Available online at www.sciencedirect.com
ScienceDirect
Radiation Physics and Chemistry 76 (2007) 1050–1057
Radiation Physics and Chemistry
www.elsevier.com/locate/radphyschem

Effect of radiation processing on antinutrients, in-vitro protein digestibility and protein efficiency ratio bioassay of legume seeds

Hania F.G. El-Niely*

Food Irradiation Research Department, National Center for Radiation Research and Technology, Atomic Energy Authority, P.O. Box 29, Nasr City, Cairo, Egypt

Received 14 June 2006; accepted 17 October 2006

Abstract

The effects of irradiation (dose levels of 5, 7.5 and 10 kGy) on nutritive characteristics of peas (*Pisum satinum* L), cowpeas (*Vigna unguiculata* L.Walp), lentils (*Lens culinaris* Med), kidneybeans (*Phaseolus vulgaris* L), and chickpeas (*Cicer arietinum* L) were examined. Analyses included proximate composition, levels of anti-nutrients (phytic acid, tannins), available lysine (AL), in vitro protein digestibility (IVPD), and protein efficiency ratio (PER) in the growing rat. The results showed that moisture, crude protein, crude fat, crude fiber, and ash were unchanged by the irradiation. Radiation processing significantly ($p < 0.05$) reduced the levels of phytic acid (PA), tannins (TN), and AL. IVPD and PER were significantly enhanced in a dose-dependent manner, relative to unirradiated control samples, for all legumes. The data sets for each legume exhibited high correlation coefficients between radiation dose and PA, TN, AL, IVPD, and PER. These results demonstrate the benefits of irradiation on the nutritional properties of these legumes.

Keywords: Legumes; Antinutrients; Phytic acid; Tannins; Radiation processing; Available lysine; Digestibility; PER; Rats

1. Introduction

Legumes are a cheap and valuable potential source of protein, and they are consumed in large quantities in Middle East countries. Legumes such as peas (*Pisum satinum* L), cowpeas (*Vigna unguiculata* L. Walp), lentils (*Lens culinaris* Med), kidneybeans (*Phaseolus vulgaris* L), and chickpeas (*Cicer arietinum* L) are consumed widely in Egypt. These legumes are valuable sources of complex carbohydrates, protein and dietary fiber, contribute significant amounts of vitamins and minerals, and high energy value (Morrow, 1991; Nielsen, 1991; Tharanathan and Mahadevamma, 2003). Protein contents in legume grains range from 17% to 40%, contrasting with 7–13% of cereals, and being equal to the protein contents of meats (18–25%) (de Almeida Costa et al., 2006). Poor nutritive values of the food legumes, due to the presence of some antinutritional substances, such as protease inhibitors, lectins, phytate, tannin and dietary fiber, including resistant starch, have been reported (Morrow, 1991; Siddhuraju et al., 2000, 2002). Tannins (TN) inhibit the digestibility of protein, whereas phytic acid (PA) reduces the bioavailability of some essential minerals (Van der Poel, 1990; Rehman and Shah, 2006). It has been observed by earlier workers, that radiation processing improves the nutritional quality of food legumes to various extents. Improvement in protein quality of soybeans and broad beans has been reported after the partial removal of trypsin inhibitor and haemaglutinin as a result of a radiation processing simple (Farag, 1998; El-Niely, 2001). PA, α-amylase inhibitor and oligosaccharids, were inactivated to a considerable extent when legume sample were irradiation Siddhuraju et al., 2000, 2002).

The literature has many reports demonstrating that thermal processing methods improve the nutritional quality of food legumes due to reduction in anti-nutrients. However, there is a scarcity of information relating to the effects of processing with ionizing energy. Therefore, the present work was undertaken to explore the effects of radiation processing on anti-nutrients and protein quality of food legumes.

*Tel.: +20 2 671 2411; fax: +202 274298.
E-mail address: elniely@hotmail.com.

0969-806X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.radphyschem.2006.10.006

2. Materials and methods

2.1. Materials

Peas (*Pisum satinum* L), cowpeas, lentils (*Lens culinaris* Med), kidneybeans (*Phaseolus vulgaris* L), and chickpeas (*Cicer arietinum* L) were obtained from the Agriculture Research Institute, Ministry of Agriculture and Land Reclamation, Giza, Egypt. The samples were cleaned to remove broken seeds, dust and other foreign matter manually.

2.2. Radiation processing

About 5 kg lots of each sample were packed in well-sealed polyethylene sleeve. They were subjected at room temperature to gamma irradiation at dose levels of 5, 7.5 and 10 kGy, as monitored by FWT-60-00^{TM} radiochromic film. The radiochromic dosimeter was purchased from Far West Technology, Inc., Goleta, California, USA (ASTM, 2002 [ISO/ASTM 51275:2002(E)]).

The irradiation facility used was Egypt's Mega Gamma-1 Type J-65000 located at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The processed and raw samples were ground to pass a 20 mesh size screen, and stored in a freezer ($-20\,^{\circ}$C) until used.

