International Food Research Journal 19(2): 639-646 (2012)

Physico-chemical and antinutritional attributes of gamma irradiated *Vigna unguiculata* (L.) Walp. subsp. *unguiculata* seeds

Tresina, P. S. and *Mohan, V. R.

Ethnopharmacology unit, Research Department of Botany,
V.O.Chidambaram College, Tuticorin-628008, Tamil Nadu, India

Abstract: Effect of gamma irradiation on *Vigna unguiculata* subsp. *unguiculata* seeds (black coloured seed coat) at various doses (2, 5, 10, 15 and 25 kGy) on the physico-chemical characteristics, proximate composition, vitamins (niacin and ascorbic acid) and antinutritional factors were investigated. No significant changes were observed in the physico-chemical characteristics of irradiated seeds. Gamma irradiation resulted in a significant increase of crude protein at all doses, while the crude lipid, crude fibre and ash content showed a dose dependent decrease. Raw seeds were rich in vitamins (niacin and ascorbic acid); when the seeds were irradiated, significant decreases were reported. Irradiation processing significantly reduces the levels of L-DOPA, phytic acid, hydrogen cyanide, trypsin inhibitor activity, oligosaccharides and phytohaemagglutinating activity. Total free phenolics, tannins and *in vitro* protein digestibility showed significant dose dependent increase. As irradiation is a physical and cold process, it may be ideal and emerge as an important technique to improve the nutritional quality of legume seeds and its products.

Keywords: Gamma irradiation, physico-chemical properties, proximate composition, vitamins, antinutrients

Introduction

Plant foods such as cereals and legumes have consistently been listed as the major potential sources of dietary protein for feeding the world of tomorrow. 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. *Vigna unguiculata* subsp. *unguiculata* (black coloured seed coat) is a less known pulse possessing high nutritional quality (Arinathan *et al*., 2003).

Radiation processing as an effective means of food preservation, has been shown to decrease antinutritional components in some proteinaceous leguminous seeds, thereby helps to provide food security (Bhat *et al.,* 2007; Alothman *et al*., 2009). Irradiated foods will be safe and consumable in regions lacking proper refrigeration facilities (FAO / IAEA, 1997).

Even though, the literatures on the nutritional and antinutritional properties of *Vigna unguiculata* seeds is available, there is a paucity of information pertaining to the effect of gamma irradiation on the under utilized legume. In this context, the present study was conducted to explore the effects of gamma irradiation on the physico-chemical and antinutritional attributes of the under utilized legume *Vigna unguiculata* (L.) Walp subsp. *unguiculata.*

Materials and Methods

Collection of seeds

The mature seed materials of *Vigna unguiculata* (L.) Walp. subsp. *unguiculata* (black coloured seed coat) were collected from Grizzled Giant Squirrel Wildlife Sanctuary, Srivilliputhur, South-Eastern slope of Western Ghats, Tamil Nadu. Soon after 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

Seed samples (each-50 g) packed in polyethylene pouches were irradiated at different doses of gamma irradiation (2, 5, 10, 15 and 25 kGy) at room temperature (25±1°C) using a Cobalt -60 Gamma cell 5000 unit at Radiological Safety Division, Indira Gandhi Center for Atomic Research, Kalpakkam, Tamilnadu. Seed samples packed similarly without irradiation served as control. A part of seeds was taken separately and the remaining seed samples were powdered and stored in screw capped bottles for further usage. The irradiated as well as unirradiated seeds were evaluated for their physico-chemical

*Corresponding author.
Email: *vrmohan_2005@yahoo.com*

characteristics and proximate composition, vitamins (niacin and ascorbic acid) and antinutritional factors.

Physico-chemical analysis

100-Seed Weight

One hundred seeds from each sample were taken at random and weighed in triplicates separately. The average weight of 100 seeds was recorded in grams (Sood *et al.,* 2002).

Seed density

Vigna unguiculata subsp. *unguiculata* seeds (100 g) were accurately weighed and transferred to a measuring cylinder. Then, 100 ml distilled water was added to it. Seed volume was recorded as total volume (ml) – 100 ml. Density was recorded as g per ml (Sood *et al.,* 2002).

Hydration capacity

Seeds weighing 100 g were counted and transferred to a measuring cylinder of 500 ml capacity and 100 ml was added. The cylinders were covered with aluminium foils and left overnight at room temperature. Next day, seeds were drained, superfluous water was removed with filter paper and swollen seeds were reweighed (Sood *et al.,* 2002). Hydration capacity per seed was determined using the following formula:

$$\text{Hydration Capacity} = \frac{\text{Weight of soaked seeds-Weight of seeds before soaking}}{\text{Number of seeds}}$$

Hydration index

Hydration index was calculated as below as per the method given by Sood *et al.* (2002).

$$\text{Hydration index} = \frac{\text{Hydration capacity per seed}}{\text{Weight of one seed (g)}}$$

Swelling capacity

Seeds weighing 100 g were counted; their volume noted and soaked in 350 ml water overnight. The volume of the seeds before and after soaking was noted with the help of a graduated cylinder (Sood et al., 2002). Swelling capacity per seed was determined using the following formula:

$$\text{Swelling capacity (ml/seed)} = \frac{\text{Volume after soaking-Volume before soaking}}{\text{Swelling of seeds}}$$

