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Effect of Gamma Radiation on Protein Profile, Protein Fraction and Solubility's of Three Oil Seeds: Soybean, Peanut and Sesame

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Abstract

The proteins properties of three oil seeds (soybean, peanut and sesame) were investigated following γ-irradiation (0.0, 0.5, 1.0, 2.0, 3.0, 5.0 and 7.5 KGy). The effect of γ-radiation on total protein solubility, albumin, globulin and SDS-ME fractions were studied using SDS-polyacrylamide gel electrophoresis. The results showed that solubility of total protein were decreased and reached to the maximum decrease using irradiation dose of 7.5 KGy compared to control. The interesting phenomena are that albumin and globulin fractions decreased in its solubility while the SDS-ME fraction increased. These phenomena may be due to the effect of gamma radiation on the protein, which may dissociate this fraction to small subunits, and rearrangement to form a complex protein even high or small molecular weight proteins solubilized only in SDS-ME fraction. The changes in protein profile were depended even on radiation dose and on the nature of oil seeds; soybean, peanut and sesame.

Keywords: electrophoresis, gamma irradiation, peanut, protein solubility, sesame, soybean

Abbreviations: SDS-sodium dodecyl sulfate; ME-2-mercapto ethanol, KGy-Kilo gray

Introduction

Soybean is a protein rich oilseed crop, which is presently the leading edible oil source throughout the world. Though soy protein ranks high compared to other vegetable proteins, it is still of poor nutritional quality compared to animal proteins. Soy protein isolates consist of 90% protein; their major components are glycinin (11S), and β-conglycinin (7S), which represent 34% and 27%, respectively (Iwabuchi and Yamaucho, 1987a, b). The rest of the proteins consist of whey proteins, such as γ-conglycinin (trimer of 170 KDa) (Hirano *et al.,* 1987), the basic 7S globulin, lipoxygenase, agglutinins, and β-amylase, which belong to the 7S fraction (Iwabuchi and Yamaucho, 1987b). It is known that the physiochemical properties of proteins are modified by irradiation as reported by Hafez *et al.* (1985), Afify and Shousha (1988) demonstrated that γ-irradiation caused molecular changes resulting in condensation or polymerization, degradation, hydrogen-bonding distribution and cleavages of intermolecular disulphide bonds (Casarett, 1968). Therefore, it is assumed that such molecular rearrangements bring about changes in secondary structure and distribution of the native conformation of soybean proteins, especially at higher dose levels. Afify and Shousha (1988) reported that SDS-PAGE proved that changes in protein subunit patterns could be identified in the 'Clark' cultivar depending to dose of γ-exposures. Three high molecular weight protein bands were detected in irradiated soybean cultivars by using Poro-PAGE 5-25% polyacrylamide.

Chiou *et al.* (1990) purified arachin and conarachin from peanut seeds by phosphate buffer and characterized on SDS-PAGE gel. Data revealed that arachin was composed of five major subunits with molecular weights (MW) of 42, 37, 35, 22 and 20 K Da and four minor subunits with MW of 66, 31, 29, and 25 K Da. Conarachin represent the major one subunit with a MW of 64 K Da and three minor subunits with a MW of 60, 32, and 21 K Da (El-Beltagi, 2011). The irradiation caused native protein aggregation might simply result from rearrangement of the composed protein subunits (Sonntage, 1987) and crosslinking of peptides or proteins to form dimers or tetramers through the action of free radicals and produced from γ-radiation. Cross-linking of proteins electrophoresis pattern was also observed in the microwave irradiation of canola meal performed by Sadeghi and Shawrang (2006). However, Lee *et al.* (2005) reported that gamma irradiation affects proteins by causing conformational changes, oxidation of amino acids, and formation of protein free radicals. Also, chemical changes in the proteins caused by gamma irradiation include fragmentation, cross-linking, aggregation and oxidation by oxygen radicals that are generated in the radiolysis of water. Gamma irradiation has a slight effect on the amino acid profile at recommended doses to foods (WHO, 1999). The chemical nature of damage in irradiated proteins has been explained by molecular rearrangements resulting in condensation or polymerization (Carroll *et al.,* 1952), degradation (Schweigert, 1959), hydrogen bond disruption and cleavages of intermolecular disulfide bonds (Casarett, 1968; Dogbevi, *et al.,* 1999a).

Sulphydryl (-SH) and disulphide (-S -S) groups in proteins are apparently more sensitive to irradiation (Lee, 1962), at very high doses of radiation, reduction in cystine content of wheat has been reported (Doguchi, 1969). The observed increase of some free amino acids in irradiated red gram, wheat, casein and shrimp could be due to a breakdown of proteins (Néné *et al.,* 1975).

