



## Effects of Gamma Irradiation on Chemical Composition, Antinutritional Factors, Ruminal Degradation and *In vitro* Protein Digestibility of Full-fat Soybean\*

M. Taghinejad\*\*, A. Nikkhah, A. A. Sadeghi, G. Raisali<sup>1</sup> and M. Chamani

Department of Animal Science, Faculty of Agriculture, Science and Research Branch  
Islamic Azad University, P.O. Box 14515-4933, Tehran, Iran

**ABSTRACT :** The aim of this study was to evaluate the effects of gamma irradiation ( $\gamma$ -irradiation) at doses of 15, 30 and 45 kGy on chemical composition, anti-nutritional factors, ruminal dry matter (DM) and crude protein (CP) degradability, *in vitro* CP digestibility and to monitor the fate of true proteins of full-fat soybean (SB) in the rumen. Nylon bags of untreated or  $\gamma$ -irradiated SB were suspended in the rumens of three ruminally-fistulated bulls for up to 48 h and resulting data were fitted to a nonlinear degradation model to calculate degradation parameters of DM and CP. Proteins of untreated and treated SB bag residues were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Digestibility of rumen undegraded CP was estimated using the three-step *in vitro* procedure. The chemical composition of raw and irradiated soybeans was similar. Results showed that phytic acid in  $\gamma$ -irradiated SB at dose of 30 kGy was eliminated completely. The trypsin inhibitor activity of 15, 30 and 45 kGy  $\gamma$ -irradiated SB was decreased ( $p < 0.01$ ) by 18.4, 55.5 and 63.5%, respectively. From *in sacco* results,  $\gamma$ -irradiation decreased ( $p < 0.05$ ) the washout fractions of DM and CP at doses of 30 and 45 kGy, but increased ( $p < 0.05$ ) the potentially degradable fractions. Gamma irradiation at doses of 15, 30 and 45 kGy decreased ( $p < 0.05$ ) effective degradability of CP at a rumen outflow rate of  $0.05 \text{ h}^{-1}$  by 4.4, 14.4 and 26.5%, respectively. On the contrary, digestibility of ruminally undegraded CP of irradiated SB at doses of 30 and 45 kGy was improved ( $p < 0.05$ ) by 12 and 28%, respectively. Electrophoretic analysis of untreated soybean proteins incubated in the rumen revealed that  $\beta$ -conglycinin subunits had disappeared at 2 h of incubation time, whereas the subunits of glycinin were more resistant to degradation until 16 h of incubation. From the SDS-PAGE patterns, acidic subunits of 15, 30 and 45 kGy  $\gamma$ -irradiated SB disappeared after 8, 8 and 16 h of incubation, respectively, while the basic subunits of glycinin were not degraded completely until 24, 48 and 48 h of incubation, respectively. It was concluded that  $\gamma$ -irradiated soybean proteins at doses higher than 15 kGy could be effectively protected from ruminal degradation. (**Key Words :** Full-fat Soybean, Gamma Irradiation, Protein Degradability, SDS-PAGE)

### INTRODUCTION

Incorporating soybeans and its byproducts into rations for dairy cattle is a fairly common practice. They are excellent sources of essential amino acids and they fit into any type of forage-based ration. Whole soybean is used as a high energy-protein supplement for dairy cows, but the

protein is highly degradable by rumen microbes (Krishnamoorthy et al., 1982). Several processing methods have been reported to enhance nutritive value of whole oilseeds including extrusion, roasting, toasting and Jet-Sploding (Arieli, 1998; Wang et al., 1999; Chen et al., 2008). Roasting and extrusion are very popular ways of feeding soybeans to dairy cows but these processing methods may adversely affect the protein digestibility and lysine availability of the final product in the small intestine (Scott et al., 1991). On the other hand, raw soybeans should be avoided in rations for calves of less than 4 months of age since raw soybeans contain various anti-nutritional factors, including trypsin inhibitor and phytic acid, which interfere with the utilization of protein and mineral in the digestive tract (Siddhuraju et al., 2002), thereby reducing growth.

Food irradiation is a process of exposing food to

\* This manuscript was obtained from PhD dissertation of first author conducted in Islamic Azad University-Science and Research Branch.

\*\* Corresponding Author: M. Taghinejad. Tel: +98-914-1010511, Fax: +98-411-6373935, E-mail: taghinejad\_mehdi@yahoo.com

<sup>1</sup> Radiation Applications Research School, Nuclear Science and Technology Research Institute, Atomic Energy Organization of Iran, P.O. Box 11365-3486, Tehran, Iran.

Received October 3, 2008; Accepted January 7, 2009

ionizing radiations, such as gamma rays emitted from radioisotopes  $^{60}\text{Co}$  and  $^{137}\text{Cs}$ , or high energy electrons and X-rays produced by machine sources (Diehl, 2002). Gamma irradiation has been recognized as a reliable and safe method for improving the nutritional value and inactivation or removal of certain anti-nutritional factors in foods and feeds (Siddhuraju et al., 2002; Farkas, 2006). In 1981, the US Food and Drug Administration (FDA) concluded that food irradiated at 10 kGy or less can be considered safe for human consumption (FDA, 1981). Gharaghani et al. (2008) reported that gamma irradiation seems to be a good procedure to improve the nutritional quality of canola meal for broiler chickens. Recently, treatment of soybean meal and canola meal with gamma irradiation was successful in reducing degradation of protein by rumen microorganisms and increasing protein intestinal digestibility (Shawrang et al., 2007, 2008).