2.3. Chemical analysis

Chemical composition (moisture, crude protein, crude fiber, ether extract and ash) of the raw and processed seed samples was determined by standard AOAC (1990) procedures. PA was extracted in 0.5 M nitric acid by shaking at room temperature for 3 h and assayed spectrophotometrically at 512 nm, as, described by Davies and Reid (1979). Tannin contents of the samples were estimated by spectrophotometer at 760 nm, using the Folin–Denis reagent after extraction with 1% hydrochloric acid in methanol (AOAC, 1990). The content of available lysine (AL) of samples was assayed according to Hurrell and Carpenter's method (1976).

2.4. In vitro protein digestibility (IVPD)

In vitro protein digestibility (IVPD) of all employed samples was determined by the extent to which the pH drops from pH 8 when the samples are subjected to sequential digestion with a multi-enzyme mixture using a modification of the multienzyme technique according to Hsu et al. (1977) and Satterlee et al. (1979). The enzymes used in the in-vitro protein digestion study were purchased from Sigma Chemical Co., St. Louis, Missouri, USA. These were porcine intestinal peptidase; porcine pancreatic trypsin (type IX); bovine pancreatic chymotrypsin (type II), and peptidase (registry number; 9031-95-3; 9002-07-7; 9004-07-3; and 9031-96-3, respectively). IVPD of the sample was then calculated using the following equation: Digestibility $\% = 234.84–22.56X$, where X is the pH recorded after a total digestion period of 20 min. The multienzyme solution was freshly prepared before each series of tests. All analyses of each sample were done in triplicate.

2.5. Protein efficiency ratio (PER) bioassay

The standard method (AOAC, 1995) for assessment of the protein efficiency ratio (PER) was followed. Male Albino rats weighing 55 ± 5 g were obtained from the animal facility of Nuclear Research Center belonging to the Egyptian Atomic Energy Authority, Inshas, Egypt. They maintained in the animal facility of NCRRT and housed individually in polypropylene cages. The cages were kept in a care room at 22–25 °C and $60\pm5\%$ relative humidity, with a photoperiod of 12 h. The rats were fed a standard pelleted diet and water, ad libitum, for an acclimatization period of 4 days. They were then randomly distributed into the experimental groups. Rats fed with casein (10%) as a source of protein served as the common control group. There were 20 experimental diets, corresponding to the 5 legumes, each at 4 dose levels (0, 5, 7.5, and 10 kGy). Ground legumes were incorporated into the respective experimental diets as a substitute for a portion of the corn starch to make up 10% protein in the final diets (Table 1). PER was carried out for 28 days according to AOAC (1994). Food consumption and body weight of rats were assessed weekly and at 10-day intervals. The PER values were corrected by a factor of 2.5 times the value for casein standard (AOAC, 1995). Animal experimentation was conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1985).

3. Results and discussion

3.1. Proximate chemical analysis

The gross compositions (moisture, crude protein, crude fat, crude fiber and ash) of studied legumes, in their raw state, exhibited that moisture content was found to be 128.8, 84.6, 98.4, 117.0, and 114.0 g kg^{-1} for peas, cowpeas, lentil, kidneybeans and chickpeas respectively. Cowpeas contained the maximum amount of crude protein (266.7 g kg^{-1}) flowed in descending order by kidneybeas (255.2 g kg^{-1}), peas (236.0 g kg^{-1}), chickpeas (218.0 g kg^{-1}), and lentil (217.7 g kg^{-1}). The crud fat content was high in chickpeas (64.3 g kg^{-1}), followed by lentil (33.6 g kg^{-1}), peas (24.9 g kg^{-1}), cowpeas (24.5 g kg^{-1}) and kidneybeas (12.5 g kg^{-1}). Peas generally contains a high amount of crud fiber (64.4 g kg^{-1}) while lentil has a low amount of crude fiber (53.3 g kg^{-1}). Among studied legume seeds the content of ash was present in kidneybeans (43.3 g kg^{-1}). The gross compositions of studied legumes, in their raw stat, were in agreement with data presented by other

Table 1
Diet composition

Ingredients ($g\,kg^{-1}$)	Casein diet	Test diets				
		Peas	Cowpeas	Lentils	Kidney beans	Chickpeas
Corn starch	700	377	420	404	410	343
Corn oil	100	100	100	100	100	100
Nonnutritive cellulose	50	50	50	50	50	50
Casein	100	—	—	—	—	—
Legumes[a]	—	423	380	396	390	457
Salt mixture[b]	40	40	40	40	40	40
Vitamin mixture[c]	10	10	10	10	10	10
Total	1000	1000	1000	1000	1000	1000

