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Effect of soaking and ionising radiation on various antinutritional factors of seeds from different species of an unconventional legume, *Sesbania* and a common legume, green gram (*Vigna radiata*)

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Abstract

Seeds of three different species of *Sesbania* (*Sesbania aculeata, S. rostrata* and *S. cannabina*) and one species of *Vigna* (*Vigna radiata*) were γ-irradiated at dose levels of 2, 4 and 6 kGy after aqueous soaking and the effects of the irradiation on the proximate composition, various antinutrients and viscosity were studied. Crude protein, crude lipid, crude fibre, ash, nitrogen free extract (NFE) and energy value did not differ appreciably between raw seeds and those soaked and irradiated after soaking, for all the samples. When compared with respective raw and soaked seed materials, irradiation significantly ($P<0.05$) increased the total phenolic contents. No significant effect was observed on the reduction of phytic acid and canavanine contents, whereas approximately 50% reduction of lectin activity was observed at the highest dose of irradiation (6 kGy) in different *Sesbania* spp. On the other hand, non protein amino acid, canavanine and lectin activities against cattle blood erythrocytes were not detected in raw, soaked, or soaked followed by irradiated seeds of *V. radiata*. The highest radiation dose level of 6 kGy in *S. aculeata* and *S. rostrata* and 4 and 6 kGy in *S. cannabina* and *V. radiata* were significantly effective ($P<0.05$) in reducing saponin content. Among the various *Sesbania* and *V. radiata* seed samples, irradiation at 2–6 kGy in *S. aculeata* significantly ($P<0.05$) reduced the trypsin inhibitor activity in a nondose-dependent manner when compared with raw and soaked samples. Particularly, the irradiation at a dose level of 6 kGy significantly ($P<0.05$) reduced viscosities of different spp. of *Sesbania* and *V. radiata* seed samples and this might be due to the depolymerisation of non-starch polysaccharides (NSPs).

Keywords: *Sesbania* spp.; Antinutrients; Canavanine; Soaking; Irradiation; Viscosity

1. Introduction

Legumes/pulses are considered to be a very important group of plant foodstuffs, particularly in the developing world, as a cheap source of protein when animal protein is scarce. In addition to proteins, they supply adequate concentration of minerals, vitamins and carbohydrates. However, the biological utilisation of existing nutrients in these crops is limited by the presence of various anti-metabolic/antiphysiological substances, such as protease inhibitors, flatulence factors, non-starch polysaccharides (NSP), lectins, phytic acid, non-protein amino acids, saponins and phenolic compounds (Liener, 1994; Siddhuraju, Becker, & Makkar, 2000). Such antinutrients, present in many conventional legume seeds, could easily be eliminated or reduced below the level of toxicity/antimetabolic activity by adopting various processing techniques, such as soaking, dry heating, boiling and sprouting. However, one or more antiphysiological substances, representing a relatively high proportion of the little-known/unconventional legumes could not be eliminated completely or even partially by the application of the earlier mentioned processing methods. Although the influence of soaking on trypsin inhibitor has been reported in some legumes, the effect of irradiation, following a soaking treatment, has not yet been studied. Haider and Chughtai (1981) found insignificant changes in trypsin inhibitor activity (TI) of green gram up to an irradiation dose of 4 kGy. On the other hand, Sattar, Durrani, Mahmood, Ahmad, and Khan (1989)

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observed decreases in the trypsin inhibitor (TI) activity during soaking and germination of irradiated green grams. Other antinutrients, namely phytic acid, α-amylase inhibitor and oligosaccharides, were inactivated to a considerable extent when the legume samples were subjected to irradiation (Siddhuraju, Makkar, & Becker, in press). Also, an irradiation dose of up to 10 kGy has been found to be effective in the depolymerisation of NSPs such as β-glucans in oat and barley, which improved the nutrient absorption in chicken by increased β-glucan solubility and reduced the extract viscosity (Campbell, Classen, & Ballance, 1986; Campbell, Sosulski, Classen, & Ballam, 1987).

The seeds of a tropical and unconventional legume, *Sesbania,* contain 30–40% crude protein (Siddhuraju, Vijayakumari, & Janardhanan, 1995) and their utilisation as animal feed is relatively meagre due to high concentrations of galactomannan-containing NSPs, saponins, trypsin inhibitor, phytic acid, lectin and phenolic constituents (Evans & Rotar, 1987; Hossain & Becker, 2001). Although, the sesbania seed samples were subjected to various domestic treatments (Hossain & Becker, 2002), no single processing method appear to be effective in completely removing the detected antinutrients. On the other hand, available information in the literature on the quantitative determination of the toxic non-protein amino acid, canavanine, in *Sesbania* seeds is also limited. In view of these facts, the present study has been made to determine the effect of soaking, and soaking followed by irradiation, on the levels of various antinutrients and the viscosity of different *Sesbania* spp. These effects in samples have been compared with a conventional legume, green gram which has been subjected to similar treatments and results are reported here.