As far as we know, there is scarcely any information in the literature about the effects of  $\gamma$ -irradiation on ruminal crude protein (CP) degradation and the type of true proteins of full-fat soybean that leave the rumen. Therefore we conducted this study in order to elucidate effects of various doses of  $\gamma$ -irradiation on chemical and anti-nutritional contents, protein degradability, intestinal digestibility of full-fat soybean, and to monitor the fate of true proteins of  $\gamma$ -irradiated soy bean in the rumen.

## MATERIALS AND METHODS

### Sample preparation and irradiation treatments

One of the most commonly used cultivars of soybean (SB) in Iran, Sahar, was provided by the Oilseed Developing and Cultivation Company (Tehran, Iran). The whole full-fat soybean seeds used in this study assayed at 910 g DM/kg. This value was determined by oven drying a 1g sample in duplicate prior to processing. The moisture content of 2 kg soybean seeds was increased by adding 160 g/kg (w/w) distilled water to 250 g/kg. The whole soybean seeds were equilibrated with the water overnight via stirring and tumbling to ensure uniform moisture absorption. Then these seeds were divided into four equal portions and placed in paper packages. Three paper packages of samples were irradiated in a gamma cell for total doses of 15, 30 and 45 kGy in the presence of air. One package (control) was left at a room temperature similar to the others. Evaporation decreased moisture content of samples (in paper packages) during the 35 h irradiation period and by leaving samples at room temperature. After completing the 45 kGy irradiation (final sample) and prior to sealing samples in plastic bags, all samples were spread in trays and allowed to air-equilibrate for 2 h. Gamma irradiation was carried out in the Radiation Applications Research School, Atomic Energy

Organization of Iran by a Gammacell 220 (AECL, 1984) research irradiator at room temperature. The dose rate determined by Fricke dosimetry was 0.36 Gy/s (Holm and Berry, 1970).

### Animals and diet

Three ruminally-fistulated bulls (416±18 kg) were used. The bulls were fed 8 kg of DM as a total mixed ration containing 700 g/kg of DM forage (70% alfalfa hay and 30% wheat straw) and 300 g/kg of DM concentrate. The concentrate consisted of ground barley, wheat bran, whole cottonseed, soybean meal, dicalcium phosphate and a vitamin+mineral premix (350, 200, 250, 170, 10 and 20 g/kg DM, respectively). Water and salt lick were available *ad libitum*. The diet was formulated according to guidelines for beef cattle by the National Research Council (NRC, 1996) to contain 144 g CP/kg of DM and was fed twice daily at 08:00 and 16:00 h.

### *In situ* ruminal degradability

Nylon bags (10 cm×20 cm; 45  $\mu\text{m}$  pore size) were filled with approximately 6 g sample (size: bag surface area of 15  $\text{mg}/\text{cm}^2$ ). The samples were ground to pass a 2 mm screen according to Nocek (1988). Duplicate bags were filled with untreated or irradiated soybean and incubated in the rumen for 0, 2, 4, 8, 16, 24 and 48 h. All bags were simultaneously placed in the rumen, just before the bulls were offered their first meal in the morning (*i.e.*, 08:00 h). After retrieval from the rumen, bags were thoroughly washed with tap water until the rinsing water was clear. The same procedure was applied to two bags to obtain the 0 h value. The residues were dried and analyzed for DM and CP to determine degradation kinetics of soybean.

### *In vitro* crude protein digestibility

Digestibility of rumen undegraded CP was estimated using the three-step *in vitro* procedure of Calsamiglia and Stern (1995). Samples of the rumen-undegradable fraction collected after the 16 h ruminal incubation period and containing 15 mg nitrogen (N) were incubated for 1 h in 10 ml of 0.1 N HCl solution containing 1 g/L of pepsin. Following incubation, pH was neutralized with 0.5 ml of 1 N NaOH and 13.5 ml of phosphate buffer (pH 7.8) containing 37.5 mg of pancreatin was added. Samples were incubated for 24 h at 38°C, and then undigested protein was precipitated using trichloroacetic acid (3 ml TCA). Afterwards, supernatants of centrifuged samples were collected and analyzed for soluble N. *In vitro* digestibility of protein was calculated as soluble N divided by amount of initial sample N (*i.e.* nylon bag residues).

