). Josephine and Janardhanan (1992), however, observed that, except for L-DOPA, all of the antinutritional factors detected in the seeds were heat-labile and hence could be eliminated by cooking. Recently, Siddhuraju et al. (1996), reporting from the same laboratory, observed that Indian *M. pruriens* seeds exhibit hemagglutinating activity. In other words, they contain lectins.

Lectins (e.g., PHAs and Con A) are one of the most important toxic and antinutritional factors in pulses. They are carbohydrate-binding proteins that are resistant to digestion when ingested (Pusztai, 1989). In addition to their ability to agglutinate the erythrocytes of numerous animal species, clump certain bacteria, and precipitate glycogen and starch from solutions, lectins negatively affect nutrient utilization by different mechanisms. They bind to the glycoproteins and glycolipids of the digestive tract mucosa (Hague, 1975; Jaffe, 1980), causing severe morphological changes in the mucosa and interfering with nutrient absorption (Liener, 1994). They induce severe reduction in feed intake (Larue-Achagiotis et al., 1992) and have even been implicated in the pathogenesis of coeliac disease (Kolberg and Sollid, 1985).

The concentrations of toxic and antinutritional factors in plants are known to be greatly influenced by climatic and ecological conditions. Recent studies by Udedibie and Carlini (1996, unpublished data) showed marked differences between *Canavalia* seeds from Brazil and Nigeria in contents of toxic and antinutritional factors. The Brazilian *M. pruriens* seeds have, however, not been subjected to laboratory tests for antinutritional factors.

This paper reports the results of a preliminary study carried out to determine the lectin and trypsin inhibitor contents of *M. pruriens* seeds from Brazil and the effect of various heat treatments on their hemagglutinating and antitryptic activities, if any.

MATERIALS AND METHODS

The *Mucuna* seeds used for the study were obtained from Agrodora in São Paulo, Brazil, a store that specializes in agricultural products.

Seed Processing. Raw seeds of *M. pruriens* were ground using a 2 mm screen. Part of the meal was stored raw. The other part was heated (with stirring) in the oven at 120 °C until it turned from whitish to light brownish color and crispy to the touch. The toasted meal was left to cool and then stored in a plastic bottle. Some of the seeds were cooked at 96 °C for 30, 60, 90, or 120 min, respectively. The cooked seeds were first dried in the sun for 2 days before oven drying for 15 min at 100 °C and ground as above and stored. Some were cooked

* Author to whom correspondence should be addressed. Permanent address: Department of Animal Production, Federal University of Technology, P.M.B. 1526, Owerri, Nigeria.

S0021-8561(97)00634-1 CCC: $15.00
Published on Web 03/19/1998

in a pressure cooker (*panela de pressão*) for 10, 20, or 30 min, respectively, dried, ground as above, and stored. Some were soaked in water for 24 or 48 h and then cooked for 30, 60, 90, or 120 min, respectively. The cooked seeds were further processed as above.

The crude protein content of all the samples was determined in duplicate with the micro-Kjeldhal method according to the AOAC (1995).

Protein extraction was done by stirring 10 g of sample in 50 mL of phosphate-buffered saline (PBS, pH 7.00) for 2 h at 4 °C. Thereafter, the mixture was centrifuged (2000*g*) and the supernatant dialyzed for 20 h, also at 4 °C. Protein concentration was determined spectrophotometrically by its absorbance at 280 nm.

Hemagglutinating Activity. Lectin content was determined by hemagglutination of human, rabbit, and pig fresh erythrocytes according to the method of Coffey et al. (1985) with some modifications. Human blood (A, O, and B groups), rabbit blood, and pig blood were respectively collected in 5 mM EDTA and centrifuged to remove soluble blood constituents. The suspended erythrocytes were then washed three times with PBS. Twenty-five microliters of the protein samples in duplicate were serially (2-fold) diluted in PBS in 96-microwell plates, and 25 μL of a 2% v/v erythrocyte suspension ($\sim 10^6$ cells) was then added to each well. Hemagglutination titers were examined after 2 h at room temperature.

