

## Effect of Gamma-Irradiation or/and Extrusion on the Nutritional Value of Soy Flour

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### Abstract

Although soybean is rich with high amount of protein, phenolic compounds and other bioactive nutrients, their bioavailability and utilization by either humans or animals are relatively low due to the presence of high proportions of various antinutrients. Therefore, this study was assessed to use gamma irradiation or/and extrusion for inactivation or removal of certain antinutritional factors as well as study the effect of these processing method on the nutritional value of soy flour (the simplest form of soy protein). Analyses included proximate composition, the level of total phenols and levels of antinutrients (phytic acid, tannins and trypsin inhibitors) of raw and processed soy flour. In addition, amino acid contents were analyzed by using high performance amino acid analyzer-Biochrom 20, gas chromatography was used for analysis of fatty acids as well as phenolic compounds were determined by high performance liquid chromatography. The results showed that moisture, crude protein, crude fat, crude fiber, and ash were unchanged by the irradiation (5 and 10 KGy) or/and extrusion except the moisture content was decreased by extrusion.  $\gamma$ -irradiation or/and extrusion processing significantly reduced the levels of phytic acid, tannins, and trypsin inhibitor while the total phenols was increased relative to unprocessed control samples. All essential amino acids, fatty acids and phenolic compound were changed by different values. From these results, it could be possible to demonstrate the benefits of  $\gamma$ -irradiation or/and extrusion processing on the nutritional properties of soy flour by reducing its antinutritional contents and improving some of functional nutrients.

**Keywords:** Gamma-irradiation; Extrusion; Soy flour; Antinutritional factors.

### Introduction

Legumes are consumed in large quantities because they are considered as poor man's meat, cheap and valuable potential source of complex carbohydrates 50 -60% , protein and dietary fiber; contribute significant amounts of vitamins and minerals, and high energy value [1,2]. Protein contents in legume grains range from 17% to 40%, contrasting with 7–13% of cereals, and being equal to the protein contents of meats 18–25% [3].

Soybeans (*Glycine max*) is a species of legumes which are becoming an important economic crop as a major source of protein, energy, polyunsaturated fats, fiber, vitamins, minerals, and other nutrients [4-6]. Its low cost and its useful health benefits are improving its use even to animal or human nutrition in different groups, in order to reduce risk factors for chronic diseases like diabetes mellitus, cancer, cardiovascular disease, osteoporosis and others [7]. On the other hand, the nutritive quality and digestibility of soy protein in both human and animals are restricted by the presence of antinutritional factors such as phytic acid, tannins and trypsin inhibitors [8].

In order to inactivate or reduce the antinutritional substances, various conventional, simple processing methods have been used such as dry heating, roasting, boiling, soaking in water [9,10]. However, none of these methods is able to completely remove all the detected antinutrients that are present in seeds, grains or feed materials. Gamma irradiation treatment and extrusion cooking of legumes may be one of the possible alternative and additional processing techniques for reducing antinutrients and improving the nutritive quality of legumes [11-13].

Soybeans irradiated at a dose level of 10 KGy, retained their normal levels of moisture, crude protein, fat and ash. This dose level does not result in the denaturation of protein, and does not affect the nitrogen containing components of the food materials [14,15]. Also, gamma-irradiation induced enhancement of isoflavones, phenols, anthocyanin

and antioxidant properties of different seed coat colored soybean genotypes has been recently reported [16].

In addition, extrusion cooking application to legume processing has developed quickly during the last decade, and can now be considered as a technology of its own right. It would allow reduction of antinutritional factors and it not only improves digestibility [17] but also improves nutrient bioavailability [13] at a cost lower than other heating systems (baking, autoclaving, etc.). Also, extrusion processing for soybeans can convert them to a high quality food product [18].

This study was designed to assess the effects of gamma irradiation or/and extrusion processing on the anti-nutritional factors, amino acid content, fatty acids and phenolic compound of soy flour.

### Material and Methods

#### Material

Freshly dried soybean seeds were obtained from the Agriculture Research Institute, Ministry of Agriculture and Land Reclamation, Giza, Egypt. Seeds were sorted by discarding damaged and immature ones. They were stored in air-tight containers at room temperature (25  $\pm$  1°C) prior to further use.

