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Starch Irradiation: Physicochemical Properties, Anti-Nutritional Factors and Enzymatic Digestibility of Native and Electron Beam Irradiated Bitter Vetch (*Vicia ervilia*) Seeds

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Abstract: *V. ervilia* seeds were exposed to EB-irradiation (10, 20 and 30 kGy) and physicochemical properties, anti-nutritional factors and subsequent effects on digestibility [RDS, SDS, RS, *in vitro* and *in vivo* digestibility] in broilers were investigated. Irradiation had significant effects ($p<0.05$) on chemical compositions so that decreased moisture, crude protein, ether extract and crude fiber contents. EB-irradiation of seeds resulted in significant dose-dependent decrease of anti-nutritional factors (total phenols, tannins, condensed tannins, canavanine and trypsin inhibitor) ($p<0.05$). EB-irradiation decreased the proportion of RDS and SDS of *V. ervilia* seeds but increased the proportions of RS. The increase in RS content indicates that the irradiation induced the structural modification besides the chain degradation. Irradiation improved ($p<0.05$) *in vivo* digestibility of dry matter, crude protein, true protein and gross energy but decreased *in vitro* and *in vivo* starch digestibility.

Key words: EB-irradiation, *V. ervilia*, anti-nutritional factors, rapidly digestible, slowly digestible, resistant starch, digestibility

INTRODUCTION

Throughout the world, many countries are producing seed crops that were adapted to their specific environment and are used as sources of protein and starch in feed for humans, animal or poultry. Some species of leguminous family are sources of cheap protein for animals. Bitter vetch (*Vicia ervilia*) is known for its high nutritional value, capacity of nitrogen fixation and ability to grow in poor soils. Its seeds contain about 22.8% CP (Farran *et al*., 2001). *V. ervilia* seeds have been used in animal feeds and when treated as an alternative source of starch and protein in poultry diet (Farran *et al*., 2001). Raw bitter vetch however is detrimental to mono-gastric animals, especially chickens. The adverse effects arise from the presence of some anti-nutritional factors in the raw seeds including L-canavanine (0.035-0.11%), trypsin inhibitor (2.14 mg g^{-1} DM) and tannin (2.01 g kg^{-1} DM). Feeding a diet with 60% raw bitter vetch has decreased weight gain and reduced feed intake in broilers and resulted in cessation of egg production of laying hens within 2 weeks post feeding. Several detoxification methods have been evaluated for leguminous seeds including soaking in water, acetic acid, sodium bicarbonate solutions (Farran *et al*., 2001) and potassium bicarbonate solution (D'Mello and Walker, 1991), boiling (Farran *et al*., 2001) and autoclaving (D'Mello and Walker, 1991). However, one or more anti-nutritional substance, representing a relatively high proportion of little known or unconventional legumes could not be eliminate completely or even partially by the application of the earlier mentioned processing methods.

Additional techniques are the application of ionizing irradiation. Compared to the physical and rheological changes, the effect of electron beam irradiation on starch and protein structure and consequent digestibility has been rarely investigated. Electron beam irradiation is often applied for the modification of food materials to change

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their physical properties (Waje and Kwon, 2007). The irradiation may generate active radicals which readily react with food components to change their molecular structure (Yu and Wang, 2007). It has also been suggested as rapid and convenient modification technique which breaks large molecules into smaller fragments and is capable of cleaving glycosidic linkages (Yu and Wang, 2007). Electron beam irradiation has also been shown to reduce or inactivate some of the anti-nutritional factors in wild leguminous seeds or meals, thereby enhancing their edibility (Bhat *et al*., 2007; Siddhuraju *et al*., 2002).

Therefore, in this study, it was aimed to evaluate effects of EB-irradiation on structural and physicochemical properties, anti-nutritional factors and subsequent effects on Englyst classification of starch and *in vitro* and *in vivo* digestibility of *V. ervilia* starch in broilers.

MATERIALS AND METHODS

The experiment was performed at the experimental farm of Agricultural, Medical and Industrial Research School, Nuclear Science and Technology Research Institute, Atomic Energy Organization of Iran, Karaj, Iran. All bird protocols were approved by the relevant Ethical Review Committee and all experimental conditions followed official guidelines for the care and management of birds.