2. Materials and methods

2.1. Seed samples

The seeds of *Sesbania aculeata* and *S. rostrata* were supplied by Dr. M. A. Hossain, Bangladesh Agricultural University, Mymensingh, Bangladesh. *S. cannabina* seeds were collected from Tamil Nadu, India and seeds of green gram (*Vigna radiata*) were procured from an Asian shop in Stuttgart. All the samples were cleaned thoroughly and the immature seeds were discarded. The cleaned seeds were stored at room temperature until further analysis.

2.2. Processing methods

Each sample (60 g) was soaked overnight in 200 ml of distilled water at room temperature (24 °C). After decanting the water, the excess water on the imbibed seeds was removed by putting the seeds between folds of filter paper and gentle pressing. These seeds were divided into four equal portions. One part was frozen immediately and the other three portions of seeds were subjected to irradiation at dose levels of 2, 4 and 6 kGy. Soon after irradiation, these samples were also frozen and, finally, all four portions (non-irradiated and irradiated) were freeze-dried.

The raw, water-soaked, and the seed samples that were irradiated following soaking, were ground into fine powder (particle size less than 0.5 mm) and then stored separately in air-tight plastic bottles at room temperature (24 °C) prior to analysis. All analyses were carried out in duplicate and reported on a dry matter basis.

2.3. Proximate composition

Moisture content of the samples was determined by oven-drying to a constant weight at 105 °C. The crude protein, crude lipid, crude fibre and ash contents were determined by following the standard AOAC method (1990). Nitrogen-free extract (NFE) was obtained by difference. Gross energy was determined by a bomb calorimeter (IKA C7000), using benzoic acid as a standard.

2.4. Total phenolics, tannins and condensed tannins

Total phenolics, tannins and condensed tannins were determined by spectrophotometric methods described by Makkar, Blümmel, Borowy, and Becker, (1993). Total phenolics were quantified by Folin-Ciocalteu reagent and, tannins, as the difference between phenolics before and after tannin removal from the extract, using insoluble polyvinylpolypyrrolidone (PVPP). Condensed tannins were determined by butanol–HCl–Fe^{+} reagent (Porter, Hrstich, & Chan, 1986). Total phenols and tannins were expressed as tannic acid equivalents and condensed tannins as leucocyanidin equivalents.

3. Quantitative determination of the non-protein amino acid, canavanine

3.1. Preparation of pentacyanoammonioferrate (PCAF) reagent

Sodium pentacyanoammonioferrate (PCAF) was prepared by a procedure described by Cacho, Garcia, and Ferrando (1989) as follows. Ten grams of sodium nitroprusside were dissolved in 55 ml of concentrated ammonia solution (32%). The solution was kept in the dark at 0 °C for 24 h. A yellow-green precipitate, containing a mixture of sodium pentacyanoammonioferrate

(II) and (III), was filtered off and the filtrate was treated with absolute ethanol until complete precipitation had occurred. This precipitate was combined with the first precipitate and washed with absolute ethanol until all the ammonia had been removed. After partial removal of the ethanol by filtration, the precipitate was dried over H_2SO_4 and stored in the dark over $CaCl_2$ contained in a desiccator. It must be used within 48 h of preparation since, after this time, the PCAF begins to decompose, turning from its characteristic yellow colour to brownish green.

3.1.1. Preparation of Sesbania samples

Two grams of a finely ground sample of different *Sesbania* seeds, which were defatted in a Soxhlet apparatus with petroleum ether, were extracted with 0.1 M HCl in the proportion of 1:25 (w/v). The mixture was stirred on a magnetic stirrer for 6 h at room temperature and left overnight. The solution was centrifuged at 10,000×***g*** for 20 min and supernatant was saved and the residue subjected to a second extraction for 6 h under the same conditions as the first. The combined extracts were adjusted to exactly pH 7.0 with 0.1 M NaOH solution and diluted to a final volume of 100 ml.

3.1.2. Determination of canavanine

One millilitre of standard canavanine (C-1625, Sigma Chemical Co., MO, USA) solution (1 mg ml^{-1}) was diluted with 0.1 M HCl to give concentrations which ranged from 0.005 to 0.08 mg ml^{-1} of canavanine. In a 10-ml volumetric flask, to 1 ml of these diluted canavanine solutions, were added 6.5 ml of 0.2 M phosphate buffer (pH 7.0), 1 ml of 1% potassium persulphate and 0.5 ml of 1% aqueous PCAF (kept in dark) and the mixture was diluted to 10 ml with distilled water. The mixture was vortexed and, after 15 min, the absorbance was measured at 520 nm. Similarly, an appropriate volume of sample solution, instead of standard canavanine, was used for the quantitative estimation. From the standard curve, the concentration of canavanine in the seed samples was determined and expressed on a dry matter basis.