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## Seeds treatment

**Gamma irradiation treatment:** Soybean seeds were packed in polyethylene bags, and sealed by heat. They were treated with 5 and 10 KGy, delivered at a dose rate of 4.75 KGy/h. The facility used was the Indian Gamma Chamber 400 A, <sup>60</sup>Co facility at the National Center for Radiation Research and Technology (NCRRT) - Nasr City, Cairo, Egypt.

**Heat processing (Extrusion):** The whole soybean seeds were cleaned, dehulled, ground to coarse particles, and then extruded by single screw extruder with three zones at the Soy processing unit, Food Technology Research Institute, Agriculture Research Center, Giza, Egypt. The heat treatment rise gradually through extruder zones for short time.

**Combination of  $\gamma$ -irradiation and extrusion:** Soybean seeds were packed in polyethylene bags, and sealed by heat. They were subjected to ambient temperature to gamma irradiation at dose levels of (5 and 10 KGy) and dose rate of 4.75 KGy/h, then followed by heat processing at high temperature (by gradual increase in temperature through extruder zones ) for short time.

## Preparation of soy flour

Either raw or treated soy flour was prepared by cleaning, dehulling the raw and treated seeds. Then the dehulled seeds were grind to obtain the flour.

## Determination of chemical composition of raw and treated soy flour

Determination of moisture, ash, crude protein, total crude lipid and crude fiber content were determined according to the method of A.O.A.C. [19].

## Determination of amino acid composition of raw and treated soy flour

This analysis was performed at the central laboratories of National Center for Radiation Researches and Technology (NCRRT) at Nasr City-Cairo, using high performance amino acid analyzer-Biochrom 20 (auto sampler version) Pharmacia Biotech.

## Procedure

### Hydrolysis procedure

Dried and defatted samples were weighed in the screw-capped tubes (50-100 mg) and 5 ml of HCL (6.0 N) was added to each tube. The tubes were attached to a system; which allows the connection of nitrogen and vacuum lines without disturbing the sample. The tubes were placed in an oven at 110°C for 24 hours [20]. The tubes were then opened and the contents of each tube was filtered and evaporated until dryness in a rotary evaporator. A suitable volume of 0.2 M sodium citrate buffer (pH 2.2) was added to dissolve the contents of each dried film of the hydrolyzed sample followed by ultrafiltration using a 0.2  $\mu$ m membrane filter [21]. Hydrolyzed sample solution was introduced to the column.

### Elution buffers and detection reagent

Amino acids were achieved using buffers with different pH and molarities. In general, higher the pH and molarities, the faster elute amino acids. Three citrate buffers were used to elute 16 amino acids, buffer 1(0.2 M, pH 3.20) and buffer 2 (0.2 M, pH 4.25) elute the acidic and neutral amino acids while buffer 3 (0.2 M, pH 6.45) elute the basic amino acids.

In addition, a loading dilution citrate buffer (0.2 M, pH 2.2) and a column-regeneration solution (0.4 M NaOH) was used. All buffers and NaOH solution were Pharmacia Biotech Chemicals. Ninhydrin detection reagent was used which consisted of ultra solve (2.0 L), ninhydrin (20 g) and hydrindantin (1.6 g). All these items were Pharmacia Biotech Chemicals.

### Analytical conditions

Amino acid analyzer equipped with stainless steel column (200x4.6 mm) packed with altropac 8 (8  $\mu$ m  $\pm$  0.5  $\mu$ m) cation exchange resin. Application of the sample is followed by stepwise elution with the aforementioned 3 buffers resolved 17 amino acids. The following program was used for the separation and detection of the amino acids:

Buffer 1 was pumped for 9 minutes followed by buffer 2 for 12 minutes and buffer 3 for 17 minutes. The column was regenerated using 0.4 M NaOH for 4 minutes followed by equilibration in buffer, for 16 min. The column was initially heated at 53°C for 9 minutes. The temperature was changed to 58°C for 13 min then changed to 95°C for 24 minutes, finally cooled down to 53°C for the remainder of the cycle (12 minutes) The cycle time from injection to injection was 58 minutes.