Sesame (*Sesamum indicum* L.) is a very ancient oilseed crop and one of the earliest domesticated oil crops in the world. It acquired importance as a source of cheap vegetable oil and proteins, good source of natural antioxidants (sesamin and sesamolin) which are unique for sesame and present in the oil (Ashri, 2007). Ionizing radiation has been routinely used to generate genetic variability for breeding and genetic studies. Mutagens used may cause genetic changes in an organism, break linkages and produce many new promising traits for the improvement of crop plants (IAEA, 1977). Induced mutation is highly instrumental in plant biology to induce genetic variability in a great number of crops (Siddiqui and Khan, 1999). Therefore different protein composition of the irradiated sesame will be studied extensively. Gamma rays cause different degrees of cell damage. Biological damage is mostly in direct, and mediated by reactive oxygen species (ROS), such as hydroxyl radical ($HO^{\cdot}$), superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide H_2O_2, single oxygen, etc., generated by the radiolysis of water (El-Beltagi *et al.,* 2011). These reactive species are known highly reactive to membrane lipids, protein and DNA. They are believed to be the major contributing factors to stress injuries and to cause rapid cellular damage (Afify and El-Beltagi, 2011; Afify *et al.,* 2011; Aly and El-Beltagi, 2010; El-Beltagi *et al.,* 2008; 2010; El-Beltagi and Mohamed, 2010; Ibrahim *et al.,* 2011; Kobeasy *et al.,* 2011; Mohamed *et al.,* 2009; O'Kane *et al.,* 1996; Salama *et al.,* 2009; Shehab *et al.,* 2010), particularly when plants are exposed to stress conditions such as chilling stress, salt stress, Fe deficiency, cadmium stress, Lead toxicity, ionizing radiation, insecticide, organisms and micro-organisms to caused degradation of important macromolecules, including DNA and cell membranes.

The main purpose of this investigation was to study changes in protein profiles in addition to total protein solubility, albumin fraction, globulin fraction and SDS-ME fraction of three oil seeds (soybean, peanut and sesame) as a results of gamma irradiation with range of 0.5 to 7.5 KGy.

Materials and methods

Soybean seeds (*Glycin Max* L. var. 'Clark'), peanut seeds (*Arachis Hypogaea* L. var. 'Giza 5') and sesame seeds (*Sesame indicum* L. var. 'Giza 32') were obtained from Food Legumes Research Department, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt, during season 2008.

Gamma iradiation treatments

Irradiation treatments were performed by using Cobalt 60 source from unit Gamma Chamber 4000; at the National Center for Research and Radiation Technology, Naser City, Cairo. Samples were irradiated at 0.5, 1, 2, 3, 5 and7.5 kGy (75%) in the gamma cell (gamma facility PX-g-30 irradiator model, Russia) at room temperature and in the presence of air. The dose rate was 4.166 rad/second (75%) as measured by ethylene mono-chloro-benzene dosimeter (ECB).

Extraction of proteins

Extraction of total protein fraction (Albumin +Globulin)

Defatted peanut and sesame meals were extracted with sodium phosphate buffer (0.2 M, pH 7.9) containing 0.02% NaN_3 at 4°C as described by Chiou and Tasi (1990). Defatted soybean meal was extracted with 0.03 M Tris-HCl pH 8.0 buffer at room temperature to yield whole buffer extract as described by Iwabuchi and Yamauchi (1987b), then centrifuged at 3000 *g* for 30 min at (Ali and Olivo, 2002).

Extraction of albumin fraction

Defatted samples were extracted with distilled water containing 0.02% NaN_3 and then centrifuged at 1500 *g* for 20 min. The extraction with water was repeated twice according to the method of Afify and Ghali (1987).

Extraction of globulin fraction

The residue from the third water extract was re-extracted with sodium phosphate buffer (0.2 M, pH 7.9) containing 0.02% NaN_3 for each peanut and sesame samples according to Chiou and Tasi (1990). While soybean samples was re-extracted with 0.03 M Tris-HCl buffer (pH 8.0) buffer in the presence of 10 mM mercapoethanol then the extracts were centrifuged according to Afify and Ghali (1987).