Collection of the seed sample: The seeds of *V. ervilia* were collected from the Agricultural and Natural Resources Research Center, Sari, Mazandaran, Iran. Soon after collection, after removing immature and damaged seeds, the mature seeds were dried in direct sunlight for 2 days and stored in plastic containers at room temperature (25°C) until further use.

Sample irradiation: Seed samples were packed in 30×40×5 cm nylon bags (0.5 mm thickness) and exposed to EB-irradiation at the Yazd radiation processing center (AEOI, Yazd center, Iran) to various doses (10, 20 and 30 kGy) at room temperature by a Rhodotron Accelerator Model TT200 (IBA Co., Belgium). All samples were irradiated at fixed beam energy of 10 MeV and the required irradiation doses were obtained by adjusting the electron beam parameters (electron beam current, conveyor speed, etc.). Double side irradiation (exposure to both sides) was performed for uniform dose delivery. The dose was determined with cellulose triacetate films. Similarly, packed seed samples without irradiation served as control. Irradiated samples evenly placed in a sifter drying in a constant temperature oven with air velocity at 0.5±0.1 m sec^{-1} and temperature at 40°C. The samples were dried until it reached a final moisture content of 14.5±0.02% (dry base) which represented the safe moisture value for grain storage.

Starch isolation: The seeds were washed thoroughly, peeled and sliced into 2 mm thick slices using a rotary slicer and the slices were kept immersed in water containing 0.5% potassium meta-bisulphite to avoid browning. Defective slices were removed. The slices were ground thoroughly in a laboratory scale grinder to get fine slurry. The slurry was filtered through a muslin cloth and the residue on the muslin cloth washed repeatedly to recover starch. The filtrate was collected in a glass jar and left overnight for the starch to settle down. The supernatant liquid was decanted and the starch layer was washed repeatedly (4-5 times) with distilled water until the supernatant became clear. The starch cake was dried in a hot-air oven at 40°C until dry. The dried starch was ground to a fine powder and kept in an airtight container at room temperature.

Chick bioassay: A total of 96 male, Ross strain broilers were selected. They were housed in pairs within 10 g in weight (at 13 days) of each other. Broilers were allotted to cages in groups of 6. Cages were 37 cm wide by 42 cm tall by 30 cm deep, contained a roost and were wire bottomed with provision for collection of excreta. Prior to the adaptation and trial period chicks were fed Chick Starter Crumb (Dodson and Horrell Ltd. Northamptonshire, UK: AME, 11.7 MJ kg^{-1}; the following in g kg^{-1}; CP, 190; Oil, 33; Fibre, 33 g; Ash, 51; Ca, 9; available P, 4.5; Lysine, 10). At day 19 the birds began an adaptation period where they were fed the assigned trial diet (Table 1). The trial period then took place between days 23 and 27, a total of 96 h. During this time, feed intake was measured and excreta collected. At all times, feed and water were provided on an *ad libitum* basis. During the trial period, temperature was maintained at 21°C and the birds were

Table 1: Experimental diets composition

Components	Amount (g/kg diet)
Bitter vetch	108.00
Wheat	700.00
Maize	60.00
Soya	110.00
Calcium phosphate	3.30
Vitamin and mineral premix[a]	12.50
Lysine	2.50
L threonine	1.20
Salt	2.50
GE (MJ kg^{-1})	17.86

[a]Content per g of premix: 0.1 g phosphorus, 0.017 g magnesium, 0.152 g calcium, 0.030 g sodium, 150 IU vitamin A, 30 IU vitamin D3, 0.2 IU vitamin E (as α-tocopherol acetate), 0.012 mg copper (as copper sulphate), 3.2 μg selenium (as selenium BCP)

kept under artificial light for 23 h day^{-1} with 1 h of dark. The air in the metabolism room was continuously circulated and humidity monitored.