### Determination of protein subunits

The protein subunits were fractionated by sodium

**Table 1.** Chemical composition and anti-nutritional factors of untreated and irradiated soybeans (n = 6)

Parameters	Untreated soybean	Gamma-irradiated soybean			SEM
		15 kGy	30 kGy	45 kGy	
Dry matter (g/kg)*	912	900	904	903	7.6
Crude protein (g/kg)	393	395	398	400	11.2
Ash (g/kg)	63	63	65	66	13.6
Neutral detergent fiber (g/kg)	212	210	212	213	13.7
Acid detergent fiber (g/kg)	15.2	150	154	155	9.2
Ether extract (g/kg)	187	185	185	184	11.4
Phytic acid (mg/100g)	234 <sup>a</sup>	94.3 <sup>b</sup>	0.0 <sup>c</sup>	0.0 <sup>c</sup>	8.9
Trypsin inhibitor factor (TIU /g)**	32.1 <sup>a</sup>	26.2 <sup>b</sup>	14.3 <sup>c</sup>	11.7 <sup>d</sup>	3.3

\* On dry matter basis. \*\* Trypsin inhibitor unit. <sup>a, b, c, d</sup> Means in the same row with different letters differ (p<0.01).

dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli (1970). Briefly, all of the ruminal undegradable fractions from each incubation period were dried, well ground and replicates were pooled together. After protein extraction as described by Sadeghi et al. (2006), 30 µl of each protein sample was loaded into the sample cell of the gel. Electrophoresis of proteins was performed on a 12% acrylamide running gel (1.0×140×190 mm<sup>3</sup>) with a 3.75% acrylamide stacking gel. Standard protein used for comparison to estimate molecular weights were β-galactosidase (116 kDa), bovine serum albumin (66.0 kDa), ovalbumin (45.0 kDa), lactate dehydrogenase (35.0 kDa), restriction endonuclease Bsp981 (25.0 kDa), β-lactoglobulin (18.4 kDa) and lysozyme (14.4 kDa).

### Chemical analyses

DM content was determined in feed samples and nylon bag residues after during at 55°C for 48 h. Nitrogen in feeds and residues after rumen and *in vitro* incubation was determined according to AOAC (Method 984.13; AOAC, 1995). Ash was determined by burning duplicate 2 g samples at 600°C for 2 h in a muffle furnace (Method 942.05; AOAC, 1995). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to the method of Van Soest et al. (1991), using an automatic fiber analyzer (Fibertec System M, Tecator, Sweden). Standard methods were also used to determine ether extract (AOAC, 1995). Trypsin inhibitor was measured using the method reported by Roy and Bhat (1974). Phytic acid was determined by the methods of de Boland et al. (1975).

### Statistical analyses

Digestion kinetics of DM or CP were determined according to the equation of Ørskov and McDonald (1979) as:

$$P = a + b(1 - e^{-ct})$$

Where, *P* is the amount degraded at a time, *a* is the washout fraction, *b* is the potentially degradable fraction, *c*

is the constant rate of disappearance of *b*, *t* is the time of incubation (h). Effective degradability (ED) was calculated using  $ED = a + bc/(c + k)$  at estimated outflow rates (*k*) of 0.02, 0.05 and 0.08 h<sup>-1</sup>.

Degradability data were analyzed as a completely randomized block design according to the general linear models procedure of SAS (1996) with the following statistical model of  $Y_{ijk} = \mu + T_i + B_j + e_{ijk}$ ; data for chemical composition and anti-nutritional factors were analyzed as a completely randomized design according to the general linear models procedure of SAS (1996) with the following statistical model of  $Y_{ijk} = \mu + T_i + e_{ijk}$ ; where  $Y_{ijk}$  is the dependent variable,  $\mu$  is the overall mean,  $T_i$  is the  $\gamma$ -irradiation effect,  $B_j$  is the animal effect, and  $e_{ijk}$  is the residual error, assumed normally and independently distributed. Least Squares Means were compared for statistical differences.

## RESULTS

### Effects on chemical composition and anti-nutritional factors of soybean

The chemical and anti-nutritional contents of the soybean (SB) are listed in Table 1. Gamma irradiation had no effect on chemical composition, but the phytic acid and trypsin inhibitor activity in  $\gamma$ -irradiated SB decreased (p<0.01) compared to untreated soybean. The trypsin inhibitor activity (TIA) of 15, 30 and 45 kGy  $\gamma$ -irradiated SB was decreased (p<0.01) by 18.4, 55.5 and 63.5%, respectively. Phytic acid in  $\gamma$ -irradiated SB at a dose of 15 kGy decreased (59%), and at doses of 30 and 45 kGy was eliminated completely.

### Effects on DM and CP degradability and *in vitro* digestibility of soybean

Ruminal degradability parameters of DM and CP and intestinal CP digestibility of untreated and  $\gamma$ -irradiated SB are shown in Table 2. Increasing irradiation dose to 30 kGy decreased the washout fractions of DM and CP by 10.9 and 21.6%, and at doses of 45 kGy by 18.8 and 37.6%,

**Table 2.** Rumen degradation parameters of dry matter and crude protein and *in vitro* crude protein digestibility of undegraded protein of untreated and irradiated soybean