3.2. Estimation of phytic acid

Phytic acid content of the sample was determined by a colorimetric procedure described by Vaintraub and Lapteva (1988). Ground samples (0.5 g each) were stirred using a magnetic stirrer in 10 ml of 3.5% HCl for 1 h. The contents were centrifuged at 3000×***g*** for 10 min to obtain supernatants. A suitable aliquot of the supernatant was diluted with 3.5% HCl to make 3 ml and then used for the assay. Results were expressed as percentage phytic acid by using commercial phytic acid (Sigma) as a standard.

3.3. Trypsin inhibitor analysis

Trypsin inhibitor activity was determined essentially according to Smith, Van Megan, Twaalhoven, and Hitchcook (1980), except that the enzyme was added last, as suggested by Liu and Markakis (1989). Defatted ground seed samples (0.25 g each) were extracted for 5 min (2×2.5 min, with intermittent cooling in between the extractions, by keeping the tubes containing the samples in an ice bath) in 12.5 ml of 0.01 M NaOH at pH 9.4–9.6 using an Ultra-Turrax macerator (20,000 rpm min^{-1}). The contents were centrifuged at 3800×***g*** for 15 min and the supernatants were collected. The supernatant was further centrifuged at 1000×***g***, following which the supernatants were collected by slowly pipetting between the residue at the bottom and the fatty layer on top. These solutions were used for the assay after appropriate dilution with water.

3.4. Estimation of total saponins

The total saponin content was determined using the spectrophotometric method described by Baccou, Lambert, and Samvaire (1977). To 0.5 g defatted ground seed samples, in a screw-capped centrifuge tube, were added 10 ml of 80% aqueous methanol. The tubes were tightly capped and the contents were stirred overnight using a magnetic stirrer. The tubes were centrifuged at 3000×***g*** for 10 min at room temperature and the supernatants were collected in 25-ml volumetric flasks. The residues were washed three times with 5 ml of 80% aqueous methanol, followed by centrifugation, and the supernatants were collected in the volumetric flasks. The final volume was made to 25 ml with 80% aqueous methanol. Aliquots of the samples from the flasks were used for saponin determination. The results were expressed as diosgenin equivalents from a standard curve of different concentrations of diosgenin in 80% aqueous methanol. The diosgenin was obtained from Roth (Art. 7044).

3.5. Phytohaemagglutinating activity

Lectin activity for the raw and treated samples was conducted by the haemagglutination method described by Gordon and Marquardt (1974) in the presence of 10 mM Mn^{2+} in round-bottom welled microtitre plates, using a 2% (v/v) trypsinised cattle blood erythrocytes suspension in saline phosphate buffer, pH 7.0 (Makkar, Becker, Abel, & Pawelzik, 1997). The haemagglutination activity was defined as the minimum amount of the seed material (in mg per ml of the assay medium) which produced agglutination. The minimum amount was the material per ml of the assay medium in the highest dilution which was positive for agglutination. One haemaglutinating unit (HU) was defined as the least

amount of material per ml in the last dilution giving positive agglutination.

3.6. Viscosity determination

For the viscosity measurements of the legume meals, 1 g of the ground sample was mixed with 100 ml of distilled water and the mixture was incubated at 85 °C for 30 min with stirring, using a magnetic stirrer. After incubation, the solution was further subjected to constant shaking by using a shaker overnight at room temperature (24 °C). The viscosity was measured using the supernatant after centrifugation ($3000\times g$, 10 min on a Rheometer at 25 °C (Neidhart, Resemann, & Carle, 2000) using a cone and plate device with 50 mm diameter and 0.0398 rad cone angle in the shear rate range from 0.25 to 1000 s^{-1} (ARES, Rheometric Scientific, USA). The viscosity (η_0) of the raw, soaked and irradiated *Sesbania* samples was determined in the Newtonian region, independent of shear rate, in the accessible shear rate range from 0.25–1000 s^{-1} whereas, in the *V. radiata* samples, shear-thinning was strong in this range and therefore the apparent viscosity (η_s) was determined from the highest viscosity in this range, which was at the lowest shear rate (0.25 s^{-1}).

3.7. Statistical analysis

Results were expressed as mean values of two replicate determinations. Data were statistically analysed using the Statistica Programme, Version 5.1. The significant difference between means was calculated by one way analysis of variance (ANOVA) using Duncan's multiple range test at $P>0.05$.