Extraction by SDS, ME 2% fraction

The residue from the sodium phosphate buffer extract was re-extracted with sodium phosphate buffer (0.2 M, pH 7.9) containing 2% (sodium dodecyl sulfate and 2-mercapto ethanol) for each peanut and sesame samples. While soybean samples was re-extracted with 0.03 M Tris-HCl buffer (pH 8.0) buffer in the presence of 10 mM 2-ME and containing 2% (sodium dodecyl sulfate and 2-mercapto ethanol) then all samples were centrifuged, according to Afify and Ghali (1987).

Protein determination

Soluble protein was estimated using the method of Comassie Brilliant Blue G-250 according to Bradford (1976).

Protein solubility

Protein solubility was expressed as the ratio of soluble protein to total protein according to Estela *et al.* (1991). Total protein of samples was determined by Kjeldahl method (N x 6.25) according to A.O.A.C. (1990).

Electrophoresis

Electrophoresis was carried on SDS-PAGE 15% polyacrylamide in Tris-glycine pH 8.3 with 0.1% SDS according to the method of Laemmli (1970).

Statistical analysis

All determinations were done in triplicate. Statistical analysis were done using SPSS (version 10) program. Mean and standard error were descriptive measures of quantitative data using the analysis of variance test (ANOVA) for independent samples. P-values <0.05 were considered significant.

Result and discussion

Soybean protein profiles

The objective of this investigation was to evaluate the effect of different doses of gamma radiation 0.5, 1.0, 2.0, 3.0, 5.0, and 7.5 KGy on soybean protein patterns. Therefore, different types of protein fractions were extracted from soybean seeds as total soluble protein, albumin, globulin and SDS/ME soluble proteins. The result in Fig. 1 shows that the electrophoretic separation of protein profiles was different according to the extraction methods. The protein profiles of total soluble protein, albumin, globulin and SDS-ME soluble proteins were affected by gamma irradiation depending on the dose of irradiation and protein fractions. Electrophoretic analysis of soybean protein after gamma radiation compared with control sample proved the presence of major protein patterns of $\dot{\alpha}$, α and β with molecular weights 79, 70 and 50 K Da, respectively corresponded to β-conglycinin.

Two subunits acidic (11S-1) and basic (11S-2) with molecular weights 38 and 26 K Da which related to glycinin (11S globulin) were identified. On the other hand, the minor protein patterns of agglutinin with molecular weight 34-36 K Da; β-amylase with molecular weight 55 K Da and Kuntiz trypsin inhibitor with molecular weight 21 K Da were identified as shown in Fig. 1. Protein globulin fraction of soybean shows an additional protein band with molecular weight 23 K Da. The intensity of the major subunit bands of 11 S-2 and 11 S-1 with MW of 38 and 26 K Da, were increased as a results of gamma radiation and reached maximum intensity with 7.5 KGy. On the other hand, most of the remaining protein did not change in its intensity by gamma radiation. It is very important to note that three major protein bands in globulin fraction with MW of 79 ($\dot{\alpha}$ conglycinin), 38 (acidic 11 S-2) and 34 K Da (agglutinin) were increased by increasing gamma irradiation and decreased to faint bands with irradiation dose of 7.5 KGy. The results showed that most of the conglycinin and the acidic and basic of glycinin are denatured by gamma radiation rather than destructive because these fractions were solubilized in the SDS-ME soluble fraction of the irradiated samples with different doses.

