Effect of Gamma Irradiation on the Molecular Composition of Semolina Soluble Protein Fractions

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ABSTRACT

The effect of gamma irradiation (0, 0.25, 1, 2.5, 5, 10 and 90 kGy) on the total protein content as well as the amino acid composition and electrophoretic patterns of the soluble proteins of semolina stored for 6 months was investigated. The protein content of the semolina sample irradiated with the high dose of 90kGy(10.2 % as is) was significantly (P< 0.05) lower than that of any of the samples irradiated with 10kGy or lower(10.9- 10.7 % as is). The latter doses had no significant (P< 0.05) effect on this variable. The amino acid content of the irradiated semolina was influenced , yet irregularly, by the irradiation dose. In general, there was an increase of tyrosine and methionine accompanied with a decrease in lysine and phenylalanine upon increasing the irradiation dose in the range of 0 to 10 kGy. On the other hand, the 90 kGy dose resulted in a drastic decrease in total amino acids as analyzed with this procedure. Electrophoresis (SDS-PAGE) separation of soluble protein fractions from semolina showed that the intensity of the bands and their numbers decreased substantially upon increasing the irradiation dose above 5 kGy.

Keywords: Semolina, Gamma irradiation, Amino acids, Electrophoresis.

Abbreviations: kGy: kilo gray; SDS-PAGE: Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis.

INTRODUCTION

Wheat is the major stable food for many peoples around the world. It is mainly used for the production of bread , pasta, biscuits and cakes. Although starch is the major constituent of wheat endosperm, protein plays the major functional role in determining the quality of bread and other products. Most wheats of the world contain protein in the range of 10-17% on wet matter basis (Kent and Evers,1994) although some samples were reported with protein content outside this range (Pmeranz, 1988). The majority of wheat protein is located in the endosperm (~80%) (Cornell and Hoveling, 1998), Osborne classified wheat proteins on the basis of solubility into two groups (Kent and Evers, 1994): soluble proteins, which consist of albumins and globulins, are soluble in water and saline solutions respectively and insoluble proteins which consist of the gliadins (alcohol soluble) and glutenins (soluble in dilute acid or base). The latter two proteins determine the viscoelastic properties of gluten (Cornell and Hoveling, 1998). The polymeric gluten is developed after adequate mixing of hydrated semolina or flour from gliadin and glutenin proteins (insoluble proteins), thus forming an insoluble network able to entrap swollen starch granules

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and gas bubbles in dough (Gianibelli *et al.*, 2001). Soluble proteins play metabolic roles in wheat kernels as they make up the endogenous amylases, inhibitors of endogenous and exogenous enzymes such as α-amylase and trypsin and proteins that clot red blood cells (phytohemaglutinins). Therefore, soluble proteins have potential physiological, nutritional, toxicological and technological significance, in addition to the role they play in the resistance of the grains to stored insect pests (Gralik and Warchalewski, 2006). Of special importance is the nutritional role they play as they contain higher levels of essential amino acids like lysine than insoluble proteins, which are low in lysine and rich in glutamic acid and proline. Insoluble proteins, on the other hand, form, in the presence of water and mineral ions, the gluten, giving the dough its structure and making it possible to make a loaf of bread by holding the fermentation gas in thier network.

Soluble proteins are heterogeneous with their fractions having molecular weights less than 40 kDa. In fact, the fastest moving wheat proteins on SDS-PAGE are albumins. Wrigley and Bietz (1988), using different pH and extraction media , showed that soluble proteins of wheat can be resolved into 21 bands in SDS-PAGE. Most of the research dealing with soluble proteins composition of durum wheat was initiated by the need to detect the adulteration of durum wheat products with common wheat (Feillet, 1988).