Swelling index

Swelling index was calculated as below as per the method given by Williams *et al*. (1983).

$$\text{Swelling index} = \frac{\text{Swelling capacity per seed}}{\text{Seed volume (ml)}}$$

Proximate composition

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

The nitrogen content was estimated by the micro-Kjeldahl method (Humphries, 1956) and the crude protein content was calculated (N x 6.25). Crude lipid content was determined using Soxhlet apparatus (AOAC, 2005). The ash content was determined by heating 2 g of the dried sample in a silica dish at 600°C for 6 hr (AOAC, 2005). Total dietary fibre (TDF) was estimated by the non-enzymatic-gravimetric method (Li and Cardozo, 1994). To determine the TDF, duplicate 500 mg ground samples were taken in separate 250 ml beakers. To each beaker 25 ml water was added and gently stirred until the samples were thoroughly wetted, (i.e. no clumps were noticed). The beakers were covered with aluminium foil and allowed to stand 90 min without stirring in an incubator maintained at 37°C. After that, 100 ml 95% ethanol was added to each beaker and allowed to stand for 1 hr at room temperature (25±2°C). The residue was collected under vacuum in a pre-weighed crucible containing filter aid. The residue was washed successively with 20 ml of 78% ethanol, 10 ml of 95% ethanol and 10 ml acetone. The crucible containing the residue was dried ≥ 2 hr at 105°C and then cooled ≥ 2 hr in a desiccator and weighed. One crucible containing residue was used for ash determination at 525°C for 5 hr. The ash-containing crucible was cooled for ≥ 2 hr in a desiccator and weighed. The residue from the remaining duplicate crucible was used for crude protein determination by the micro-Kjeldahl method as already mentioned. The TDF was calculated as follows.

$$TDF\% = 100 \times \frac{Wr - [(P+A)/100]\,Wr}{Ws}$$

Where *Wr* is the mg residue, *P* is the % protein in the residue, *A* is the % ash in the residue, and *Ws* is the mg sample.

The nitrogen free extract (NFE) was obtained by difference (Muller and Tobin, 1980). The energy value of the seed (kJ) was estimated by multiplying the percentages of crude protein, crude lipid and NFE by the factors 16.7, 37.7 and 16.7, respectively (Siddhuraju *et al*., 1996).

Vitamins Analysis

Ascorbic acid and niacin contents were extracted and estimated as per the method given by Sadasivam and Manickam (1996). For the extraction of ascorbic acid, 3 g air-dried powdered sample was ground with 25 ml of 4% oxalic acid and filtered. Bromine water was added drop by drop to 10 ml of the filtrate until it turned orange-yellow to remove the enolic hydrogen atoms. The excess of bromine was expelled by blowing in air. This filtrate was made up to 25 ml with 4% oxalic acid and used for ascorbic acid estimation. Two ml of the extract was made up to 3 ml with distilled H_2O in a test tube. One ml of 2% 2, 4-dinitrophenyl hydrazine reagent and a few drops of thiourea were added. The contents of the test tube were mixed thoroughly. After 3 hr incubation at 37°C, 7 ml of 80% H_2SO_4 was added to dissolve the osazone crystals and the absorbance was measured at 540 nm against a reagent blank.

For the extraction of niacin, 5 g air-dried powdered sample was steamed with 30ml concentrated H_2SO_4 for 30 min. After cooling, this suspension was made up to 50 ml with distilled H_2O and filtered. Five millilitres of 60% basic lead acetate was added to 25ml of the filtrate. The pH was adjusted to 9.5 and centrifuged to collect the supernatant. Two ml of concentrated H_2SO_4 was added to the supernatant. The mixture was allowed to stand for 1 hr and centrifuged. The 5 ml of 40% $ZnSO_4$ was added to the supernatant. The pH was adjusted to 8.4 and centrifuged again. Then the pH of the collected supernatant was adjusted to 7 and used as the niacin extract. For estimation, 1ml extract was made up to 6 ml with distilled water in a test tube; 3 ml cyanogen bromide was added and shaken well, followed by addition of 1 ml of 4% aniline. The yellow colour that developed after 5 min was measured at 420 nm against a reagent blank. The ascorbic acid and niacin contents present in the sample were calculated by referring to a standard graph and expressed as milligrams per 100 grams of powdered samples.

Analyses of antinutritional compounds

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 quantified. 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 10 ml of reaction mixture at 410 nm. Trypsin inhibitor activity has been defined in terms of trypsin units inhibited per mg protein.

Extraction, TLC separation and estimation of oligosaccharides

Extraction of oligosaccharides was done following the method of Somiari and Balogh (1993). Five g of raw and irradiated seed flours were extracted with 50 ml of 70% (v/v) aqueous ethanol and kept on an orbital shaker at 130 rpm for 13 hr and then filtered through Whatman No. 1 filter paper. Residue was further washed with 25 ml of 70% (v/v) ethanol. The filtrates obtained were pooled and vacuum-dried at 45°C. The concentrated sugar syrup was dissolved in 5 ml of double-distilled water.