Chemical composition: Moisture content was determined from the mass of samples before and after they were stored overnight in an oven at 105°C (Methods 925.09; AOAC, 1995). Nitrogen was determined by using a Dosimat-776 Metrohm apparatus (Metrohm Co., Switzerland) according to AOAC (Method 984.13; AOAC, 1995). The instrument was calibrated each time with ammonium sulphate as a nitrogen standard. Starch contents were determined on a spectrophotometer at 510 nm after extraction with boiling water. Fat content was determined with a Solvent Extractor (Behr Labour-Technik, Dusseldorf, Germany) equipped with six Soxhlet posts. The ether extract was determined according to the method 920.39 (AOAC, 1995). Ash was determined by burning duplicate 2 g samples at 540°C for 3 h in a muffle furnace (Method 942.05; AOAC, 1995). Crude fiber was determined by treating an oil-free sample by sulphuric acid (0.26 N) and potassium hydroxide (0.23 N) solution using an automatic fiber analyzer (Velp Scientifica, Milan, Italy) followed by oven drying and muffle furnace incineration (AOAC, 1995). Gross energy of grain and excreta samples were determined by adiabatic bomb calorimeter using Parr-4 Model 1241 Calorimeter. The True protein of the samples was quantitatively estimated following the Method of Bradford (1976). The protein contents of the samples were calculated using a calibration curve obtained for bovine serum albumin standards (0-1.5 mg) treated in the same way. Two extractions were carried the per sub-sample and each sample was analyzed in duplicate.

Antinutritional features

Phenolics and tannins: Total phenolics of the seed flours were assayed by adapting the method outlined by Porter *et al*. (1986). A known amount of the seed flour was extracted twice with methanol (50%, 5 mL) in a water bath (95°C, 10 min). The pooled extract was made up to 10 mL, the extract (0.5 mL) was mixed with an equal quantity of distilled water and treated with 5 mL Na_2CO_3 (in 0.1 N NaOH). After 10 min, 0.5 mL Folin-Ciocalteu's reagent (diluted 1:1 with distilled water) was added and the color developed was read at 725 nm. The phenolics determined were expressed as Gallic Acid Equivalents (GAE).

The Vanillin-HCl Method was adapted to determine tannins in the seed flours (Porter *et al*., 1986). A known amount of the seed flour (1 g) was extracted with methanol (10 mL, 28°C, 12 h), vortexed and decanted. This process was repeated and the supernatant was pooled and made up to 25 mL. The extract (1 mL) was treated with reagent mixture (5 mL) (4% vanillin in methanol and 8% concentrated HCl in methanol, 1:1). After 20 min, the color developed was read at 500 nm (Spectronic 21, Miltonroy, USA) using catechin (50-250 μg) as standard. Condensed tannins were determined by butanol-HCl-Fe^{+} reagent (Porter *et al*., 1986). Condensed tannins were expressed as leucocyanidin equivalents.

Determination of canavanine

Preparation of Pentacyanoammonioferrate (PCAF) reagent: Sodium Pentacyanoammonioferrate (PCAF) was prepared by a procedure described by Cacho *et al*. (1989) as follows. Total 10 g of sodiumnitroprusside 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 pentacyano ammonioferrate (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 color to brownish green.

Preparation of *V. ervilia* samples: The 2 g of a finely ground sample of *V. ervilia* 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.

Determination of canavanine: About 1 mL 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 0.005-0.08 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.

Trypsin inhibitor analysis: Trypsin inhibitor activity was determined essentially according to Smith *et al.* (1980). 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.

Englyst classification of starch: The digestibility of starch was analyzed according to the procedure of Englyst *et al.* (1992) with a slight modification. To prepare enzyme solution I, amyloglucosidase solution (0.14 mL) was diluted to 6.0 mL with deionized water. Enzyme solution II was prepared by suspending porcine pancreatic α-amylase (12.0 g) in water (80.0 mL) with magnetic stirring for 10 min, centrifuging the mixture for 10 min at 1500×g and then transferring a portion (54.0 mL) of the supernatant into a beaker. Enzyme III was prepared immediately before use by mixing water (4.0 mL), enzyme solution I (6.0 mL) and enzyme solution II (54.0 mL). A starch sample (200 mg) was dissolved in phosphate buffer (15 mL, 0.2 mol L^{-1} and pH 5.2) by vortexing. After equilibrated at 37°C for 5 min, seven glass balls (10 mm diameter) and enzyme solution III (5.0 mL) were then added followed by incubation in a water bath at 37°C with shaking (150 rpm). Aliquots of hydrolyzed solution (0.5 mL) were taken at different time intervals and mixed with 4 mL of absolute ethanol to deactivate the enzymes. The glucose content of the hydrolyzates was determined using glucose oxidase/peroxidase assay kits. Percentage of hydrolyzed starch was calculated by multiplying a factor of 0.9 with the glucose content. Each sample was analyzed in triplicate. The values of different starch fractions of RDS, SDS and RS were obtained by combining the values of G20 (glucose released after 20 min), G120 (glucose released after 120 min), FG (free glucose) and TG (total glucose) and using the following equations:

$$RDS\ (\%) = (G120-FG)\times 0.9\times 100$$

$$SDS\ (\%) = (G120-G20)\times 0.9\times 100$$

$$RS\ (\%) = (TG-FG)\times 0.9\times 100-(RDS+SDS)$$

***In vitro* digestibility:** Enzymatic digestibility by α-amylase was investigated for non-irradiated and irradiated *V. ervilia* using the method described by Zhang *et al.* (1995) with some modifications. Starch (1 g, dry basis) was mixed with $KHPO_4/K_2PO_4$ buffer (40 mL, 0.2 M, pH 6.9) in a test tube. The mixture was heated in a temperature regulated water bath at 90°C for 40 min. It was cooled to 25°C and 320 units of bacterial α-amylase Bacillus licheniformis (2 units mg^{-1}, Fluka) were added. Five replicate preparations were made for each sample, in order to monitor enzymatic digestibility with time. The tubes were placed in water bath and they were incubated at 30°C between 10 and 26 h. H_2SO_4 (1.0%, w/v, 5 mL) was added to stop the enzymatic digestion. Samples were then centrifuged at 11,000 rpm for 15 min. The residue was washed with ethanol (50 mL, 85%) and it was centrifuged again. The resulting residue was scooped out, oven dried at 100°C to a constant weight. In each case, a blank starch without enzymatic hydrolysis was included to correct for initial concentration of soluble sugars. Starch digestibility was expressed as percent weight loss after α-amylase digestion.

***In vivo* digestibility:** Broilers were used in this study as a model for determining dry matter, starch, crude protein, true protein and gross energy digestibilities of untreated and irradiated samples. The experimental diets were given to their respective. The experiment was carried out with 3 days adaptation period, 2 days starvation for depleting digestive tract then 1 day feeding and following 2 days starvation for complete excretion of undigested material (Shawrang *et al*., 2011). The samples of dropping avoided during final 72 h period were collected, weighted and frozen (-18°C). Analyses of dry matter, starch, crude protein, true protein and gross energy of untreated and treated samples were conducted and calculations were carried out.

Carboxyl content: Carboxyl content was determined as per the procedure of Mattisson and Legendre. To 0.5-1.0 g of starch, 25 mL, 0.1 mol equi L^{-1} HCI was added and the mixture was allowed to stand for 30 min with occasional stirring. The slurry was filtered through a fritted glass crucible and washed with distilled water until it was free from chlorine. The starch was then transferred to a 500 mL beaker to which 300 mL distilled water was added. It was then boiled for 5-10 min for complete gelatinization followed by titration with 0.1 mol equi L^{-1} NaOH solutions with phenolphthalein as indicator. A blank test was also performed with unmodified starch. Carboxyl content was calculated as follows:

$$\text{Milli-Eq. of acidity/100 g starch} = \frac{(A-B)\times 0.1 \text{ mol equil(NaOH)}}{W}\times 100$$

Where:
A = Titer value for sample
B = Titer value for blank
W = Weight of dry sample in grams

$$\text{Apparent percent carboxyl} = \text{Milli-equivalents of acidity/100 g starch}\times 0.045$$

Viscosity determination: For the viscosity measurements of the *V. ervilia* seeds, 1 g of the ground sample were 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×g, 10 min on a Rheometer at 25°C using a cone and plate device with 50 mm diameter and 0.0398 rad cone angle in the shear rate range from 0.25-1000 sec^{-1} (ARES, Rheometric Scientific, USA). The viscosity ($\eta 0$) of the raw and irradiated *V. ervilia* samples was determined in the Newtonian region, independent of shear rate, in the accessible shear rate range from 0.25-1000 sec^{-1}.