Gamma irradiation (0.2-1.0 kGy) is used for effective control of insect infestation in cereals and their products (IAEA, 1991) . Food irradiation may replace the use of highly toxic fumigants like methyl bromide and phosphine gas for disinfestation of cereal grains , fruits and vegetables (Agundez-Arvizu *et al.*, 2006). The Joint FAO/ IAEA/ WHO Expert Committee on the Wholesomeness of Irradiated Foods (JECFI) declared foods irradiated up to 10 kGy as safe, presenting no toxicological hazard and no special nutritional or microbiological problems (Anonymous, 1981). However, later in September 1997, a study group appointed by WHO concluded that " even foods treated with doses greater than 10 kGy can be considered safe and nutritionally adequate when produced under established good manufacturing practices" (De Bruyn, 2000). Lee *et al.* (2005) reported that gamma irradiation affects proteins by causing conformational changes, oxidation of amino acids, rupturing of covalent bonds and formation of protein free radicals. The same authors reported that 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.

However, no detailed studies have been reported on the effects of gamma irradiation on the properties of soluble proteins and the amino acid profile of semolina. Hence, this study was undertaken to elucidate the effect of gamma irradiation on the electrophoretic pattern of the soluble proteins, amino acid profile and protein content of semolina.

MATERIALS AND METHODS

Durum Wheat Samples

Jordanian durum wheat, *Horani 27* cultivar, harvested in summer of 2006 season was obtained from Maru experiment station North of Jordan and brought to the Cereal Processing Laboratory at the University of Jordan in locally – produced polypropylene knit bags. The durum samples were milled to semolina on the Quadrumate I junior mill (Brabender, Duisberg, Germany). One and a half kilogram semolina samples were packed individually in transparent polyethylene food grade bags before irradiation.

Gamma Irradiation of Semolina

The irradiation facility is located at the Jordan Atomic Energy Agency (JAEA) (Amman, Jordan).

Samples were irradiated using the gamma cell (Gamma facility PX-γ-30, Russia) at room temperature and in the presence of air. Gamma cell was loaded with Co^{60} pencil type source and the dose rate was 1.214 kGy/hr. Ethylene mono-chloro-benzene dosimeter (ECB) was used to calibrate the irradiator. The exposure time was calculated to treat the samples with irradiation doses of 0.25, 1, 2.5, 5, 10 and 90 kGy. Irradiated semolina was stored at room temperature (between 20-26ºC) and about 20-35% relative humidity for a period of six months before running the analysis.

Protein Content

Total crude nitrogen of semolina samples was determined using Micro-Kjeldahl method according to the approved method No. 46-13 of the American Association of Cereal Chemists (AACC)- (AACC, 2000). All treatments were run in duplicates.

RP – HPLC Analysis of Amino Acids

Semolina proteins were prepared for amino acid hydrolysis by the method of total acid catalyzed hydrolysis (6.0 N HCl, 24 hr, 110 ºC) (Provimi Expert Department, 2007) followed by derivatization with FMOC reagent. Then 80% n-pentane and 20% ethyl acetate solutions were added and 1 ml from the lower layer of the prepared sample was filtered by a Teflon filter (0.45 µm) and 20 µl portion injected in the instrument. This test was performed in duplicate to obtain an average value of each amino acid.

Reversed-Phase HPLC from Waters Company (Milford, MA, USA) was used to determine the amino acid composition of semolina according to the method of the Association of Official Analytical Chemists (AOAC, 1995). The RP-HPLC instrument was equipped with an auto-injector (Waters 2695, separation module) and the separation of amino acids was conducted in a Nova-Pack RP-C_{18} ,4 µm particle size ,(3.9mm×150mm) column (Waters, Milford, MA, USA) coupled with a Waters 2996- Photodiode Array Detector.

Analysis was run under the following conditions: mobile phase consisted of three parts (A, B and C). A: acetate buffer (pH 4.2), B: methanol (HPLC grade) and C: acetonitrile (HPLC grade). The flow rate was set at 1.06 ml/min and the column temperature maintained at 30.2 ºC. The eluted and derivatized amino acids were detected by monitoring their fluorescence at 263 nm(AOAC, 1995). The gradient elution of A, B and C solutions is shown in Table 1.

