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The Effect of Irradiation Treatment on the Non-Enzymatic Browning Reaction in Legume Seeds

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ABSTRACT

The present study was conducted to evaluate the effects of gamma irradiation treatment, at room temperature, on the non-enzymatic browning reaction (Millerd reaction products, MRPs) generated in soybeans, broad beans and dried peas seeds at dose levels of 10, 30 and 60 kGy and their effects on the chemical constituents, soluble protein, available lysine and *in vitro* protein digestibility. The formation of MRPs in the studied legumes was assayed by monitoring the formation of brown pigments (browning intensity) by spectrophotometric method.

The results revealed that the chemical composition of irradiated legumes showed non-significant differences relative to the raw one. A dose dependent decrease in soluble proteins and available lysine in the three legumes were observed. The non-enzymatic browning reaction was significantly increased with increasing the radiation dose, which was proved by changes in browning index tests. At the same time, the *in vitro* protein digestibility was increased after irradiation up to 60 kGy. Irradiation of dried peas with 60 kGy produced higher browning index than the other legumes. A positive correlation was observed between the radiation dose and the browning index for soybeans (R^2= 0.96), broad beans (R^2 = 0.81) and dried peas (R^2 = 0.97) which means that 96%, 81% and 97 of the variation in the incidence of non-enzymatic browning reaction in soybean, broad bean and dried peas, respectively, are due to the effect of irradiation treatments.

The present study suggests that the formation of non-enzymatic browning reaction did not impair the nutritional quality of legumes, therefore, the process of irradiation was helpful in increasing the *in vitro* protein digestibility of studied legumes. These results clearly indicated that gamma irradiation processing at the studied doses can add valuable effects to the studied legumes.

Key words : Non-enzymatic browning reaction/ Mailard reaction/ Gamma irradiation/ Legumes.

INTRODUCTION

Grain legumes occupy an important place in human nutrition, especially in the dietary pattern of low income groups of people in developing countries. They are normally consumed after processing, which not only improves palatability of foods but also increases the bioavailability of nutrients(1). Legumes contain two to three folds more proteins than cereals, besides being good sources of dietary carbohydrates. Plant proteins are now identified biologically as active and functionally versatile dietary components and are cheaper substitutes than animal proteins[2]. However, they frequently lose a part of their functional properties due to several circumstances such as pest infestation and pathogens during harvest and storage or when subjected to the conventional cooking processes including heat treatment. Moreover, the presence of the antinutritional factors can affect their nutritional functions, therefore, several methods are extensively used to minimize or to inactivate these antinutritional factors, among them gamma radiation is the one[3-7].

Irradiation is an ecofriendly technology utilized for improving the hygienic quality, nutritional safety and security. The technology involves ionizing radiation which ensure the food safety and extend the shelf-life of a wide variety of foods[8, 9]. In addition to the control of microorganisms of numerous food commodities, ionizing irradiation can be used to improve their flavour, palatability and enhance legumes flour protein digestibility, and may also affect legumes protein functionality[10, 11] and also increased the bioavailability of nutrients by inactivating antinutritional factors such as gossypol[3], trypsin, haemagglutinins[7, 12], growth inhibitors, tannin inhibitors[13] and flatulence factors[2], and it can be used to reduce several carcinogenic agents[14]. On top of that, irradiation was used in the drying procedure of food commodities[15] and in the development of traditional fermented foods[16].

The majority of chemical changes caused due to irradiation of food are similar to those by other preservation methods like heat. The chemical changes taking place during irradiation are the result of the direct effect of radiation on the food components or by indirect action through reactive intermediates formed by radiolysis of water[17]. Ionizing radiation, through the production of free radicals, can affect proteins by promoting reactions such as protein–protein association, deamination, cleavage of peptide and disulphide bonds and by association of aromatic and heterocyclic residues[18]. These changes depend on factors such as dose, pH, hydration state and temperature during irradiation[19] as well as the presence or absence of oxygen[20]. The safety and wholesomeness of irradiated foods have been well established and reviewed from time to time[17, 19].

The Maillard reaction or non-enzymatic browning is one of the most important modifications in foods that contain proteins and reducing carbohydrates. It is corresponded to a set of reactions resulting from the initial condensation between an available amino group and a carbonyl containing moiety, usually reducing sugar. This reaction is known to be responsible for the attractive flavour and brown colour of some cooked foods[21]. It is one of the major reactions taking place during thermal processing, cooking, and storage of foods[22]. A myriad of products are formed, which have a direct impact on nutritional and sensory qualities of foods. Other studies observed that the colour intensity of irradiated foods was increased as the irradiation dose increased[23-25]. Nicoli *et al.*[26] suggested that irradiation leads to non-enzymatic browning reactions similar to those induced in heat treated food. They hypothesized that these observations may be due to the formation of colored compounds by the Maillard reaction products (MRPs), since brown pigments are formed in advanced stages of browning reactions. The colour measurement provides a useful index for evaluating the intensity of browning reactions induced on food processing[27]. Ionizing radiation was reported to produce non-enzymatic browning or MRPs in aqueous glucose/lysine solutions. The non-enzymatic browning reaction that resulted as a result of gamma irradiation was influenced by the conditions of a system such as the reactant type, pH or medium and this was similar to the browning reaction during heat processing or storage[28, 29]. The loss of lysine availability and the decrease of protein digestibility are the main nutritional consequences of Maillard reaction during heat treatment and storage of foods[30, 31]. However, it was noticed that the antioxidant potential of MRPs formed by irradiation of whey proteins has been reported recently[29] to be similar to the antioxidative properties of MRPs produced by heat treatment of amino acid–sugar in model systems[32]. However, information on the formation of MRPs by irradiation in dry legumes is scanty, therefore, the present study was conducted to investigate radiation-induced changes in dry legumes employed throughout this investigation in terms of changes in soluble protein, available lysine and formation of non-enzymatic browning reaction, which occurred as a result of gamma irradiation alongside their *in vitro* protein digestibility. These legumes were the soybeans (*Glycine max* L.) variety of Crowford, broad beans (*Vicia faba*), variety Giza-461 and dried peas (*Pisum sativum* L.) variety Master pea.