Separation of oligosaccharides was done by TLC. Thirty g of cellulose-G powder was dissolved in 45 ml of double distilled water and shaken well until the slurry was homogeneous. TLC plates were coated with the slurry and air-dried. Spotting of the sugar samples was done by using micropipettes. Five µl aliquots of each sample were spotted thrice separately. The plates were developed by using a solvent system of n-propanol, ethyl acetate and distilled water (6:1:3), and dried (Tanaka *et al.,* 1975). The plates were sprayed with α –naphthol reagent (1%, w/v). Plates were dried in a hot-air oven. The separated spots were compared with standard sugar spots. A standard sugar mixture containing raffinose, stachyose and verbascose (procured from sigma chemical co., St. Louis, USA). Separated sugars that appeared were verbascose, stachyose and raffinose. The sugar spots were scrapped, eluted in 2 ml of distilled water kept overnight and filtered through Whatman No. 1 filter paper. The filtrates were subjected to quantitative estimation.

The eluted individual oligosaccharides were estimated by the method of Tanaka *et al.* (1975). One ml of the eluted and filtered sugar solution was treated with one ml of 0.2 M thiobarbituric acid and one ml of concentrated HCl. The tubes were boiled in a water bath for exactly 6 min. After cooling, the oligosaccharide contents were quantified in a Elico UV-Spectrophotometer model SL 150 at 432 nm. Average values of triplicate estimations were calculated and the content of oligosaccharides was expressed on dry weight basis.

Quantitative determination of phytohaemagglutinating (lectin) activity

Lectin activity was determined by the method of Almedia *et al.* (1991). One g of air-dried seed flour was stirred with 10 ml of 0.15 N sodium chloride

solution for 2 hrs and the pH was adjusted to 4.0. The contents were centrifuged at 10,000 X g for 20 min. and the supernatants were collected separately. The protein content was estimated by Lowry *et al.* (1951) method. Human blood (blood groups A, B and O) was procured from the blood bank of Jothi Clinical Laboratory, Tuticorin.

Blood erythrocyte suspensions were prepared by washing the blood samples separately with phosphate-buffered saline and centrifuged for 3 min at low speed. Supernatants were removed with Pasteur pipettes. The washing procedure was repeated three times. The washed cells were diluted by one drop of cells with 24 drops of phosphate – buffered saline.

The determination of lectin was done by the method of Tan *et al.* (1983). Clear supernatant (50 µl) was poured into the depression (pit) on a micro-titration plate and serially diluted 1:2 with normal saline. The human blood erythrocyte (A, B and O blood groups) suspensions (25 µl) were added to each of the twenty depressions. The plates were incubated for 3 hrs at room temperature. After the incubation period, the titre values were recorded. One haemagglutinating unit (HU) is defined as the least amount of haemagglutinin that will produce positive evidence of agglutination of 25 µl of a blood group erythrocyte after 3 hr incubation at room temperature. The phytohaemagglutinating activity was expressed as haemagglutinating units (HU) / mg protein.

Determination of in vitro *protein digestibility (IVPD)*

This was determined using the multi-enzyme technique (Hsu *et al.,* 1977). The enzymes used for IVPD were purchased from Sigma Chemical Co., St. Louis, MO, USA. Calculated amounts of the control (casein) and sample were weighed out, hydrated in 10 ml of distilled water and refrigerated at 5°C for 1 hr. The samples containing protein and enzymes were all adjusted to pH 8.0 at 37°C. The IVPD was determined by the sequential digestion of the samples containing protein with a multi-enzyme mixture (trypsin, ∝-chymotrypsin and peptidase) at 37°C followed by protease at 55°C. The pH drop of the samples from pH 8.0 was recorded after 20 min of incubation. The IVPD was calculated according to the regression equation $Y = 234.84 – 22.56X$, where Y is the % digestibility and X the pH drop.

Statistical analyses

The above said data were estimated on triplicate determinations. Analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were used for analysis (SPSS software for windows release 11.5; SPSS/Inc., Chicago IL, USA) of any significant difference in chemical compositions among the gamma irradiated legumes. Significance was accepted at $p < 0.05$.

Results and Discussion

Physico-chemical analyses

The results of the physico-chemical analysis of the unirradiated and irradiated seeds are compiled in Table 1. No significant changes were observed in the seed weight, seed density and seed volume of the gamma irradiated *Vigna unguiculata* subsp. *unguiculata* seeds. Hydration capacity, hydration index, swelling capacity and swelling index have increased with increasing irradiation doses. This was not statistically significant. Similar results were also reported in *Phaseolus vulgaris* and *Cicer arietinum* (Koksel and Celik, 2002). In this study, the water absorption properties of *Vigna unguiculata* subsp. *unguiculata* seeds as measured both gravimetrically (hydration capacity and hydration index) and volumetrically (swelling capacity and swelling index) might also be related to the degradation of starch.