Standard amino acids solution from Sigma-Aldrich AA-S-18 type (Saint Louis, MO, USA) was dissolved in 9-fluorenyl methyl chloroformate (FMOC) and n-pentane. However, a known weight of DL-methionine and L-cystine (Merck, Darmstadt, Germany) was treated with a mixture of formic acid and hydrobromic acid to oxidize bound and free cystine and methionine into cysteic acid and methionine sulphonic acid, respectively. Oxidation of sulfur containing amino acids in semolina samples was done with the same acids mixture.

Table 1. Gradient program employed for the separation of amino acids from semolina soluble proteins.

Time (min)	A	B	C
0-10	75	0	25
10-50	20	30	50
50-52	0	100	0
52-60	0	100	0
60-60.1	75	0	25

Electrophoresis SDS-PAGE of Soluble Proteins

Soluble proteins were extracted according to the method described by Wrigley and Bietz (1988) and modified by Singh and Skerritt (2001) as follows: One gram semolina was extracted with five volumes of 0.5 N NaCl. The contents were then centrifuged in the

ultracentrifuge (Centrikon T-1080, Kentron Instruments, Italy) for 10 minutes at 20,000 *g*. Soluble proteins were analyzed by SDS-PAGE according to the method described by Laemmli (1970) using 12% polyacrylamide resolving gel and 4% polyacrylamide stacking gel. The electrophoretic separations were performed using a Mini-PROTEAN®3 Cell (Bio-Rad Laboratories, Hercules, CA, USA). Molecular weights of the proteins were estimated using of PageRuler Prestained Protein Ladders (Fermentas, USA), and the reference band protein of 70 kDa is colored with an orange dye. The gel containing protein bands was scanned using a normal computer scanner to quantify the bands using TINA 2.0 computer software program.

Statistical Analysis

The protein content results were analyzed using statistical analysis system (SAS, 2000). Analysis of variance (ANOVA) was performed using complete randomized design (CRD). Means of each treatment variable were compared for significance at 5% probability level using least significant differences (LSD) test.

RESULTS AND DISCUSSION

Table 2 shows that the protein content of irradiated semolina (10.7-10.9%) was not significantly ($P \leq 0.05$) affected by the irradiation dose up to 10 kGy.

Table 2. Protein content of irradiated semolina.

	Irradiation dose (kGy)						
	0	0.25	1	2.5	5	10	90
Protein% (N×5.7)	10.8[a]	10.9[a]	10.8[a]	10.9[a]	10.7[a]	10.7[a]	10.2[b]

- Each mean is the average of two replicates.
- Means in the same row with different superscripts are significantly ($P \leq 0.05$) different according to LSD.
- Values in the above table are expressed on (as is) basis.

This result is in agreement with MacArther and D'Appolonia (1983), Kanemaru *et al.* (2005) and Azzeh and Amr (2009) who found that low and medium doses of gamma irradiation did not affect the protein content of flour and semolina. However, applying a 90 kGy irradiation dose decreased the protein content significantly ($P \leq 0.05$) to attain 10.2%. This significant decrease could be due to partial nitrogen destruction at such dose. Similar effect was not observed at lower doses.