MATERIALS AND METHODS

Legume samples and irradiation treatment:

The dry legume seeds used in the current study were the soybeans (*Glycine max,* L.) variety of Crowford, broad beans (*Vicia faba*) variety Giza-461 and dried peas (*Pisum sativum,* L.) variety Master pea supplied by the Field Crops Research Institute, Ministry of Agriculture and Land Reclamation, Giza, Egypt. Broken and damaged seeds and foreign materials were hand removed from the samples. Cleaned legume seeds were packed, in 500 g portions, into polyethylene bags and sealed by heat and irradiated at the ambient temperature with gamma rays from ^{60}Co source at the National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt. The facility used was the Indian Gamma Chamber 400 A. The applied doses were 10, 30 and 60 kGy delivered at a dose rate of 3.952 kGy/h at the time of experimentation. The absorbed dose during the irradiation was monitored by FWT-60-00™ radiochromic film(33).

Preparation of the seed flour:

Raw and irradiated legume seeds were powdered in a mill to 60 mesh size with suitable precaution to avoid contamination of samples. The powdered samples were stored in closed polyethylene bags and stored at 4 °C until further use.

Proximate analysis:

Proximate analysis (moisture, crude protein, crude fiber, crude fat and ash) was determined according to the method of AOAC(34) for the raw and irradiated legumes. The method of Müller and Tobin(35) was used to calculate total crude carbohydrates (nitrogen free extract, NFE):

Total crude carbohydrates (%) = 100 – [crude protein (%) + crude fat (%) + crude fibre (%) + ash (%)]

The gross energy of raw or irradiated seeds were calculated based on the formula used by Ekanayake *et al.* (36):

Gross energy (kJ 100 g^{-1} DM)= (crude protein x 16.7) + (crude fat x 3.7) + (carbohydrates x 16.7)

Soluble protein and available lysine assay:

The determination of soluble proteins was carried out according to the method of Bradford(37) using bovine serum albumin as a standard. The content of available lysine (AL) of samples was assayed according to Hurrell and Carpenter's method(38).

Browning index determination

Browning index (BI) of raw or irradiated seeds was determined according to Hwang *et al.* (39) on 1 g sample. The method for browning index measurement depends on the liberation of the brown pigment formed due to the occurrence of Maillard reaction using suitable reagents according to materials to be tested such as $CaCl_2$/Tris base buffer pH 7.0 (v/v). The optical density (OD) of centrifuged clear filtrate of samples was read on a spectrophotometer at wave length 440 (early Maillard reaction products) and 550 nm (late Maillard reaction products). Water was used as a blank and browning index was calculated as:

Browning index = absorption at 440 – absorption at 550 nm

For practical purposes, BI was expressed as OD/g dry solids[40].

In vitro protein digestibility (IVPD) assay:

In vitro protein digestibility (IVPD) of all employed samples was determined by the extent to which the pH drops from 8 when the samples are subjected to sequential digestion with a multienzyme mixture using a modification of the multienzyme technique according to Hsu *et al.*[41] and Satterlee *et al.*[42]. The protein solutions of employed seeds (6.25 mg ml^{-1} in distilled water) were adjusted to pH 8.0 with 0.1 N NaOH while stirring at 37°C in a water bath. 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 numbers: 9031-95-3, 9002-07-7, 9004-07-3 and 9031-96-3, respectively). The multienzyme solution was added to the protein solution at a ratio of 1:l0 (v/v). IVPD of the samples 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.

Statistical analysis:

The results were analyzed for all variables using the general linear models (GLM) of SAS software version 9.1.3, Service Pack 4[43] and Duncan's Multiple Range Test with the main effect (radiation dose level). The least square means were compared if a significant F (5% level of P) was detected by ANOVA. Linear, quadratic and cubic orthogonal polynomial contrasts were used to evaluate the effect of radiation dose level. Pearson’s correlation coefficient (r) and linear regression analysis in SAS were also utilized to define the relationship between different parameters and irradiation dose (kGy) when a linear response was observed. All the analyses were done in triplicate and the results were reported as mean ± standard error on a dry weight basis.

RESULTS AND DISCUSSION

Effect of gamma irradiation on the proximate chemical constituents:

The major chemical constituents (dry matter, moisture, ash, crude protein, crude fat, crude fiber, total crude carbohydrates and gross energy) of raw and irradiated soybeans, broad beans and dried pea as affected by gamma irradiation at dose levels of 10, 30 and 60 kGy are presented in table 1. Data regarding the chemical composition of the raw starting materials are in agreement with those published in the literature[44, 45]. Raw broad beans and dried peas were similar in moisture, protein, fat, carbohydrates, crude fiber, ash and gross energy. On the other hand, raw soybean showed higher protein and fat at the expense of moisture, carbohydrate and gross energy.

It is evident from tables 1 and 2 that there was non-significant effect of radiation treatment on the macronutrient content of the soybeans, broad beans and dried peas. Regression analysis of the moisture, ash, crude protein, crude fat, total crude carbohydrate and gross energy in relation to the applied irradiation dose revealed that the chemical constituents of investigated beans were non-significantly affected by gamma irradiation up to 60 kGy. The linear, quadratic and/or cubic effects were non-significant (table 2). An exception for this observation was for total crude carbohydrate and gross energy of dried peas which were increased linearly as a function of radiation dose (tables 1 and 2). Furthermore, it was observed that radiation treatment reduced the quantities of crude fiber of the soybeans, broad beans and dried peas linearly as compared with corresponding raw samples (tables 1 and 2). It would be interesting to determine total, soluble and insoluble dietary fibre fractions in raw and gamma irradiated legume seeds to gain a better insight into the fibre contents. The increase in carbohydrates might be attributed to radiation-induced breakdown of complex sugars (polysaccharides) into simple extractable forms (e.g. free sugars)[(46)], therefore, gross energy of dried peas was altered significantly as a function of radiation dose (tables 1 and 2).

The non-significant effects of the applied radiation doses used in this study can be attributed to the relatively limited amount of water content of the three studied legumes. The present findings are in good agreement with the data previously obtained by El-Niely[(47)] who showed that irradiation of broad beans (*Vicia faba*) at levels of 2.5, 5, 10 and 20 kGy did not induce any significant change in the chemical composition. Moreover, irradiation of full-fat soybeans irradiated at a dose levels of 5, 15, 30 and 60 kGy retained their normal levels of dry matter, moisture, ash, crude protein, crude fat and crude fibre. These dose levels did not result in the denaturation of protein and did not affect the nitrogen containing components of the food materials[(48)].