Trypsin Inhibitor Activity. Trypsin inhibitor activity was determined according to the method of Erlanger et al. (1961) with some modifications. To measure 100% trypsin activity, 20 μL of 1 mg/mL trypsin was mixed with 100 μL of 0.1 mM of Tris buffer (pH 8.5) and made up to 450 μL with distilled water. This was then mixed with 50 μL of 4 mM L-BAPNA (benzyol-L-arginyl *p*-nitroanilide), and the rate of liberation (per minute) of *p*-nitroaniline from L-BAPNA by trypsin was determined with a spectrophotometer at 405 nm absorbance. Trypsin inhibitor activity was measured by mixing 100 μL of the buffer, 20 μg in 20 μL of trypsin, 5−20 μL of protein extract, and corresponding volumes of distilled water so as to inhibit up to 60−70% of the trypsin activity. The mixture was then allowed to incubate for 15 min before 50 μL of L-BAPNA was added. The rate of liberation of *p*-nitroaniline was determined as earlier stated. One unit of trypsin inhibitor (TIU) was the amount of material [microunits per milligram of protein] inhibiting 1 mg of trypsin under the experimental condition.

RESULTS AND DISCUSSION

The data on crude protein content and hemagglutinating and antitryptic activities of the variously processed *M. pruriens* seeds are presented in Table 1. The crude protein values are in agreement with the values in the literature. Raw *M. pruriens* seeds from Nigeria have been reported to contain 303.3 g kg^{-1} (Emenalom, 1996) and seeds from India, 314.4 g kg^{-1} (Siddhuraju et al., 1996). Cooking tended to reduce the crude protein contents due to solubilization of some nitrogenous compounds during cooking. The proteins of the *Mucuna* seeds appeared to be extremely soluble as indicated by the values of extractable fractions (141.4−242.3 mg/mL). Similar treatment of other legume seeds (*Canavalia ensiformis*, *Glycine max*, *Vigna unguiculata*, *Vicia faba*, *Phaseolus vulgaris*, and *Phaseolus angularis*) resulted in extraction of 26−78 mg of proteins/mL (Udedibie and Carlini, 1997, unpublished data).

The proteins of the seeds failed to agglutinate any of the three types of human erythrocytes. This treatment was repeated along with the proteins (Con A) of *C. ensiformis* as the positive control. The three types of human erythrocytes were agglutinated by *C. ensiformis* but not by *M. pruriens*. In view of the fact that strains and cultivars of a species may differ in their specificity toward the erythrocytes of various species of animals and blood groups (Liener, 1986), we decided to try rabbit blood. There was no hemagglutination. There was also no hemagglutination when pig blood was used. The *Mucuna* seeds from India agglutinated the erythrocytes of the three human blood groupings (Siddhuraju et al., 1996).

Table 1. Effects of Processing Methods on Crude Protein Content and Hemagglutinating and Antitryptic Activities of *M. pruriens* Seeds from Brazil

sample	crude protein (g/kg)	extr protein (mg/mL)	hemaggl activity (HU/g)	TIU (g^{-1} of seed)	recov (%)[a]
raw seed	321.5	172.0	NH[b]	11864.8	100.0
toasted seed	334.2	87.9	NH	6979.3	58.8
cooked 30 min	298.4	196.7	NH	746.6	6.3
cokked 60 min	293.0	228.8	NH	NI[c]	0.0
cooked 90 min	287.2	197.1	NH	NI	0.0
cooked 120 min	286.3	242.3	NH	NI	0.0
pressure cooked 10 min	303.5	161.2	NH	159.8	1.3
pressure cooked 20 min	300.6	190.4	NH	NI	0.0
pressure cooked 30 min	293.3	192.6	NH	NI	0.0
soaked in water 24 h					
cooked 30 min	310.1	147.2	NH	138.4	1.2
cooked 60 min	299.0	152.9	NH	NI	0.0
cooked 90 min	287.5	158.6	NH	NI	0.0
cooked 120 min	284.5	141.4	NH	NI	0.0
soaked in water 48 h					
cooked 30 min	304.3	163.5	NH	NI	0.0
cooked 60 min	288.7	196.3	NH	NI	0.0
cooked 90 min	287.2	191.3	NH	NI	0.0
cooked 120 min	283.1	189.9	NH	NI	0.0

[a] Percent of TIU left in the sample after treatments. [b] No hemagglutination. [c] No inhibition.