The flow rate was 25 ml/hr for ninhydrin reagent and 35 ml/hr for the buffers. The reaction between the amino acids and ninhydrin occurred at 135°C in a 10 ml PTFE reaction coil (0.3 mm I.D) immersed in silicon oil. Detection was performed at two wavelengths (570 and 440 nm). The data of each chromatogram was analyzed by EZ. Chrom-Chromatography Data system Tutorial and user's Guide- Version 6.7.

### Determination of fatty acid composition of raw and treated soy flour

The Fatty Acids (FA) were analyzed using Chromatograph-Mass at NCRRT. Separation was obtained by using a selective detector instrument "GC -MS" type HP, 6890 series, equipped with a flame ionization detector and innowax-cross linked polyethylene glycol fused silica column was used for characterization of fatty acids.

### Determination of total phenols of raw and treated soy flour

Total phenolic contents were measured by using the Folin Ciocalteu colorimetric method [22].

### Determination of phenolic compounds of raw and treated soy flour

Grounded dry powder of soy flour (10 g) was weighed into a test tube. A total of 100 ml of 80% aqueous methanol was added, and the suspension was stirred slightly. Tubes were sonicated twice for 15 min and one left at room temperature (~20°C) for 24 h. The extract was centrifuged for 10 min (10 min, 1500xg), and supernatants were filtered through a 0.2  $\mu$ m millipore membrane filter then 1-3ml was collected in avial for the HPLC analysis of phenolic compounds.

Phenolic standard were determined by HPLC according to the method of Goupy et al. [23]. Phenolic compounds of soy flour were analyzed at the Agriculture Research Center, Giza, Egypt by using HPLC Hewlett Packard (series 1050) equipped with autosampling injector, solvent degasser, Ultraviolet (UV) detector set at 280 nm and quarter HP pump (series 1050). The column temperature was maintained at 35°C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/min. The Phenolic standard from sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used

to calculate phenolic compounds concentration by the data analysis of Hewlett Packard software.

### Determination of antinutritional factors of raw and treated soy flour

Tannins content were measured by using the vanillin-HCl method [24], phytic acid content was determined by the method described of Wheeler and Ferrel [25], as well as trypsin inhibitors were determined by using benzoyl-DL-arginine-p-nitroanilide (BAPA) as substrate according to Hamerstrand et al. [26].

### Statistical Analysis

Statistical analyses were performed using computer program Statistical Packages for Social Science [27] and values compared with each other using one-way analysis of variance [ANOVA].

### Results

The major chemical components of raw and treated soy flour were obtained at Table 1. It is evident that contents of raw soy flour not significantly affected by  $\gamma$ -irradiation at both dose 5 & 10 KGy, extrusion and by the combination of irradiation and extrusion except the moisture content was significantly decreased under the effect of extrusion.

Results in Figure 1 showed the amino acid contents of raw and treated soy flour. The most abundant essential amino acids of raw soy flour were leucine, histidine, lysine and phenylalanine.

Arginine, aspartate, proline and glutamate were the most abundant nonessential amino acids of raw flour. In case of  $\gamma$ -irradiation, extrusion and irradiation+extrusion, all essential amino acids were increased by different values while histidine was slightly decreased in all treated soy flour except in extruded flour its value was slightly increased. However, all nonessential amino acids were aroused by the applying treatments on raw soy flour, but only proline was decreased, as well as the amount of serine was increased by  $\gamma$ -irradiation (5 KGy). In addition, cysteine not found in the raw flour but it was observed in all treated samples by different amount.

Raw soy flour contains unsaturated fatty acids oleic (47.99%) of total identified fatty acids, palmitic (37.11%) and stearic (14.90%) as saturated fatty acid of total identified fatty acids. The total Saturated Fatty Acids (SFA), total UnSaturated Fatty Acids (USFA) and Unsaturated to Saturated ratio (U/S ratio) was 52.01%, 47.99% and 0.92 respectively. It can be seen from the result that, the percentage of palmitic (C16:0), stearic (C18:0) and oleic (C18:1) of raw flour were declined by  $\gamma$ -irradiation at 5 and 10 KGy or/and extrusion.