The radiolytic compounds that can formed after an irradiation treatment, from the compounds already present in the foodstuff, depend directly on the water content and because of this, when dried legumes or dehydrated materials are irradiated, the expected modifications are much less but the damage inflicted by irradiation on the peptide bond depends, in addition to the degree of hydration of the material, greatly on the oxygen content since this bond is highly stable and is not normally broken by the irradiation doses generally applied to foodstuffs[(49)]. Moreover, the crude protein and fat in a complex matrix of foodstuffs have been reported to be more resistant to radiation than in the pure state[(12, 50)]. The moisture content of the raw soybeans, broad beans and dried peas samples in this study was low (7.1, 9.7 and 7.6 g/100 g, respectively) and does not produce enough water free radicals needed to induce significant change in the gross composition of studied beans, therefore, it was to be expected that the crude protein, crude fat, ash, carbohydrate and gross energy would remain largely unaltered throughout the assay for all the treatments applied.

Table 1: Proximate chemical constituents of raw and irradiated samples of soybeans (*Glycine max*, L.), broad beans (*Vicia faba*) and dried peas (*Pisum sativum,* L.) (on dry weight basis).

Parameters	Irradiation dose (kGy)			
	0	10	30	60
Soybeans (*Glycine max,* L.)				
Dry matter (%)	92.9±0.03	92.9±0.03	93.0±0.23	92.9±0.15
Moisture (%)	7.1 ±0.03	7.1±0.03	7.0±0.23	7.1 ±0.15
Ash (%)	6.3±0.12	6.3 ±0.13	6.3 ±0.20	6.3 ±0.03
Crude protein (%)	31.7±0.58	31.4±0.58	31.7±0.38	31.8±0.54
Crude fat (%)	25.6±0.27	25.9±0.39	25.9±0.20	25.8±0.17
Crude fiber (%)	6.1±0.27	6.1±0.15	5.7 ±0.15	5.6±0.12
Total crude carbohydrates (%)	30.3±0.80	30.3±1.12	30.5±0.71	30.4±0.35
Gross energy (kJ 100/ g DM)	1131.2±3.00	1127.2±8.80	1133.3±4.80	1134.9±2.80
Broad beans (*Vicia faba*)				

Dry matter (%)	90.3±0.07	90.2±0.09	90.2±0.07	90.2±0.15
Moisture (%)	9.7±0.07	9.8±0.09	9.9±0.07	9.8±0.15
Ash (%)	3.7±0.18	3.9±0.19	3.7±0.13	3.7±0.15
Crude protein (%)	25.3±0.34	25.5±0.42	25.2±0.29	25.5±0.31
Crude fat (%)	1.9±0.06	1.9±0.06	1.9±0.06	1.9±0.03
Crude fiber (%)	7.8±0.12	7.5±0.06	7.5±0.06	7.2±0.27
Total crude carbohydrates (%)	61.2±0.07	61.3±0.13	61.6±0.35	61.8±0.43
Gross energy (kJ 100/ g DM)	1452.7±4.50	1455.5±4.60	1457.7±3.30	1464.3±4.40
Dried peas (*Pisum sativum*, L.)				
Dry matter (%)	92.4±0.09	92.4±0.07	92.4±0.10	92.4±0.07
Moisture (%)	7.6±0.09	7.6±0.07	7.6±0.10	7.6±0.07
Ash (%)	3.9±0.07	3.8±0.09	3.9±0.07	3.9±0.03
Crude protein (%)	23.6±0.12	23.6±0.06	23.7±0.15	23.6±0.09
Crude fat (%)	1.8±0.03	1.83±0.09	1.9±0.03	1.9±0.03
Crude fiber (%)	6.1±0.20	5.5±0.06	4.7±0.06	4.1±0.10
Total crude carbohydrates (%)	64.5±0.29	65.2±0.09	65.9±0.18	66.5±0.03
Gross energy (kJ 100/g DM)	1478.6±3.40	1490.3±1.70	1502.7±1.60	1512.7±1.20

- Total crude carbohydrates was calculated by difference, and gross energy according to Ekanayake *et al.*[36].
- Each value represents mean ± standard error of three determinations.

Table 2: Orthogonal polynomial (linear, quadratic and cubic) contrasts analysis for the main response of chemical constituents of legumes related to the effect of radiation dose level.

Parameters	Orthogonal polynomial contrasts (Probability)		
	Linear	Quadratic	Cubic
Soybeans (*Glycine max*, L.)			
Dry matter			
Moisture	0.8527	0.8527	0.8527
Ash	0.7515	0.8131	0.9157
Crude protein	0.8269	0.6702	0.8058
Crude fat	0.5792	0.5948	0.8520
Crude fiber	0.0227	0.5693	0.3428
Total crude carbohydrates	0.9276	0.9676	0.9676
Gross energy	0.5007	0.6206	0.5597
Broad beans (*Vicia faba*)			
Dry matter			
Moisture	0.8238	0.8681	0.9407
Ash	0.7575	0.6236	0.6291
Crude protein	0.8653	0.8496	0.5847
Crude fat	0.6826	0.7599	0.8910
Crude fiber	0.0245	0.9163	0.3837
Total crude carbohydrates	0.1668	0.8670	0.6728
Gross energy	0.0871	0.6679	0.8030
Dried peas (*Pisum sativum*, L.)			
Dry matter			
Moisture	0.9295	0.8434	0.7911
Ash	0.8232	0.6195	0.6565
Crude protein	0.6931	0.6931	0.6002
Crude fat	0.5871	0.5871	0.7845
Crude fiber	0.0001	0.8681	0.4234
Total crude carbohydrates	0.0001	0.9260	0.9009
Gross energy	0.0001	0.7054	0.7587

Effect of gamma irradiation on protein solubility:

Protein solubility is accepted as one of the easiest and quickest physical indexes to evidence the nutritional value of legumes(51). Protein solubility values between 75% and 85% are considered appropriate to obtain an acceptable body weight gain in growing laboratory animals(52, 53). Drastic gamma irradiation treatments at 10, 30 and 60 kGy lead to a decrease in protein solubility in the studied legumes by 17.42%, 23.35% and 29.49% for soybeans, 14.82%, 21.13% and 27.84% for broad beans and by 13.66%, 24.28% and 30.32% for dried peas as compared with their respective control sample (table 3). These results indicated that the irradiated soybeans, broad beans and dried pea at the applied radiation doses had a proper solubility value that could support the animal growth (table 3).

Table (3): Effect of gamma irradiation on soluble protein and available lysine content in soybeans, broad beans and dried peas seeds.