4. Results and discussion

4.1. Proximate composition

Proximate composition and energy value of raw, water-soaked seed samples of *S. aculeata, S. rostrata, S. cannabina* and *V. radiata* and those soaked followed by irradiation are presented in Table 1. The gross composition, such as dry matter, crude protein, crude lipid, crude fibre ash, gross energy value and nitrogen-free extract of raw, soaked and irradiated (2, 4 and 6 kGy) samples were found to be similar. Among the different *Sesbania* spp. the observed level of crude protein in the raw seeds sample of *S. aculeata* (34.5%) and *S. rostrata* (33.7%) were higher than *S. cannabina* (29.2%). These values are comparable with those reported previously in *S. aculeata* and *S. rostrata* (Hossain & Becker, 2001). The irradiation treatment did not produce any substantial change in the proximate composition (Table 1). Similarly, irradiation of full-fat soybean, at 5, 15, 30 and 60 kGy, did not induce any significant change in their chemical composition (Farag, 1998). This might be due to the amount of water molecules in the samples. The level of radiation dosage does not favour the production of enough radiolytic products and ultimately does not induce significant changes in the gross composition.

4.2. Phenolic constituents

The results of soaking, and soaking followed by irradiation, on various antinutritional factors of different *Sesbania* spp. and *V. radiata* seed samples are presented in Table 2. The total phenolics in raw *Sesbania* seeds ranged from 2.0 to 3.0% and these concentrations are comparable to those in previous reports on different *Sesbania* spp. (Hossain & Becker, 2001). However, these values were much higher than the value found in the conventional legume, *V. radiata* (0.45%). Also, the levels of tannins (1.32–1.97%) and condensed tannins (2.24–3.31%) in different raw *Sesbania* samples were higher than the values in *V. radiata* (0.21 and 0.16%, respectively). Neither soaking, nor soaking followed by irradiation treatments, reduced total phenolics, tannins or condensed tannins. On the other hand, the soaking followed by irradiation treatments increased all the phenolic substances. This might be due to the increased extractability of phenolics by depolymerisation and dissolution of cell wall polysaccharides after irradiation. Leonhardt, Henning, Nehring, Baer, Flachowsky, and Wolf (1983) showed that the digestibility of wheat, oat, barley and rye straws can be increased by up to 80% by treatment with gamma rays or accelerated electrons which depolymerise cellulose and hemicellulose. Nonetheless, phenolics in legumes are located in the seed coat and thus they could be removed by dehulling processes.

4.3. Canavanine

The potent antimetabolic properties of canavanine result primarily from its ability to function as a highly effective antagonist of arginine metabolism due to its structural similarity to this protein amino acid. It is also believed to function in maintaining nitrogen requirements of developing plants and to contribute significantly to plant chemical defence. The arginine-like structure enables canavanine to bind many enzymes that usually interact with arginine, and it is incorporated into polypeptide chains, resulting in structurally aberrant canavanine-containing proteins. Canavanine is subject to hydrolytic cleavage by arginase, yelding urea and L-canaline (Rosenthal, 1977). Although canavanine was only slightly toxic to adult and neonatal rats following single subcutaneous. injections, multiple injections caused growth inhibition, alopecia, appetite depression and weight loss. The pancreas was affected

more than other organs that were evaluated (Thomas & Rosenthal, 1987a, 1987b). Monkeys fed alfalfa sprouts developed a systemic lupus erythematosus-like syndrome, which was attributed to canavanine toxicity (Montanaro & Bardana, 1991). Human consumption of alfalfa seeds gives an initial reduction in serum cholesterol levels, but prolonged ingestion of alfalfa seeds has been associated with pancytopenia, anemia, leukopenia, and with the development of antinuclear antibodies (Montanaro & Bardana, 1991). Canavanine-sensitive organisms, mainly insects, metabolise it by incorporating it into protein macromolecules. Synthesis and utilisation of such aberrant and impaired canavanine-containing protein adversely affects various developmental processes in insects, manifesting itself as toxic to these organisms. In the present study, the level of canavanine in the different species of *Sesbania* was found to be 0.94–1.16% and this value is lower than the value (2.65%) reported in the Jack bean (*Canavalia ensiformis*) by Natelson (1985). There was no significant change in the canavanine content of the raw *Sesbania* seeds following the soaking and irradiation treatments. As has been reported by Turner and Harborne (1967) for *Vigna* spp., canavanine was also not detected in the *V. radiata* in the present study. Based on the above observations, it may be concluded that the toxic amino acid concentration could not be reduced by soaking or soaking followed by irradiation up to 6 kGy.

4.4. Phytic acid

Another anti-nutrient, phytic acid, in legumes and cereals, is also of major concern as it chelates mineral cations and interacts with proteins forming insoluble complexes which lead to reduced bioavailability of trace minerals and reduced digestibility of proteins (Reyden

Table 1
Proximate composition and energy value of raw, soaked and soaked, followed by irradiated seeds, of three different species of *Sesbania* and *Vigna radiata* samples (g 100 g^{-1} DM)