As shown in Table 3, cystine, methionine, glycine, alanine, leucine, serine, threonine, tyrosine, asparatic acid and glutamic acid increased; while arginine, lysine, valine, isoleucine and phenylalanine decreased as the irradiation dose was increased up to a 10 kGy dose. At 10 kGy, tyrosine exhibited the highest increase, whereas phenylalanine suffered the highest reduction. The effect of gamma irradiation on the amino acids of semolina could be related to the structure of amino acids; i.e. simple amino acids, such as glycine may increase due to their formation as a result of the radiolytic breakdown of the more complex aliphatic amino acids. Aliphatic amino acids with longer chain length provide additional C-H bonds for interaction with OH radicals that reduce the extent of oxidative deamination (Simic, 1983). In the presence of thiol (-SH) or disulfide (S-S) groups, oxidation of sulfur occurs, and in aromatic and heterocyclic amino acids hydroxylation of aromatic ring is the principal reaction (Matloubi *et al.*, 2004). Wang and Von Sonntag (1991) concluded that sulfur-

containing as well as aromatic amino acids are, in general, the most sensitive ones to irradiation, while simple amino acids could be formed by the destruction of other amino acids. Sulfur-containing amino acids (cystine and methionine) increased as the irradiation dose increased; a finding that is not in full agreement with those of previously mentioned authors. Lysine, the most important amino acid in cereal grains, decreased by about 5% using 10 kGy irradiated semolina. Remarkably, there was a 52% reduction in lysine content by applying a 90 kGy irradiation dose. The results indicate that total amino acids increased upon increasing the irradiation dose up to 10 kGy. This result is compatible with that of Erkan and Ozden (2007) who reported that amino acid composition of sea bream was increased after irradiation. This can be attributed to better hydrolysis of proteins, by irradiation, with the consequence of more efficient extraction of their amino acids. However, the semolina sample irradiated with 90 kGy showed a drastic reduction in the total amino acid content which could be due to the radiolytic effect of gamma rays on amino acids which results in the formation of byproducts not detected by this analytical technique.

Table 3. Amino acid content of irradiated semolina proteins.

Amino acid	Irradiation dose (kGy)						
	0	**0.25**	**1**	**2.5**	**5**	**10**	**90**
Cystine	0.175 ±0.011	0.182 ±0.021	0.180 ±0.016	0.187 ±0.012	0.189 ±0.021	0.184 ±0.002	0.173 ±0.023
Methionine	0.119 ±0.012	0.118 ±0.010	0.124 ±0.021	0.121 ±0.019	0.126 ±0.002	0.120 ±0.013	0.099 ±0.021
Arginine	0.452 ±0.012	0.441 ±0.020	0.434 ±0.014	0.438 ±0.013	0.436 ±0.014	0.400 ±0.006	0.408 ±0.014
Lysine	0.273 ±0.009	0.261 ±0.007	0.267 ±0.003	0.263 ±0.010	0.265 ±0.006	0.260 ±0.011	0.131 ±0.012
Glycine	0.210 ±0.012	0.214 ±0.006	0.208 ±0.012	0.215 ±0.023	0.224 ±0.012	0.227 ±0.008	0.183 ±0.014
Alanine	0.331 ±0.026	0.341 ±0.025	0.338 ±0.022	0.348 ±0.013	0.352 ±0.009	0.356 ±0.005	0.341 ±0.013
Valine	0.451 ±0.022	0.447 ±0.021	0.451 ±0.010	0.438 ±0.021	0.435 ±0.007	0.448 ±0.004	0.430 ±0.012
Leucine	0.734 ±0.017	0.742 ±0.012	0.758 ±0.006	0.755 ±0.004	0.764 ±0.008	0.760 ±0.011	0.705 ±0.011
Isoleucine	0.359 ±0.014	0.336 ±0.012	0.357 ±0.021	0.351 ±0.006	0.333 ±0.021	0.340 ±0.019	0.329 ±0.013
Serine	0.385 ±0.022	0.385 ±0.016	0.365 ±0.024	0.396 ±0.013	0.374 ±0.011	0.413 ±0.016	0.389 ±0.024