Proximate composition analyses

The data related to the proximate composition of gamma irradiated and unirradiated *Vigna unguiculata* subsp. *unguiculata* seeds are furnished in Table 2. A significant decrease was noted in the moisture content of the gamma irradiated seeds of *Vigna unguiculata* subsp. *unguiculata,* when compared to the unirradiated seeds. Crude protein is the major chemical constituent of the presently investigated wild legume. Irradiation resulted in a significant increase in the crude protein content at all the doses. The crude protein content of the unirradiated seeds was higher than that of the *Vigna unguiculata* subsp. *cylindrica* and *Vigna radiata* var. *sublobata* (Arinathan *et al*., 2009). A significant dose - dependent decrease was observed in the crude lipid, crude fibre and ash content on irradiation. The currently investigated unirradiated seeds contained higher lipids than those in other wild legumes like *Cicer arietinum*, *Vigna mungo* and *Vigna radiata* (Bravo *et al.,* 1999) and five varieties of *Lablab purpureus* (Kala *et al*., 2010). Low crude fibre is nutritionally valued as it traps less proteins and carbohydrates (Balogun and Fetuga, 1986). The decrease in fibre has been attributed to depolymerization and delignification of the plant matrix (Sandev and Karaivanov, 1977). Irradiation did not significantly alter the NFE values. The calorific value of the present investigation is higher than those of the other legumes (moth bean, peas,

Table 1. Physiochemical properties of *Vigna unguiculata* subsp. *unguiculata* seeds treated with gamma irradiation

Component	Dose					
	Raw	2 kGy	5 kGy	10 kGy	15 kGy	25 kGy
Seed weight (g 100 seed^{-1})	2.834 ± 0.78^{a}	2.826 ± 0.56^{a}	2.812 ± 0.92^{a}	2.824 ± 0.48^{a}	2.814 ± 0.38^{a}	2.801 ± 0.54^{a}
Seed density (gmL^{-1})	0.0206 ± 0.01^{a}	0.0201 ± 0.02^{a}	0.0201 ± 0.02^{a}	0.0202 ± 0.03^{a}	0.0206 ± 0.01^{a}	0.0260 ± 0.01^{a}
Seed volume (mL^{-1} seeds)	42.21 ± 1.08^{a}	41.12 ± 1.56^{a}	41.33 ± 1.16^{a}	42.34 ± 1.06^{a}	42.56 ± 1.32^{a}	41.24 ± 1.14^{a}
Hydration capacity (g^{-1} seed)	0.0245 ± 0.04^{a}	0.0247 ± 0.06^{a}	0.0262 ± 0.08^{a}	0.0298 ± 0.07^{a}	0.0321 ± 0.09^{a}	0.0338 ± 0.07^{a}
Hydration index	0.8657 ± 0.04^{a}	0.8727 ± 0.03^{a}	0.9323 ± 0.08^{a}	1.0567 ± 0.03^{a}	1.1423 ± 0.04^{a}	1.2071 ± 0.09^{a}
Swelling capacity (mLseed^{-1})	0.0530 ± 0.01^{a}	0.0541 ± 0.01^{a}	0.0562 ± 0.02^{a}	0.0593 ± 0.02^{a}	0.0601 ± 0.01^{a}	0.0623 ± 0.01^{a}
Swelling index	0.0013 ± 0.001^{a}	0.0013 ± 0.001^{a}	0.0014 ± 0.001^{a}	0.0014 ± 0.001^{a}	0.0014 ± 0.001^{a}	0.0015 ± 0.001^{a}

Means ± SE (N = 3) means in the column with same superscript differs non significantly ($p<0.05$)

Table 2. Proximate composition of *Vigna unguiculata* subsp. *unguiculata* seeds treated with gamma irradiation (g 100 g^{-1})

Component	Dose					
	Raw	2 kGy	5 kGy	10 kGy	15 kGy	25 kGy
Moisture	6.28 ± 0.13^{a}	6.10 ± 0.21^{a}	5.94 ± 0.17^{b}	5.54 ± 0.11^{b}	5.12 ± 0.14^{c}	5.06 ± 0.15^{c}
Crude protein (kjeldhal N x 6.25)	22.10 ±0.17^{e}	24.24 ± 0.16^{db}	25.19 ± 0.24^{c}	25.78 ± 0.54^{bc}	26.48 ± 0.37^{ab}	26.98 ± 0.28^{a}
Crude lipid	4.68 ± 0.03^{f}	4.30 ± 0.07^{e}	4.10 ± 0.09^{d}	3.86 ± 0.10^{c}	3.74 ± 0.03^{b}	3.21 ± 0.02^{a}
Total Dietary Fibre	5.58 ± 0.03^{f}	5.12 ± 0.06^{e}	4.94 ± 0.05^{d}	4.30 ± 0.07^{c}	4.12 ± 0.05^{b}	3.54 ± 0.07^{a}
Ash	4.64 ± 0.01^{a}	4.10 ± 0.03^{b}	3.54 ± 0.04^{b}	3.24 ± 0.03^{c}	2.68 ± 0.02^{d}	2.24 ± 0.02^{d}
NFE (Nitrogen Free Extractives)	67.68 ± 2.78^{bc}	66.54 ± 1.94^{cd}	66.37 ± 1.52^{d}	66.68 ± 2.38^{bcd}	66.72 ± 3.13^{b}	67.24 ± 2.16^{a}
Calorific value (kJ100^{-1} DM)	1675.762±10.26^{b}	1680.775±8.36^{ab}	1682.954±3.48^{b}	1689.604±12.36^{a}	1697.438±7.34^{ab}	1694.491±9.35^{ab}

Means ± SE (N = 3) means in the column with unlike superscript differs significantly ($p<0.05$)

green gram and horse gram), where in calorific value ranged between 1318 and 1394 kJ/100 g (Rao *et al*., 1989) and five varieties of *Lablab purpureus,* where in calorific value ranged between 1524 and 1604 kJ/100 g (Kala *et al*., 2010).