[a]Irradiated samples were incorporated at the same level as the non-irradiated samples.
[b]Salt mixture: $CaCO_3$, 78.6 g; $Ca_3C_{12}H_{10}O_{14}\cdot 4H_2O$, 308.3 g; $CaHPO_4\cdot 2H_2O$, 112.8 g; K_2HPO_4, 218.8 g; KCl, 124.7 g; NaCl, 77.1 g; $MgSO_4$, 38.3 g; $MgCO_3$, 35.2 g; $Fe(C_6H_{17}N_3O_7)$, 15.3 g; $MnSO_4\cdot H_2O$, 0.201 g; $CuSO_4\cdot 5H_2O$, 0.078 g; KI, 0.041 g; $AlNH_4(SO_4)_2\cdot 12H_2O$, 0.507 g.
[c]Vitamin mixture: vitamin A, 1000 IU; vitamin D, 100 IU; vitamin E, 10 IU; vitamin K, 0.5 mg; thiamine, 0.5 mg; riboflavin, 1 mg; pyridoxine, 0.4 mg; pantothenic acid, 4 mg; niacin, 4 mg; choline, 200 mg; inositol, 25 mg; Para-aminobenzoic acid, 10 mg; vitamin B_{12}, 2 μg; biotin, 0.02 mg; folic acid, 0.2 mg; added cellulose to make up to 1 g.

authors (Kutos et al., 2003; Ratnayake et al., 2001). No appreciable variation in chemical composition occurred within processed samples owing to the applied irradiation dose (up to and including 10 kGy). The present findings are in good agreement with the data previously obtained on raw soybeans irradiated at 10 kGy (Farag, 1998), broad beans irradiated at 2.5, 5, 10 and 20 kGy (El-Niely, 1996) or irradiated peanut kernels at 5, 7.5 and 10 kGy (El-Niely, 2001). Different explanations have been proposed for the non-significant effects of radiation processing elsewhere (Farag, 1998; El-Niely, 2001).

3.2. *Antinutritional constituents and their stability to gamma irradiation*

Table 2 summarizes the data for PA and TN of raw and radiation processed peas, cowpeas, lentil, kidneybeans and chickpeas at dose levels of 5, 7.5 and 10 kGy. PA in legumes is one of major concern as it chelates mineral cations and interacts with proteins forming insoluble complexes which lead to reduced bio-availability of minerals and reduced digestibility of protein (Reyden and Selvendran, 1993). The PA concentration in the peas, cowpeas, lentil, kidneybeans, and chickpeas was 9.02, 6.83, 11.5, 10.99, and 8.40 $mg\,g^{-1}$, respectively (Table 2) and these values are comparable with those of previous reports (Abd El-Hady and Habiba, 2003).

Radiation treatment at dose levels of 5, 7.5 and 10 kGy significantly reduced the PA content of peas by 8.9%, 11.4%, and 17.2%, of cowpeas by 8.6%, 11.4%, and 14.8%, of lentil by 15.1%, 25.2%, and 32.7%, of kidneybeans by 7.5%, 14.2%, and 26.9% and of chickpeas by 6.5%, 11.5%, and 20.2%, when compared with respective raw seed materials, respectively. The extent of reduction increased linearly with increase in radiation dose.

Regarding to TN content of studied legumes, significant variations were noticed in the contents of TN of the studied legumes (Table 2). Raw lentil contained the highest value (920 mg 100 g^{-1}) while kidneybeans showed the least (237 mg 100 g^{-1}). Radiation treatment resulted in a moderate significant reduction in TN in all legumes compared to controls (Table 2). About 13.6%, 19.9% and 27.8% of the TN content of peas was reduced at irradiation dose levels of 5, 7.5 and 10 kGy, respectively. The reduction in TN content of cowpeas was 13.4%, 21% and 22.9%, of lentil by 7.6%, 12% and 21.7%, of kidneybeans by 11.2%, 16.7% and 25% and of chickpeas by 6.3%, 16.4% and 28.1% as a result of subjected to the above mentioned irradiation doses respectively.

Treatment of soybean seeds with irradiation (1.0 kGy), alone or in combination with soaking reduced the level of phytate compared to controls (Sattar et al., 1990). This reduction might be due to chemical degradation of phytate to the lower inositol phosphates and inositol by the action of free radicals produced by the radiation (De Boland et al., 1975). Another possible mode of phytate loss during irradiation could have been through cleavage of the phytate ring itself. In the present study, the ineffectiveness of the irradiation on the reduction/cleavage of PA might be governed by the medium radiation dose (5–10 kGy).

The little reduction in TN of studied legume seeds might be due to the relative stability of phenolics in studied legumes for the applied radiation doses (up to 10 kGy). However, reduction rate linearly increased with the increase in radiation dose.

The observations about PA and TN in these legumes tend to suggest that radiation processing up to 10 kGy had little effects on their value and therefore to achieve grater reduction in these antinutrients, legumes need to receive higher radiation dose above 10 kGy. The required radiation doses were computed from the linear regression equations, presented in Table 5.