Parameters	Irradiation dose (kGy)			
	0	10	30	60
Soybeans (*Glycine max*, L.)				
Soluble protein[1] (g /16 g N)	42.83^{a} ± 0.145	35.37^{b} ±0.273	32.83^{c} ±0.145	30.20^{d} ±0.503
Range of variation ($X_{max} – X_{mim}$)	0.500	0.900	0.500	1.600
Losses rates %	----	17.42	23.35	29.49
Available lysine [2], (g 16/ g N)	6.60^{a} ±0.058	6.37^{b} ±0.013	6.23^{c} ±0.027	5.43^{d} ±0.038
Range of variation ($X_{max} – X_{mim}$)	0.200	0.040	0.090	0.130
Losses rates %	----	3.48	5.61	17.73
Broad beans (*Vicia faba*)				
Soluble protein[3] (g /16 g N)	40.70^{a} ±0.153	34.67^{b} ±0.120	32.10^{c} ±0.252	29.37^{d} ±0.504
Range of variation ($X_{max} – X_{mim}$)	0.500	0.400	0.800	1.700
Losses rates %	----	14.82	21.13	27.84
Available lysine [4], (g /16 g N)	4.53^{a} ±0.067	4.44^{b} ±0.019	4.34^{c} ±0.013	4.13^{d} ±0.038
Range of variation ($X_{max} – X_{mim}$)	0.200	0.060	0.040	0.120
Losses rates %	----	1.99	4.19	8.83
Dried peas (*Pisum sativum*, L.)				
Soluble protein[5] (g /16 g N)	41.43^{a} ±0.273	35.77^{b} ±0.120	31.37^{c} ±0.384	28.87^{d} ±0.536
Range of variation ($X_{max} – X_{mim}$)	0.900	0.400	1.300	1.800
Losses rates %	----	13.66	24.28	30.32
Available lysine [6] (g /16 g N)	4.79^{a} ±0.047	4.50^{b} ±0.015	4.32^{c} ±0.012	3.87^{d} ±0.067
Range of variation ($X_{max} – X_{mim}$)	0.160	0.050	0.040	0.200
Losses rates %	----	6.05	9.81	19.21

Means of each sample in the same row with the same letter are not significantly different ($P < 0.05$).

1 Dose level linear (P=0.0001), quadratic (P=0.0001), cubic (P=0.0060)

2 Dose level linear (P=0.0001), quadratic (P=0.0001), cubic (P=0.0021)

3 Dose level linear (P=0.0001), quadratic (P=0.0006), cubic (P=0.0260)

4 Dose level linear (P=0.0001), quadratic (P=0.2206), cubic (P=0.5927)

5 Dose level linear (P=0.0001), quadratic (P=0.0024), cubic (P=0.7059)

6 Dose level linear (P=0.0001), quadratic (P=0.4479), cubic (P=0.1469)

The present study confirmed the conclusion of El-Niely[47] that protein solubility of broad beans was reduced significantly as a function of gamma irradiation dose where the reduction in soluble protein was 4, 12, 18 and 29% when the broad beans was irradiated at dose levels of 2.5, 5, 10 and 20 kGy, respectively. The major effect of gamma irradiation is the production of free radicals, that produced by the splitting of water molecules (water radiolysis) then free radicals have the potential to interact with individual amino acids as well as the secondary, tertiary and quaternary structures of proteins[54].

Radiation-induced reactions in proteins are strongly influenced by their complex structure which includes the folding of peptide chains, disulphide linkages between the chains, hydrogen bonds, hydrophobic bonds and ionic bonds. Disulphide bonds in the tertiary structure may be cleaved by the action of free radicals action which may cause protein degradation[55]. Radiation usually causes degradation of fibrous proteins to smaller protein fractions, which may involve breakage of C–N bonds in the backbone of the peptide chain or splitting of disulphide bonds[55].

The amino acid composition, particularly at the protein surface, influences protein solubility and higher solubility is related to the presence of low numbers of hydrophobic residues[56]. Irradiation at high doses has been found to alter protein structure and may lead to formation of smaller peptides exposing hydrophilic groups which increase the interactions of hydrophilic amino acids with water molecules. Such alterations can change the protein solubility pattern at different doses due to alterations in amino acids at the surface as well as length of polypeptides[57]. Pednekar *et al.* [11] reported that the protein produced from the 20 kGy treated soybean (*Glycine max*) showed least solubility than the control samples. As a result, the reduction in soluble protein content may be due to the fact that irradiation of globular protein causes formation of protein aggregates and consequently, aggregation would be expected to decrease solubility of protein[58]. Urbain[59] reported that radiation induced protein aggregation and degradation and globular proteins usually undergo aggregation because of disturbances in secondary and tertiary structures that expose reactive groups to the action of radiolytic products of water.

Effect of gamma irradiation on the available lysine content:

The determination of available lysine is now accepted as a routine procedure in many laboratories[60]. Table 3 summarizes the data obtained for the available lysine contents of raw and irradiated investigated legumes at 10, 30 and 60 kGy. The available lysine contents in non-irradiated soybeans, broad beans and dried peas seeds were 6.60, 4.53 and 4.79 g /16g N, respectively. The orthogonal polynomial contrasts of the available lysine values among the applied radiation doses showed linearly significant effects of the applied radiation doses. The data showed that gamma irradiation of studied legumes induced a significant decrease in available lysine content with increasing radiation dose. Reduction of 3.48%, 5.61% and 17.73% for soybeans, 1.99%, 4.19% and 8.83% for broad beans and 6.05, 9.81 and 19.21% for dried peas, respectively, were occurred with 10, 30 and 60 kGy radiation. The conclusive decrease in the average values of the available lysine contents in soybeans, broad beans and dried peas seeds was found at dose of 60 kGy (linear, quadratic and/or cubic effects were significant) to reach its minimum (5.43 g/16g N for soybeans, 4.13 g/16g N for broad beans and 3.87 g/16g N for dried peas seeds). The available lysine reduction apparently followed the same pattern as protein solubility. The lysine molecule with a free e-amino group is nutritionally available and during irradiation, some of these free e-groups were combined with active substances in these legumes to form unavailable complexes and thus decrease the amount of available lysine[48].