The raw *Mucuna* seeds exhibited an extremely high concentration of trypsin inhibitors (11 865 TIU g^{-1} of seed). This is >6 times the value for *C. ensiformis* (1864 TIU g^{-1} of seed) obtained under the same experimental condition. The trypsin inhibitors were, however, completely inactivated when the seeds were subjected to 1 h of cooking at 96 °C. The inhibitors were also completely eliminated in 30 min of cooking following 48 h of soaking in water. Toasting of the seeds appeared to be highly inefficient as a method of eliminating the inhibitors. Only ~42% of the inhibitors could be inactivated by the treatment. Similar observations had been made on other legume seeds (Babar et al., 1988; Bressani and Sosa, 1990; Udedibie et al., 1994).

Much less is known about the feeding value of *Mucuna* beans. Dietary raw *Mucuna* seeds have been reported to reduce the growth rate of broiler chicks and the egg production of laying hens (Harms et al., 1961; Afolabi et al., 1985; Olaboro et al., 1990). Recent trials in Nigeria (Emenalom, 1996) have also shown that broiler chicks could not tolerate >10% dietary level of toasted or cooked *Mucuna* seeds, although birds on cooked beans tended to perform better than those on toasted beans.

Some authors have tried to blame the toxicity of the *Mucana* seeds on L-DOPA (Pieris et al., 1980; Afolabi et al., 1985; Josephine and Janardhanan, 1992). That assumption does not seem to have strong scientific basis. L-DOPA has recently gained a prominent place in the treatment of Parkinsonism. If L-DOPA is actually responsible for the poor performance of these animals, similar effects are likely to occur in patients of Parkinsonism who consume L-DOPA on a regular basis. L-DOPA has also been shown to be toxic only in individuals with glucose-6-phosphate dehydrogenase (G-6-PD) deficiency in their erythrocytes (Nechama and

Edward, 1967). Recent studies in India (Siddhuraju et al., 1996) have shown that the Indian *Mucuna* seeds contain lectins, which could not be completely eliminated by autoclaving for 15 min. It appears therefore that the proponents of the L-DOPA theory seemed to have ignored the strong toxic and antinutritional activities of lectins on nonruminants, particularly under an ad libitum feeding system (Jayne-Williams, 1973; Hague, 1972; Jaffe, 1980; Pusztai, 1989; Larue-Achagiotis et al., 1992; Liener, 1994). The poor performance of the experimental birds on raw or insufficiently cooked seeds is most likely to be due to additive or synergistic effects of lectin and protease inhibitor activities.

CONCLUSION

The high crude protein content and the absence of hemagglutinating activity of the Brazilian *M. pruriens* seeds are nutritional factors indicative of strong potential of the strain as protein supplement in human foods or livestock feeds and call for further investigation in that direction. The introduction of the strain in other similar agroecological areas is also worthy of trial and is therefore strongly recommended.

LITERATURE CITED

Afolabi, O. A.; Oshuntogun, B. A.; Adewusi, S. R.; Fapojuwo, O. O.; Ayorinde, F. O.; Grissom, F. E.; Oke, O. L. Preliminary nutritional and chemical evaluation of raw seeds from *Mucana solanei*. *J. Agric. Food Chem.* **1985**, *33*, 122−124.

AOAC. *Official Methods of Analysis*, 16th ed.; Association of Official Analytical Chemists: Washington, DC, 1995.

Arora, R. K. Native food plants of the North-eastern Tribals. In *Glimpses of Indian Enthonobotany*; Jain, K. S., Ed.; Oxford and IBH Publishing: New Delhi, India, 1981.

Babar, V. S.; Chava, J. K.; Kadam, S. S. Effects of heat treatments and germination on trypsin inhibitor activity and polyphenols in jackbean (*C. ensiformis*). *Plant Food Hum. Nutr.* **1988**, *38*, 319−324.

Bressani, R.; Sosa, J. L. Effects of processing on the nutritive value of *Canavalia* jackbean (*C. ensiformis*). *Plant Food Hum. Nutr.* **1990**, *40*, 207−214.

Coffey, D. G.; Uebersax, M. A.; Hosfield, G. L.; Brunner, J. R. Evaluation of hemagglutinating activity of low-temperature cooked kidney beans. *J. Food Sci.* **1985**, *50*, 78−81.

Emenalom, O. O. Determination of the value of *Mucuna pruriens* (velvet bean) as feed ingredient in poultry diets. MSc. Thesis, Federal University of Technolgy, Owerri, Nigeria, 1996.

Erlanger, B. F.; Kokowsky, N.; Cohen, W. The preparation and properties of two new chromogenic substrates of trypsin. *Arch. Biochem. Biophys.* **1961**, *95*, 271−278.