Table 2
Radiation processing effects on phytic acid and tannins of food legume seeds

Legumes contents	Radiation dose (kGy)			
	0.0	5.0	7.5	10.0
Peas				
Phytic acid, (mg g^{-1})	9.02 ± 0.039	8.22 ± 0.040	7.99 ± 0.052	7.47 ± 0.145
Tannins, (mg 100 g^{-1})	320.7 ± 5.81	277.0 ± 1.00	257.0 ± 0.578	231.7 ± 2.18
Cowpeas				
Phytic acid, (mg g^{-1})	6.83 ± 0.067	6.24 ± 0.083	6.05 ± 0.029	5.82 ± 0.025
Tannins, (mg 100 g^{-1})	246.7 ± 1.76	213.7 ± 1.86	195.0 ± 2.52	190.3 ± 1.86
Lentil				
Phytic acid, (mg g^{-1})	11.50 ± 0.58	9.76 ± 0.145	8.60 ± 0.058	7.73 ± 0.088
Tannins, (mg 100 g^{-1})	920.0 ± 2.89	850.0 ± 5.77	810.0 ± 5.77	720.0 ± 5.77
Kidneybeans				
Phytic acid, (mg g^{-1})	10.99 ± 0.047	10.17 ± 0.088	9.43 ± 0.393	8.03 ± 0.033
Tannins, (mg 100 g^{-1})	237.7 ± 3.93	211.0 ± 0.58	198.0 ± 7.23	178.0 ± 1.15
Chickpeas				
Phytic acid, (mg g^{-1})	8.40 ± 0.054	7.85 ± 0.053	7.43 ± 0.088	6.70 ± 0.100
Tannins, (mg 100 g^{-1})	264.7 ± 2.40	248.0 ± 1.53	221.3 ± 4.37	190.3 ± 0.88

Mean values ± SE of triplicate determinations, on dry matter basis.

3.3. *Effect of radiation processing on the AL content*

The AL content of raw peas, cowpeas, lentil, kidneybeans and chickpeas, as assayed according to Hurrell and Carpenter's method (1976), was found to be 1.57, 1.50, 1.60, 1.54, and 1.27 g 16 g^{-1} N, respectively (Table 3). Statistical analysis of the data showed that radiation processing of raw peas at dose levels of 5, 7.5 and 10 kGy reduced AL content by 12.7%, 29.9% and 31.8%, respectively. Statistical analysis indicated that there was no significant difference between peas processed at 7.5 and 10 kGy. Same trend was observed when cowpeas subjected to the applied radiation doses, where the reduction in its AL content was 31.3%, 36.9% and 42.7%, respectively, with 5, 7.5 and 10 kGy, radiation. In respect to the effect of the employed radiation doses on lentil, the analysis of the data showed that irradiation at 5 kGy did not result in any significant changes in AL content. However, a reduction of 20.6% and 37.5%, respectively, occurred with 7.5 and 10 kGy.

Radiation treatment of kidneybeans and chickpeas at dose levels of 5, 7.5 and 10 kGy decreased AL by 10.4%, 12.3% and 20.8% for kidneybeans and 18.9%, 23.4% and 24.4% for chickpeas, respectively. The statistical analysis showed that there are no significant differences between kidneybeans processed at 5 and 7.5 kGy. Meanwhile, the statistical analyses showed that there is no significant analysis between chickpeas processed at 5, 7.5 and 10 kGy (Table 3).

In the present study, the radiation dose from 5 to 10 kGy linearly reduced the availability of lysine in the experimental beans and the reduction in it was correlated with the applied radiation doses (Table 3). As only the lysine molecule with a free ε-amino group is nutritionally available, it is likely that during radiation some of these free ε-groups combine with active substances in these beans to form unavailable complexes and thus lower the amount of AL.

3.4. *In vitro protein digestibility*

The data presented in Table 3 show that the in vitro apparent protein digestibility varied greatly among the tested legume seeds. Raw peas, cowpeas, lentil, kidneybeans, and chickpeas was 74.10%, 49.97%, 51.07%, 70.47%, and 73.67% digestible, respectively. After the effect of irradiation treatment, there were some changes ensued as a function of irradiation dose (Table 4). Digestibility of the peas significantly increased approximately by 1.7%, 3.1% and 5.3% as a result of 5, 7.5 and 10 kGy radiation dose level, respectively. Also, the cowpeas increased by 10%, 16.7% and 27.7%, the lentil increased by 15.4%, 20.2% and 27.9%, the kidneybeans increased by 8.9%, 11.6% and 15.9% and also slight, but significant increase in IVPD of chickpeas was amounted 3.6%, 5.7% and 10.5% upon the effect of irradiation treatment at 5, 7.5 and 10 kGy, respectively.