Parameters	Untreated soybean	Gamma-irradiated soybean			SEM
		15 kGy	30 kGy	45 kGy	
<b>Dry matter</b>					
<i>a</i> (g/kg)	402 <sup>a</sup>	390. <sup>a</sup>	333 <sup>b</sup>	275 <sup>c</sup>	13.3
<i>b</i> (g/kg)	541 <sup>c</sup>	542 <sup>c</sup>	607 <sup>b</sup>	680 <sup>a</sup>	14.3
<i>a+b</i> (g/kg)	943	932	940	955	8.1
<i>c</i> (h) <sup>-1</sup>	0.091 <sup>a</sup>	0.080 <sup>b</sup>	0.064 <sup>c</sup>	0.054 <sup>d</sup>	0.0022
<b>Effective rumen degradation (g/kg)</b>					
0.02 h <sup>-1</sup>	845 <sup>a</sup>	825 <sup>b</sup>	795 <sup>c</sup>	769 <sup>d</sup>	6.7
0.05 h <sup>-1</sup>	750 <sup>a</sup>	725 <sup>b</sup>	673 <sup>c</sup>	625 <sup>d</sup>	7.9
0.08 h <sup>-1</sup>	689 <sup>a</sup>	662 <sup>a</sup>	603 <sup>b</sup>	547 <sup>c</sup>	9.0
<b>Crude protein</b>					
<i>a</i> (g/kg)	346 <sup>a</sup>	336 <sup>a</sup>	281 <sup>b</sup>	216 <sup>c</sup>	14.5
<i>b</i> (g/kg)	662 <sup>c</sup>	665 <sup>c</sup>	716 <sup>b</sup>	761 <sup>a</sup>	12.0
<i>a+b</i> (g/kg)	1,007 <sup>a</sup>	1,001 <sup>a</sup>	997 <sup>a</sup>	977 <sup>b</sup>	6.10
<i>c</i> (h) <sup>-1</sup>	0.103 <sup>a</sup>	0.081 <sup>b</sup>	0.061 <sup>c</sup>	0.046 <sup>d</sup>	0.0034
<b>Effective rumen degradation (g/kg)</b>					
0.02 h <sup>-1</sup>	900 <sup>a</sup>	870 <sup>b</sup>	823 <sup>c</sup>	747 <sup>d</sup>	3.2
0.05 h <sup>-1</sup>	792 <sup>a</sup>	749 <sup>b</sup>	678 <sup>c</sup>	582 <sup>d</sup>	4.5
0.08 h <sup>-1</sup>	721 <sup>a</sup>	674 <sup>b</sup>	596 <sup>c</sup>	496 <sup>d</sup>	5.4
<b><i>In vitro</i> crude protein digestibility (g/kg)</b>	570 <sup>a</sup>	585 <sup>a</sup>	638 <sup>b</sup>	730 <sup>c</sup>	9.5

<sup>a, b, c, d</sup> Means in the same row with different letters differ (p<0.05).

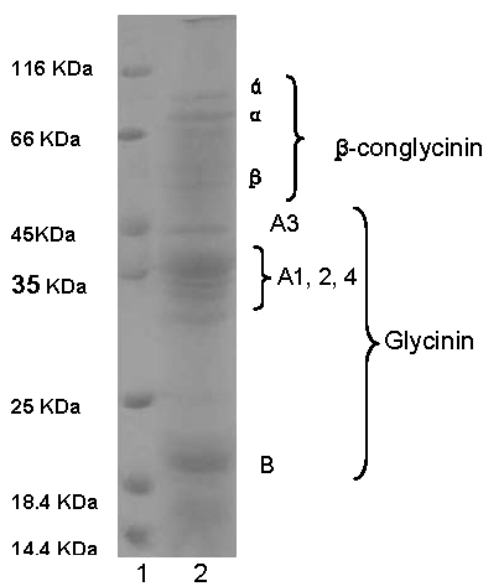
*a*: the washout fraction (g/kg); *b*: the potentially degradable fraction (g/kg); *c*: the rate of degradation.

respectively, compared to untreated soybean (p<0.05). Increasing irradiation dose to 30 kGy increased the *b* fraction of DM and CP by 12.1 and 8.2%, and at doses of 45 kGy by 25.7 and 15%, respectively compared to untreated soybean (p<0.05). At doses of 15, 30 and 45 kGy, degradation rate of the *b* fraction of CP decreased 21, 40 and 55%, respectively (p<0.05). Decrease in degradation