Statistical analysis Treatments were analyzed as a completely randomized design under the general model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:
Y_{ij} = The dependent variable
μ = The general mean
T_i = The treatment i = 1-3
e_{ij} = The experimental error

calculated using the GLM procedure of the SAS Software. The broilers were the experimental units for all analyses. Treatment means were compared using the Duncan Method, an α-value of 0.05 was used to assess significance and orthogonal polynomial contrast were performed to find a linear or quadratic response.

RESULTS AND DISCUSSION

Proximal features: EB-irradiation caused significant loss of moisture of raw *V. ervilia* seeds ($p<0.05$) (Table 2). Low moisture content will be advantageous in maintenance and improvement of shelf life. The high protein content in *V. ervilia* seeds emphasizes their value as a vital source of nutrients. EB-irradiation significantly decreased the crude protein and crude fiber of seeds. It would be interesting to determine total, soluble and insoluble dietary fiber fractions in raw and EB-irradiated *V. ervilia* seeds, to gain a better insight into the fiber contents. Fiber levels reduced in direct proportion to the level of irradiation that may be due to depolymerisation of fiber. It appears that radiation resulted in random depolymerisation and decomposition of cellulose and seriously weakens the cellulosic fiber. The quantity of ash in any seed sample assumes importance, as it determines the nutritionally important minerals. *V. ervilia* seeds contained a high amount of carbohydrates which might be due to low lipid content. The increase in carbohydrates might be attributed to radiation-induced breakdown of complex sugars (polysaccharides) into simple extractable forms (e.g., free sugars) (Gopalan *et al*., 1989).

Table 2: Chemical composition of irradiated *V. ervilia* seed (as g/100 g dry matter)

Irradiation Dose (kGy)	Moisture	Ash	Crude protein	Ether extract	Crude fiber	NFE
Control	6.070^{a}	5.950^{c}	22.800^{a}	3.020^{a}	5.230^{a}	56.93^{c}
10	5.220^{b}	6.750^{a}	19.600^{c}	2.120^{b}	4.280^{b}	62.03^{b}
20	5.260^{b}	6.370^{b}	20.400^{b}	2.060^{b}	4.000^{c}	61.91^{b}
30	5.270^{b}	6.490^{b}	19.300^{c}	2.110^{b}	3.860^{c}	62.97^{a}
SEM	0.005	0.001	0.005	0.001	0.001	0.01

Table 3: Effect of radiation on the tannin concentration, canavanine (mg/100 g DM) and trypsin inhibitor (mg/g DM) in the *V. ervilia* seeds

Irradiation dose (kGy)	Total phenols	Tannins	Condensed tannins	Canavanine	Trypsin inhibitor
Control	202.0^{a}	188.0^{a}	230.0^{a}	78.00^{a}	2.030^{a}
10	96.0^{b}	155.0^{b}	180.0^{b}	77.10^{a}	1.640^{b}
20	68.0^{c}	128.0^{c}	142.0^{c}	76.30^{a}	1.040^{c}
30	46.0^{d}	94.0^{d}	94.0^{d}	71.50^{b}	0.340^{d}
SEM	2.5	1.2	1.5	0.35	0.003

$^{a-d}$Values followed by the different superscripts letter within a column differ significantly ($p<0.05$) from each other; SEM: Standard Error of the Means

Anti-nutritional features

Phenolics, tannins and condensed tannins: Total phenolics, tannins and condensed tannins of EB-irradiated *V. ervilia* seeds revealed a significant dose-dependent decrease ($p<0.05$) compared to the control (Table 3). About 18, 32 and 50% of the tannin content of *V. ervilia* was reduced at irradiation dose levels of 10, 20 and 30 kGy, respectively. Reduction of phenolics in *V. ervilia* was 52, 66 and 77%, respectively. These reductions for condensed tannins were 22, 38 and

59%, respectively. Although, the effects of EB and gamma irradiation on phenolics and tannin contents have been reported, there is no information available in literature on the effect of ionizing irradiation on tannin contents of *V. ervilia*. Reduction in the tannin contents is very favorable, once this anti-nutritional factor presents the capacity of decreasing the protein digestibility. When this anti-nutritional factor is found at the proportion of 5:1 tannin/protein, all protein is precipitated due to the tannin action. Reduction of phenolics and tannin by EB-irradiation in the present study is consistent with some earlier studies (Shawrang *et al*., 2011).

