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The Effects of Processing on the Proximate and Phytochemical Compositions of *Mucuna pruriens* Seeds (Velvet Beans)

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Abstract: The effects of processing on the proximate and phytochemical compositions of Mucuna pruriens seed extract were investigated. The proximate analysis of the hulled seeds revealed the following nutrients and antinutrients: Crude lipid (2.69%), crude fiber (3.86%), crude protein (28.23%), ash (5.26%), carbohydrate (60.03%) and moisture content (12.32%). The boiled or cooked samples exhibited the following results: Crude lipid (2.51%), crude fiber (3.83%), protein (22.17%), ash (4.24%), carbohydrate (67.92%) and moisture content (12.32%). After soaking prior to boiling or cooking at 98°C for one hour, the results were: Crude lipid (2.51%), crude fiber (3.81%), protein (22.05%), ash (3.10%), carbohydrate (68.52%) and moisture content (11.92%). Quantitative phytochemical analysis of the raw seed extract indicated: Flavonoids (0.42%), alkaloids (1.07%), saponins (0.47%), tannins (0.28%), hydrogen cyanide (12.69 mg/kg), phenol (2.82%) and phytate (0.43%). The concentrations of the phytochemicals in the boiled samples were as follows: Flavonoids (0.35%), alkaloids (0.88%), saponins (0.39%), tannins (0.09%), hydrogen cyanide (6.59 mg/kg), phenols (0.33%) and phytate (0.24%). The soaked and boiled samples showed the following result: Flavonoids (0.31%), alkaloids (0.88%), saponins (0.37%), tannins (0.08%), hydrogen cyanide (5.54 mg/kg), phenols (0.32%) and phytate (0.22%). The effects of processing involving soaking, soaking and boiling, have remarkable detoxification effect, anti-nutrient and nutrient composition reduction on Mucuna pruriens seeds. The most highly affected anti-nutrient and toxicant in the seed is hydrogen cyanide, which showed a reduction from 12.67 mg/kg for the raw sample to 5.54 mg/kg for the soaked and boiled samples; a reduction of 56.34%. Another anti-nutrient, phytate was reduced by more than 48.84%; phenols from 2.83% to 0.32%, a reduction of 88.69%; we therefore conclude that the effects of processing vis a vis the methods employed. have significant effects in the reduction of the concentrations of the anti-nutrients and toxicants present in the seeds of this under-utilized legume. We recommend that these novel processes be employed by individuals, farmers and food processing companies wishing to include this all important legume as part of family dietary as well as in poultry, animal feeds or in the formulation of diets for the undernourished and low income groups.

Key words: Proximate analysis, phytochemicals, anti-nutrients Mucuna pruriens, effect of processing

INTRODUCTION

Bridging the gap between the teeming population and food production is one of the most important tasks of developing countries. Most developing tropical countries have depended on major conventional legumes and animal based sources as key protein concentrates for livestock feeding as well as for human nutrition. The major conventional legumes are soybean (Glycine max.) and groundnut or peanut (Arachis hypogea). The demand for these items has given rise to a disproportionate increase in their prices and consequently on the cost of livestock and feeds. Protein-Energy Malnutrition (PEM) has therefore been recognized as the most common form of malnutrition in regions where people depend on starch based diets for survival (FAO, 1994; Pellelier, 1994; Michaelson and Henrik, 1998). There is therefore, the need for identification and exploitation of other novel legumes, which fortunately are in abundance in these regions to fulfill the growing need of plant based proteins and under utilized legumes as inexpensive and good sources of protein than the conventional sources of protein (Chel-Guerrero et al., 2002; Krause et al., 1996; Siddhuragu et al., 2000). Mucuna pruriens commonly known as velvet beans in Nigeria, Australia, South Africa, Brazil, USA, Pica in Venezuala and Bengal beans in India, is a highly productive black seeded tropical legume that is obscurely known and utilized as human and animal feed. In many tropical countries, it is valuable only as green manure or cover crop. The presence of some anti-nutritional factors in the raw seeds has limited its use in non-ruminant animals. Mucuna prureins has been reported to contain trypsin inhibitors. phytate, cyanogenic glycosides, tannins and L-3,4dihydroxyphenylalanine (L-DOPA) (Marv and Jonardhanama, 1992; Udedibie and Carlini, 1998). However, the concentrations of the toxic and anti-nutrient factors in the plant are known to be influenced by climatic and ecological conditions. A study conducted by Udedibie and Carlini (1998), has shown that Brazilian Mucuna pruriens lack haemagglutinating activity contrary to the report that the Indian variety contain lectins. In Nigeria, Mucuna pruriens seeds have however attracted little attention as a potential source of protein and energy. Mucuna pruriens belong to the leguminous family, Fabaceace, genus Mucuna and specie Pruriens. The legume family (Fabaceae) is the third largest among flowering plants consisting of approximately 650 genera and 20,000 species (Doyle, 1994). Many of the legumes possess multiple uses such as food, fodder and pharmaceuticals. Some seeds of legumes contain anticancerous compounds that retard the growth of cancer cells. For example, the alkaloid "genistein" derived from Kudzu, retards or arrests the growth of cancer cells. Four Indian accessions of Mucuna consists of high amount of crude protein of 20.2-29.6% (Vedivet and Janardhan, 2000), Ezeagu et al. (2003) reported the concentration of carbohydrates in the range of 55.20-64.88 g/100 g, having the highest concentration. Legumes constitute a rich source of minerals particularly potassium, magnesium, iron, zinc and calcium. The presence of phytochemicals and toxic factors in legumes decrease the overall nutritional qualities. Seeds of Mucuna contain several antinutritional factors which include- indole-3-alkylamines, N,N-dimethyltryptamine and bufotenine, some of which are controlled substances in many countries. They are psychoactive in humans at extremely low doses and are unacceptable in foods (Duke, 1981).