Effect of gamma irradiation on the non-enzymatic browning reaction:

Non-enzymatic browning or Maillard reaction describes a complex series of reactions between reactive carbonyl groups in reducing sugars and free amino groups in proteins, which contain basic amino acid such as lysine. The Maillard reaction can be considered as one of the most important chemical reaction taking place during food processing. Its influence on food quality attributes colour, flavour and nutritional value which includes desired as well as unwanted effects, and requires the consideration of processing conditions as well as physicochemical properties of the food material[61]. Browning index test was performed to assess the extent of non-enzymatic browning reaction (Maillard reaction) due to radiation treatment of the investigated legumes.

Table 4 shows the brown color developed in soybeans, broad beans and dried peas after gamma irradiation at various radiation doses (10, 30 and 60 kGy). Irradiation of the three legumes under investigation showed significant variation in the generation of brown colour of the irradiated legumes. The browning index was dramatically increased as a function of radiation dose (table 4).

From the present results, it was observed that browning index values of irradiated legumes were increased with increasing the carbohydrate contents in seeds of soybeans, broad beans and dried peas, where the carbohydrate content was 30.3%, 61.2% and 64.5%, respectively (table 1). The present study indicated that dried peas was the most sensitive legume for irradiation treatment at 10, 30 and 60 kGy to develope brown colour which had the highest value of browning index as compared with soybeans and broad beans. The orthogonal polynomial contrasts of the browning index values among the applied radiation doses showed that the radiation dose level had linear (P=0.0001), quadratic (P=0.0004) and/or cubic (P=0.0004) effects (table 4). These results are agreed with Krumhar and Berry[49], Cunha *et al.*[62] and Fombang *et al.*[63] who reported that the increase in yellow colour of sorghum flour with irradiation and cooking may be indication to the occurrence of Maillard reaction. They were not certain to what extent the colour changes are related to the formation of Maillard products. The yellow coloration was highest in 50 kGy dry irradiated flours and could therefore represent the onset of Maillard browning. The non-enzymatic browning reaction generated by gamma irradiation has been hypothesized to be the result of the breakdown of the glycosidic and peptidic linkages which being promoted during irradiation and then the breakdown product such as the carbonyl and amino compounds can react to form colored compounds[64].

Effect of gamma irradiation on in vitro protein digestibility:

Low digestibility of the seed's protein is one of the main draw backs limiting the nutritional quality of food legumes. Since the *in vivo* techniques are time consuming as well as expensive and the results from *in vitro* studies are equally reliable, the *in vitro* methods have been successfully used in assessing the protein digestibility of foods[44].

The effect of gamma irradiation at dose levels of 10, 30 and 60 kGy on the *in vitro* protein digestibility (IVPD) of investigated seeds by the multienzyme technique (the enzymes were porcine intestinal peptidase, porcine pancreatic trypsin (type IX), bovine pancreatic chymotrypsin (type II), and peptidase) is shown in table 4. The *in vitro* protein digestibility of the non-irradiated legumes came in the following order: soybeans > peas > broad beans. Raw soybeans, broad beans and dried peas seeds exhibit 79.80%, 72.30% and 75.87% IVPD respectively, which is comparable to that of many common legumes like pigeon pea (69%), mung bean (67%), soybeans (71%) and *Phaseolus angularis* (69%) which were higher than rice bean (58%) and faba bean (53%)[65-68].

Table (4): Effect of gamma irradiation on browning index and *in vitro* protein digestibility (IVPD) in soybeans, broad beans and dried peas seeds.

Parameters	Irradiation dose (kGy)			
	0	10	30	60
Soybeans (*Glycine max*, L.)				
Browning index [1]	0.2590 [d] ±0.003	0.2743 [c] ±0.003	0.3223 [b] ±0.005	0.3577 [a] ±0.0019
Range of variation ($X_{max} - X_{mim}$)	0.008	0.010	0.200	0.400
Changes %	----	5.91	24.44	38.11
In vitro protein digestibility [2] (IVPD) %	79.80 [d] ±0.115	84.23 [c] ±0.133	86.40 [b] ±0.058	87.57 [a] ±0.120
Range of variation ($X_{max} - X_{mim}$)	0.400	0.400	0.200	0.400
Gain%	----	5.55	8.27	9.74
Broad beans (*Vicia faba*, L.)				
Browning index [3]	0.1610 [d] ±0.0006	0.3000 [c] ±0.0101	0.3703 [b] ±0.0030	0.4256 [a] ±0.0120
Range of variation ($X_{max} - X_{mim}$)	0.0020	0.0320	0.0100	0.04000
Gain %	----	86.34	130.0	164.35
In vitro protein digestibility [4] (IVPD) %	72.30 [d] ±0.115	74.33 [c] ±0.418	76.70 [b] ±0.153	78.50 [a] ±0.173
Range of variation ($X_{max} - X_{mim}$)	0.4000	1.3000	0.5000	0.6000
Gain%	----	2.81	6.09	8.58
Dried peas (*Pisum sativum*, L.)				
Browning index [5]	0.2503 [d] ±0.0026	0.3660 [c] ±0.0064	0.4290 [b] ±0.0021	0.6023 [a] ±0.0069
Range of variation ($X_{max} - X_{mim}$)	0.0090	0.0220	0.0070	0.0230
Changes %	----	46.22	71.39	140.63
In vitro protein digestibility [6] (IVPD) %	75.87 [d] ±0.437	77.63 [c] ±0.318	78.60 [b] ±0.116	79.57 [a] ±0.120
Range of variation ($X_{max} - X_{mim}$)	1.400	1.00	0.400	0.400
Gain%	----	2.32	3.60	4.88

Means of each sample in the same row with the same letter are non-significantly different ($P < 0.05$).

1 Dose level linear (P=0.0001), quadratic (P=0.0153), cubic (P=0.0143)
2 Dose level linear (P=0.0001), quadratic (P=0.0001), cubic (P=0.0335)
3 Dose level linear (P=0.0001), quadratic (P=0.0008), cubic (P=0.1642)
4 Dose level linear (P=0.0001), quadratic (P=0.7929), cubic (P=0.4870)
5 Dose level linear (P=0.0001), quadratic (P=0.0004), cubic (P=0.0004)
6 Dose level linear (P=0.0001), quadratic (P=0.1950), cubic (P=0.5447)

The relatively low protein digestibility of raw legume seeds may be due to the presence of antinutritional compounds and their structural characteristics. Numerous studies have indicated that globulin is the major storage protein, which is quite resistant to be attacked by proteolytic enzymes[(69)]. Antinutritional factors in raw beans may also inhibit the enzymatic digestion of protein resulting in lower protein digestibility values than in irradiated legumes[(70)].