Component	Raw	Soaked	S + 2 kGy	S + 4 kGy	S + 6 kGy
Sesbania aculeata					
Dry matter	92.61 (0.08)	93.26 (0.14)	93.01 (0.22)	92.99 (0.01)	92.54 (0.05)
Crude protein	34.5 (0.14)	35.7 (0.17)	33.9 (0.37)	33.3 (0.42)	34.4 (0.04)
Crude lipid	6.10 (0.17)	6.17 (0.01)	6.36 (0.07)	6.04 (0.05)	6.29 (0.01)
Crude ash	3.65 (0.00)	3.72 (0.07)	3.78 (0.08)	3.44 (0.20)	3.81 (0.02)
Crude fibre	11.22 (0.19)	12.12 (0.23)	12. 00 (0.09)	12.18 (0.74)	12.17 (0.37)
Gross energy (MJ kg^{-1})	19.3 (0.23)	19.6 (0.18)	19.83 (0.18)	19.8 (0.29)	20.0 (0.17)
NFE	44.6	42.3	44.0	45.1	43.3
Sesbania rostrata					
Dry matter	90.68 (0.09)	92.79 (0.03)	92.95 (0.04)	93.81 (0.06)	93.60 (0.11)
Crude protein	33.7 (0.11)	32.4 (0.06)	32.8 (0.34)	32.6 (0.18)	32.6 (0.17)
Crude lipid	5.39 (0.02)	5.53 (0.04)	5.41 (0.08)	5.51 (0.22)	4.89 (0.06)
Crude ash	3.07 (0.10)	2.84 (0.04)	2.92 (0.16)	3.20 (0.01)	3.02 (0.11)
Crude fibre	12.4 (0.02)	13.9 (0.20)	13.0 (0.57)	12.4 (0.21)	12.9 (0.16)
Gross energy (MJ kg^{-1})	19.8 (0.29)	19.9 (0.28)	19.9 (0.21)	19.9 (0.31)	20.3 (0.08)
NFE	45.6	45.4	45.9	46.3	46.6
Sesbania cannabina					
Dry matter	93.13 (0.04)	93.90 (0.11)	94.69 (0.11)	94.23 (0.08)	94.57 (0.07)
Crude protein	29.2 (0.04)	29.1 (0.01)	29.3 (0.05)	28.9 (0.03)	29.7 (0.01)
Crude lipid	5.68 (0.33)	5.65 (0.04)	5.91 (0.11)	5.37 (0.38)	5.98 (0.16)
Crude ash	3.77 (0.10)	3.56 (0.06)	3.56 (0.14)	3.44 (0.08)	3.82 (0.03)
Crude fibre	13.0 (0.35)	12.9 (0.22)	13.0 (0.04)	14.1 (1.17)	12.6 (0.74)
Gross energy (MJ kg^{-1})	19.7 (0.17)	19.8 (0.20)	19.8 (0.24)	18.7 (0.12)	20.4 (0.10)
NFE	48.4	48.8	48.2	48.3	47.9
Vigna radiata					
Dry matter	91.85 (0.04)	93.23 (0.02)	92.92 (0.06)	92.96 (0.08)	92.68 (0.11)
Crude protein	26.6 (0.09)	26.5 (0.38)	27.2 (0.04)	27.5 (0.05)	27.5 (0.07)
Crude lipid	0.80 (0.03)	0.87 (0.09)	0.91 (0.11)	0.81 (0.06)	0.98 (0.03)
Crude ash	3.43 (0.01)	3.23 (0.06)	3.11 (0.08)	3.16 (0.05)	3.20 (0.06)
Crude fibre	5.10 (0.32)	4.97 (0.18)	5.14 (0.37)	4.89 (0.09)	5.19 (0.06)
Gross energy (MJ kg^{-1})	18.2 (0.26)	18.8 (0.02)	18.9 (0.04)	18.3 (0.12)	19.1 (0.05)
NFE	64.0	64.4	63.7	63.7	63.1

DM, dry matter basis; values in parentheses are ±standard deviation of mean. NFE, nitrogen free extract = [100−(crude protein + crude fibre + crude lipid + ash)]; S, soaked.

& Selvendran, 1993). The phytic acid concentrations in the *S. aculeata, S. rostrata* and *S. cannabina* were 2.05, 1.54 and 1.91%, respectively (Table 2) and these values are comparable with those of previous reports in the same species of *Sesbania* (Hossain & Becker, 2001). However, the value (1.20%) found in common pulse, *V. radiata*, appears to be lower than those observed in the unconventional legumes. When subjected to treatments such as soaking, and soaking followed by irradiation at dose levels of 2, 4 and 6 kGy, no significant differences ($P > 0.05$) were observed between the raw seeds and those subjected to various treatments. Duodu, Minnaar, and Taylor (1999) reported that cooking did not decrease phytic acid in sorgum porridge, but irradiation (10 kGy) caused a significant decrease. Similarly, treatment of soybean seeds with irradiation (1.0 kGy), alone or in combination with soaking reduced the level of phytate compared to controls (Sattar, Neelofar, & Akhtar, 1990). This reduction might be due to chemical degradation of phytate to the lower inositol phosphates and inositol by the action of free radicals produced by the radiation (De Boland, Garner, & O'Dell, 1975). Another possible mode of phytate loss during irradiation could have been through cleavage of the phytate ring itself. In the present study, the ineffectiveness of the irradiation on the reduction/cleavage of phytic acid