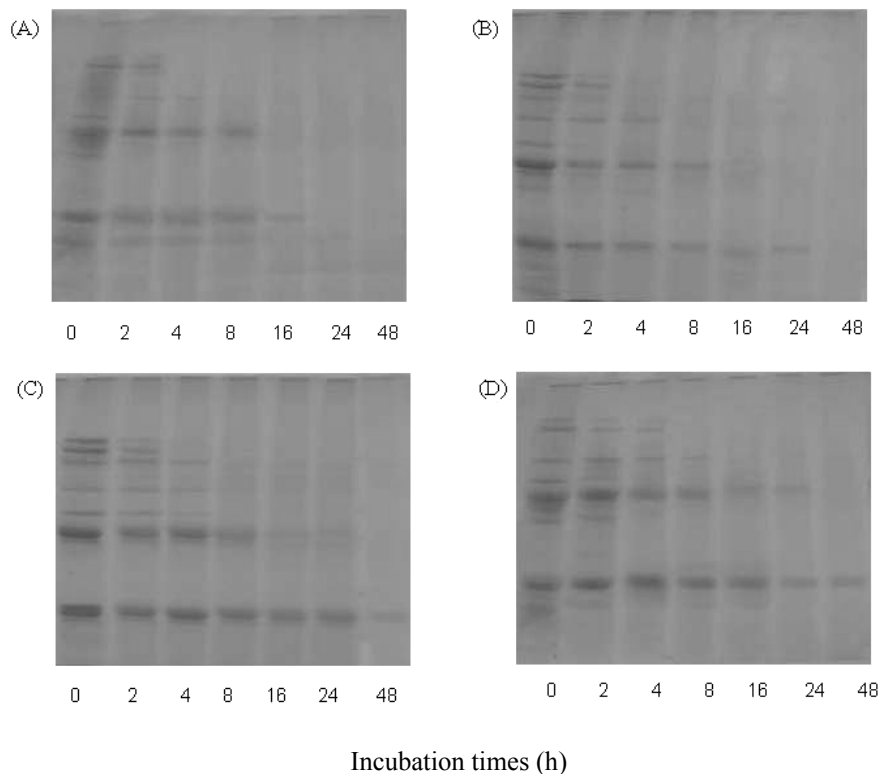
rate of the *b* fraction of DM at doses of 15, 30 and 45 kGy was 12, 29.7 and 40.1%, respectively. Effective degradability (ED) of DM and CP decreased as irradiation dose increased (p<0.05). At irradiation doses of 15, 30 and 45 kGy, ED of CP at rumen a outflow rate of 0.05 h<sup>-1</sup> was decreased by 4.4, 14.4 and 26.5%, and ED of DM was decreased by 3.3, 10.3 and 15.9%, respectively, compared to the untreated sample. Gamma irradiation increased *in vitro* digestibility of CP at doses of 30 and 45 kGy by 12 and 28%, respectively (p<0.05).

**Effects on electrophoretic profiles of soybean proteins**

The SDS-PAGE analysis of soybean protein subunits is presented in Figure 1. Soybean was primarily composed of β-conglycinin (α: 92.4 kDa; α: 78.5 kDa; β: 55.5 kDa) and glycinin (A<sub>3</sub>: 44.1 kDa; A<sub>1,2,4</sub>: 35.7 kDa; B: 20.3 kDa). The electrophoretic patterns of untreated, 15, 30 and 45 kGy-irradiated SB proteins incubated in the rumen are depicted in Figure 2. Electrophoretic analysis of untreated soybean proteins (Figure 2A) incubated in the rumen revealed that β-conglycinin subunits had disappeared after 2 h of incubation time, whereas the subunits of glycinin were more resistant to degradation until 16 h of incubation. From the SDS-PAGE patterns, acidic subunits of 15, 30 and 45 kGy γ-irradiated SB disappeared after 8, 8 and 16 h of incubation, respectively, while the basic subunits of glycinin were not degraded completely until 24, 48 and 48 h of incubation, respectively (Figure 2B, C and D).



**Figure 1.** The molecular weight of standard protein (lane 1) and protein subunits of soybean (lane 2) (A; acidic and B; basic subunit).



**Figure 2.** Electrophoretic patterns of untreated (A), 15 kGy (B), 30 kGy (C) and 45 kGy (D) gamma-irradiated soybean proteins incubated in the rumen. Each lane shows an incubation time (i.e., 0, 2, 4, 6, 8, 16, 24 and 48 h of incubation in the rumen).

## DISCUSSION

### Chemical composition and anti-nutritional content of soybean

Gamma irradiation had no effect on chemical composition of SB. The present findings are in agreement with the data previously obtained on soybeans irradiated up to 60 kGy (Diaa El-Din and Farag, 1998). The main proteins responsible for the low nutritional value of raw soybean are trypsin inhibitors. Trypsin inhibitors (TI) may actually cause an increase in the secretion of digestive enzymes, including trypsin, chymotrypsin and elastase by inducing hypertrophy and hyperplasia of the pancreas in animals, particularly monogastrics, and can depress their growth (Liener et al., 1994). The results observed in this study indicate that the irradiation treatment has a substantial effect on the anti-tryptic activity naturally present in soybeans. Farag (1989) reported that the detoxification dose needed for complete inactivation of all the anti-nutritional factors naturally present in soybeans seemed to be higher than the maximal dose level of 10 kGy recommended by the JECFI in 1980. Results in the current study are consistent with those of Diaa El-Din and Farag (1998) and de Toledo et al. (2007), who studied the effects of various  $\gamma$ -irradiation doses on inactivation of trypsin inhibitor activity (TIA) in

soybean and concluded that as radiation dose increased the TIA decreased. Inactivation of TI in irradiated samples could be attributed to the destruction of sulfhydryl and disulphide (-S-S-) groups that are liable to become damaged by radiation, particularly in the legumes (Siddhuraju, 2002). Therefore, it seems that irradiation treatment of soybean could break down the structure of TI, which will lead to reduction of TIA in the soybean.