The *in vitro* protein digestibility (IVPD) was found to be 84.23, 86.40 and 87.7% when the soybean seeds was irradiated at 10, 30 and 60 kGy, respectively. The improvement was 5.55, 8.27 and 9.74 %, respectively, over the control (79.80%) when soybeans seeds was irradiated at the studied doses. Also, irradiation treatment at the applied doses significantly (P <0.05) affect the IVPD of broad beans as shown in table 4 where the IVPD of broad beans irradiated at 10, 30 and 60 kGy was informed by 2.81, 6.09 and 8.58%, respectively. In case of irradiation of dried pea at the studied doses, maximum IVPD (79.57%) was observed at 60 kGy treatment (table 4).

The present data showed that all radiation treatments up to 60 kGy positively affect the IVPD of the three legumes (tables 4) and thus, improved its protein quality. The positive improvement of *in vitro* protein digestibility of these legumes in all irradiation doses may be attributed not only to the removal/reduction of antinutrients but also to the structural disintegration of dietary fiber and the native protein, including enzyme inhibitors and lectins(47, 71).

The improvement in IVPD has been described as the result of a reduction in the trypsin inhibitory activity in legume seeds and the tannin content of cereals caused by irradiation(72, 73). Another factor that may influence the bioavailability of dietary protein is the dietary fiber content(74). Melito and Tovar(75) showed that disruption of the cell wall structure by irradiation increased the protein digestibility of legume seeds. Moreover, irradiation treatment may result in an increase in the amount of soluble dietary fiber because it breaks glycosidic linkages of polysaccharides producing a solubilization of high and low molecular weight substances from insoluble polysaccharides(76). However, it should be noted that the non-enzymatic browning reaction occurred in the studied legumes after gamma irradiation treatment (non-thermal treatment), and increased as a function of radiation dose, did not have pronounced effects on the *in vitro* protein digestibility of these legumes. Fagbemi *et al.*(63) and Hsu *et al.*(41) reported that dry heat treatment reduced protein digestibility due to non-enzymatic browning (Maillard reaction) between the reducing sugars from starch hydrolysis and the proteins as well as the thermal cross linking that occurred during dry heating.

Correlation between different parameters:

Table 5 shows the Pearson's correlation coefficient (r) between irradiation treatment (dose levels), soluble protein (SP), available lysine (AL), browning index (BI) and *in-vitro* protein digestibility (IVPD) of soybeans, broad beans and dried peas. Data in this table clearly demonstrated that SP and AL contents were negatively correlated with irradiation dose and there was a strong inter-relationship among them, meanwhile, both BI and IVPD values were significantly increased as a function of irradiation dose for all the studied legumes (Table 5). From these results, it could be concluded that 88%, 90% and 92%; 97%, 93% and 93%; 98%, 90% and 98%, and 88%, 96% and 89% of the variations in SP, AL, BI and IVPD of soybean, broad bean and dried peas, respectively, are due to the effect of irradiation dose (kGy). Meanwhile, the formation of non-enzymatic browning reaction (brown colour) in studied legumes can be explained by the variations in SP and AL where there is strong inter-relationship among them.

Table 5. Pearson's correlation coefficient (r) between irradiation treatment (RT), soluble protein (SP), available lysine (AL), browning index (BI), and in-vitro protein digestibility (IVPD) of soybeans (*Glycine max*, L.), broad beans (*Vicia faba*) and dried peas (*Pisum sativum*, L.).

Soybeans (*Glycine max*, L.)				
	SP	**AL**	**BI**	**IVPD**
RT	- 0.87647	- 0.96750	0.98073	0.87569
SP		0.82181	- 0.88604	- 0.99179
AL			- 0.91127	- 0.80271
BI				- 0.91127
Broad beans (*Vicia faba*)				
RT	- 0.90458	- 0.93048	0.90145	0.96187
SP		0.87218	- 0.98825	- 0.95583

AL			- 0.84056	- 0.90097
BI				- 0.84056
Dried peas (*Pisum sativum*, L.)				
RT	- 0.92080	- 0.96150	0.98270	0.89386
SP		0.95543	- 0.93880	- 0.94817
AL			- 0.97506	- 0.93799
BI				0.92681

The present results were analyzed by a linear regression model as a function of radiation dose (kGy). The analysis indicated that the correlation coefficients between radiation dose and SP, AL, BI or IVPD were significant for soybeans, broad beans and dried peas. The linear equations that verify the previous influence of soybeans, broad beans and dried peas are as the following:

Soybeans:

$Y_{SP} = 39.83 - 0.181\ X$ ($R^2 = 0.7682$, $P = 0.0002$)

$Y_{AL} = 6.63 - 0.019\ X$ ($R^2 = 0.9361$, $P = 0.0001$)

$Y_{BI} = 0.2612 + 0.002\ X$ ($R^2 = 0.9618$, $P = 0.0001$)

$Y_{IVPD} = 81.66 + 0.113\ X$ ($R^2 = 0.7668$, $P = 0.0002$)

Broad beans:

$Y_{SP} = 38.37 - 0.166\ X$ ($R^2 = 0.8183$, $P = 0.0001$)

$Y_{AL} = 4.52 - 0.0064\ X$ ($R^2 = 0.8658$, $P = 0.0001$)

$Y_{BI} = 0.2162 + 0.0039\ X$ ($R^2 = 0.8126$, $P = 0.0001$)

$Y_{IVPD} = 72.96 + 0.1001\ X$ ($R^2 = 0.9252$, $P = 0.0001$)

Dried peas:

$Y_{SP} = 39.16 - 0.193\ X$ ($R^2 = 0.8478$, $P = 0.0001$)

$Y_{AL} = 4.52 - 0.0064\ X$ ($R^2 = 0.8658$, $P = 0.0001$)

$Y_{BI} = 0.2753 + 0.0055\ X$ ($R^2 = 0.9657$, $P = 0.0001$)

$Y_{IVPD} = 76.53 + 0.0556\ X$ ($R^2 = 0.7990$, $P = 0.0001$)

where Y_{SP}, Y_{AL}, Y_{BI} and Y_{IVPD} denote the soluble protein (g/16 g N), available lysine (g/ 16 g N), browning index (OD/g) and *in vitro* protein digestibility (%), respectively, and X is the dose in kGy.