Whereas, some fatty acids were observed by using the above mentioned treatments, such as linoleic acid (C18:2). Unsaturated to saturated ratio (U/S ratio) was changed by  $\gamma$ -irradiation at 5 and 10 KGy or/and extrusion to 0.99, 1.27, 3.60, 1.77 and 1.51, respectively (Figure 2).

Figure 3 showed the different mean value of 13 phenolic compounds in the raw and treated soybean flour. The main content of phenolic compounds of raw soy flour was pyrogallol, syringic, catachin and vanillic acid. Both of catachin and P-benzoic were increased under the effect of  $\gamma$ -irradiation, extrusion and the combination of both while these processing methods reduced pyrogallo, gallic and syringic acid.

As shown in Figure 4, the total phenol of raw soy flour (7.25 mg/g) was significantly increased by gamma irradiation at dose level (5 &

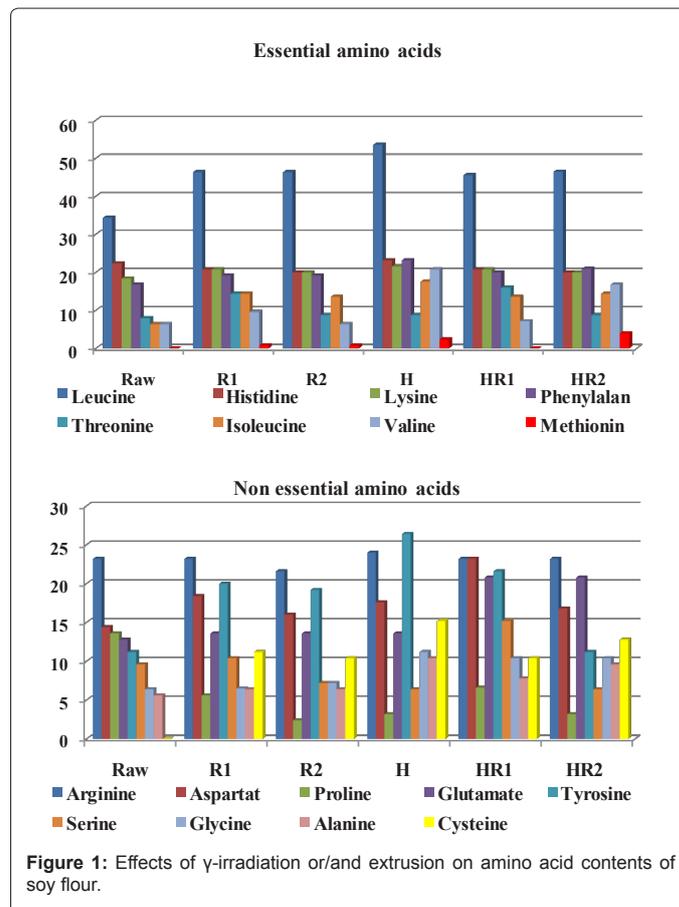


Figure 1: Effects of  $\gamma$ -irradiation or/and extrusion on amino acid contents of soy flour.

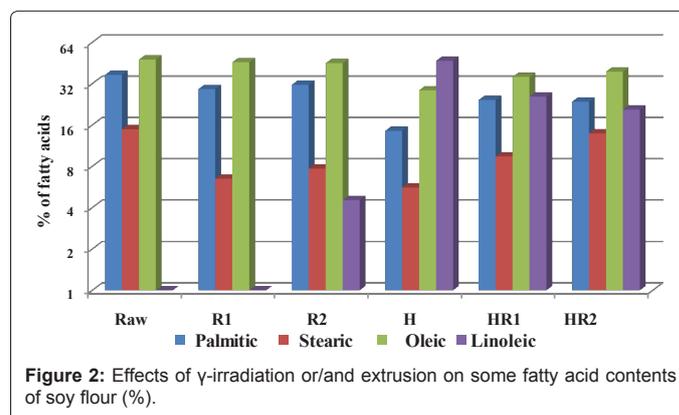


Figure 2: Effects of  $\gamma$ -irradiation or/and extrusion on some fatty acid contents of soy flour (%).