Numerous studies have led to the conclusion that phytic acid can bind essential dietary minerals, thus making them unavailable or only partially available for absorption. Phytate chelates with certain metal ions such as calcium, magnesium, zinc, copper and iron, to form insoluble complexes that are not readily broken down and may pass through the digestive tract unchanged (Al-Kaiesy et al., 2003). In the present study,  $\gamma$ -irradiation decreased the phytic acid content of SB and it was completely eliminated at doses of 30 and 45 kGy. Bhat et al. (2007) reported that phytic acid of velvet seed was completely eliminated on exposure to doses of 15 and 30 kGy. This reduction in phytic acid content is probably due to chemical degradation of phytate to the lower inositol phosphates and inositol by the action of free radicals produced by the radiation or cleavage of the phytate ring itself (de Boland et al., 1975; Siddhuraju, 2002).

### Ruminal degradation and intestinal digestibility of irradiated soybean

Gamma irradiation at a dose of 15 kGy did not significantly change the washout and potentially degradable fractions of DM and CP but at doses of 30 and 45 kGy reduced the washout fraction of DM and CP and increased the potentially degradable fraction of DM and CP. Decrease in solubility of protein observed in the current study is in agreement with the results of Lacroix et al. (2002) and Abu et al. (2006) who demonstrated  $\gamma$ -irradiation decreases protein solubility due to denaturation, occurring through cross-linking of chains and protein aggregation. Ham et al. (2009) reported that irradiation of plain yogurt at dose of 10 kGy reduced the allergenicity of milk proteins by structural denaturation and creating changes in their binding ability against allergens.

The degradation rate of the b fraction of DM and CP significantly decreased as irradiation dose increased. Irradiation at doses of 15, 30 and 45 kGy decreased the effective protein degradability of soybean at a ruminal outflow rate of 0.05 h<sup>-1</sup> by 4.4, 14.4 and 26.5%, respectively, compared to untreated soybean. Indeed, bacteria are the principal microorganisms involved in protein degradation in the rumen and most bacterial proteases are associated with the cell surface (Kopečný and Wallace, 1982). Therefore, the initial step in protein degradation by ruminal bacteria is adsorption of soluble proteins to bacteria (Wallace, 1985). Consequently  $\gamma$ -irradiation may have reduced the protein degradability of soybean protein by reducing its solubility. Insoluble nitrogen-containing components may have a special significance for the ruminant because solubility generally (but not necessarily) renders the nitrogen more available for microbial metabolism (Van Soest, 1994).

Results in the present work are consistent with Lee et al. (2005), who tried to use ionizing radiation as a cross-linking agent to improve functional properties of soybean protein films (as biodegradable packaging of foods). Based on a SDS-PAGE study they showed that  $\gamma$ -irradiation below 16 kGy was not effective in formation of high molecular weight aggregates of soybean proteins, but this increased at higher doses. Also, our results were consistent with a study by Shawrang et al. (2007), in which ruminal effective degradability of CP and DM of  $\gamma$ -irradiated soybean meal decreased as irradiation dose increased from 25 to 50 and 75 kGy.

Protein denaturation as a result of  $\gamma$ -irradiation increased *in vitro* CP digestibility of irradiated SB at doses of 30 and 45 kGy by 12 and 28%, respectively. The protein in raw, whole soybeans suffers reduced intestinal digestibility because of trypsin inhibitor (Aldrich et al., 1997). The results observed in this study, indicates that irradiation treatment has a substantial effect on the antitryptic activity naturally present in soybeans. Moreover, Ressouany et al.

(1998) demonstrated that cross-linked proteins are hydrophobic, therefore are compact and could pass to the intestine. Irradiation may induce unfolding of the protein and denaturation, exposing hydrophobic amino acids (especially aromatics) that are positional groups for the active site of pepsin and trypsin enzymes (Murray et al., 2003).

### Electrophoresis patterns of irradiated soybean proteins

Two major protein components in soybean proteins are  $\beta$ -conglycinin and glycinin. Three components of  $\beta$ -conglycinin are  $\alpha$ ,  $\alpha$  and  $\beta$ , and glycinin is composed of an acidic subunit, with sub fractions of A<sub>3</sub> and A<sub>1,2,4</sub>, and a basic subunit. Molecular weights measured in the current study are in agreement with molecular weights of 91.2, 72.1, 55.0, 37.2 and 21.1 kDa subunits which were recently determined in a study by Shawrang et al. (2007).

In untreated SB, subunits of the  $\beta$ -conglycinin disappeared after 2-4 h of incubation, but subunits of the glycinin were resistant to degradation. The resistance of glycinin to ruminal degradation with  $\beta$ -conglycinin is probably associated with its chemical and physical structure and higher intermolecular disulfide bonds that join its basic and acidic polypeptides compared with  $\beta$ -conglycinin (Romagnolo et al., 1990). SDS-PAGE profiles of untreated and  $\gamma$ -irradiated SB at low dose (15 kGy), showed similar disappearance patterns of protein subunits (Figure 2A and B) whereas  $\gamma$ -irradiated SB at doses of 30 and 45 kGy had different disappearance patterns of protein subunits compared to untreated and  $\gamma$ -irradiated SB at dose of 15 kGy (Figure 2C and D). Radiation produces active species such as hydroxyl radicals (OH) and hydrated electrons (e<sub>aq</sub>) through action on the water molecules which in turn react with the protein molecules (Yamamoto, 1992). The chemical changes caused by irradiation in proteins include disruption of the ordered structure of protein molecules as well as degradation, cross-linking, and aggregation of the polypeptide chains due to oxygen radicals (Gaber, 2005). As mentioned above, Lee et al. (2005) demonstrated that  $\gamma$ -irradiation below 16 kGy was not effective in formation of high molecular weight aggregates in soybean proteins. Therefore,  $\gamma$ -irradiation at 15 kGy caused slight breakdown of polypeptide chains and therefore, less formation of high molecular weight protein, but at higher dose (30 and 45 kGy) there were cross-linked products of the degraded protein molecules that could not penetrate the running gel. This result confirmed our current observations on ruminal CP degradability of untreated and  $\gamma$ -irradiated SB that showed  $\gamma$ -irradiation at doses above 15 kGy could significantly decrease the washout fraction and increase the potentially degradable fraction of CP compared to untreated SB (Table 2).