CONCLUSION

Based on the data available in the literature and the results of present study, gamma irradiation produced free radicals and radiolysis products (Millerd reaction products; MRPs) of certain food constituents. These products and free radicals may condense to produce coloured products in foods which were observed in the irradiated legumes as assayed by browning index. The formation of browning colour could be explained on the basis that irradiation promotes the breakdown of glycosidic linkages of the disaccharide and sucrose of food carbohydrates.

Available lysine retention after irradiation treatments of soybeans, broad beans and dried peas was significantly influenced by the level of irradiation dose. Minimum available lysine was estimated at 60 kGy and in contrast, increase in the irradiation dose increased the browning index. However, the minimum value of non-enzymatic browning reaction for irradiated legumes was estimated at 10 kGy. Under the conditions of the present study, the results showed that the non-enzymatic browning reaction, MRPs, are probably produced and increased with increasing the irradiation dose (kGy) and accompanied by the reduction in soluble protein and available lysine. All of these aspects were occurred without altering the proximate composition except for fiber content, and the reduction in soluble protein and available lysine may be contributed in the formation of MRPs. The losses in soluble protein and available lysine due to irradiation treatment did not impair its nutritive value, therefore, the present study suggests that the formation of non-enzymatic reaction did not also impair the nutritional quality of legumes and accordingly, the irradiation treatment was helpful in increasing the *in vitro* protein digestibility of studied legumes. These results clearly indicated that gamma irradiation can add valuable effects to the studied legumes.

The application of radiation processing, the non-thermal technology ‘‘cold pasteurization and sterilization" for microbial and enzymatic inactivation as well as the alteration of the properties of food ingredients allows to overcome the necessity of heating the food matrix and therefore, reduces heat-induced changes in product quality including nutritional quality. From the obtained results of this study, it can be concluded that irradiation treatment can be used safely for obtaining enhanced protein quality with such legumes, which could be used to assist in the preparation of legumes products used as weaning foods to prevent protein-energy malnutrition.

REFERENCES

(1) R. N. Tharanathan, and S. Mahadevamma; Trends in Food Science and Technology; 14:507 (2003).
(2) J.P. Machaiah, and M.D. Pednekar; Food Chemistry; 79, 293–301 (2002).
(3) H. M. Abu-Tarboush; Journal of Agriculture and Food Chemistry; 46, 2698–2702 (1998).
(4) J. P. F. D’Mello; “Tropical legumes in animal nutrition”. Wallingford, UK: CAB International; 135–172 (1995).
(5) S. Khokhar, and B. M. Chauhan; Journal of Food Science; 52, 1083–1084 (1986).
(6) R. Rattansi, and M. Dikshit; J. Am. Oil Chemists’ Society; 74(9), 1161–1164 (1997).
(7) H. F. G. El-Niely; Radiation Physics and Chemistry*; 76*(6), 1050-1057(2007).
(8) R.B. Parens, and A.H . Lichtenstein; Nutr Clin Care; 7 (4):149 (2004).
(9) M. T. Al-Kaisey, M. A. Mohammed, M. A. Alwan, and M. H. Mohammed, Radiation Physics and Chemistry; 63, 295–297 (2002).
(10) M. Lacroix, T. C. Le, B. Ouattara, H. Yu, M. Letendre, and S. F. Sabato; Radiation Physics and Chemistry; 63, 827–832 (2002).
(11) M. Pednekar, A. K. Das, V. Rajalakshmi, and A. Sharma; Radiation Physics and Chemistry*; 79*(4), 490-494 (2010).
(12) P. Siddhurajua, H.P.S. Makkarb, and K. Beckera; *Food Chemistry; 78*, 187-205 (2002).
(13) T. C. F de Toledo, S. G. Canniatti-Brazaca, V. Arthur, and S. M. S. Piedade; Radiation Physics and Chemistry; 76, 1653–1656 (2007).
(14) X. Fan, and K. Mastovska; Journal of Agriculture and Food Chemistry; 54, 8266–8270 (2006).
(15) J. Wang, and Y. Du; International Journal of Food Science and Technology; 40, 75–82 (2005).
(16) M.-W. Byun, C. Jo, and J.-W Lee; “Food irradiation research and technology”. Oxford: Blackwell ; 249–262 (2006).
(17) J. F. Diehl; Radiation Physics and Chemistry; 63, 211–215 (2002).
(18) Y. Cho, J. S. Yang, and K. B. Song; Food Research International; 32, 515–519 (1999).
(19) I. S. Arvanitoyannis; “Irradiation of Food Commodities: Techniques, Applications, Detection, Legislation, Safety and Consumer Opinion”. Academic Press, Elsevier Inc; (2010).
(20) M. Giroux, and M. Lacroix; Food Research International; 31, 257–264 (1998).
(21) H. Jing, and D. D. Kitts; Food and Chemical Toxicology; 40, 1007–1015 (2002)..
(22) J. O'Brien, and P. A. Morrissey; Crit Rev. J. Food Sci. Nutr.; 28(3), 211 (1989).
(23) M. Roushdi, A. Harras, A. El-Meligi, and M. Bassim; Cereal Chem.; 58 (2), 110–112 (1981).
(24) J.H. Kim, H. J. Ahn, H. J. Yook, K .S. Kim, K. S. Rhee, G. H. Ryu, and M. W. Byun; Radiat. Phys. Chem.; 69 (2), 179–187 (2004).