10 KGy) to 9.6 mg/g and 10.5 mg/g and by irradiation (5 & 10 KGy) and extrusion to 9.5 and 10 mg/g, respectively. While the extrusion significantly decreased the total phenols to 6.5 mg/g.

The results observed in table 2 summarized the mean values of Tannin (TN), phytic Acid (PA) and trypsin inhibitor of raw and processed soy flour. A significant reduction was noticed in values of these antinutritional factors by processing of raw sample and the highest reduction was observed in irradiated (10 KGy)+extruded soy flour.

### Discussion

Soybean and its products are economically valued because their

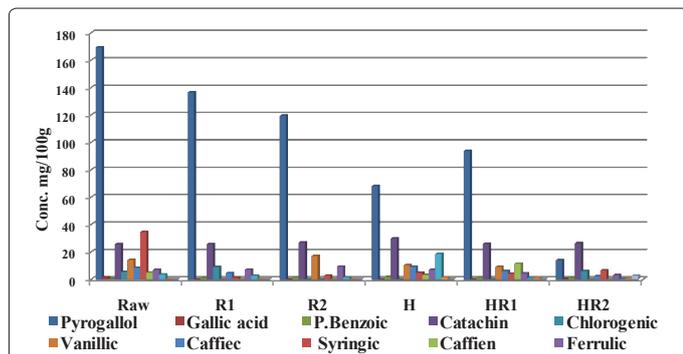


Figure 3: Effects of  $\gamma$ -irradiation or/and extrusion on some phenolic compounds of soy flour.

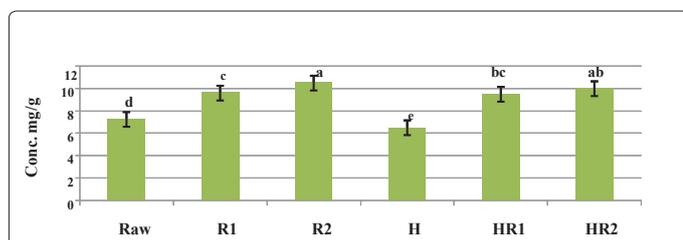


Figure 4: Effects of  $\gamma$ -irradiation or/and extrusion on total phenols of soy flour.

Treatment	Moisture	Ash	Fat	Fiber	Crude protein	NFE	Dray matter
Raw	6.40 ± 0.057 <sup>a</sup>	6.50 ± 0.057 <sup>a</sup>	20.76 ± 0.33 <sup>a</sup>	6.30 ± 0.057 <sup>a</sup>	43.60 ± 0.15 <sup>a</sup>	16.53 ± 0.33 <sup>a</sup>	93.60 ± 0.057 <sup>c</sup>
R <sub>1</sub>	6.40 ± 0.057 <sup>a</sup>	6.10 ± 0.057 <sup>a</sup>	20.33 ± 0.33 <sup>a</sup>	6.23 ± 0.033 <sup>a</sup>	43.50 ± 0.06 <sup>a</sup>	17.43 ± 0.29 <sup>a</sup>	93.60 ± 0.057 <sup>c</sup>
R <sub>2</sub>	6.47 ± 0.033 <sup>a</sup>	6.30 ± 0.057 <sup>a</sup>	20.67 ± 0.33 <sup>a</sup>	6.17 ± 0.057 <sup>a</sup>	43.46 ± 0.14 <sup>a</sup>	16.83 ± 0.40 <sup>a</sup>	93.53 ± 0.033 <sup>c</sup>
H	4.70 ± 0.057 <sup>b</sup>	6.50 ± 0.057 <sup>a</sup>	22.00 ± 0.57 <sup>a</sup>	6.20 ± 0.033 <sup>a</sup>	43.43 ± 0.08 <sup>a</sup>	17.17 ± 0.63 <sup>a</sup>	95.30 ± 0.057 <sup>a</sup>
HR <sub>1</sub>	4.60 ± 0.057 <sup>b</sup>	6.13 ± 0.033 <sup>a</sup>	21 ± 0.57 <sup>a</sup>	6.13 ± 0.033 <sup>a</sup>	43.36 ± 0.06 <sup>a</sup>	18.77 ± 0.63 <sup>a</sup>	95.4 ± 0.057 <sup>a</sup>
HR <sub>2</sub>	4.87 ± 0.057 <sup>b</sup>	6.23 ± 0.033 <sup>a</sup>	20.76 ± 0.33 <sup>a</sup>	6.07 ± 0.033 <sup>a</sup>	43.36 ± 0.03 <sup>a</sup>	18.61 ± 0.45 <sup>a</sup>	94.13 ± 0.033 <sup>b</sup>
Probability	0.0001	0.0763	0.2260	0.2006	0.5880	0.7707	0.0001