Canavanine: The potent anti-metabolic 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. 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 (Siddhuraju *et al*., 2002). There was significant change in the canavanine content of the raw *V. ervilia* seeds following the irradiation treatments. Based on the above observations, it may be concluded that the toxic amino acid concentration could be reduced by irradiation (Table 3).

Trypsin inhibitor activity: EB-irradiation had a substantial effect on the antitrypsin activity naturally present in *V. ervilia* (Table 3). Controls presented the highest values followed by doses of 10 and 20 kGy and dose of 30 kGy presented the lowest value. Trypsin inhibitor may decrease crude protein digestibility of feed particularly in mono-gastric animals and can depress their growth. Reduction of trypsin inhibitor activity by irradiation was proportional to the dose. Only 50-60% of reduction on the trypsin inhibitory activity is required to avoid pancreatic hypertrophy in rats and the inactivation of 70-80% resulted in a maximum value of Protein Efficiency Rate (PER) of diet containing trypsin inhibitor activity (Siddhuraju *et al*., 2002). In the present research, radiation with dose of 10 kGy promoted reduction of 19.21% in average on the trypsin inhibitory activity, dose of 20 kGy reduced 48.76% and 30 kGy reduced 83.25%. Published studies about the effects of EB-irradiation on the trypsin inhibitor activity of *V. ervilia* are scarce but studies on other legume seeds showed that the reduction in antitrypsin activity is due to the breakage of the trypsin inhibitor structure by irradiation so that inactivation of trypsin inhibitor in irradiated samples could be attributed to the destruction of disulphide (-S-S-) groups (Siddhuraju *et al*., 2002). Researchers observed that sulfhydryl (-SH) and disulphide (-S-S-) groups in proteins are apparently highly susceptible to irradiation.

Englyst classification of starch: RDS and SDS content of *V. ervilia* starch were decreased with increasing irradiation dose (Table 4). It was assumed that the proportion of SDS might be partially transformed to RS, since the RS content was increased by irradiation. Only a few legume starches have been analyzed for their RDS, SDS and RS contents and no data were found by researchers regarding the effect of irradiation on the Englyst classification of *V. ervilia*. Furthermore, due to the different methods used for this analysis, it is difficult to make a meaningful comparison of the levels of RDS, SDS and RS among legume starches. SDS and RS levels have been shown to be influenced by factors such as amylose content, crystallinity and amylopectin structure (Chung and Liu, 2009). The results are in accordance with other reports which observed a reduction in starch digestibility (Chung and Liu, 2009, 2010; Rombo *et al*., 2004). Rombo *et al*. (2004) and Chung and Liu (2009) suggested that an increase in the proportion of β-bonded and carboxyl groups starch after irradiation as a result of transglucosidation might result in inhibition of enzyme attack and induce decreased starch digestibility. This assumption might be supported by the present study in which the content of carboxyl groups was considerably increased by irradiation of *V. ervilia* starches.

The increase of RS by EB-irradiation of *V. ervilia* starches in the present study may indicate that changes in molecular structure such as the production of β-bonded starch, the increase in carboxyl groups and the formation of physically less accessible packed structure occurred which might lead to decreased starch hydrolysis. The susceptibilities of legume starches towards hydrolysis by α-amylase reported in the literature cannot be compared due to differences in α-amylase source (bacterial, fungal, pancreatic), enzyme concentration, time of hydrolysis and enzyme purity. From the present and earlier studies, it could be suggested that irradiation increased the proportion of RS content and decreased the proportion of

Table 4: The amounts of Rapidly Digestible Starch (RDS), Slowly Digestible Starch (SDS) and Resistant Starch (RS) of EB-irradiated bitter vetch seed starches