Evidence from iv vitro studies indicates that digestion of native legume seed storage protein is limited because of the structure and conformation of the protein (Carbonaro et al., 2000). Also, in vitro studies have shown that phytate–protein complexes are insoluble and less subject to attack by proteolytic enzymes than the same protein alone (Ravindran et al., 1995) and subsequently affect the functional properties of protein. Moreover, the partial removal of TN and PA probably created a large space

Table 3
Radiation processing effects on available lysine, in vitro protein digestibility and protein efficiency ratio bioassay of certain food legume seeds

Parameters	Radiation dose (kGy)			
	0.0	5.0	7.5	10.0
Peas				
AL, g $(16\,g\,N)^{-1}$	1.57±0.037	1.37±0.033	1.10±0.058	1.07±0.088
IVPD (%)	74.10±0.21	75.37±0.04	76.37±0.09	78.03±0.318
PER	0.790±0.007	0.860±0.015	0.917±0.003	1.02±0.042
Cowpeas				
AL, g $(16\,g\,N)^{-1}$	1.50±0.58	1.03±0.067	0.947±0.020	0.860±0.006
IVPD (%)	49.97±0.09	54.96±0.11	58.33±0.32	63.83±0.09
PER	0.592±0.004	0.647±0.015	0.747±0.003	0.800±0.006
Lentil				
AL, g $(16\,g\,N)^{-1}$	1.60±0.058	1.53±0.033	1.27±0.033	1.00±0.058
IVPD (%)	51.07±0.38	58.93±0.24	61.40±0.02	65.33±0.449
PER	0.56^{d}±0.657	0.698^{c}±0.011	0.783^{b}±0.003	0.824^{a}±0.018
Kidneybeans				
AL, g $(16\,g\,N)^{-1}$	1.54±0.006	1.38±0.012	1.35±0.007	1.22±0.058
IVPD (%)	70.47±0.15	76.77±0.39	78.67±0.33	81.67±0.88
PER	0.793±0.007	0.867±0.007	0.900±0.006	0.960±0.006
Chickpeas				
AL, g $(16\,g\,N)^{-1}$	1.27±0.067	1.03±0.037	0.973±0.007	0.960±0.006
IVPD (%)	73.67±0.33	76.3±0.33	77.90±0.27	81.40±1.14
PER	0.783±0.012	0.837±0.015	0.910±0.015	0.963±0.015

AL, available lysine; IVPD, in vitro protein digestibility; PER, protein efficiency ratio.
Mean values±SE of triplicate determinations.

within the matrix, which increased the susceptibility to enzymatic attack (Rehman and Shah, 2001) and consequently improve the digestibility of protein after irradiation treatment up to 10 kGy. Higher protein digestibility after irradiation treatment may be due to increased accessibility of the protein to enzymatic attack. However, this effect could also be due to inactivation of proteinaceous antinutritional factors (Van der Poel., 1990).

The amelioration in IVPD can be attributed to destruction of antinutritional factors, such as protease inhibitors and lectin which lead to an improvement in the digestibility of the protein (Liener, 1978). The in vitro apparent digestibility of protein data indicated a beneficial effect for radiation when the in vitro digestibility of the studied legume seeds was considered. These results are in good agreement with those obtained by Rady et al. (1987) who reported that the nutritional value of all varieties of beans, based on chick growth, was significantly improved by irradiation. Pusztai et al. (1979) found that raw kidneybeans was of poor nutritive value and they attributed that to some lethal toxic factors of the ingested kidneybeans, which were due to relatively large amounts of hemagglutinating activity. Arora (1983) and Bressani et al. (1987) attributed the poor nutritive value of legumes to the presence of some forms of proteins which inhibit the digestive enzymes such as trypsin, chymotrypsin inhibitors and hemagglutinins. The protein quality of legumes influenced also by seed polyphenols (Singh, 1993).

The apparent improvement in vitro digestibility that being ensured through radiation treatment, may be attributed to appreciable effect of irradiation on the antinutritional factors present naturally in non-irradiated studied legumes which are more sensitive to enzyme action.

It is well known that radiation could induce and (or) stimulate other factors. Molecular rearrangement and changes in peptide linkages between the amino groups of amino acids could affect the nutritive availability and the biological utilization of the irradiated proteins. Such changes could interfere with the protein digestibility and/or its biological value. Thus, protein digestibility may be decreased and/or increased without incurring amino acid destruction (El-Hakeim et al., 1991).

Therefore, it could be concluded that the irradiation process offers a good treatment for legumes to reduce or eliminate their antinutritional factor(s) with consequent increase in their digestibility and thereby increase the utilization of their proteins.

3.5. PER bioassay

PER bioassay reflects the ability of a protein to support body weight gain of animals. The radiation processing significantly ($P<0.05$) affected PER values of the studied legumes as presented in Table 4. As expected, rats fed the raw peas, cowpeas, lentil, kidneybeans and chickpeas showed lower PER than those fed irradiated peas,