Vitamins analyses

Table 3 summarizes the vitamins (niacin and ascorbic acid) content of both irradiated and unirradiated seeds of *Vigna unguiculata* subsp. *unguiculata.* A significant decrease in vitamins (niacin and ascorbic acid) occurred only at high doses of irradiation. The presently investigated under utilized legume exhibited highest level of niacin content than that of an earlier report in *Vigna catjang and Vigna* spp. (Rajyalakshmi and Geervani, 1994) and *Vigna unguiculata* subsp. *cylindrica* (Arinathan *et al.,* 2009); and lower when compared with *Vigna radiata* var. *sublobata* (Arinathan *et al.,* 2009). In an earlier report, niacin content decreased significantly in mung bean treated with gamma irradiation (Khattak and Klopfenstein, 1989). The tribal pulse registered high level of ascorbic acid than *Cicer arietinum* (Fernandez and Berry, 1988); *Teramnus labialis* (Arinathan *et al*., 2009) and five varieties of *Lablab purpureus* (Kala *et al*., 2010) and low level when compared to *Vigna radiata* var. *sublobata* and *Vigna unguiculata* subsp. *cylindrica* (Arinathan *et al.,* 2009).

Analyses of antinutritional factors

The presence of antinutritional factors is one of the major drawbacks limiting the antinutritional and food qualities of the legumes (Salunkhe, 1982). For this reason, a preliminary evaluation of some of these factors in the tribal pulse *Vigna unguiculata* subsp. *unguiculata* is made (Table 4). A definite significant dose-dependent increase of total free phenolics was noted in the currently investigated tribal pulse indicating enhancement of phenylalanine-ammonia lyase activity rather than more extractability (Bhat *et al*., 2007). The present values of phenolics were higher when compared to *Vigna capensis* and *Vigna sinensis* (Mohan and Janardhanan, 1993). Siddhuraju *et al.* (2002a) found increased phenolics in *Vigna radiata* and *Sesbania* seeds on soaking followed by irradiation. Although phenolics are considered as one of the major antinutrients, considerable interest has been recently shown in their possible antioxidant activities and potential health benefits. Epidemiological studies have correlated the consumption of plant produce with high phenolics to reduce cardio-cerebrovascular disease and cancer mortality (Hertog *et al*., 1997). The tannin content of the investigated legume significantly increased dose-dependently. Elevation of tannins in *Vigna unguiculata* subsp. *unguiculata* seeds by gamma irradiation may be attributed to their higher extractability. The tannin content of

Table 3. Vitamins (niacin and ascorbic acid) content of *Vigna unguiculata* subsp. *unguiculata* seeds treated with gamma irradiation (mg 100 g^{-1})

Components	Raw	Dose				
		2 kGy	5 kGy	10 kGy	15 kGy	25 kGy
Niacin	16.33 ± 0.21 [a]	16.04 ± 0.18 [a]	15.92 ± 0.38 [a]	15.47 ± 0.41 [a]	13.38 ± 0.23 [b]	11.42 ± 0.30[c]
Ascorbic acid	42.10 ± 1.28 [a]	41.89 ± 0.79[ab]	41.78 ± 0.62[ab]	40.12 ± 0.58 [b]	38.10 ±0.52[c]	36.28 ± 0.68[d]

Means ± SE (N = 3) means in the column with unlike superscript differs significantly ($p<0.05$)

Table 4. Data on *in vitro* protein digestibility and antinutritional factors of *Vigna unguiculata* subsp. *unguiculata* seeds treated with gamma irradiation

Component	Dose					
	Raw	2 kGy	5 kGy	10 kGy	15 kGy	25 kGy
In Vitro Protein Digestibility	74.21 ±1.76[e]	76.30 ± 1.24[de]	78.20 ± 1.30[cd]	80.46 ± 1.14[bc]	82.10 ± 1.04[b]	82.10 ± 1.04[a]
Total free phenolics ($g100g^{1}$)	1.24 ± 0.06[c]	1.36 ± 0.04[bc]	1.48 ± 0.16[bc]	1.52 ± 0.08[b]	1.68 ± 0.27[a]	1.94 ± 0.34[a]
Tannins ($g100g^{-1}$)	0.34 ± 0.03[b]	0.39 ± 0.02[b]	0.42 ± 0.01[b]	0.48 ± 0.03[b]	0.56 ± 0.03[a]	0.72 ± 0.06[a]
L-DOPA ($g100g^{-1}$)	2.26 ± 0.24[a]	2.04 ± 0.11[ab]	1.96 ± 0.14[b]	1.54 ± 0.07[bc]	1.36 ± 0.13[bc]	1.04 ± 0.08[c]
Phytic acid ($mg100g^{-1}$)	378.40 ± 1.26[f]	336.10±1.34[e]	296.14±1.08[d]	204.52±0.98[c]	124.16±0.88[b]	107.56±0.92[a]
Hydrogen cyanide ($mg100g^{1}$)	0.28 ± 0.07[a]	0.26 ± 0.03[a]	0.22 ± 0.04[ab]	0.19 ± 0.03[ab]	0.15 ± 0.03[bc]	0.11 ± 0.01[c]
Trypsin inhibitor activity (TIU mg^{-1} protein)	24.28 ± 0.12[a]	23.10 ± 0.38[b]	22.46 ± 0.28[c]	20.16 ± 0.41[d]	16.30 ± 0.23[e]	13.54 ± 0.26[f]
Oligosaccharides($g100g^{-1}$)						
Raffinose	0.48 ± 0.06[a]	0.42 ± 0.03[ab]	0.39 ± 0.03[ab]	0.32 ± 0.02[abc]	0.28 ± 0.01[bc]	0.21 ± 0.01[c]
Stachyose	1.74 ± 0.18 [a]	1.28 ± 0.06[ab]	1.01 ± 0.05[bc]	0.86 ± 0.03[cd]	0.52 ± 0.08[de]	0.34 ± 0.06[f]
Verbascose	1.21 ± 0.24 [a]	1.01 ± 0.17[a]	0.94 ± 0.11[a]	0.61 ± 0.08[b]	0.44 ± 0.07[b]	0.16 ± 0.03[c]
Phytohaemagglutinating activity (Hu mg^{-1} protein)[b]						
A group	56	32	26	22	17	12
B group	128	110	86	64	41	18
O group	14	10	6	-	-	-

