

Impact of Gamma Irradiation on the Nutritional and Antinutritional Qualities of *Mucuna Deeringiana* (Bort) Merrill: An Underexploited Food Legume

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Abstract:- Impact of gamma irradiation on *Mucuna deeringiana*, the tribal legume seeds at various doses (2, 5, 10, 15 and 25 kGy) were assessed for its proximate composition, vitamins (niacin and ascorbic acid) and antinutritional factors. Gamma irradiation resulted in an increase in crude protein at all doses, whereas the crude lipid, crude fibre and ash confirmed a decrease in dose-dependent. The unirradiated seed samples of *M. deeringiana* were rich in niacin and ascorbic acid content. They were significantly decreased on irradiation at all the doses. Impact of gamma irradiation revealed a dose-dependent increase in total free phenolics and tannins while the rest of the antinutritional factors like L-DOPA, phytic acid, hydrogen cyanide, trypsin inhibitor activity, oligosaccharides and phytohaemagglutinating activity were significantly reduced. Increased in vitro protein digestibility was noted in the irradiated seeds. Findings of the current study expose that use of gamma irradiation does not affect the overall nutritional composition and can be used as an effectual method of preservation.

Key Words: *Mucuna deeringiana*, gamma irradiation, niacin, ascorbic acid, anti-nutritional factors.

INTRODUCTION

Plant foods such as cereals and legumes play a key role in overcoming protein-energy malnutrition in developing countries, where paucity of animal protein prevails. Edible legumes fulfill the basic nutritional requirements as they are rich in proteins, essential minerals, unsaturated fatty acids and vitamins. In contrast, the extent of production of legumes has failed to keep speed with the needs of ever-increasing populations (Ali & Kumar, 2000).

To fulfill the growing constraints of plant-based proteins for humans and livestock, wide-ranging research is in progress on the potential of employing underutilized legumes (Vadivel & Janardhanan, 2000). But with increasing interest in new food sources and in improved genetic diversity within domesticated lines, the seeds of wild plants including tribal pulses are now receiving more attention. (Vijayakumari et al., 1996).

Though most of the tribal pulses are rich in proteins and other nutrients, certain antinutrients are associated with them; latter have to be eliminated for effective utilization of the pulse nutrients. To achieve this, several processing methods such as germination, soaking and cooking and dry heat treatment have been used. Radiation processing as an effectual means of food preservation, has been shown to lessen anti-nutritional factors in various proteinaceous leguminous seeds, thus helps to offer food safety (Bhat et al., 2008; Allothman et al., 2009; Tresina and Mohan, 2011).

The genus *Mucuna*, belonging to the family Fabaceae is rich in crude protein, essential fatty acids, starch content, and certain essential amino acids. In contrast, it also contains various antinutritional factors and some cyclitols with antidiabetic effects. All parts of the *Mucuna* plant acquire medicinal properties and reveal an extensive variety of pharmacological effects, including antidiabetic, antiinflammatory, neuroprotective and antioxidant properties. The seeds are used in traditional medicine to prevent the toxic effects of snake bites (Lampariello et al., 2012). In spite, the literatures on the nutritional and antinutritional features of *Mucuna deeringiana* seeds is existing, information on the impact of processing with gamma irradiation is inadequate. Therefore, the present exploration was instigated to explore the impact of gamma irradiation on the nutritional and antinutritional factors of the tribal pulse, *M. deeringiana*.

MATERIALS AND METHODS

Collection of seeds

The mature seed materials of *Mucuna deeringiana* (Bort) Merrill were collected from Maruthuvazhmalai, Kanyakumari District, Tamil Nadu. Soon after the collection, the seeds were sun dried for 2-3 days and were surface cleaned with muslin cloth and physically damaged, immature and insect infested seeds were eliminated.

Irradiation

At Radiological Safety Division, Indira Gandhi Center for Atomic Research, Kalpakam, Tamil Nadu, seed samples (each-50g) packed in polyethylene pouches were irradiated at different doses of gamma irradiation (2, 5, 10, 15 and 25 kGy) at room temperature ($25\pm 1^\circ\text{C}$) using a Cobalt -60 Gamma cell 5000 unit. Seed samples packed in the same way without irradiation served as control. In screw capped bottles, the seed samples were powdered and stored for further usage.