Table 4
Correlation coefficient between different parameters

	Phytic acid	Tannins	AL	IVPD	PER
Peas					
Irradiation dose	−0.973	−0.990	−0.901	0.953	0.902
Phytic acid		0.971	0.902	−0.952	−0.940
Tannins			0.876	−0.949	−0.910
AL				−0.836	−0.899
IVPD					0.910
Cowpeas					
Irradiation dose	−0.955	−0.977	−0.938	0.980	0.956
Phytic acid		0.886	0.873	−0.982	−0.921
Tannins			0.942	−0.930	−0.922
AL				−0.878	−0.867
IVPD					0.966
Lentil					
Irradiation dose	−0.994	−0.965	−0.880	0.993	0.932
Phytic acid		0.963	0.901	−0.987	−0.927
Tannins			0.923	−0.959	−0.925
AL				−0.865	−0.904
IVPD					0.892
Kidneybeans					
Irradiation dose	−0.923	−0.929	−0.924	0.981	0.980
Phytic acid		0.852	0.889	−0.891	−0.945
Tannins			0.859	−0.925	−0.919
AL				−0.867	−0.937
IVPD					0.947
Chickpeas					
Irradiation dose	−0.956	−0.943	−0.871	0.924	0.936
Phytic acid		0.981	0.772	−0.898	−0.947
Tannins			0.731	−0.928	−0.943
AL				−0.764	−0.747
IVPD					0.862

AL, available lysine; IVPD, in vitro protein digestibility; PER, protein efficiency ratio.

Table 5
Computed radiation dose (kGy) needed for complete deactivation of antinutritional factors present naturally in the studied food legume seeds

Antinutrient	Peas	Cowpeas	Lentil	Kidneybeans	Chickpeas
	kGy				
Phytic acid	59.7	49.0	34.9	40.0	52.3
Tannins	36.5	41.5	49.1	48.1	37.3

cowpeas, lentil, kidneybeans, and chickpeas at dose levels 5, 7.5 and 10 kGy (Table 3).

From these results, it could be noticed that the PER values increased significantly after irradiation treatment at dose levels 5, 7.5 and 10 kGy indicating a beneficial effect for radiation processing. These results are in good agreement with those obtained by El-Wakeil et al. (1994) who found that irradiation treatment at 10 kGy, has significantly improved the PER values of raw soybeans, kidneybeans, broad beans, chickpeas and lupines. The PER values were significantly increased after irradiation treatment by 37.82%; 12.79%; 46.73%; 60.67% and 33.73% for the above mentioned legumes, respectively. Mahmoud (1983) reported that the PER of raw soybeans was found to be lower than those irradiated at dose levels 5, 10, 20 kGy. The PER was reported to increase by increasing the irradiation dose levels.

The data (Table 3) showed that radiation processing of raw legume seeds improved the nutritional quality as measured by PER in rats. The enhancement of PER of processed legume seeds, at the maximum radiation dose (10 kGy) as a recommended dose for food preservation under the current regulation (WHO, 1981, 1994; 1999), may be attributed to the partial radio-inactivation of the antinutritional factors which could interfere with the normal intestinal digestion and/or absorption of the studied legume seeds and consequently their protein availability.

3.6. Association between different parameter

Correlations and linear regression analysis were used for testing any relationship between irradiation treatments and the evoked response. The data presented in Table 4 demonstrated the closeness of the association between the irradiation and the contents of PA, TN, AL, in vitro digestibility (IVPD) and PER of peas, cowpeas, lentil, kidney beans and chickpeas. Data in this Table clearly demonstrated that PA, TA and AL contents were negatively correlated with irradiation dose and that there was a strong inter-relationship among them, as well as high inter- relationship among antinutritional factors (PA and TN) of each legume and their nutritive value (AL, IVPD and PER) (Table 4). Meanwhile, IVPD and PER values were significantly increased as a function of irradiation dose.

On the basis of such linear regression equations the necessary doses were computed at which the respective antinutrient factor is essentially reduced. Among these food legume seeds the total inactivation of PA and TN would be expected to occur at 59.7 kGy for peas and 49.1 kGy for lentil, respectively (Table 5). These computed irradiation doses seemed to be higher than the maximal dose level of 10 kGy recommended by the JECFI in 1980 (WHO, 1981). This is attributing to the lower water (85–129 g kg^{-1}) content of studied legume seeds, which not favour the production of enough radiolytic products for the denaturation of all antinutritional factors. Such irradiation doses did not impair the sensory proprieties and the nutritional value of legumes.

The degradation of antinutrients, on exposure to gamma rays, was directly proportional to the radiation dose. In soybeans the level of inactivation of trypsin inhibitor activity (TIA) increased linearly with increase in radiation dose (41.8%, 56.3%, 62.6% and 72.5% loss of TIA activity at radiation level of 5, 15, 30 and 60 kGy, respectively; Farag, 1998). The author suggested that effective deactivation of the antinutritional factors present in soybeans could be safely accomplished through irradiation processing at dose levels up to 60 kGy. On the other hand, TIA activity in *Pongamia glabra* oil cake was reduced by 84% on exposure to 50 kGy. (Rattansi and Dikshit, 1997). A chick bioassay test showed that radiation treatment (180 kGy) improved the nutritive value and metabolisability of lentils (Daghir et al., 1983). Improving the nutritional properties of the studied legumes, which are widely grown and consumed around the world, would be useful in helping to popularize food uses of these processed beans and support their physiological benefits such as reducing plasma cholesterol. It also a has positive influence on blood glucose and insulin concentration in both normal and diabetic patients (Slavin, 1988).