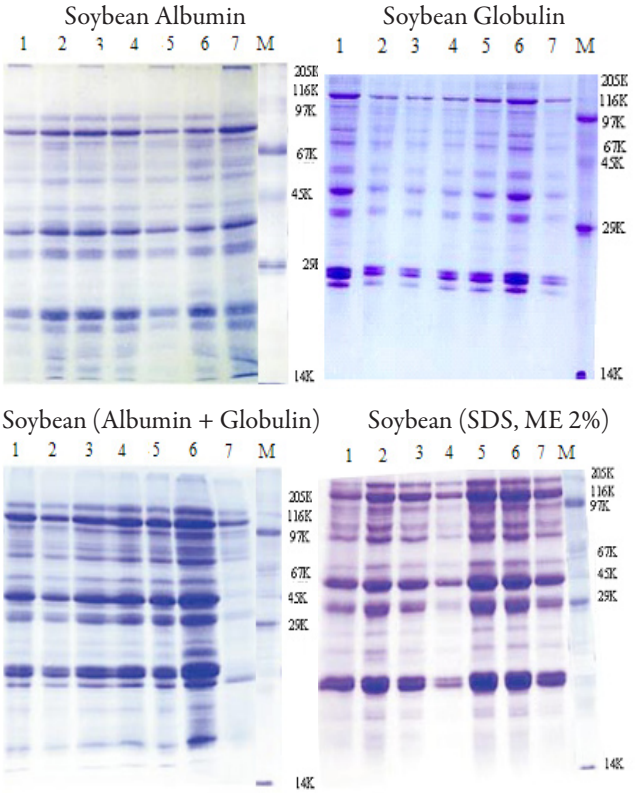

Fig. 1. Electrophoretic separation of protein subunits of soybean in SDS-PAGE 5 and 15% PAA in Tris-glycine buffer pH 8.3 with 0.1% SDS. γ-Radiation treatments; 1-control, 2-0.5 KGy, 3-1.0 KGy, 4-2.0 KGy, 5-3.0 KGy, 6: 5 KGy, 7-7.5 KGy, M-Molecular weight protein markers

Since radiation is known to cause disintegration and aggregation of protein molecule this may be occur in the present investigation (Kumta and Tappel, 1961), which will effect on the protein solubility. While it is known that gamma radiation is generally attributed to direct breakdown in protein or enhance the activity of lysozomal (proteolytic) enzyme, which help to aggregate the protein and decrease its solubility (Van Huystee and Verma, 1969). Therefore the irradiation dose used considered very important, since this dose could unfold protein to increase its solubility through the deamination of the protein (Rahma and Mostafa, 1988; Taha and Mohamed, 2004) or aggregate protein during disintegration through the decrease of the sulphahydryl group and increase the disulphide bond (Dogbevi *et al.,* 1999b) and rearrangement of the small molecular weight protein to a high molecular weight and causes decrease in protein solubility (Afify and Shousha, 1988).

Tab. 1. Protein solubility of different fractions from irradiated Soybean with gamma rays

Irradiation dose (KGy)	Total protein	Total soluble protein		Albumin fraction		Globulin fraction		SDS/MS soluble protein	
		% Protein	% Solubility	% Protein	% Solubility	% Protein	% Solubility	% Protein	% Solubility
0.0 KGy	44.6 ± 0.10	34.0 ± 0.50^{a}	76.2	19.5 ± 0.23^{a}	43.7	10 ± 0.23^{a}	22.4	4.5 ± 0.15^{a}	10.1
0.5 KGy	44.3 ± 0.07	33.5 ± 0.33^{a}	75.6	18.7 ± 0.23^{ab}	42.2	9.5 ± 0.10^{ab}	21.4	4.7 ± 0.07^{ab}	10.6
1.0 KGy	44.2 ± 0.07	32.5 ± 0.29^{ab}	73.5	18.0 ± 0.30^{bc}	40.7	8.5 ± 0.44^{bc}	19.2	4.9 ± 0.08^{bc}	11.1
2.0 KGy	44.0 ± 0.10	32.0 ± 0.33^{ab}	72.7	17.5 ± 0.17^{bcd}	39.7	8.3 ± 0.17^{bcd}	18.9	5.0 ± 0.15^{cd}	11.4
3.0 KGy	43.7± 0.12	30.0 ± 0.68^{bc}	68.7	17.0 ± 0.12^{cd}	38.9	8.0 ± 0.25^{cd}	18.3	5.7 ± 0.17^{d}	13.0
5.0 KGy	43.2 ± 0.09	29.0 ± 0.58^{c}	67.1	16.5 ± 0.44^{d}	38.2	7.0 ± 0.32^{d}	16.2	6.0 ± 0.18^{d}	13.9
7.5 KGy	42.7 ± 0.05	28.0 ± 0.58^{c}	65.6	14.5 ± 0.23^{e}	34.0	6.5 ± 0.23^{d}	15.2	6.5 ± 0.03^{d}	15.2
LSD 0.05		2.564		1.341		1.489		0.707	

Data represent the mean ± S.E. of three experimental replicates. Values with different letters in the same experiment and same column are significantly different ($P \leq 0.05$)