(25) Y.S. Lee, Y.S. Oh, J. W. Lee, J. W. Kim, C. O. Rhee, C. O. Lee, and M. W. Byun; J. Korean Soc. Food Sci. Nutr.; 33 (3), 582–586 (2004).
(26) M. C. Nicoli, M. C. Casadei, M. E. Guerzoni, and C. R. Lerici; Appl. Radiat. Isot.; 45 (3), 389 (1994).
(27) P. Fernandez-Artigas, E. Guerra-Hernandez, and B. Garcia-Villanova, J. Food Chem., 74:499(1999).
(28) S. P. Chawla, R. Chander, and A. Sharma; Food Chemistry; 103,1297–1304 (2007).
(29) S. P. Chawla, R. Chander, and A. Sharma; FoodChemistry; 116, 122–128(2009).
(30) R. F. Hurrell; "In: P. A. Finot, H. U. Aeschbacher, R. F. Hurrell, & R. Liardon (Eds.), The Maillard reaction in food processing, human nutrition and physiology" Basel: Birkh.auser Verlag.; 245–258 (1990).
(31) H. E. Swaisgood, G. L. and Catignani; Advances in Food and Nutrition Research; 35, 185–236 (1991).
(32) K. Jayathilakan, and K. Sharma; Food Chemistry; 95, 620–626 (2006).
(33) ASTM, American Society for Testing Materials. Standard dosimetry for radiation process, ISO/ ASTM (E). Annual Book of ASTM Standards, ASTM, Philadelphia, PA; 40-44 (2002).
(34) AOAC, Official Methods of Analysis, 18th Ed., revision 1. Association of Official Analytical Chemists, Washington DC, USA; (2006).
(35) H. G. Müller, and G. Tobin; "Nutrition and food processing", London: Croom Helm Ltd; 302 (1980).
(36) S. Ekanayake, E. R. Jansz, and B. Nair; Food Chemistry; 66, 115–119(1999).
(37) M. M. Bradford; Analytical Biochemistry; 72, 248-254 (1976).
(38) R.F. Hurrell, and K. J., Carpenter; Proc. Nutr. Soc.; 35, 23A (1976).
(39) Hwang Jean- Yu, Yeong- Shin Shue, and Hung- Min Chang; Food Res. Inter.; 34, 639 (2001).
(40) R. Palombo, A. Gertler, and I. Saguy; Journal of Food Science; 49, 1609–1613(1984).
(41) H. W. Hsu, D. L. Vavak, L. D. Satterlee, and G. A. Miller; J. Food Sci.; 38, 126-130 (1977).
(42) L. D. Satterlee, H. F. Marshall and J. M. Tennyson; J. Am. Oil Chem. Soc.; 56, 103-108 (1979).
(43) SAS Institute Inc., SAS/ STAT 9.1 User's Guide., Cary, NC, USA; (2004).
(44) R. Y. Khattab, S. D. Arntfield, and C. M. Nyachoti; LWT - Food Science and Technology; 42(6), 1107-1112 (2009).
(45) H. Schumacher, H. Paulsen, A. E. Gau, W. Link, H. U. Jürgens, O. Sass, and R. Dieterich; Plant Breeding*; 130* (2), 156-164 (2011).
(46) R. Bhat, and K. R. Sridhar; *Food Chemistry; 107*(1), 174-184(2008).
(47) H. F. G. El-Niely, Chemical constituents and digestibility of broad beans subjected to irradiation treatment, MSc. Thesis, Women's College, Ain Shams University, Cairo, Egypt; (1996).
(48) M. Diaa El-Din H. Farag; J. Animal Feed Sci. Tech.; 73:319 (1998).
(49) K. C. Krumhar, and J. W. Berry; Journal of Food Science; 55(4), 1127–1132 (1990).
(50) P. Sánchez-Bel, I. Egea, F. Romojaro, and M. C. Martínez-Madrid; *LWT - Food Science and Technology; 41*(3), 442-449 (2008).
(51) M. C. Olguina, N. Hisanob, A.E. D'Ottaviob, M. I. Zingalea, G. C. Revelanta, and S. A. Calderaric; Journal of Food Composition and Analysis; 16 441–449 (2003).
(52) N. Dale, O. W. Charles, and S. Duke; Poultry Science; 65, 164–170(1985).
(53) C. M. Parsons, K. Hashimoto, K. Wedekind, and D. H. Baker; Journal of Animal Science; 69, 2918–2924 (1991).
(54) A. El-Rawas, A. Hvizdzak, M. Davenport, S. Beamer, J. Jaczynski, and K. Matak; *Food Chemistry; 133*(1), 212-219 (2012).
(55) E. M. Stewart; "Food Irradiation Principles and Applications". New York, John Wiley Sons Inc.; 37–75 (2001).

(56) A. Moure, A. Sineiro, H. Dominguez, and J.C. Parajo; Food Research International; 39, 945–963 (2006).
(57) F. R. Bautista, F. R. Thomson, and R. F. Cain; Journal of Food Science; 26, 15–20 (2006).
(58) P. Alexander, M. Fox, K. A. Stracey, and D. Rosen; Nature; 178, 846-848 (1956).
(59) W. M. Urbain; "Food Irradiation". Orlando: Academic Press; (1986).
(60) P. McDonald, R. A. Edwareds, and J. F. D. Greenhalgh; "Animal Nutrition", 2nd ed., English Language Book Society (ELBS), England;(1979).
(61) P. Sharma and H. S. Gujral; *Food Research International; 44*(1), 235-240 (2011).
(62) M. F. Cunha, V. C. Sgabieri and M. H. Damasio; J. Agric. and Food Chem.; 41: 1710 (1993).
(63) T. N. Fagbemi, A. A. Oshodi, and K. O. Ipinmoroti; Pakistan J. of Nutr.; 4, 250–256(2005).
(64) J.W. Lee, S.H. Oh, J.H. Kim, E. H. Byun, M. Ree Kim, M. Baek, and M.W. Byun; *Radiation Physics and Chemistry; 76*(5), 886-892 (2007).
(65) C. F. Chau, and C. K. Cheng; J. Agricultural and Food Chemistry, 45, 4773–4776 (1997).
(66) U. Chitra, V. Vimala, U. Singh, and P. Geervani; Plant Foods for Human Nutrition; 47, 163–172(1995).
(67) K. Saharan, N. Khetarpaul, and S. Bishnoi; J. Food Sci. Tech.; 39, 418–421 (2002).
(68) P. Siddhuraju, and K. Becker; J. Agri. Food Chemistry; 49, 3058–3067 (2001).
(69) S. S. Deshpande; Critical Reviews in Food Science and Nutrition; 32, 333–363 (1992).
(70) S. S. Nielson; Food Technology; 45, 112–118 (1991).
(71) H. F. G. El-Niely; Biochemical and nutritional studies on radiation processed peanuts. Ph.D. dissertation, Department of Biochemistry and Nutrition, Women's College, Ain Shams University. Cairo, Egypt; (2001).
(72) M. Shastry, and E. John; J. Sci. Food Agric.; 55, 529-538(1991).
(73) I. A. G. Hassan, and A. El Tinay; Food Chem.; 53, 149-151(1995).
(74) D. Gaudard de Week, C. Hischenhuber, and J. Kruseman, Trav. Chim. Aliment. Hyg.; 85, 317-339 (1994).
(75) C. Melito, and J. Tovar; J. Food Sci.; 59, 833-838 (1995).
(76) M. Nyman, T. Nylander, and N. G. Asp; Food Chem.; 47, 169-176 (1993).