The potential effect of such irradiation doses on the reduction of various antinutrients could open avenues for the possible utilization of under-utilized and non-conventional legumes as potential additional food and feed sources in the near future.

4. Conclusion

Irradiation processing has been used as a means to inactivate antinutritional factors naturally present in the five legumes used in this study. The present work clearly shows that irradiation processing of peas, cowpeas lentil, kidneybeans and chickpeas can improve their nutritional quality as assayed by the AL, IVPD and PER method with growing albino rats due to the inactivation of antinutritional factors. The results suggest that:

(a) irradiation processing of studied legumes at dose levels of 5–10 kGy is effective in upgrading the nutrient quality of peas, cowpeas lentil, kidney beans and chickpeas; and
(b) the loss in AL due to irradiation treatment did not impair its nutritive value.

Maximum improvement in protein quality (i.e. IVPD and PER) was observed at the higher radiation (10 kGy). However, gradual decline in PA and TN was observed, as a function of radiation dose. Reductions in the levels of antinutrients from different food legumes were also observed as a result of different radiation treatments. In view of these results, it is suggested that food legumes should be subjected to radiation processing to gain maximum improvement in protein digestibility over the benefit of irradiation as a phytosanitary method to prevent the introduction or spread of regulated pests.

References

Abd El-Hady, E.A., Habiba, R.A., 2003. Effect of soaking and extrusion conditions on antinutrients and protein digestibility of legume seeds. Lebesm-Wiss. U.-Technol. 36, 285–293.

AOAC, 1990. Official Methods of Analysis, 15th ed. Association of Official Agricultural Chemists, Washington, DC, p. 342 and 1230.

AOAC, 1995. Official Methods of Analysis. 16th ed., vol. II. Association of Official Agricultural Chemists, Washington, DC, p. 58 (Chapter 45).

Arora, S.K., 1983. Chemistry and Biochemistry of Legumes. Published in India by Oxford and IBT Publishing Co.

ASTM, 2002. American Society for Testing Materials. Standard dosimetry for radiation process, ISO/ASTM 51275:2002 (E). Standard practice for use of a radiochromic film dosimetry system. Annual Book of ASTM Standards, ASTM, Philadelphia, PA, pp. 40–44.

Bressani, R., Brenes, R.S., Garcia, A., Elias, L.G., 1987. Chemical composition, amino acid content and protein quality of Canavalia spp.seeds. J. Sci. Food Agric. 40, 17–23.

Carbonaro, M., Grant, G., Cappelloni, M., Pusztai, A., 2000. Perspectives into factors limiting in vivo digestion of legume proteins: antinutritional compounds or storage protein? J. Agric. Food Chem. 48, 742–749.

Daghir, N.J., Sell, J.L., Mateos, G.G., 1983. Effect of gamma irradiation on nutritional value of lentils (*Lens culinaris*) for chicks. Nutr. Rep. Int. 27, 1087–1093.

Davies, N.T., Reid, H., 1979. An evaluation of phytates, zinc, copper, iron and manganese content of and availability from soya based textured vegetable protein. Meat substitute of meat extruder. Br. J. Nutr. 41, 579–582.

de Almeida Costa, G.E., de Silva Queiroz-Monici, K., Reis, S.M.P.M., de Oliveira, A.C., 2006. Chemical composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes. Food Chem. 94, 327–330.

De Boland, A.R., Garner, G.B., O'Dell, B.L., 1975. Identification and properties of 'phytate' in cereal grains and oilseed products. J. Agric. Food Chem. 23, 1186–1189.

El-Hakeim, N.F., Yousri, R.M., Roushdy, H., Farag, M.D.El-D.H., 1991. Nutritional evaluation of irradiated animal protein by-products. Isotopenpraxis 27 (3), 104–108.

El-Niely, H.F.G., 1996. Chemical constituents and digestibility of broad beans subjected to irradiation treatment. M.Sc. Thesis, Department of Biochemistry and Nutrition. Women's College, Ain Shams University. Cairo, Egypt.

El-Niely, H.F.G., 2001. Biochemical and nutritional studies on radiation processed peanuts. Ph.D. dissertation, Department of Biochemistry and Nutrition, Women's College, Ain Shams University. Cairo, Egypt.

El-Wakeil, F.A., Sharabash, M.T.M., Farag, M.D.El-D.H., Mahrous, S.R., 1994. Influence of radiation processing on the nutritive value of some legumes. Egypt. J. Rad. Sci. Appl. 7 (2), 294.

Farag, M.D.El-D.H., 1998. The nutritive value for chicks of full-fat soybeans irradiated at up to 60 kGy. Animal Feed Sci. Tech. 73, 319–328.

Hsu, H.W., Vavak, D.L., Satterlee, L.D., Miller, G.A., 1977. A multienzyme technique for estimating protein digestibility. J. Food Sci. 38, 126–130.

Hurrell, R.F., Carpenter, K.J., 1976. An approach to the rapid measurement of reactive lysine in food by dye binding. Proc. Nutr. Soc. 35, 23A.