Analyses of Proximate Composition and Vitamins (Niacin and Ascorbic acid)

By drying 50 transversely cut seed in an oven at 80°C for 24 hours the moisture content (%) was determined and is expressed on a percentage basis. For further investigation the air-dried samples were powdered partly in a Wiley mill (Scientific Equipment, Delhi, India) to 60-mesh size and accumulated in screw capped bottles at room temperature.

By the micro-Kjeldahl method the nitrogen content was estimated (Humphries, 1956). In addition to that the crude protein content was calculated ($\text{N} \times 6.25$). Crude lipid content was found out using Soxhlet apparatus (AOAC, 2005). The ash content was found out by heating 2g of the dried sample in a silica dish at 600°C for 6hr (AOAC, 2005). Using the non-enzymatic-gravimetric method the total dietary fibre (TDF) was calculated (Li and Cardozo, 1994). The nitrogen free extract (NFE) was obtained by difference (Muller and Tobin, 1980). By multiplying the percentages of crude protein, crude lipid and NFE by the factors 16.7, 37.7 and 16.7, correspondingly, the energy value of the seed (kJ) was estimated (Siddhuraju et al., 1996). As per the method given by Sadasivam and Manickam, 1996, ascorbic acid and niacin contents were extorted and estimated.

In vitro protein digestibility and Analyses of antinutritional compounds

Using the multi-enzyme techniques In vitro protein digestibility (IVPD) of unprocessed and processed seed samples was determined (Hsu et al., 1977). The antinutritional compounds, total free phenolics (Bray and Thorne, 1954), tannins (Burns, 1971), the non-protein amino acid, L-DOPA (3, 4-dihydroxyphenylalanine) (Brain, 1976), phytic acid (Wheeler and Ferrel, 1971) and hydrogen cyanide (Jackson, 1967) were enumerated. Trypsin inhibitor activity was determined by the enzyme assay of Kakade et

al. (1974) by using benzoil-DL-arginin-p-nitroanilide (BAPNA) as a substrate. One trypsin inhibitor unit (TIU) has been expressed as an increase of 0.01 absorbance units per 10mL of reaction mixture at 410nm. One unit of activity corresponds to that amount of trypsin inhibitor in μg protein which gives 50% inhibition of enzyme activity under experimental conditions. Trypsin inhibitor activity has been defined in terms of trypsin units inhibited per mg protein. Extraction of TLC separation and estimation of oligosaccharides were done following the method of Somiari and Balogh (1993) and Tanaka et al. (1975). By the method of Almedia et al. (1991) and Tan et al. (1983) Lectin activity was determined.

Statistical Analysis

The above said data were estimated using triplicate determinations. Investigation of variance (ANOVA) and Paired samples -t' test were used for analysis (SPSS software for windows release 17.0; SPSS/Inc., Chicago IL, USA) of any significant difference in chemical compositions among the gamma irradiated legumes. Significance was accepted at $p < 0.05$ and $p < 0.01$.

RESULTS AND DISCUSSION

Analyses of Proximate Composition and Vitamins (Niacin and Ascorbic acid)

Table 1 recapitulates the proximate composition and vitamins (niacin and ascorbic acid) of gamma irradiated and unirradiated *M. deeringiana* seeds. The moisture content of the *M. deeringiana* seeds significantly declined ($p < 0.01$), as the dose increased in contrast to the unirradiated seeds. Crude protein and carbohydrates comprise the chief chemical constituents of the seeds. Gamma irradiation resulted in an increment in crude protein at all the irradiated doses (Raw, 22.78%; 25kGy, 28.39%). The unirradiated seeds contained higher crude protein content when compared with the earlier report of *Cajanus cajan* (Kumar et al., 1991), *Cicer arietinum* (Khatoun and Prakash, 2006) and *Lablab purpureus* var. Co12 (Kala et al., 2010), tribal pulses like *Dolichos trilobata*, *Rhynchosia cana*, *R. suaveolens*, *Vigna radiata* var. sublobata and *V. unguiculata* subsp. *cylindrica* (Arinathan et al., 2009). The crude lipid, crude fibre and ash on irradiation showed a dose-dependent decrease in the current study, which was important ($p < 0.05$) at 15 and 25kGy. The present results were similar to the earlier report of *Mucuna* seeds (Bhat et al., 2007).