Means ± SE (N = 3) means in the column with unlike superscript differs significantly ($p<0.05$)
[*] Values are means of two determinations

unirradiated seeds was lower than *Vigna capensis* and *Vigna sinensis* (Mohan and Janardhanan, 1993). In the currently investigated seeds, the content of non-proteinaceous amino acid, L-DOPA showed a significant dose-dependent decline. These results were in consonance with the earlier report on *Mucuna* beans (Bhat *et al.,* 2007). The observation made in the present study showed that, the phytic acid content significantly reduced dose-dependently. Duodo *et al*. (1999) indicated that, phytic acid degradation by irradiation is due to cleavage in the structure of phytic acid itself, which may also be true in the present study also. Kaisey *et al.* (2003) reported that, irradiation treatment reduces the phytic acid content. The values of phytic acid of the present study was found to be low when compared with commonly consumed grain legumes like *Vigna mungo* (Kataria *et al*., 1988) and *Vigna radiata* (Kataria *et al*., 1989). Hydrogen cyanide is known to cause acute or chronic toxicity. Hydrogen cyanide of *Vigna unguiculata* subsp. *unguiculata* seeds showed a significant dose-dependent decline in gamma irradiated seeds than the unirradiated seeds. The content of hydrogen cyanide level in the presently investigated pulse is far below the lethal level i.e. 36mg/100 mg (Oke, 1969) and comparable with those of *Vigna sinensis* and *Pisum sativum* (Montgomery, 1980) and *Dolichos lablab* var. *vulgaris* (Vijayakumari *et al*., 1995). Irradiation processing significantly reduced the trypsin inhibitor activity which was proportional to the irradiation dose. Inactivation of trypsin inhibitor in irradiated samples could be attributed to the destruction of disulphide (-S-S-) groups (El-Morsi *et al*., 1992). Khattak and Klopfenstein (1989) also showed that, sulphur containing amino acids were liable to become damaged by irradiation particularly in legumes. The irradiated seeds registered a dose-dependent significant decrease in oligosaccharides when compared to the unirradiated seeds. The oligosaccharide contents were inactivated to a considerable extent when legume samples were irradiated (Siddhuraju *et al*., 2002b). In case of broad bean, a complete destruction of raffinose and stachyose was observed at 7.5 kGy radiation dose (Kaisey *et al.,* 2003). Regarding phytohaemagglutinating activity, the human erythrocytes of 'B' blood group registered highest level of phytohaemagglutinating activity in unirradiated seeds when compared to 'A' blood group and 'O' blood group. Blood group 'O' in our study exhibited a much lower activity than did other blood groups and this observation is in agreement with earlier studies of *Mucuna* seeds (Siddhuraju *et al.,* 1996; Vijayakumari *et al.,* 1996). In earlier study, when soy bean was subjected to radiation dose of

10 kGy, the phytohaemagglutinating activity was reduced by 50% (Farag, 1989) which is a significantly higher reduction than with soaking and dehulling (Liener, 1994). Our current investigation revealed a significant ($p<0.05$) and rapid dose-dependent increase was noted in the *in vitro* protein digestibility activity. However, these values were higher when compared to the earlier reports of Bhat *et al.* (2007) in *Mucuna* bean seeds.

Conclusion

The results of the present investigation reveals that *Vigna unguiculata* subsp. *unguiculata* seed is a valuable source of nutrition due to high protein and carbohydrate with an adequate quantity of vitamins (niacin and ascorbic acid). The seeds exhibited good physico-chemical properties, which is of immense value in food industries in production of quality food formulation. Application of gamma irradiation as a method of preservation of *Vigna unguiculata* subsp. *unguiculata* seed quality did not show any adverse effect on the nutritional composition. In view of the importance of *Vigna unguiculata* subsp. *unguiculata* seeds as food and feed, it may be necessary to set an ionizing radiation to a specific dose to achieve optimum benefits or to eliminate phenolics, tannins, phytic acid, L-DOPA and hydrogen cyanide. As irradiation is a physical and cold process, it may emerge as one of the important techniques for improving the nutritional quality of *Vigna unguiculata* subsp. *unguiculata* seeds.