Amino acid	**Irradiation dose (kGy)**						
	0	**0.25**	**1**	**2.5**	**5**	**10**	**90**
Threonine	0.600	0.614	0.608	0.643	0.651	0.649	0.622
	±0.011	±0.022	±0.011	±0.020	±0.009	±0.014	±0.009
Phenylalanine	0.490	0.499	0.476	0.469	0.453	0.419	0.458
	±0.013	±0.003	±0.010	±0.022	±0.016	±0.017	±0.014
Tyrosine	0.553	0.574	0.575	0.567	0.633	0.646	0.440
	±0.010	±0.013	±0.018	±0.017	±0.017	±0.019	±0.009
Aspartic acid	0.657	0.662	0.658	0.675	0.684	0.694	0.631
	±0.022	±0.009	±0.022	±0.016	±0.021	±0.021	±0.016
Glutamic acid	3.81	3.86	4.06	4.04	3.92	4.08	3.86
	±0.026	±0.022	±0.031	±0.011	±0.016	±0.034	±0.022
Total	**9.599**	**9.676**	**9.859**	**9.906**	**9.839**	**9.996**	**9.199**

- Each mean is the average of two replicates.
- All values are expressed as g amino acid /100g semolina.
- Means ± S.D.

The electrophoretic patterns of semolina soluble proteins are shown in Figure 1. Number of bands for each lane of the electrophoresis gel was 19 bands for the 5 kGy irradiated sample and lower treatments (Table 4). Irradiation with a 10 kGy dose reduced the number of bands, and the 90 kGy irradiation treatment further reduced the band numbers. Gralik and Warchalewski (2006) studied the influence of gamma irradiation on the electrophoresis patterns of wheat grain albumin fractions. They found that the electrophoresis pattern of the albumin sample at 0.1 kGy showed 15 protein bands, while in the control sample there were only 11 bands. The lower protein bands (5 bands) were detected in wheat grain samples irradiated by 10 kGy. The band intensity decreased with increasing the irradiation dose, as seen in Table 4, except in the irradiation dose of 1 kGy. These data show that soluble proteins' components (enzymes, enzymatic inhibitors and others) were stable up to 5 kGy irradiation dose, while a partial damaging effect to soluble proteins came into view after applying a 10 kGy irradiation dose and higher. MacArthur and D'Appolonia (1983) studied the effects of low gamma irradiation doses (up to 3 kGy) on the activity of α-amylase using Grain Amylase Analyzer (GAA), model 191. The GAA values showed very little change within a particular wheat variety as the level of irradiation was increased, which means that α-amylase activity was not affected by low irradiation doses. Urbian (1986) reported that the enzymes, in general, present in cereal grains are unaffected by irradiation. Also, the irradiation up to 4.65 kGy had no effect on α-amylase activity as compared to the control sample with an enzymatic activity of 0.027 SKB units/g for these samples; whereas 9.3 and 27.9 kGy doses decreased the enzymatic activity to 0.024 and 0.020 SKB units/g, respectively, which means that large doses had a slight effect on enzymatic activity that decreased meaningfully with increasing the irradiation dose. These effects of gamma irradiation on food proteins may have, on the other hand, several applications on improving the nutritional quality of foodstuff. As reported by Byun *et al.* (2002) and Zhenxing *et al.* (2007), irradiated protein extracts exhibited

a decrease in allergenicity with the increase of irradiation dose in comparison with non-irradiated foods. In addition, Machaiah *et al.* (1999) and Siddihuraju *et al.* (2002) used low doses of gamma irradiation to reduce the quantity or activity of various antinutritional factors found in legumes such as lectin activity, saponin content and trypsin inhibitors. In this study, irradiation of semolina did not influence its protein content. At the same time, there was some change in the amino acid content in a manner which needs to orient the studies of food irradiation on this area.