Protein solubility of soybean seeds

The results in Tab. 1 showed that, the total protein content was not affected by irradiation, while the solubility of total protein fraction was decreased and reached to the maximum decrease using irradiation dose 7.5 KGy (65.6%) compared to the control (76.2%). The interesting phenomena are that albumin and globulin fractions decreased in its solubility while SDS-ME fraction increased to reach 15.2% compared to control 10.1%. Significant differences showed between the total soluble protein and irradiation treatments especially with 3, 5 and 7.5 KGy. The statistical analysis of the albumin, globulin and SDS-ME soluble protein showed significant changes in protein solubility started from irradiation dose 1 KGy. The levels of the significant differences were dependent on the irradiation dose and the methods of protein extraction and have LSD 1.39, 1.48 and 0.707 respectively. These results are in agreement with those found by Diehl *et al.* (1978) amd Manjaya *et al.* (2007), cited that irradiation of globular protein causes formation of protein aggregates which effect on its solubility. Byun and Kang (1994) reported that irradiation of soybean proteins caused decrease in the 7 S and 11 S fractions and corresponding increase in the 2 S and 15 S fractions. Therefore partial degradation of 7 S globulin into 2 S protein and aggregation of 11 S globulin to 15 S component were proposed as result of ionizing radiation. Disulfide bonds and hydrophobic interactions have been shown to be responsible for decreased protein solubility during extrusion of soy. While significant increasing in protein solubility were noted in SDS-ME soluble fraction after irradiation treatment and reached to its maximum at 7.5 KGy (15.2%) compared to control sample (10.1%). These phenomena may be due to the direct effect of free radical produced from gamma radiation and affect the protein composition.

Radiation may dissociate these protein fractions to small subunits and rearrangement to form a complex protein even high or small molecular weight compounds (Afify and Shousha, 1988), solubilized only in SDS-ME fraction (Carroll *et al.*, 1952; Néné *et al.*, 1975; Schoweigert, 1959). Gamma irradiation decreased the water soluble fraction and increased the potentially degradable fraction and decreased the degradation rate of the protein. A decrease in protein solubility resulting from irradiation, observed in the present work gives evidence of the occurring cross-linking of chains and proteins aggregation (Delincee and Pushpa, 1981; Nisizawa, 1988; Zabielski *et al.,* 1984). Solubility of proteins relates to surface hydrophobic (protein-protein) and hydrophilic (protein-solvent) interaction (Taha and Mohamed, 2004). Irradiation induces the unfolding of the protein and denaturation, thus exposing non-polar groups that were previously blocked. Thus, gamma-irradiation increased hydrophobicity by exposing non-polar groups. On the other hand, when secondary and tertiary structures of a protein are unfolded, the hydrophobic groups interact and reduce water binding. Moreover, hydrophobic interactions lead to aggregation, followed by coagulation and precipitation (Englard and Seifter, 1990).

Peanut protein profiles

The results in Fig. 2 showed that, electrophoretic separation of protein profiles were different according to extraction method. The protein profiles of total soluble protein, albumin, globulin and SDS-ME soluble proteins were affected by gamma irradiation depending on the dose of irradiation and protein fractions. Since arachin and conarachin, the two major globulins in peanut comprise more than 85% of the proteins (Johns and Jones, 1916). The intensity of the five major subunits of the storage protein, arachin with MW 42, 37, 35, 22 and 20 K Da (Chiou *et al.,* 1990) were changed in its intensity according to the extraction methods and irradiation dose. Albumin was slightly decreased while most globulin protein fraction bands showed strong intensity especially with 7.5 KGy (Fig. 2). On the other hand the four minor subunits of arachin protein with MW 66, 31, 29 and 25 K Da did not change in its intensity by gamma radiation. While the intensity of conarachin major subunit with MW 64 K Da in the albumin fraction was decreased after different doses of irradiation treatments. The three minor subunits with MW 60, 32 and 21 K Da were increased after irradiation