## CONCLUSION

The results show that  $\gamma$ -irradiation of full-fat soybean was effective in decreasing ruminal degradability of its protein and increasing *in vitro* CP digestibility. In addition, the results indicate that  $\gamma$ -irradiation decreased the phytic acid content and trypsin inhibitor activity of full-fat soybean. Consequently, this study suggests that  $\gamma$ -irradiation can be used to improve nutritive value of full-fat soybeans at doses higher than 15 kGy. Further study is needed to determine economic benefits of this processing in comparison with other processing methods.

## ACKNOWLEDGMENT

We would like to express thanks for financial support provided by the Islamic Azad University, Science and Research Branch (Tehran, Iran). Moreover, the authors gratefully acknowledge Dr. P. Shawrang for her helpful comments, Mr. S. R. Ebrahimi and Dr. S. A. Mirhadi for their cooperation, and Mr. Kazemzadeh and Mr. Assareh for laboratory assistance.

## REFERENCES

- Abu, J. O., K. Muller, K. G. Duodu and A. Minnaar. 2006. Gamma irradiation of cowpea (*Vigna unguiculata* K. Walp) flours and pastes: Effects on functional, thermal and molecular properties of isolated protein. *Food Chem.* 95:138-147.
- AECL Technical Publication 1984. Operators and Accessories Manual of Gammacell 220.
- AOAC. 1995. Official methods of analysis, 16th ed. Association of Official Analytical Chemists, Arlington, Virginia.
- Aldrich, C. G., S. Ingram and J. R. Coldfelter. 1997. Assessment of post-uminal amino acid digestibility of roasted and extruded whole soybeans with the precision-fed rooster assay. *J. Anim. Sci.* 75:3046-3051.
- Al-Kaiesy, M. T., H. A. Abdul-Kader, M. H. Mohammad and A. H. Saeed. 2003. Effect of  $\gamma$ -irradiation on antinutritional factors in broad bean. *Radiat Phys. Chem.* 67:493-496.
- Arieli, A., 1998. Whole cottonseed in dairy cattle feeding: a review. *J. Dairy Sci.* 72:97-110.
- Bhat, R., K. R. Sridhar and K. T. Yokotani. 2007. Effect of ionising radiation on antinutritional features of velvet seed bean (*Mucuna pruriens*). *Food Chem.* 103:860-866.
- Calsamiglia, S. and M. D. Stern. 1995. A three-step *in vitro* procedure for estimating intestinal digestion of protein in ruminants. *J. Anim. Sci.* 73:1459-1465.
- Chen, P., P. Ji and S. L. Li. 2008. Effects of feeding extruded soybean, ground canola seed and whole cottonseed on ruminal fermentation, performance and milk composition and fatty acid profile in early lactation dairy cow. *Asian-Aust. J. Anim. Sci.* 21:204-213.
- Cho, Y. and K. B. Song. 2000. Effect of  $\gamma$ -irradiation on molecular properties of BSA and  $\beta$ -lactoglobulin. *J. Biochem. Mol. Biol.* 33:133-137.
- De Boland, A. R., G. B. Garner and B. L. O'Dell. 1975. Identification and properties of phytate in cereal grains and oil seed products. *J. Agric. Food Chem.* 23:1186-1189.
- De Toledo, T. C. F., S. G. Canniatti-Brazaca, V. Artur and S. M. S. Piedade. 2007. Effects of gamma radiation on total phenolics, trypsin and tannin inhibitors in soybean grains. *Radiat. Phys. Chem.* 76:1653-1656.
- Diaa El-Din, M. and H. Farag. 1998. The nutritive value for chicks of full-fat soybeans irradiated at up to 60 kGy. *J. Anim. Feed Sci. Technol.* 73:319-328.
- Diehl, J. F. 2002. Food irradiation-past, present and future. *Radiat. Phys. Chem.* 63:211-215.
- Farkas, J. 2006. Irradiation for better foods. *Trends in Food Science and Technology* 17:148-152.
- Food and Drug Administration 1981. Irradiation in the production, processing, and handling of food; final rule. 21CFR Part 179. *Federal Register* 51:13376-13399.
- Gaber, M. H. 2005. Effect of  $\gamma$ -irradiation on molecular properties of bovine serum albumin. *J. Biosci. Bioengin.* 100:203-206.
- Gharaghani, H., M. Zaghari, G. shahhoseini and H. Moravej. 2008. Effect of gamma irradiation on antinutritional factors and nutritional value of canola meal for broiler chickens. *Asian-Aust. J. Anim. Sci.* 21:1479-1485.
- Ham, J. S., S. G. Jeog, S. G. Lee, G. S. Han, A. Jang, Y. M. Yoo, H. S. Chase, D. H. Kim, H. J. Kim, W. K. Lee and C. Jo. 2009. Quality of irradiated plain yogurt during storage at different temperatures. *Asian-Aust. J. Anim. Sci.* 22:289-295.
- Kopečný, J. and R. J. Wallace. 1982. Cellular location and some properties of proteolytic enzymes of rumen bacteria. *Appl. Environ. Microbiol.* 43:1026-1033.
- Krishnamoorthy, U., T. V. Muscato, C. J. Sniffen and P. J. Van Soest. 1982. Nitrogen fractions in selected feedstuffs. *J. Dairy Sci.* 65:217-229.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680.
- Lacroixa, M., T. C. Lea, B. Ouattaraa, H. Yua, M. Letendrea, S. F. Sabatoc, M. A. Mateescub and G. Patterson. 2002. Use of  $\gamma$ -irradiation to produce films from whey, casein and soya proteins: structure and functionals characteristics. *Radiat. Phys. Chem.* 63:827-832.
- Lee, S. L., M. S. Lee and K. B. Song. 2005. Effect of  $\gamma$ -irradiation on the physicochemical properties of gluten films. *Food Chem.* 92:621-625.
- Liener, I. E. 1994. Implications of antinutritional components in soybean foods: Critical Reviews. *Food Sci. Nut.* 34:31-67.
- Murray, R. K., D. K. Granner, P. A. Mayes and V. W. Rodwell. 2003. *Harper's Biochemistry*, 26th ed. McGraw-Hill, New York, New York.
- National Research Council 1996. Nutrient requirements of beef cattle. 7th Ed. National Academy Press, Washington, DC.
- Nocek, J. E. 1988. *In situ* and other methods to estimate ruminal protein and energy digestibility: a review. *J. Dairy Sci.* 71:2051-2069.
- Ørskov, E. R. and I. McDonald. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J. Agric. Sci. (Camb.)* 92:499-503.