Various processing methods have been adopted by researchers/investigators and food processing companies to reduce the level of L-DOPA and other toxic substances present in Mucuna puriens. Most of the methods include: The use of water to leach out the toxicants, chemicals and thermal treatment for transformations to biologically inactive and non-toxic components (Bressani, 2002). Hydrothermal treatment, fermentation and germination have been shown to be most effective in reducing the anti-nutrients in Mucuna pruriens seeds. Others have suggested cracking the seeds and soaking them in running water for 36 h (Bressani, 2002). All parts of Mucuna plant are known to possess high medicinal value. The seeds have been known to contain many pharmaceuticals antiepileptic and anti-neoplastic activities. Roots of Mucuna are used in Ayureda, an indigenous medicine to constipation, nephropathy, strangury, dysmenorrhea, amenorrhea, elephantiasis, dropsy and ulcers (Sridhar and Bhat, 2007). Seeds possess anti-Parkinsonian activity, anti-inflammatory, antispasmodic,

anti-vemin, anabolic. androgenic, analgesic, hypocholesterolemic, aphrodisiac, febrifuge, hypoglycemic, CNS stimulant, diuretic, hypotensive, menstrual stimulant and vermifuge effects (Bressani, 2002). Mucuna seeds have been in high demand in international markets since after the discovery of L-DOPA, a potential drug for the treatment of Parkinsonism. Alcoholic extracts of Mucuna pruriens seeds have anti-oxidant properties in vitro and in vivo (Rajeshwar et al., 2005). Methanol extract of Mucuna pruriens show strong anti-oxidant activity through the inhibition of alpha, alpha-diphenyl-beta-picrylhydrazyl (DPPH) and hydroxyl radicals, nitric oxide and scavanges superoxide anion and hydrogen peroxide. Seeds of Mucuna constitute a source of food for some ethnic groups of Asia and Africa (Dako and Hill, 1977; lyayi and Eghareuba, 1998). Immature pods and leaves serve as vegetables. We are poised to investigate the nutritive value of this legume and to determine its proximate composition and phytochemical contents and moreover, the effects of processing on these parameters and making recommendation for its widespread consumption as a staple food for human and animal nutrition.

MATERIALS AND METHODS

Chemicals and sources: Most materials used in the research work included :The electron Spectrophotometer (Jenway, 6061, England), Steam bath, Electric hot plate shaker (Clifton CE25696, USA), ethanol, ethyl acetate, nitric acid, Diethylether (BDH-England). Tetraoxosulphate (vi) acid (H₂SO₄) and picric acid, all purchased from BDH, England. Laboratory mill, oven, aqueous ammonia, HCl and muffle furnace. *Mucuna prurien* seeds were supplied by the Department of Crop Science, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Sample preparation: Two hundred grams (200 g) of Mucuna pruriens seeds were purchased from the grocery of the department of Crop Science, Michael Okpara University of Agriculture, Umudike in Abia State of Nigeria. The seeds were first sorted and divided into three groups of 50 grams (50 g) each. Group A seeds were soaked in water for 12 h and hulled, sun dried, milled into flour and stored in a container, labeled "Raw Mucuna Seed" (RMS). Group B seeds were soaked in water for 24 h and hulled. The hulled seeds were soaked in distilled water for 24 h, boiled or cooked at 98°C for 60 min, sun dried, milled into fine flour, stored in a container labeled "Boiled Mucuna Seed" (BMS). The third group was soaked in water for 48 h, hulled and boiled as above. The boiled seeds were then dried on the sun, milled into flour to produce the "Soaked and Boiled Mucuna Seeds" sample (SABMS) which was labeled accordingly.