Table 2
Effect of soaking and soaking followed by irradiation on the various heat-stable antinutrients in different species of *Sesbania* and *Vigna radiata* (g $100g^{-1}$ DM)

Treatment	*Sesbania aculeata*	*Sesbania rostata*	*Sesbania cannabina*	*Vigna radiata*
Total phenolics[a]				
Raw	2.04b (0.19)	2.69b (0.32)	3.00b (0.08)	0.45c (0.02)
Soaked	2.00ab (0.26)	2.72b (0.14)	3.11ab (0.00)	0.48bc (0.03)
S + 2 kGy	2.70a (0.20)	3.46a (0.11)	3.42a (0.04)	0.58a (0.01)
S + 4 kGy	2.32ab (0.16)	2.94b (0.01)	3.38ab (0.32)	0.52b (0.01)
S + 6 kGy	2.42ab (0.16)	2.94b (0.03)	3.42a (0.08)	0.52b (0.01)
Tannins[a]				
Raw	1.32 (0.11)	1.90 (0.32)	1.97 (0.08)	0.21c (0.01)
Soaked	1.48 (0.27)	1.98 (0.17)	2.22 (0.01)	0.28b (0.01)
S + 2 kGy	1.69 (0.21)	2.34 (0.11)	2.22 (0.03)	0.32a (0.01)
S + 4 kGy	1.40 (0.12)	1.95 (0.06)	2.20 (0.27)	0.28b (0.01)
S + 6 kGy	1.44 (0.14)	1.90 (0.05)	2.20 (0.01)	0.27b (0.01)
Condensed tannins[b]				
Raw	2.24 (0.61)	3.31ab (0.28)	3.23c (0.10)	0.16d (0.01)
Soaked	2.47 (0.11)	3.69ab (0.25)	4.34a (0.35)	0.41a (0.02)
S + 2 kGy	2.29 (0.05)	3.71a (0.09)	3.85ab (0.11)	0.34b (0.00)
S + 4 kGy	2.01 (0.06)	3.29ab (0.16)	3.84ab (0.18)	0.29c (0.02)
S + 6 kGy	2.12 (0.07)	3.13b (0.23)	3.69bc (0.23)	0.30c (0.01)
Canavanine				
Raw	1.07 (0.01)	0.94 (0.00)	1.16 (0.01)	ND
Soaked	0.95 (0.01)	1.01 (0.07)	1.13 (0.07)	ND
S + 2 kGy	1.03 (0.01)	0.93 (0.05)	1.11 (0.00)	ND
S + 4 kGy	1.04 (0.08)	0.95 (0.01)	1.09 (0.02)	ND
S + 6 kGy	1.08 (0.07)	0.93 (0.01)	1.07 (0.02)	ND
Phytic acid				
Raw	2.05 (0.48)	1.54 (0.06)	1.91 (0.06)	1.20 (0.01)
Soaked	2.06 (0.06)	1.39 (0.00)	2.17 (0.09)	1.14 (0.08)
S + 2 kGy	2.01 (0.21)	1.41 (0.23)	1.87 (0.09)	1.13 (0.01)
S + 4 kGy	2.05 (0.18)	1.65 (0.12)	1.87 (0.05)	1.19 (0.00)
S + 6 kGy	1.98 (0.01)	1.72 (0.13)	2.11 (0.45)	1.23 (0.02)
Saponins[c]				
Raw	2.48a (0.48)	2.41a (0.04)	2.60ab (0.04)	1.51a (0.16)
Soaked	2.51a (0.28)	2.54a (0.20)	2.89a (0.19)	1.50a (0.05)
S + 2 kGy	2.48a (0.34)	1.97ab (0.20)	2.43b (0.15)	1.41a (0.08)
S + 4 kGy	1.89ab (0.13)	2.06ab (0.03)	1.59c (0.14)	0.90b (0.35)
S + 6 kGy	1.52b (0.01)	1.52b (0.03)	1.44c (0.14)	0.70b (0.04)

Values in parentheses are ±standard deviation of mean. DM, dry matter basis; ND, not detected. Values with different letters in the same column differ significantly at $P < 0.05$. S, soaked.

[a] As tannic acid equivalents.

[b] As leucocyanidin equivalents.

[c] Diosgenin equivalents.

might be governed by the low radiation dose (2–6 kGy) and, further, effectiveness of irradiation lies more in the combined hydrothermal and germination processing methods, than in the radiation treatment alone.