Table 1. Proximate composition and Vitamin (niacin and ascorbic acid) content of *Mucuna deeringiana* seeds untreated and treated with gamma irradiation (g 100g-1)

Component	Dose					
	Raw	2 kGy	5 kGy	10 kGy	15 kGy	25 kGy
Moisture	8.24±0.03	7.82±0.06 ^a	7.32±0.07 ^{ab}	6.86±0.05	6.44±0.03 ^{abc}	6.07±0.01 ^{abce}
Crude protein	22.78±0.11	23.68±0.12	24.50±0.13	25.40±0.16	27.14±0.18	28.39±0.06
Crude lipid	7.26±0.06	7.01±0.04 ^a	6.78±0.06 ^{ab}	6.52±0.03 ^{abc}	6.11±0.05 ^{abcd}	5.96±0.03 ^{abcdj}
Total Dietary Fibre	7.01±0.11	6.96±0.10 ^a	6.32±0.13 ^{ab}	5.96±0.08 ^{abc}	5.54±0.03 ^{abcd}	5.12±0.03 ^{abcde}
Ash	5.11±0.05	4.88±0.09 ^a	4.11±0.06 ^{ab}	3.68±0.11 ^{abc}	3.11±0.02 ^{abcd}	2.66±0.04 ^{abcde}
Nitrogen Free Extractives	57.84±0.78	57.47±0.69	58.29±0.54	58.44±0.34	58.10±0.76	57.90±0.36
Calorific Values (kJ / 100g DM)	1620.11±1.38	1619.48±1.24	1638.19±2.10	1645.93±1.96	1653.86±1.75	1665.74±1.66
Niacin (mg 100g ⁻¹)	20.09±0.06	19.54±0.16 ^a	18.24±0.11 ^{ab}	17.06±0.08 ^{abc}	16.21±0.20 ^{abcd}	15.11±0.26 ^{abcde}
Ascorbic acid (mg 100g ⁻¹)	38.76±0.11	37.11±0.08 ^a	36.06±0.14 ^{ab}	35.66±0.14 ^{abc}	34.14±0.13 ^{abcd}	33.32±0.17 ^{sabcde}

36.06±0.14ab
 34.14±0.13abcd

radiata (Tresina et al., 2014). In the present study, the contents of niacin and ascorbic acid decreased drastically ($p < 0.01$) at a dose rate of 15 and 25 kGy respectively. In an earlier study, niacin and ascorbic acid content significantly diminished in mung bean and *Vigna unguiculata* subsp. *unguiculata* treated with gamma irradiation (Khattak and Klopfenstein, 1989; Tresina and Mohan, 2011).

Means ± SE (N = 3) a denotes significance at ($p < 0.01$) between untreated and treated seeds; b-e denotes significance at ($p < 0.01$) between the treated seeds; j denotes significance at ($p < 0.05$) between the treated seeds

The lipid content of *M. deeringiana* seeds was elevated when compared to that of *Cicer arietinum* (Srivastava and Ali, 2004) and *Cajanus albicans* (Murthy and Emmanuel, 2011). The dose-dependent decrease in fibre on irradiation has been attributed to depolymerization and delignification of the plant matrix (Sande and Karaivanov, 1977). Irradiation did not significantly modify the NFE values and vaguely increased at 10kGy (58.44%). The calorific value increased above 5kGy. The calorific value of the current study exceeds the energy values of Lablab purpureus varieties Co1, Co2, Co9 and Co11 (Kala et al., 2010), tribal pulses *Rhynchosia cana*, *R. suaveolens*, *Tamarindus indica*, *Teramnus labialis* and *Vigna radiata* var. *sublobata* (Arinathan et al., 2009).