Proximate analysis

Determination of moisture: The moisture content of the samples were determined by the methods described by Pearson (1976) and James (1995).

Determination of crude lipid: The crude lipid was determined by the continuous solvent extraction method in a Soxhlet apparatus as described by James (1995).

Determination of crude fiber: The crude fiber was determined by the Weende method described by both Pearson (1976) and James (1995).

Determination of protein: The protein content of the samples were determined by the Kjeldhal method reported by James (1995). The total nitrogen was determined and multiplied by the factor, 6.25 to obtain the protein concentration or content.

Determination of the carbohydrate: The carbohydrate content of the test samples was determined by estimation using the arithmetical difference method described by Pearson (1976) and James (1995). The carbohydrate content was calculated and expressed as the nitrogen free extract.

Determination of the ash: The ash content of the samples was determined by the method described by James (1995).

Phytochemical screening (Qualitative analysis)
Test for flavonoids: The presence of flavonoids in the test samples was determined by the acid/alkaline test described by Harborne (1973).

Test for alkaloids: The presence of alkaloids in the test samples was investigated using the test described by Harborne (1973).

Test for tannins: The procedure employed was the ferric chloride test described by Harborne (1973).

Quantitative analysis of the phytochemicals

Determination of flavonoids: This was carried out by the gravimetric procedure of Harborne (1973).

Determination of alkaloids: The quantitative determination of alkaloids was carried out by the alkaline precipitation using the gravimetric method described by Harborne (1973).

Determination of tannins: The Follin-Dennis Spectrophotometric method of Pearson (1976) was used.

Determination of saponins: The saponin content of the sample was determined by double solvent extraction gravimetric method (Harborne, 1973).

Determination of phytate: The spectrophotometric method of Griffiths and Thomas (1981) was used.

Determination of cyanogenic glycosides (HCN): This was carried out by the procedure described by AOAC (1990).

Determination of phenols: The Follin-Dennis method described by Pearson (1976) was used to determine the phenol content.

RESULTS AND DISCUSSION

The results of all analyses are shown in Tables 1 and 2 respectively and involved the proximate composition and phytochemical analyses of the sample.

The results are shown in the Table 1 and 2. Table 1 shows the proximate composition of the raw, boiled and soaked and boiled seeds. Table 2 shows the phytochemical composition of the same samples in Table 1. The data on proximate composition of the differently processed seeds of Mucuna pruriens showed that the seeds are good sources of proteins (22.05-28.23%), fat (2.51-2.69%), crude fiber (3.81-3.86%) and carbohydrate (60.02-68.53%) respectively. The raw sample (RMS) recorded the highest protein concentration (28.23±0.20). This is in agreement with the findings reported in India of Mucuna pruriens (20.2-29.6 %) crude protein (Vedivet and Janardhan, 2000) and from Sri Lanka, 24.0-31.3% (Ravindran and Ravindran, 1988). The crude protein content of Mucuna pruriens surpassed those of many wild legumes (Atylosia scarbaeoides, 17.3%; Erythrina indica, 21.5%; Tamarindus indica, 14% (Arinathan et al., 2003). The concentration of protein in the seeds analyzed suggests that it is quite close to the daily protein need of 23.6 g for adult human beings as recommended by the USA National Research Council (1974). This protein content is much higher than those of popularly consumed legumes such as chicken pea (Cicer anetinum), green pea (Pisum sativum), common bean (Phaseolus vulgaris), pigeon pea (Cajanus cajan) and Lentil (Lens culinarus) with a range from 18.5-21.9% for the raw grains (Costa et al., 2006; Kumar et al., 1991; Mugendi et al., 2010) except for soybean that contains an average of 38% crude protein (Augustin and Klein, 1989) Soaking and boiling reduced the protein content of the seeds as was seen in the boiled sample which recorded a protein content of 22.17±0.20 g; while the soaked and boiled sample gave a protein content of 22.05±0.10 g. Soaking, soaking and boiling prior to cooking reduced the protein content by 21.47% and 21.89% respectively. This may be due to denaturation or solubilization of some nitrogenous compounds during cooking. The seeds were generally found to be low in crude lipid (2.69 ± 0.1) ; (2.51 ± 0.01) and (2.51 ± 0.01) respectively for the raw, boiled, soaked and boiled Mucuna pruriens. This is similar to the reported value of 2.8-4.9% of

Table 1: Proximate composition of raw, boiled, soaked and boiled *Mucuna pruriens* seeds. Values are expressed in % per 100 g of sample

Sample	Protein	Crude fat	Crude fiber	Ash	Moisture	Carbohydrate
RMS	28.23±0.20	2.69±0.10	3.86±0.02	5.26±0.02	12.32±0.21	60.03±0.20
BMS	22.17±0.20	2.51±0.01	3.83±0.02	4.24±1.10	11.93±0.00	67.92±0.10
SABMS	22.05±0.10	2 51+0 01	3 81+0 01	3.10±0.02	11 92+0 00	68 53+0 00