- Ressouany, M., C. Vachon and M. Lacroix. 1998. Irradiation dose and calcium effect of the mechanical properties of cross-linked caseinate films. *J. Agric. Food Chem.* 46:1618-1623.
- Romagnolo, E., C. E. Polan and W. E. Barbeau. 1990. Degradability of soybean meal protein fractions as determined by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Dairy Sci.* 73:2379-2385.
- Roy, D. N. and R. V. Bhat. 1974. Trypsin inhibitor content in some varieties of soybean (*Glycine max* L.) and sunflower seeds (*Helicenthus annuus* L.). *J. Sci. Food Agric.* 25:765.
- Sadeghi, A. A., A. Nikkhah, P. Shawrang and M. M. Shahrehabak. 2006. Protein degradation kinetics of untreated and treated soybean meal using SDS-PAGE. *Anim. Feed Sci. Technol.* 126:121-133.
- SAS Institute Inc. 1996. Statistical Analysis System (SAS) User's Guide. SAS Institute, Cary, NC, USA.
- Scott, T. A., D. K. Combs and R. R. Grummer. 1991. Effects of roasting, extrusion, and particle size on the feeding value of soybeans for dairy cattle. *J. Dairy Sci.* 74:2555-2562.
- Shawrang, P., A. Nikkhah, A. Zare-Shahneh, A. A. Sadeghi, G. Raisali and M. Moradi-Shahrehabak. 2007. Effects of gamma irradiation on protein degradation of soybean meal. *Anim. Feed Sci. Technol.* 134:140-151.
- Shawrang, P., A. Nikkhah, A. Zare-Shahneh, A. A. Sadeghi, G. Raisali and M. Moradi-Shahrehabak. 2008. Effects of gamma irradiation on chemical composition and ruminal protein degradation of canola meal. *Radiat. Phys. Chem.* 77:918-922.
- Siddhuraju, P., H. P. S. Makkar and K. Becker. 2002. The effect of ionising radiation on antinutritional factors and the nutritional value of plant materials with reference to human and animal food. *Food Chem.* 78:187-205.
- Van Soest, P. J., J. B. Robertson and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583-3597.
- Van Soest, P. J. 1994. Nutritional ecology of the ruminants. 2nd Edition. Cornell University Press. NY. USA. p. 293.
- Wallace, R. J. 1985. Adsorption of soluble proteins to rumen bacteria and the role of adsorption in proteolysis. *Br. J. Nutr.* 53:399-408.
- Wang, Y., T. A. McAllister, M. D. Pickard, Z. Xu, L. M. Rode and K. J. Cheng. 1999. Effect of micronizing full fat canola seed on amino acid disappearance in the gastrointestinal tract of dairy cows. *J. Dairy Sci.* 82:537-544.
- Yamamoto, O. 1992. Effect of radiation on protein stability. In: Stability of protein pharmaceuticals (Ed. T. J. Ahern and M. C. Manning), New York. NY, USA: Plenum Press. pp. 361-419.