The presently investigated seeds of *M. deeringiana* seeds revealed the highest level of niacin content which was found to be higher than that of the previous report in *Cicer arietinum* (Alajaji and El-Adawy, 2006), *Vicia faba*, *Phaseolus vulgaris* (Vega et al., 2010), *Vigna mungo* varieties (Tresina et al., 2010) and *V. radiata* (Tresina et al., 2014). The ascorbic acid content of *M. deeringiana* seeds also recorded elevated level compared to *Phaseolus vulgaris*, *Cajanus cajan*, *Vigna radiata*, *V. mungo* (Kakati et al., 2010), *V. mungo* varieties (Tresina et al., 2010) and *V.*

***In vitro* protein digestibility and Analyses of antinutritional compounds**

The present study revealed a significant and rapid dose-dependent enhancement in the *in vitro* protein digestibility. It is well known that radiation could induce and/or stimulate other factors. Molecular rearrangement and changes in peptide linkages between the amino groups of amino acids could change the nutritive availability and the biological utilization of the irradiation proteins. Such changes could interfere with the protein digestibility and/or its biological value. Hence, protein digestibility might be decreased and/or increased without incurring amino acid destruction (El-Hakeim et al., 1991).

A dose-dependent increment in total phenolics was noted in the investigated tribal legume. Siddhuraju et al. (2002a, b) found increased phenolics in *Sesbania* and *Vigna radiata* seeds on soaking, followed by irradiation. In a previous research study, *Mucuna pruriens* seeds confirmed a dose-dependent increase in phenolic compounds (Bhat et al., 2007). They attributed such increase in phenolics to higher extractability by depolymerization and dissolution of cell wall polysaccharide by irradiation.

The tannin content of the *M. deeringiana* seeds acquired a dose-dependent increase. Elevation of tannins in underutilized legumes seeds by gamma irradiation may be attributed to their higher extractability. Comparable results were noted previously in gamma irradiated *Mucuna pruriens* seeds (Bhat et al., 2007). Some reports specify that, irradiation lessen tannins in seeds (El-Niely, 2007). Such disparity may be attributed to the differential response, variability in the genetic constituents (strains and varieties), geographical origin and other biological factors of legumes. The L-DOPA of *M. deeringiana* seeds also showed a dose-dependent turn down. These observations were in consonance with the former work on *Mucuna* beans (Bhat et al., 2007). On the other hand, a significant ($p < 0.01$) loss in the content of L-DOPA was noted to the level of 4.54% and 3.38% at the irradiation dose of 15 kGy and 25 kGy respectively. It was detected that, irradiation significantly lessened the levels of L-DOPA in all the earlier examined accessions of velvet bean (Gurumoorthi et al., 2007). To remove L-DOPA from the nutritional point of view, the irradiation process is desirable and comparable with the earlier reports. It may be possible to set the ionizing radiation to a specific dose to retain optimum levels of L-DOPA in *Mucuna* seeds for preferred nutritional or pharmaceutical uses.

Irradiation resulted in a significant ($p < 0.01$) dose-dependent diminution in the phytic acid content in the *M. deeringiana* seeds compared to the unirradiated seeds. Utmost reduction was noted at the dose of 25 kGy. In an earlier study, phytic acid of *Mucuna pruriens* seeds was completely removed when exposed to doses of 15 and 30 kGy (Bhat et al., 2007).

Kaisey et al. (2003) registered that, irradiation treatment reduced the phytic acid content. Hydrogen cyanide of *M. deeringiana* seeds proved a significant ($p < 0.01$) dose-dependent decline in gamma irradiated seeds. The content of hydrogen cyanide level in *M. deeringiana* seeds is far below the lethal level i.e. 36 mg/100g (Oke, 1969).

The assessment made in this study revealed that, the trypsin inhibitor activity reduced significantly ($p < 0.01$) after irradiation treatment. In the subsequent dose of irradiation, the decrease in trypsin inhibitor activity was proportional to the irradiation dose. Toledo et al. (2007) noted reduced level of trypsin inhibitor after different doses of gamma irradiation. It is proposed that, reduction in trypsin inhibitor may be due to the breakage of protein structure of trypsin inhibitor with gamma irradiation. The irradiated underutilized legume seeds above the dose level of 2 kGy recorded a significant ($p < 0.01$) dose-dependent decrease in oligosaccharides when compared to the unirradiated seeds. The greater degradation of oligosaccharides in irradiated legumes could be a radiation-related phenomenon or attributable to improved activity of the associated degradative enzymes (Machaiiah and Pednekar, 2002) and gamma irradiation may break glycosidic linkages in oligosaccharides to produce more sucrose and decreases the content of oligosaccharides (Dixit et al., 2010). Corresponding to all the tested blood groups; as the dose increased, the phytohaemagglutinating activity decreased significantly ($p < 0.01$) in the irradiated seeds of *M. deeringiana*.