The values in the table above are means ± Standard deviation from triplicate determinations. RMS = Raw Mucuna Seed; BMS = Boiled Mucuna Seed, SABMS = Soaked and Boiled Mucuna Seeds

Table 2: Phytochemical composition of the raw, boiled, soaked and boiled *Mucuna pruriens* seeds. Values are expressed in % per 100 g of sample

	Raw	Boiled	Soaked and boiled
Phytochemical	(RMS)	(BMS)	(SABMS)
Flavonoids	0.42±0.02	0.35±0.02	0.31±0.02
Alkaloids	1.07±0.01	0.88±0.01	0.37±0.02
Saponins	0.47±0.02	0.39±0.02	0.37±0.02
Tannins	0.28±0.00	0.09±0.00	0.08±0.00
Phenols	2.82±0.01	0.33±0.01	0.32±0.01
Carbohydrates	0.43±0.02	0.24±0.00	0.22±0.01
HCN (mg/kg)	12.69±0.01	6.59±0.01	5.54±0.01

The values are Means±SD from triplicate determinations (n = 3)

Mucuna crude lipid by (Ravindran and Ravindran, 1988; Siddhuragu et al., 2000), but differed from 8.47-14.0% reported by some other workers. Mucuna seeds show wide variation in crude lipid content. Mucuna pruriens is a vegetable protein rich seed and has the advantage of low fat content over animal protein sources. These seeds may be used as energy-reduction diet. Soaking and boiling seem to have no tangible effect on the crude lipid concentration of the seeds probably as a result of the hydrophobic nature of lipids which make them insoluble in aqueous solutions.

The crude fiber content of the seeds ranged from 3.81±0.01 - 3.86±0.02%. This varied slightly from 4.19% in Mucuna cochinchinensu reported by Ezeagu et al. (2003) and 6.30% in Mucuna utilis (Ravindran and Ravindran, 1988). High crude fiber in diets is known to enhance digestibility, slows down the release of glucose into the blood stream, reduce blood cholesterol levels, prevent bowel cancers and atherosclerosis (Anderson et al., 1995; Salvin et al., 1997). The ash content of raw Mucuna pruriens obtained was 5.26±0.02%. This is in agreement with the values in some literature: Ash in Mucuna seeds range from 2.9-5.0% (Ravindran and Ravindran, 1988; Vedivet and Janardhan, 2000). From Table 1, boiling, soaking and boiling increased the carbohydrate content of the raw seeds from (60.03±0.20%) to 67.92±0.10 for RMS and BMS samples; then to 68.53±0.00 for SABMS, showing a percentage increase of 13.14 and 14.16% respectively. This finding has equally been reported by other researchers on the starch content of raw and cooked peas as well as other legumes (Costa et al., 2006). This could probably be explained in terms of the significant reduction in protein concentration and moreover, the increased hydrolysis of the carbohydrate

store in the seeds, making it available and ready for utilization. The values obtained for carbohydrates are similar to the range of values (59.20-4.88%) reported by Ezeagu et al. (2003). Table 2 shows the results of the quantitative and qualitative assays of the phytochemicals in the samples. In each case, it could be observed that the raw Mucuna seed samples recorded the highest values; while soaking and boiling reduced the concentrations to low levels, with the least level observed for the samples soaked for 48 h prior to boiling. Soaking prior to boiling reduced the concentrations of the flavonoids, alkaloids, saponins, tannins, hydrogen cyanide, phenols and phytate by 26.19%, 21.28%, 71.43%, 48.07%, 88.65% respectively. Boiling did not have any significant effect on the proximate composition of the seeds except the protein content that was reduced. Soaking in water or periodic changing of the water during soaking, would give a more appreciable reduction in the phytochemical concentrations. The effects of soaking and boiling on the HCN concentration were very significant when compared with the results of the raw sample (raw, 12.69±0.01; soaked, 6.59±0.01; soaked and boiled 5.54±0.01). These processes can be seen as procedures for detoxification of the seeds. It may also be possible to extend the period of soaking for more than 48 h with periodic changing of the water and the period of boiling increased to more than 2 h, this would nonetheless, be a more novel approach than chemical detoxification that would actually leave the consumers of such foods in fear of toxicants and additives.

From the results of various analyses, it could be concluded that adequate processing would go a long way in reducing the level of anti-nutrients/toxicants (phytochemicals) present in the seeds of the plant in order to maintain minimum safe consumption levels, thus making *Mucuna prurient* an ideal food for human and animal nutrition. More over, for its medicinal values, these processing techniques making it an important cash crop, highly priced internationally.

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