a,b,c,...Means within same column followed by different letters are significantly different at (P<0.05). Values are means of three replicates (± SE).

Raw→ raw soy flour.

R<sub>1</sub>→ irradiated at dose 5KGy. R<sub>2</sub>→ irradiated at dose 10KGy. H→ extruded.

HR<sub>1</sub>→ irradiated (5 kGy) +Extruded. HR<sub>2</sub>→ irradiated (10KGy) +Extruded.

NFE→Nitrogen Free Extract.

Table 1: Effects of  $\gamma$ -irradiation, extrusion and their combinations on the chemical composition of soybean flour (%).

nutrient and phytochemical characteristics, which also classifies it as a food of high nutritional value and functional claims. Also, they contain several antinutritional factors, which could limit their consumption content and removal of these undesirable components is essential to improve the nutritional quality of soy [9].

In this study, the results of raw soy flour chemical composition were in line with Khan et al. [28], who analyzed soy and observed that moisture (6.27%), protein (41.56%), crude fat (23.68%), crude fiber (6.825) and ash (4.54%). The results confirmed that these chemical compositions were not significantly affected by  $\gamma$ -irradiation at both dose 5 & 10 KGy which in agreement with El-Niely [29] and that can be attributed to the relatively limited amount of water content of soy flour,

Treatment	Tannic acid mg/g	Phytic acid mg/g	Trypsin inhibitor IU/g
Raw	0.46 ± 0.018 <sup>a</sup>	5.23 ± 0.088 <sup>a</sup>	75.37 ± 1.01 <sup>a</sup>
R <sub>1</sub>	0.32 ± 0.011 <sup>b</sup>	4.53 ± 0.176 <sup>b</sup>	65.60 ± 0.70 <sup>b</sup>
R <sub>2</sub>	0.26 ± 0.008 <sup>b</sup>	4.43 ± 0.088 <sup>b</sup>	49.47 ± 0.41 <sup>c</sup>
H	0.23 ± 0.005 <sup>b</sup>	3.40 ± 0.057 <sup>c</sup>	32.87 ± 0.32 <sup>d</sup>
HR <sub>1</sub>	0.21 ± 0.005 <sup>b</sup>	3.27 ± 0.033 <sup>c</sup>	29.23 ± 0.20 <sup>d</sup>
HR <sub>2</sub>	0.18 ± 0.005 <sup>c</sup>	3.17 ± 0.033 <sup>c</sup>	28.03 ± 0.12 <sup>d</sup>
Probability	0.0130	0.0020	0.0003

a,b,c,...Means within same column followed by different letters are significantly different at (P<0.05). Values are means of three replicates (± SE).

Raw→ raw soy flour.

R<sub>1</sub>→ irradiated at dose 5KGy. R<sub>2</sub>→ irradiated at dose 10KGy. H→ extruded.

HR<sub>1</sub>→ irradiated (5 kGy) +Extruded. HR<sub>2</sub>→ irradiated (10KGy) +Extruded.

NFE→Nitrogen Free Extract.