4.5. Saponins

Saponins are a diverse group of compounds containing an aglycone moiety linked to one or more sugar or oligosaccharide residues. Some of the major biological effects of saponins in animal include erythrocyte haemolysis, depressed growth, reduced feed intake and effects on nutrient absorption and cholesterol and bile acid metabolism (Cheeke, 1996). The total saponin contents in the different *Sesbania* seeds ranged from 2.41 to 2.60% (Table 2). These values are higher than those reported by Hossain and Becker (2001) in *S. aculeata* and *S. rostrata*. However, the value observed in the *V. radiata* (1.5%) was higher than the value reported by Fenwick and Okaenfull (1983) in the same legume (0.57%). No significant reduction of the total saponin concentration was observed between the raw and the water-soaked seed samples, whereas soaking, followed by irradiation, increased the reduction of saponin levels in a dose-dependent manner. Interestingly, at the irradiation dose of 6 kGy, a significant reduction of saponin was observed. A possible mechanism for the reduction is the delinking of the carbohydrate moiety from the aglycone of steroids/triterpenoids bound through glycosidic linkages under the radiation process. Similarly, in the oligosaccharides, α-1–6 glycosidic linkages have been degraded under irradiation conditions and subsequently increased the levels of free sugars, as has been reported in mung bean (Machaiah, Pednekar, & Thomas, 1999). However, further research into the radiolytic products of saponins under the irradiation treatment is needed.

4.6. Phytohaemagglutinating and trypsin inhibitor activities

Lectins, otherwise referred to as phytohaemagglutinins, are mainly glycoproteins, which in vitro agglutinate red blood cells. The toxic effect of lectins can be related to their ability to bind to some specific receptor sites on the surface of the epithelial cells lining the intestine, thus leading to non-specific interference with the absorption or transport of nutrients across the intestinal wall. Trypsin inhibitors cause an increase in the secretion of the digestive enzyme, trypsin by inducing hypertrophy and hyperplasia of the pancreas. This has led to the hypothesis that the growth depression caused by the trypsin inhibitor was the consequence of an endogenous loss of amino acids in the form of enzymes being secreted by a hyperactive pancreas (Liener, 1994). The effects of soaking, and soaking followed by irradiation, on the phytohaemagglutinating and trypsin inhibitor activities are shown in the Table 3. Lectin activity against cattle blood erythrocytes was not observed in the green gram. However, a substantial lectin activity has been found in the different *Sesbania* spp and similar results have also been reported by Hossain and Becker (2001) for *S. aculeata* and *S. rostrata*.

Both soaking and soaking followed by irradation up to 4 kGy had no effect on the lectin activity. However, in the samples processed at 6 kGy, the phytohaemagglutinating activity was reduced by 50%. Similarly, for soybeans irradiated at dose levels of 15, 30, and 60 kGy, reductions of lectin activity of 50, 75 and 94%, respectively, against rabbit red blood cells were reported by Farag (1998). Such incomplete removal of lectin activity may be due to the low radiation dosages (up to 6 kGy) used and the presence of a high content of galactomannan-containing non-starch polysaccharides with intact seed coat on the surface of cotyledons, which

Table 3
Effect of soaking and soaking followed by irradiation on the heat-labile antinutrients in different species of *Sesbania* and *Vigna radiata*

Sample	Raw	Soaked	S + 2 kGy	S + 4 kGy	S + 6kGy
Haemagglutinating activity (mg ml^{-1})[a]					
S. aculeata	0.59 (1.71)[b]	0.59 (1.71)	0.59 (1.71)	0.59 (1.70)	1.18 (0.85)
S. rostrata	0.58 (1.73)	0.59 (1.70)	0.59 (1.70)	0.59 (1.70)	1.18 (0.85)
S. cannabina	1.18 (0.85)	1.18 (0.85)	1.19 (0.84)	0.60 (1.67)	1.21 (0.83)
V. radiata	ND	ND	ND	ND	ND
Trypsin inhibitor activity (mg of pure Trypsin inhibited g^{-1} sample)					
S. aculeata	8.60a (0.04)[c]	8.49a (0.03)	6.32b (0.01)	6.35b (0.05)	6.37b (0.01)
S. rostrata	8.78a (0.03)	8.59ab (0.00)	8.45b (0.17)	8.44b (0.10)	8.51b (0.01)
S. cannabina	34.5 (0.43)	34.0 (0.00)	33.9 (0.38)	33.8 (0.24)	33.8 (0.62)
V. radiata	21.6 (0.18)	21.3 (0.13)	21.3 (0.01)	21.3 (0.24)	21.4 (0.16)

DM, dry matter basis; ND, not detected; values with different letters in the same row differ significantly at $P < 0.05$. S, soaked.

[a] Minimum amount of the seed sample (mg) per ml assay medium which showed haemagglutination.

[b] Values in parentheses indicate the haemagglutinating unit (HU) mg^{-1} sample.