References

Almedia, N.G., Calderon de la Barca, A.M. and Valencia, M.E. 1991. Effect of different heat treatments on the anti-nutritional activity of *Phaseolus vulgaris* (variety ojo de Carbra) lectin. Journal of Agricultural and Food Chemistry 39: 1627-1630.

Alothman, M., Bhat , R. and Karim, A.A. 2009. Effects of radiation processing on phytochemicals and antioxidants in plant produce. Trends in Food Science Technology 20: 201-212.

AOAC, 2005. Official Methods of Analysis (18th edn.). Association of Official Analytical Chemists. Washington. DC.

Arinathan, V., Mohan, V.R., John De Britto, A. and Murugan, C. 2003. Chemical composition of certain tribal pulses in South India. International Journal of Food Science and Nutrition 54: 209-217.

Arinathan, V., Mohan, V.R., Maruthupandian, A. and Athiperumalsami, T. 2009. Chemical evaluation of raw seeds of certain tribal pulses in Tamil Nadu, India. Tropical and Subtropical Agroecosystems 10: 287-294.

Balaogun, A.M. and Fetuga, B.L. 1986. Chemical composition of some underexploited leguminous crop seeds in Nigeria. Journal of Agriculture and Food Chemistry 34: 189-192.

Bhat, R., Sridhar, K.R. and Sahadevan Seena. 2007. Nutritional quality evaluation of velvet bean seeds (*Mucuna pruriens*) exposed to gamma irradiation. International Journal of Food Science and Nutrition 59: 261-278.

Brain, K.R. 1976. Accumulation of L-DOPA in cultures from *Mucuna pruriens.* Plant Science Letters **7**: 157-161.

Bravo, L., Siddhuraju, P. and. Sauvo-Calixto, F. 1999. Composition of under exploited Indian pulses. Comparison with common legumes. Food Chemistry. 64: 185-102.

Bray, H.G. and Thorne, W.V. 1954. Analysis of phenolic compounds methods. Biochemical Analyst. 1: 27-52.

Burns, R.B.1971. Methods of estimation of tannin in the grain, sorghum. Agronomy Journal 63: 511 -512.

Duodu, K.G., Minnaar, A. and Taylor, J.R.N. 1999. Effect of cooking and irradiation on the labile vitamin and antinutrients content of a traditional African Sorghum porridge and Spinach relish. Food Chemistry 66**:** 21-27.

El-Morsi, E.R., Abdel-Salam, H.S., Ismail, H.A., Aboul-Fetouch, S.A. and Fawzay, A.M. 1992. Effect of gamma irradiation on trypsin inhibitor and subunit structure of proteins in field bean (*Vicia faba*) seeds during germination. Minia Journal of Agriculture and Research Development 14: 849-871.

FAO/IAEA, 1997. Technology transfer of food irradiation to reduce post-harvest food losses in Africa. Second FAO/IAEA Research co-ordination meeting on the Co- ordinated Research Programme, Tangier, Morocco.

Farag, M.D.E.H. 1989. Radiation deactivation of antinutritional factors: trypsin inhibitor and haemagglutinin in soybeans. Egyptian Journal of Radiation Science and Applications 6: 207-215.

Fernandez, M.L., and Berry, J.W. 1988. Nutritional evaluation of chick pea and germinated chickpea flours. Plant Foods for Human Nutrition 38: 127-134.

Hertog, M.G.L., Sweetnam, P.M., Fehily, A.M., Elwood, P.C. and Kromhout, D. 1997. Antioxidant flavonols and ischaemic heart disease in a welsh population of men the Caerphilly study. American Journal of Clinical Nutrition 65: 1489-1494.

Hsu, H.W., Vavak, D.L., Satterlee, L.D. and Miller, G.A. 1977. A multi-enzyme technique for estimating protein digestibility. Journal of Food Science 42: 1269 – 1271.

Humphries, E.C. 1956. Mineral composition and ash analysis In: Peach K. and Tracey, M.V. (eds.) Modern Methods of Plant Analysis Vol.1, Springer-Verlag, Berlin, pp: 468-502.

Jackson, M.L. 1967. Cyanide in Plant tissue. In: Soil Chemical Analysis. Asia Publishing House, New Delhi, India. pp. 337.

Kaisey, M.T.A.L., Alwan, A.K.H.., Mohammad, M.H.

and Saeed, A.H. 2003. Effect of gamma irradiation on antinutritional factors in broad bean. Radiation Physics and Chemistry 67: 493-496.

Kakade, M.L., Rackis, J.J., McGhce, J.E. and Puski, G. 1974. Determination of Trypsin inhibitor activity of soy products: a collaborative analysis of an improved procedure. Cereal Chemistry 51: 376 -38

Kala, K. B., Tresina Soris, P., Mohan, V.R. and Vadivel, V. 2010. Nutrient and chemical evaluation of *Lablab purpureus* (L.) Sweet. Advances in Bioresearch 1: 44-53.

Kataria, A., Chauhan, B.M. and Gandhi, S. 1988. Effect of domestic processing and cooking on the antinutrients of black gram. Food Chemistry 30, 149-156.