Irradiation dose (kGy)	RDS (%)	SDS (%)	RS (%)
Control	10.30^{a}	34.60^{a}	55.10^{d}
10	8.50^{b}	31.80^{b}	59.70^{c}
20	7.90^{bc}	27.60^{c}	64.50^{b}
30	6.80^{c}	24.30^{d}	68.90^{a}
SEM	0.08	0.11	0.08

$^{a-d}$Values followed by the different superscripts letter within a column differ significantly ($p<0.05$) from each other; SEM: Standard Error of the Means

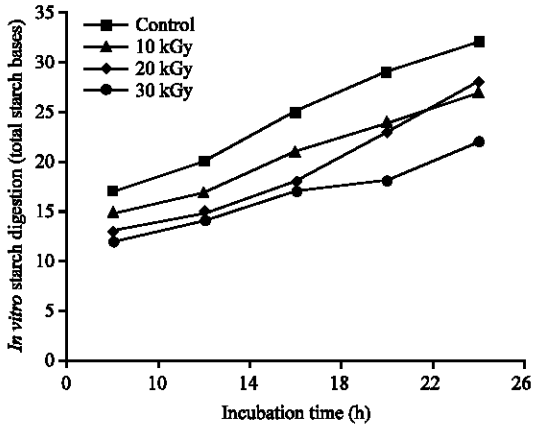

Fig. 1: Time course of *in vitro* starch digestion (proportion of total starch) of *V. ervilia* seed

Table 5: Effects of electron beam irradiation on bitter vetch seed *in vivo* digestibility

Irradiation dose (kGy)	*In vivo* digestibility (%)				
	Starch	Dry matter	Gross energy	Crude protein	True protein
Control	43.50^{a}	61.42^{d}	37.80^{c}	76.22	81.43^{d}
10	42.20^{b}	63.50^{c}	38.40^{c}	76.98	83.83^{c}
20	39.70^{c}	64.70^{b}	40.60^{b}	79.20	86.30^{b}
30	37.60^{d}	67.30^{a}	44.50^{a}	82.60	90.40^{a}
SEM	0.07	0.04	0.04	0.04	0.03

$^{a-d}$Values followed by the different superscripts letter within a column differ significantly ($p<0.05$) from each other; SEM: Standard Error of the Means

SDS content, regardless of crystalline type but no consistent trends in the proportion of RDS were observed.

***In vitro* starch digestibility:** The results indicate pronounced decreases in enzymatic digestibility as the level of irradiation dose increased (Fig. 1). On the other hand, enzymatic digestibility increased progressively as the period of incubation increased. As mentioned earlier, a reasonable explanation to substantiate decreases in enzymatic digestibility is the increase of β-bonded starch after irradiation as a result of transglucosidation. Result obtained from this method is supported by Englyst *et al.* (1992) Method and *in vivo* starch digestibility results achieved from this study.

Effects on *in vivo* digestibility: The results of *in vivo* digestibility of untreated and irradiated *V. ervilia* grains are shown in Table 5. With increase in doses, digestibility of dry matter, crude protein, true protein and gross energy increased significantly compared to control but digestibility of starch decreased significantly. No data were found by researchers regarding the effect of irradiation on *in vivo* digestibility of *V. ervilia.*

Duodu *et al.* (2003) reported that tannins have a detrimental effect on the ileal digestibility of proteins. Due to their hydroxyl groups, tannins may interact with and form complexes with proteins which may lead to precipitation because of the large size of the tannins. In addition to possibly causing a change in protein conformation, study of Siddhuraju *et al.* (2002) showed that the tannins may also exert steric effects (due to their large size) and prevent enzymes access to the proteins. Therefore, in this study it seems that the partial removal of tannin probably created a large space within the matrix which increased the susceptibility to enzymatic attack and consequently improved the digestibility of protein after irradiation treatment. The apparent *in vivo* digestibility of protein data obtained from this experiment indicated a beneficial effect for radiation when the *in vivo* digestibility of the studied legume seed was considered. Another possible reason for increasing in protein digestibility is modification in the three dimensional structure of proteins due to irradiation. Studies of Shawrang *et al.* (2008) illustrated that protein denaturation occured by irradiation that leads to improvement in intestinal protein digestion. Existence of Non Starch Polysaccharides (NSPs) in seeds causes the intestinal contents to become viscous and interfere with nutrient assimilation and the general well-being (Yoon *et al.*, 2010). Researchers showed that irradiation of seeds containing NSPs, fed to chicks improved the apparent absorption of fat, amino acids and starch. It has been suggested that this increase was induced by structural degradations in NSPs which allowed easy access of the digestive enzymes to starch (Yoon *et al.*, 2010). Results obtained from this study regarding Viscosity of *V. ervilia* seeds confirm this claim that irradiation causes reduction of NSPs and consequently decrease of Viscosity. In contrast, results achieved from *in vivo* and *in vitro* digestibility of *V. ervilia* showed that starch digestibility decreased by EB-irradiation. As mentioned above, results obtained from some *in vitro* studies suggested that an increase in the proportion of β-bonded starch after irradiation as a result of transglucosidation might induce decreased starch digestibility.