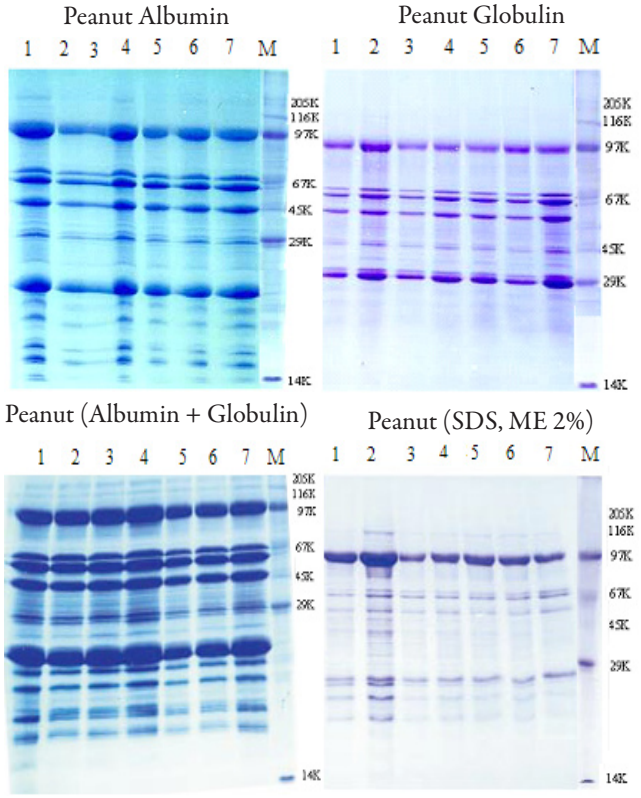

Fig. 2. Electrophoretic separation of protein subunits of peanut in SDS-PAGE 5 and 15% PAA in Tris-glycine buffer pH 8.3 with 0.1% SDS. γ-Radiation treatments; 1-control, 2-0.5 KGy, 3-1.0 KGy, 4-2.0 KGy, 5-3.0 KGy, 6-5 KGy, 7-7.5 KGy, M-Molecular weight protein markers

treatments. Because of irradiation effect there is a minor band with MW 16 K Da was appeared in irradiated samples in the total soluble protein (albumin + globulin) and its intensities were increased with the increasing in irradiation dose (Fig. 2). The results showed that most of major and minor bands of arachin and conarachin are denatured by gamma radiation rather than destructive because these fractions were re-solubilized in the SDS-ME fraction, especially the conarachin with MW of 64 K Da (Fig. 2). The irradiation caused native protein aggregation might simply result from rearrangement of the composed protein subunits (Chiou *et al.,* 1990, 1988; Sonntage, 1987) through a crosslinking of peptides or proteins to form dimers or tetramers by the action of free radicals. (Van Huystee, 1968) the question arose whether this fragmentation was brought about indirectly by enhanced lysozomal (proteolytic) activity, or by a direct effect of radiation such as ionization of the protein chain at the hit sites and perhaps by free radical reactions. Van Huystee (1971), Haskill and Hunt (1967) observed that the results of gel filtration of the irradiated protein arachin with MW 330 K Da showed increase in small molecular fragments and causes the formation of disulfide bridges between molecular strands in protein and causes aggregation of the pieces.

Protein solubility of peanut seeds

The results in Tab. 2 showed that, the total protein content was not affected by irradiation. While the solubility of total protein fraction was decreased and reached to its maximum decrease using irradiation dose 7.5 KGy (60.0%) compared to control (82.8%). Results showed significant differences between irradiation treatments and control samples, in the four-protein fractions. The significant differences of the total soluble protein started from dose 2 KGy, while albumin fraction started at 3 KGy. High significant differences could be noted even in globulin or in SDS-ME protein fraction, which started with irradiation dose of 1 KGy. The results showed that albumin was decreased in its solubility to 30% compared to control (43.1%) while globulin fractions decreased in its solubility from control (31.5%) compared with 22.1% in irradiated dose of 7.5 KGy. On the other hand total soluble protein was decreased by 20% as a result of gamma radiation to 7.5 KGy. These results are in agreement with Diehl (1978), Maity *et al.* (2009), cited that irradiation of globular protein, caused formation of protein aggregates, and caused decrease in protein solubility.

Tab. 2 Protein solubility of different fractions from irradiated peanut with gamma rays

Irradiation dose (KGy)	Total protein	Total soluble protein		Albumin fraction		Globulin fraction		SDS/MS soluble protein	
		% Protein	% Solubility	% Protein	% Solubility	% Protein	% Solubility	% Protein	% Solubility
0.0 KGy	30.2 ± 0.10	25.0 ± 0.33^{a}	82.8	13.0 ± 0.54^{a}	43.1	9.5 ± 0.44^{a}	31.5	4.3 ± 0.60^{a}	14.2
0.5 KGy	29.8 ± 0.13	23.5 ± 0.17^{ab}	78.9	12.5 ± 0.33^{a}	42.0	9.0 ± 0.58^{a}	30.2	4.4 ± 0.12^{a}	14.8
1.0 KGy	29.6 ± 0.13	22.5 ± 0.45^{abc}	76.0	12.3 ± 0.40^{a}	41.5	7.8 ± 0.27^{ab}	26.3	4.6 ± 0.18^{ab}	15.5
2.0 KGy	29.4 ± 0.03	21.5 ± 0.45^{bc}	73.1	12.0 ± 0.44^{a}	40.8	7.5 ± 0.19^{b}	25.5	5.5 ± 0.17^{abc}	18.7
3.0 KGy	29.3 ± 0.07	20.0 ± 0.67^{cd}	68.3	11.5 ± 0.21^{ab}	39.2	7.0 ± 0.07^{b}	23.9	5.7 ± 0.32^{bcd}	19.5
5.0 KGy	29.1 ± 0.12	18.0 ± 0.58^{d}	62.0	10.0 ± 0.29^{bc}	34.4	7.0 ± 0.20^{b}	23.4	6.0 ± 0.20^{cd}	20.6
7.5 KGy	29.0 ± 0.03	17.3 ± 0.35^{d}	60.0	8.7 ± 0.15^{c}	30.0	6.4 ± 0.22^{b}	22.1	6.1 ± 0.19^{d}	21.0
LSD 0.05		3.163		1.889		1.702		0.404	