Kataria, A., Chauhan, B.M. and Punia, D. 1989. Antinutrients and protein digestibility (*in vitro*) of mungo bean as affected by domestic processing and cooking. Food Chemistry 32: 9-17.

Khattak, A.B. and. Klopfenstein, C.F. 1989. Effect of gamma irradiation on the nutritional quality of grain and legume. I. Stability of niacin, thiamin and riboflavin. Cereal Chemistry 66: 169-170.

Koksel, H. and Celix, S. 2001. Quality evaluation of gamma irradiated food legumes. GIDA. 26: 275-280.

Li, B.W. and Cardozo, M.S. 1994. Determination of total dietary fibre in foods and products with little or no starch, non-enzymatic gravimetric method: collaborative study. Journal of Association and Official and Analytical Chemists International 77: 687 -689.

Liener, I.E. 1994. Implications of antinutritional components in Soybean foods. Critical Reviews of Food Science and Nutrition 34: 31-67.

Lowry, O.H., Rorebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with folin phenol reagent. Journal of Biological Chemistry 193: 265 – 275.

Mohan, V.R. and Janardhanan, K. 1993. Chemical composition and nutritional evaluation of two little-known species of *Vigna.* Food Chemistry 48: 367-371.

Montgomery, R.D. 1980. Cyanogens. In: Liener IE, editor. Toxic constitutes of Plant Food Stuffs. 2nd ed. New York: Academic Press. pp.158 – 160

Muller, H.G. and Tobin, G. 1980. Nutrition and food processing, London : Croom Helm Ltd.

Rao, B.S.N., Deosthale, Y.G. and Pant, K.C. 1989. Nutritive Value of Indian Foods. Hyderabad, India: National Institute of Nutrition, Indian Council of Medical Research 47-91.

Oke, O.L. 1969. The role of hydrocyanic acid in nutrition. World Review of Nutrition and Dietics 11: 118-147.

Rajyalakshmi, P. and Geervani, P. 1994. Nutritive value of the foods cultivated and consumed by the tribals of South India. Plant Foods for Human Nutrition 46: 53-61.

Sadasivam, S. and Manickam, A. 1996. Biochemical methods, new age International (P) limited publishers, New Delhi, India.

Salunkhe, D.K., Sathe, S.K. and Reddy, N.R. 1982. Legume lipids. In: S.K. Arora. (ed , (pp.51-107). Oxford and IBH Publishing Co., New Delhi, India.

Sandev, S. and Karaivanov, I. 1977. The composition and digestibility of irradiated roughage treatment with gamma irradiation. Tierernahrung Fuetterung, 10, 238-242.

Siddhuraju, P., Osoniyi, O., Makkar, H.P.S and Becker, K. 2002a. Effect of soaking and ionizing radiation on various antinutritional factors of seeds from different species of an unconventional legume, *Sesbania* and a common legume, green gram (*Vigna radiata*). Food Chemistry 179: 273-281.

Siddhuraju, P., Makkar, H.P.S and Becker, K. 2002b. The effect of ionizing radiation on antinutritional factors and the nutritional value of plant materials with reference to human and animal food. Food Chemistry 78: 187-205.

Siddhuraju, P., Vijayakumari, K. and Janardhanan, K. 1996. Chemical composition and protein quality of the little known legume velvet bean (*Mucuna pruriens* L.D.C.) Journal of Agricultural and Food Chemistry 44: 2636-2641.

Somiari, R.T. and Balogh, E 1993. Effect of soaking, cooking and alpha- galactoside treatment on the oligosaccharide content of cowpea flours. Journal of Science Food and Agriculture 61: 339 – 343.

Sood, M., Malhotra, S.R. and Sood, B.C. 2002. Effect of processing and cooking on proximate composition of chick pea (*Cicer arietinum*) varieties. Journal of Food Science and Technology 39: 69-71.

Tan, N.H., Rahim, Z.H.A., Khor, H.T. and Wong, K.C. 1983. Winged bean (*Psophocarpus tetragonolobus*). Tannin level, phytate content and Haemagglutinating activity. Journal of Agricultural and Food Chemistry 31: 916 – 917.

Tanaka, M., Thanankul, D., Lee, T.C. and Chichester, L.O. 1975. A simplified method for the quantitative determination of sucrose, raffinose and stachyose in legume seeds. Journal of Food Science 40: 1087–1088

Vijayakumari, K., Siddhuraju, P. and Janardhanan, K. 1996. Effect of different post-harvest treatments on antinutritional factors in seeds of the tribal pulse, *Mucuna pruriens* (L.)DC. International Journal of Food Science and Nutrition 47: 263-272.

Vijayakumari, K., Siddhuraju, P. and Janardhanan, K. 1995. Effects of various water or hydrothermal treatments on certain antinutritional compounds in the seeds of the tribal pulse, *Dolichos lablab* var. *vulgaris* L. Plant Foods for Human Nutrition 48: 17-29.

Wheeler, E.L. and Ferrel, R.E. 1971. A method for phytic acid determination in wheat and wheat fractions. Cereal Chemistry 48: 312–320.

Williams, P.C., Nakoul, H. and Singh, K.B. 1983. Relationship between cooking time and some physical characteristics in chickpeas (*Cicer arietinum* L.). Journal of Science Food and Agriculture 34: 492-496.