Table 2 Data on in vitro protein digestibility and antinutritional factors of *Mucuna deeringiana* seeds untreated and treated with gamma irradiation

Component	Dose					
	Raw	2 kGy	5 kGy	10 kGy	15 kGy	25 kGy
<i>In vitro</i> protein digestibility (%)	70.12 ± 0.58	72.14 ± 0.48	78.30 ± 0.76	82.56 ± 0.65	84.16 ± 0.62	88.13 ± 0.36
Total free phenolics (g 100g ⁻¹)	2.74 ± 0.14	2.96 ± 0.10	3.12 ± 0.08	3.24 ± 0.16	3.50 ± 0.21	3.76 ± 0.14
Tannins (g 100g ⁻¹)	0.16 ± 0.01	0.18 ± 0.01	0.21 ± 0.03	0.34 ± 0.3	0.42 ± 0.01	0.52 ± 0.03
L-DOPA (g 100g ⁻¹)	6.55 ± 0.42	6.06 ± 0.21 ^a	5.34 ± 0.38 ^{ab}	5.01 ± 0.17 ^{abc}	4.54 ± 0.30 ^{abcd}	3.38 ± 0.24 ^{abcde}
Phytic acid (mg 100g ⁻¹)	510.12 ± 1.24	468.30 ± 1.11	396.18 ± 1.06 ^f	328.10 ± 2.12	295.20 ± 2.42 ^{ab}	245.91 ± 2.04 ^{abc}
Hydrogen cyanide (mg 100g ⁻¹)	0.18 ± 0.03	0.16 ± 0.03 ^a	0.13 ± 0.01 ^{ab}	0.11 ± 0.03 ^{abc}	0.08 ± 0.01 ^{abcd}	0.05 ± 0.01 ^{abcde}
Trypsin inhibitor activity (TIU mg ⁻¹ protein)	46.16 ± 1.12	43.06 ± 0.98 ^f	38.72 ± 0.78 ^{ag}	36.50 ± 0.67 ^{abc}	33.10 ± 0.54 ^{abcd}	31.14 ± 0.38 ^{bcde}
Oligosaccharides(g100g ⁻¹)						
Raffinose	0.98 ± 0.03	0.82 ± 0.12 ^a	0.76 ± 0.08 ^a	0.52 ± 0.06 ^{abc}	0.48 ± 0.05 ^{bcd}	0.22 ± 0.03 ^{abcde}
Stachyose	1.14 ± 0.04	0.96 ± 0.03 ^a	0.84 ± 0.03 ^{ab}	0.52 ± 0.02 ^{abc}	0.36 ± 0.04 ^{abcd}	0.28 ± 0.01 ^{abc}
Verbascose	4.24 ± 0.09	3.41 ± 0.08	2.96 ± 0.11	2.24 ± 0.12	1.54 ± 0.18	1.12 ± 0.04
Phytohaemagglutinating activity (Hu)						

mg ⁻¹ protein*						
A group	168	152	130	96	52	31
B group	68	51	46	30	26	12
O group	12	09	06	-	-	-

Means \pm SE (N = 3) *values are means of two determinations. a denotes significance at (p<0.01) between untreated and treated seeds; b-e denotes significance at (p<0.01) between the treated seeds; f denotes significance at (p<0.05) between untreated and treated seeds; g-j denotes significance at (p<0.05) between the treated seeds

CONCLUSION

It is evident from the present study that *M. deeringiana* seeds are a valuable source of nutrition. *M. deeringiana* seeds possess a variety of antinutritional factors which may/may not affect the consumers. Concerning the necessity of cutback in antinutritional factors in raw *M. deeringiana* seeds in food and industrial uses, further studies have to be initiated to standardize an appropriate gamma irradiation dose required to ensure maximum benefits of *M. deeringiana* seeds and their products. Therefore, it could be concluded that the irradiation process offers a good treatment for legumes to reduce or eliminate their antinutritional factors with subsequent increase in the digestibility and thereby, increase the utilization of their protein.

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