Hague, D. Studies of storage proteins of higher plants. 1. Concanavalin A from three species of the genus *Canavalia*. *Plant Physiol.* **1975**, *55*, 636−642.

Harms, R. I.; Simpson, C. F.; Waldroup, P. W. Influence of feeding various levels of velvet beans to chicks and laying hens. *J. Nutr.* **1961**, *75*, 127−134.

Jaffe, W. G. Hemagglutinins (lectins). In *Toxic Constituents of Plant Foodstuffs*; Liener et al., Eds.; Academic Press: New York, 1980; pp 525−552.

Jayaweera, D. M. A. *Medical Plants Used in Ceylon*; National Science Council of Sri-Lanka: Colombo, 1981.

Jayne-Williams, D. J. The influence of dietary jackbean (*C. ensiformis*) and concanavalin A on the growth of conventional and gnotobiotic Japanese quail. *Nature* **1973**, *243*, 150−151.

Josephine, R. M.; Janardhanan, K. Studies on the chemical composition and antinutritional factors in three germplasm seed materials of the tribal pulse, *Mucuna pruriens* (L) DC. *Food Chem.* **1992**, *43*, 13−18.

Kolberg, J.; Sollid, L. Lectin activity of gluten identified as wheat germ agglutinin. *Biochem. Biophys. Res. Commun.* **1985**, *130*, 867−872.

Larue-Achagiotis, C.; Pichard, M.; Louis-Sylvestre, J. Feeding behaviour of rats on a complete diet containing concanavalin A. *Reprod. Nutr. Dev.* **1992**, *32*, 343−350.

Liener, I. E. Nutritional significance of lectins in the diet. In *The Lectins*; Liener et al., Eds.; Academic Press: New York, 1986; pp 525−552.

Liener, I. E. Implications of antinutritional components in soybean foods. *Crit. Rev. Food Sci. Nutr.* **1994**, *34*, 31−67.

Nechama, S. I.; Edward, R. K. Does 3,4-dihydroxyphenylalanine play a part in favism? *Nature* **1967**, *215*, 285−286.

Olaboro, G.; Okot, M. W.; Mugerwa, J. S.; Latshawa, J. D. Growth-depressing factors in velvet beans fed to broiler chicks. *E. Afr. Agric. For. J.* **1991**, *57*, 103−110.

Pieris, N.; Jansz, E. R.; Dharmadasa, H. M. Studies on *Mucuna* species of Sri-Lanka. *Agriculturist (Colombo)* **1980**, *107*, 15−20.

Pusztai, A. Biological effects of dietary lectins. In *Recent Advances of Research in Antinutritional Factors in Legume Seeds*; Huismay et al., Eds.; Pudoc: Wageningen, The Netherlands, 1989; pp 17−29.

Ravindran, V.; Ravindran, G. Nutritional and antinutritional characteristics of *Mucuna (Mucuna utilis*) bean seeds. *J. Sci. Food Agric.* **1988**, *46*, 71−79.

Siddhuraju, P.; Vijayakumari, K.; Janardhanan, K. Chemical composition and protein quality of the little known legume, velvet bean (*Mucuna pruriens* (L) DC.). *J. Agric. Food Chem.* **1996**, *44*, 2636−2641.

Udedibie, A. B. I.; Esonu, B. O.; Obaji, C. N.; Durunna, C. S. Dry urea treatment as a method of improving the nutritive value of the jackbean (*C. ensiformis*) for broilers. *Anim. Food Sci. Technol.* **1994**, *48*, 335−345.

Vasudeva, R.; Shanpru, M. K. Some plants in the life of the Garos of Meghalaya. In *Glimpses of Indian Enthnobotany*; Jain, S. K., Ed.; Oxford and IBH Publishing: New Delhi, India, 1981; pp 153−160.

Vijayakumari, K. Studies on the antinutritional factors in tribal pulses. Ph.D. Thesis, Bharathiar University, Coimbatore, India, 1994.

Received for review July 21, 1997. Revised manuscript received December 29, 1997. Accepted December 30, 1997. We are grateful to CNPQ (Conselho Nacional de Desenvolvimento Cientifico e Technologico, Brasilia, Brasil) and FINEP (Financiadora de Estudo e Projeto, Rio de Janeiro, Brazil) for financial support of this work and for CNPQ sponsorship of one year sabbatical leave of the first author in Brazil.

JF970634Y