Data represent the mean ± S.E. of three experimental replicates. Values with different letters in the same experiment and same column are significantly different (P ≤ 0.05)

Tab. 3. Protein solubility of different fractions from irradiated Sesame with gamma rays

Irradiation dose (KGy)	Total protein	Total soluble protein		Albumin fraction		Globulin fraction		SDS/MS soluble protein	
		% Protein	% Solubility	% Protein	% Solubility	% Protein	% Solubility	% Protein	% Solubility
0.0 KGy	28.6 ± 0.07	24.0 ± 0.33^{a}	83.9	9.0 ± 0.22^{a}	31.5	13.0 ± 0.17^{a}	45.5	2.3 ± 0.10^{a}	8.0
0.5 KGy	27.8 ± 0.03	22.0 ± 0.38^{ab}	79.1	8.4 ± 0.12^{ab}	30.2	12.5 ± 0.12^{a}	45.0	3.2 ± 0.20^{a}	11.5
1.0 KGy	27.5 ± 0.13	21.0 ± 0.5^{ab}	76.7	8.2 ± 0.15^{ab}	29.8	12.3 ± 0.38^{ab}	44.7	3.5 ± 0.12^{a}	12.7
2.0 KGy	27.3 ± 0.07	19.6 ± 0.62^{bc}	71.8	6.7 ± 0.24^{c}	24.5	11.7 ± 0.52^{b}	42.9	5.5 ± 0.03^{b}	20.0
3.0 KGy	27.1 ± 0.10	18.0 ± 0.29^{bc}	66.4	6.5 ± 0.21^{c}	24.0	11.5 ± 0.17^{b}	42.4	6.5 ± 0.12^{c}	24.0
5.0 KGy	27.0 ± 0.07	17.7 ± 0.35^{bc}	65.6	6.4 ± 0.19^{c}	23.7	11.0 ± 0.32^{b}	40.7	6.7 ± 0.06^{d}	24.8
7.5 KGy	26.5 ± 0.10	17.0 ± 0.33^{c}	64.2	6.0 ± 0.33^{c}	22.6	10.7 ± 0.21^{b}	40.4	6.9 ± 0.19^{d}	26.0
LSD 0.05		3.808		0.708		1.474		0.692	

Data represent the mean ± S.E. of three experimental replicates. Values with different letters in the same experiment and same column are significantly different (P ≤ 0.05)

Sesame protein profiles

The results in Fig. 3 showed that, some changes were detected after the gamma irradiation treatment in the protein profiles of albumin, globulin and SDS-ME soluble protein fractions. On the other hand, no changes were noted in total soluble protein fraction. In albumin fraction, two bands with high molecular weight 118, 76 K Da have been detected in seeds irradiated with the high doses of gamma irradiation (3, 5, 7.5 KGy) while one minor protein band with molecular weight 18 K Da was detected at the same doses. These three bands could not be detected ether in control or in seeds irradiated with low doses of gamma irradiation (0.5, 1 and 2 KGy). The appearance of high molecular weight protein was in agreement with the results of aggregation of a special protein to produce high molecular weight proteins as a result of γ-irradiation (Diehl *et al.,* 1978). On the other hand, the intensities of two minor bands with molecular weight 16, 20 K Da were decreased at gamma irradiation dose 2, 3 and 5 KGy. A new protein band with MW of 40 K Da could be detected in sesame irradiated with 3 KGy. The results are in agreement with the finding of Afify *et al.* (1992a), Gralik and Warchalewski (2006) who showed that, irradiation of an aqueous solution of serum albumin or wheat albumin produced destruction in its amino acid content. The present results were in agreement with (Afify and Shousha, 1988) who reported that irradiation caused slight differences in albumin protein band intensities and inactivation of enzyme and polymerization and aggregation of simple protein. In globulin fraction, the intensities of the three major subunits with molecular weight 35, 30 and 20 K Da was increased as a result of gamma radiation doses and reached to maximum intensity with 7.5 KGy.