Kutos, T., Golob, T., Kac, M., Plestenjak, A., 2003. Dietary fiber content of dry and processed beans. Food Chem. 80 (2), 231–235.

Liener, I.E., 1978. Protease inhibitor and other toxic factors in seeds. In: Norton, G. (Ed.), Plant Proteins. Butter worth's, London, p. 117.

Mahmoud, S.Z., 1983. Nutritive value of some feed stuffs treated with radiation. M.Sc. Thesis, Fac. of Agric. Ain Shams. Univ., Egypt.

Morrow, B., 1991. The rebirth of legumes: legume production, consumption and export are increasing as more people become aware of legumes nutritional benefits. Food Technol. 45, 96–121.

National Research Council, 1985. Guide for the Care and The Use of Laboratory Animals. NIH Publication, National Institute of Health, Bethesda, Maryland.

Nielsen, S.S., 1991. Digestibility of legume protein: studies indicate that the digestibility of heated legume protein is affected by the presence of other seed components and the structure of the protein. Food Technol. 45 (9), 112–114.

Pusztai, A., Clarke, E.M.N., King, T.P., Stewart, T.C., 1979. Nutritional evaluation of kidney bean (*Phosealus vulgaris*): Chemical composition, lectin content and nutritional value of selected cultivars. J. Sci. Food Agric. 30, 843.

Rady, A.H., Mostafa, M.M., Rahma, E.H., 1987. Effect of humid and dry heat application on the quality of soybean oil and cake residue. Egypt. J. Food Sci. 15, 37.

Ratnayake, W.S., Hoover, R., Shahid, F., Perera, C., Jane, J., 2001. Composition, molecular structure, and physicochemical properties of starches from four field peas (*Pisum sativum*) L. cultivars. Food Chem. 74, 189–202.

Rattansi, R., Dikshit, M., 1997. Protease inhibitors and in vitro digestibility of karanja (Pongamia glabra) oil seed residue. A comparative study of various treatments. J. Am. Oil Chem. Soc. 74, 1161–1164.

Ravindran, V., Bryden, W.L., Kornegay, E.T., 1995. Phyates: occurrence, bioavailability and implications in poultry nutrition. Poult. Avian Biol. Rev. 6, 125–143.

Rehman, Z.U., Shah, W.H., 2001. Tannin contents and protein digestibility of black grams (*Vigna mungo*) after soaking and cooking. Plant Food Human Nutr. 56, 265–273.

Rehman, Z.U., Shah, W.H., 2006. Thermal heat processing effects on antinutrients, protein and starch digestibility of food legumes. Food Chem. 91, 327–331.

Reyden, P., Selvendran, R.R., 1993. Phytic acid: properties and determination. In: Macrae, R., Robinson, R.K., Sadler, M.J. (Eds.), Encyclopedia of Food Science, Food Technology and Nutrition. Academic Press, London, pp. 3582–3587.

Sattar, A., Neelofar, X., Akhtar, M.A., 1990. Effect of radiation and soaking on phytate content of soybean. Acta Aliment. 19, 331–336.

Satterlee, L.D., Marshall, H.F., Tennyson, J.M., 1979. Measuring protein quality. J. Am. Oil Chem. Soc. 56, 103–108.

Siddhuraju, P., Becker, K., Makkar, H.P.S., 2000. Studies on the nutritional composition and antinutritional factors of three different germplasm seed materials of an under-utilized tropical legume, Mucuna pruriens var. utilis. J. Agric. Food Chem. 48, 6048–6060.

Siddhuraju, P., Makkar, H.P.S., Becker, K., 2002. The effect of ionising radiation on antinutritonal factors and the nutritional value of plant materials with reference to human and animal food. Food Chem. 78 (2), 187–205.

Singh, U., 1993. Protein quality of pigeon pea as influenced by seed polyphenols and cooking process. Plant Food Human Nutr. 43, 171–179.

Slavin, J.L., 1988. Nutritional value of soybean fiber. In: McCann, L. (Ed.), Soybean Utilization Alternative Crops and Products. University of Minnesota, St Paul Feb. 16–19.

Tharanathan, R.N., Mahadevamma, S., 2003. Grain legumes: a boon to human nutrition. Trends Food Sci. Technol. 14, 507–518.

Van der Poel, A.F.B., 1990. Effect of processing on antinutritional factors and protein nutritional value of dry beans. J. Anim. Feed Sci. Technol. 2, 179–208.

WHO, 1981. Wholesomeness of irradiated foods. Report of a Joint FAO/IAEA/WHO Expert Committee, Technical Report Series, No. 659. World Health Organization, Geneva Switzerland.

WHO, 1994. Safety and Nutritional Adequacy of Irradiated Food. World Health Organization, Geneva Switzerland.

WHO, 1999. High-dose irradiation: wholesomeness of irradiated food with doses above 10 kGy. Report of a Joint FAO/IAEA/WHO Study Group, Technical Report Series, No. 890. World Health Organization, Geneva, Switzerland.