Chung and Liu (2009) claimed that the increase in carboxyl groups by irradiation resulted in inhibition of enzyme attack. This assumption might be supported by the present study in which the content of carboxyl groups was considerably increased by irradiation of *V. ervilia* starches. From compare of results achieved regarding viscosity, *in vitro* and *in vivo* digestibility of *V. ervilia* starch, it could be concluded that the effect of EB-irradiation on starch granules was stronger than effects on NSPs that finally caused in decrease of starch digestibility.

Table 6: Carboxyl content and viscosity of *V. ervilia* seeds

Irradiation dose (kGy)	Carboxyl content (g/100 g)	Viscosity (mPa.s)
Control	0.000[d]	7.72[a]
10	0.060[c]	6.28[b]
20	0.090[b]	4.36[c]
30	0.130[a]	3.85[d]
SEM	0.001	0.01

[a-d]Values followed by the different superscripts letter within a column differ significantly ($p<0.05$) from each other; SEM: Standard Error of the Means

Carboxyl content: The carboxyl content increased as the irradiation dose was increased in *V. ervilia* starches (Table 6). The radiation degradation of starch is initiated by the generation and transformation of free radicals and follows the low-molecular products with the number of carboxylic acids and aldehydes (Sharpatyi, 2003). Therefore, the main degradation products formed during irradiation of native starch were carboxylic acids which resulted in an increase in carboxyl content of all starch samples. Similar findings were reported by Chung and Liu (2009) with irradiated bean starch.

Viscosity: EB-irradiation at 10, 20 and 30 kGy significantly reduced the viscosity of *V. ervilia* when compared with the control samples (Table 6). The high viscosity in *V. ervilia* is due to the presence of NSPs, i.e., galactomannan and it has also been reported to interfere in the nutrient metabolism of mono-gastrics (Yoon *et al*., 2010).

As above mentioned, these carbohydrate polymers cause the intestinal contents to become viscous and interfere with nutrient assimilation and general well-being and depolymerisation of such non-starch polysaccharides by irradiation significantly improves the growth parameters in chicks. 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 utilization studies through *in vivo* approaches are needed on *V. ervilia* samples.

CONCLUSION

Irradiation processing has been used as a means to inactivate anti-nutritional factors and increase of nutritional quality of *V. ervilia* seeds. The present research clearly shows that irradiation processing of *V. ervilia* at dose levels of 10, 20 and 30 kGy can improve its nutritional quality. Maximum improvement in protein quality (i.e., *in vivo* protein digestibility) was observed at the higher radiation (30 kGy). EB-irradiation decreased tannin, canavanine, trypsin inhibitor activity and starch digestibility (approximately 50, 9, 83 and 13% reduction, respectively) of *V. ervilia* seeds. EB-irradiation altered the physicochemical properties including an increase in carboxyl content and a decrease in viscosity. Further studies are needed to evaluate the definite effect of EB-radiation on anti-nutritional factors using pure molecules. Unlike chemical treatments which are time consuming, irradiation can be a quick and efficient method for modifying the properties of different seeds.

NOMENCLATURE

EB-irradiation = Electron Beam irradiation
RDS = Rapidly Digestible Starch
SDS = Slowly Digestible Starch
RS = Resistant Starch

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