On the other hand the intensities of the four minor bands with molecular weight 33, 16, 15 and 14 K Da was increased also as a result of gamma irradiation and reached to its maximum with 7.5 KGy. The low molecular weight proteins of 17, 18 and 19 K Da were increased in their intensity especially after 0.5 KGy and the intensities of the bands were decreased by increasing the irradiation dose. The changes in globulin fraction were probably due to radiation induced protein denaturation and degradation of the insoluble protein fraction as stated by Chow and Subha (1985), Afify and Shousha (1988), Afify *et al.* (1992b). The slight changes in protein patterns after SDS-PAGE separation in sesame seeds after irradiation may be due to slight changes in bonds stabilized protein conformation or due to slight destructive in some aromatic and heterocyclic amino acid residues (Taub *et al.,* 1976), and the possibility of rearrangement (Sonntage, 1987).

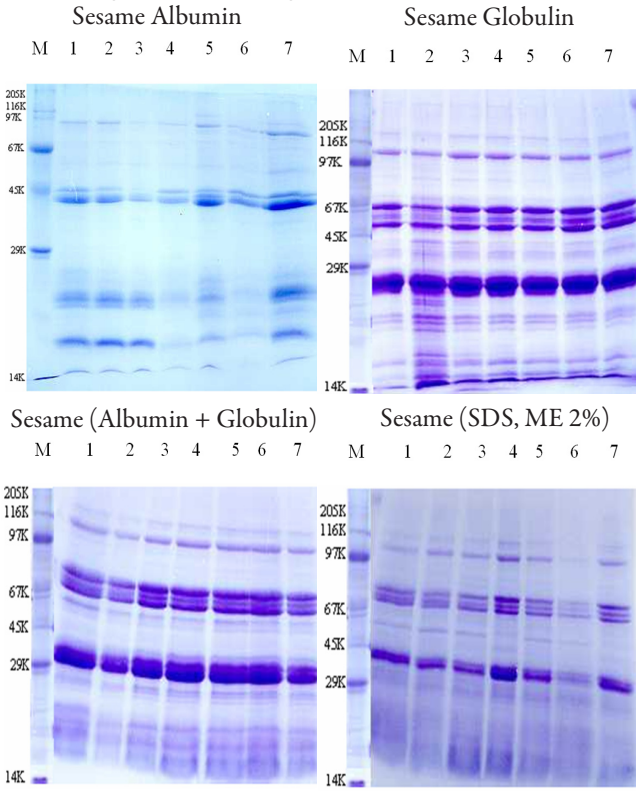

Fig. 3. Electrophoretic separation of protein subunits of sSesame in SDS-PAGE 5 and 15% PAA in Tris-glycine buffer pH 8.3 with 0.1% SDS. γ-Radiation treatments; 1-control, 2-0.5 KGy, 3-1.0 KGy, 4-2.0 KGy, 5-3.0 KGy, 6-5 KGy, 7-7.5 KGy, M-Molecular weight protein markers

Protein solubility of sesame seeds

The results in Tab. 3 showed that, the total protein content was not affected by irradiation, while the solubility of total protein fraction were decreased and reached to the maximum decrease using irradiation dose 7.5 KGy (64.2%) compared to the control (83.9%). Significant differences could be noted by comparing statistical analysis especially LSD and coefficient of variation of irradiated seeds for the three protein fractions; total soluble protein, albumin and SDS-ME soluble protein. On the other hand globulin fraction showed no significant differences. The significant differences in three protein fractions started at the same level of irradiation dose of 2 KGy. Therefore this dose considered the very important one and taken in the consideration during the irradiation of sesame to keep the protein quality and quantity of the irradiated seeds for commercial use. These results are in agreement with those found by Afify *et al.* (1992a). Such protein solubility reduction was probably due to radiation induced protein denaturation of sesame (Chow and Subha, 1986) and aggregations of protein (Sonntage, 1987).

Conclusions

One of the interests in this investigation was to determine the mechanisms might be causes fragmentation of protein during radiation exposure of oil seeds peanut, soybean and sesame seeds. The direct breakdown of the molecule by ionization capacity of the passing photon may be one possibility. In addition, radiation normally cause the formation of disulphide bridge between polypeptide chain which may be effect on the aggregation of the low molecular weight protein and effect on its conformation and solubility. While the other the enzymatic breakdown of the protein and enzyme molecule due to the stimulation of hydrolytic activity by radiation. It is concluded that implementing gamma radiation up to 7.5 kGy on the three oil seeds, will affect total soluble protein, albumin and globulin fractions as well as SDS/ME fraction. As a general trend intensity of bands was decreased with the increased gamma dose.

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