[c] Values in parentheses are ±standard deviation of mean.

have protective effects. The TI activities in *S. aculeata* and *S. rostrata* were 8.60 and 8.78 TI g^{-1} sample, respectively, and these values were lower than that of the conventional pulse, *V. radiata* (21.6 TI g^{-1} sample). However, the TI activity observed in the seeds of *S. cannabina* (34.5 TI g^{-1} sample) appears to be similar to the reported value for soybean (Makkar et al., 1997). Soaking and soaking followed by irradiation (2 kGy) significantly reduced the TI activities in *S. aculeata*. Increase in irradiation dose from 2 kGy to 6 kGy had no further effect. Similar observations were made for *S. rostrata*. No changes were observed in the trypsin inhibitor (TI) activities between the irradiated, soaked and raw samples of *S. cannabina* and *V. radiata*, respectively. Similarly, reduction in the total trypsin inhibitor activity of dry seeds was not observed at irradiation doses of 3 kGy in field bean (El-Morsi, Abdel-Salam, Ismail, Aboul-Fetouh, & Fawzay, 1992). However, the same authors have reported that irradiation and subsequent germination of seed samples caused inactivation of 5.6 and 10.4% of the total TI activity at dose levels of 1 and 2 kGy, respectively. The inactivation of trypsin inhibitor in irradiated samples could be attributed to the destruction of disulphide (–S–S–) bonds. Lee (1962) observed that sulfhydryl (–SH) and disulphide (–S–S–) groups of proteins are apparently highly susceptible to irradiation. However, Iyer, Salunkhe, Sathe, and Rockland (1980) reported that gamma irradiation (1–5 kGy) of dehydrated samples, followed by cooking, was effective in reducing TI activity in three varieties of beans. Hence, it may be concluded that, even at low levels of irradation, the inactivation of lectin and trypsin inhibitor activities could be achieved by additional cooking processes.

4.7. Viscosity

Levels of viscosity in raw, soaked, and seeds soaked followed by irradiation, at dose levels of 2, 4 and 6 kGy of seeds of *Sesbania* spp. and *V. radiata*, are shown in Table 4. Gamma irradiation at 2–6 kGy significantly reduced the viscosity of *S. rostrata, S. cannabina* and *V. radiata* when compared with the soaked samples. However, no significant change in viscosity for *S. aculeata* was observed between the soaked and the irradiated samples, although the viscosity was reduced after soaking. Nonetheless, the viscosity (η_0) values found in different *Sesbania* samples seemed to be much higher than the value of apparent viscosity (η_s) observed in the green gram. The higher viscosity in *Sesbania* spp is due to the presence of NSPs, i.e. galactomannan, and it has also been reported to interfere in the nutrient metabolism of monogastrics (Smits & Annison, 1996). These carbohydrate polymers cause the intestinal contents to become viscous and interfere with nutrient assimilation and general well-being in chicken. The depolymerisation of such non-starch polysaccharides in oats by irradiation significantly improved the growth parameters in chicks (Campbell et al., 1986). In this regard, further extensive studies of the effect of higher doses of irradiation on the viscosity nature of NSP, its solubility parameters and nutrient utilisation studies through in vivo approaches are needed on Sesbania samples.

Table 4
Effect of soaking and soaking followed by irradiation on the viscosity of seed samples from different species of *Sesbania* and *Vigna radiata* [mPa·s] at 25 °C

Treatments	*S. aculeata*[a]	*S. rostrata*[a]	*S. cannabina*[a]	*V. radiata*[b]
Raw	7.65a (0.062)	8.53b (0.077)	3.22c (0.033)	11.5d (6.06)
Soaked	3.57dc (0.104)	9.26a (0.279)	4.81a (0.022)	152.5a (26.81)
S + 2 kGy	3.82bc (0.118)	5.55c (0.219)	4.11b (0.150)	81.0bc (29.95)
S + 4 kGy	4.08b (0.061)	4.57d (0.081)	3.18c (0.161)	101.8b (5.18)
S + 6 kGy	3.49d (0.163)	4.56d (0.078)	2.94c (0.185)	59.6c (14.32)

Values with different letters in the same column differ significantly at $P < 0.05$. S, soaked. Values in parentheses are ± standard deviation of mean.

[a] Viscosity (η_0): viscosity in the Newtonian region, independent of shear rate.

[b] Viscosity (η_s): apparent viscosity measured as highest viscosity at the lowest shear rate (0.25 s^{-1}).

5. Conclusion

Irradiation treatment from 2 to 6 kGy did not induce any significant changes in the proximate composition parameters, such as crude protein, crude fibre, ash and crude lipid contents. On the other hand, presently admitted dose levels appear to be ineffective in the reduction of various antinutrients, except saponins. A significant ($P < 0.05$) reduction of viscosity level in the *S. rostrata, S. cannabina* and *V. radiata* samples, noted after irradiation (2–6 kGy) treatment of soaked seeds, suggested substantial reduction in NSPs. Further, studies on impact of higher radiation doses (> 6 kGy), combined with hydrothermal processing, on various antinutrients, including NSPs and possible biological effects of radiolytic products, are required.

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