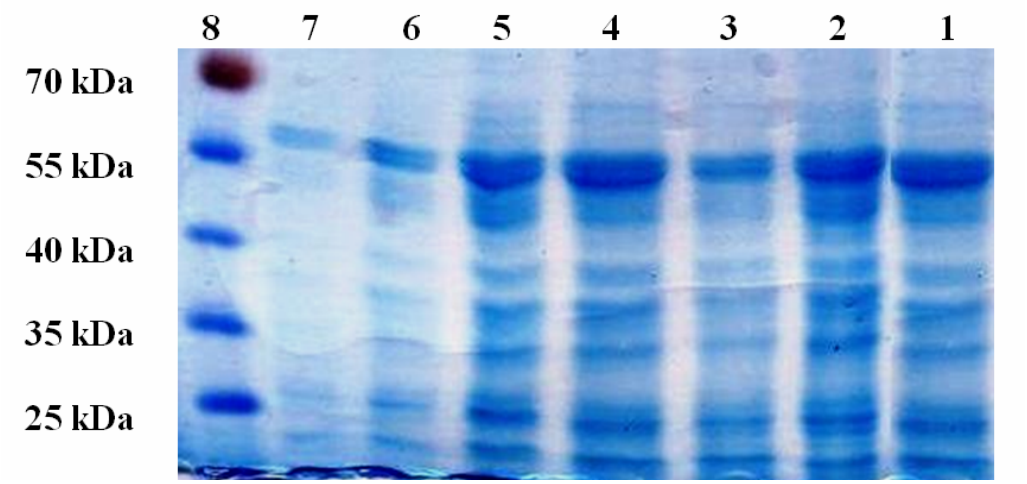

Figure 1. SDS-PAGE analysis of soluble proteins
(Lane 1: 0 kGy, Lane 2: 0.25 kGy, Lane 3: 1 kGy, Lane 4: 2.5 kGy, Lane 5: 5 kGy, Lane 6: 10 kGy, Lane 7: 90 kGy and Lane 8: standard molecular weights)

Table 4. Effect of semolina irradiation on the SDS-PAGE electrophoretic patterns of its soluble proteins .

Irradiation dose (kGy)	Optical density	Reduction (%)	Number of bands
0	1688.51	----	19
0.25	1731.03	----	19
1	930.49	44.89	19
2.5	1653.85	2.05	19
5	1348.08	20.16	19
10	626.91	62.87	13
90	242.61	85.63	11

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تأثير معاملة السميد بأشعة جاما على التركيب الجزيئي لبروتيناته الذائبة

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ملخـص

تمت دراسة تأثير جرعات مختلفة من أشعة جاما (0 و0.25 و1 و2.5 و5 و10 و90 كيلوجراي) على كمية البروتين والأحماض الأمينية ونمط الرحلان الكهربائي للبروتينات الذائبة في السميد المخزن لمدة 6 شهور. أظهرت النتائج أن كمية البروتين في السميد لم تتأثر حتى جرعة تشعيع مقدارها 10 كيلوجراي، بينما قلت كمية البروتين بشكل معنوي ($P<0.05$) في عينة السميد المعاملة بجرعة 90 كيلوجراي (10.2 %) عن نظيراتها المعاملة بجرعات 10 كيلوجراي أو أقل (10.7-10.9%) . لوحظ عدم وجود نمط ثابت لتأثير الزيادة في جرعة التشعيع (10 كيلوجراي أو أقل) على محتوى السميد من الأحماض الأمينية. وأشارت نتائج تحليل الأحماض الأمينية إلى أنه بزيادة الجرعات المستعملة حتى 10 كيلوجراي سجل الحامضان الأمينيان تايروسين وميثايونين أكثر زيادة بينما سجل الحامضان فينيل-ألانين واللايسين أكثر نقص. أما بالنسبة لنتائج الرحلان الكهربائي للبروتينات الذائبة بطريقة *SDS-PAGE* فقد تبين أن كثافة الحزم وعددها قل بشكل ملحوظ مع زيادة جرع التشعيع فوق 5 كيلوجراي.

الكلمات الدالة: سميد، التشعيع بأشعة جاما، التركيب الجزيئي للبروتينات الذائبة، أحماض أمينية، رحلان كهربائي.

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