Table 2: Effects of  $\gamma$ -irradiation, extrusion and their combinations on antinutritional factors of soy flour.

so it would not be easily to be radiolyzed by irradiation to produce enough free radicals that could induce significant changes in gross composition of this material. 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 [30]. On the other hand, only the moisture content was decreased by extrusion processing and that may be due to the release of water during extrusion processing produced extrudates with lower moisture content than raw flour. Nearly the same results were reported by Alonso et al. [31] who observed that extrusion processing at high temperature and short time resulted in water and volatile compounds evaporation and that cause release of water and decrease in moisture content of seeds.

It was necessary in the present investigation undertaking amino acid analysis to assess if any alteration has been occurred in the protein quality due to  $\gamma$ -irradiation exposure and extrusion treatment. The results of amino acid analysis of both raw and treated soy flour obtained that some essential and non essential amino acids of treated soy flour had higher level than those of raw one. However, histidine was increased only in case of extrusion while serine was aroused by  $\gamma$ -irradiation at dose 5 KGy as well as proline value was declined under the effect of all different treatments. In addition, both of cysteine and methionine was not observed in the raw flour but appeared by applying the different processing methods. The results of amino acids of  $\gamma$ -irradiated soy flour were in agreement with those found by Abd-Elkalik et al. [32]. The changes in the concentration of amino acids induced by irradiation may probably be due to free radicals that might be formed in association with splitting of the peptide bonds, deamination and decarboxylation reactions of amino acids followed by chains of chemical reactions forming other new radicals [33]. Similarly, the extrusion cooking has some unique features compared to other heat processes and is able to break covalent bonds in biopolymers, and the intense structural disruption and mixing facilitate reactions otherwise limited by diffusion of reactants and products [34,35].

In regard to the fatty acid profile of raw and processed soy flour, the results indicated that oleic acid (C18:1) was the most abundant fatty acid in raw soybean followed by palmitic (C16:0) and stearic (C18:0). It was observed that  $\gamma$ -irradiation at different dose levels (5 & 10 KGy) caused different changes in the fatty acid composition which may be due to molecular structure change in fatty acids, formation of free radicals

induced by gamma irradiation [36]. Al-Kahtani et al. [37] observed that irradiation at 1.5–10 KGy caused a decrease in some fatty acids (C14:0, C16:0 and C16:1). Maxwell and Rady [38] reported an increase in oleic acid with increasing doses of gamma irradiation. However, Hafez et al. [39] did not find changes in the fatty acids (C16:0, C18:1 and C18:2) of soybeans at different radiation doses (1, 5, 10, 20, 40, 60, 80 and 100 KGy). Similarly, Byun et al. [40] showed no significant changes in the fatty acid composition, and trans fatty acids content of soybean oil extracted from gamma irradiated soybean (0–10 kGy).

Additionally, the data in the present study obtained that extrusion processing and irradiation + extrusion increased the total Unsaturated Fatty Acid (USF) as well as these processing methods resulted in appearance of linoleic acid (C18:2). Rokey and Plattner [41] observed that fatty acid composition can be affected during extrusion as a result of hydrogenation, isomerization, polymerization and lipid oxidation. Also, Žilić et al. [42] reported that depending on the temperature and applied heat treatments, the content of linoleic and oleic fatty acid oscillated.

Results in the study obtained that treatment of soy flour with gamma irradiation (5 & 10 KGy) caused different changes in phenolic contents by increasing the amount of P-benzoic and ferrulic and decreasing of pyrogallol, syringic and chrisin. Villavicencio et al. [43] presented higher contents of phenolic compounds in irradiated samples when compared with raw samples and attributed this result due to decomposition of some large insoluble phenolic compounds into small soluble phenolic molecules and may also be beneficial for the antioxidant properties of the plant seeds.

In the effect of extrusion, the contents of selected phenolic compounds before and after extrusion cannot give univocal conclusions. In this study, the observed increase in some free phenolic compounds during extrusion could be due to the increased release of these bioactive compounds from the matrix due to extrusion thus accessible in the extraction [44]. In addition, reduction of total phenols during extrusion could be attributed to thermal degradation and denaturation, changes in chemical reactivity or to formation of insoluble complexes during heating [45].

The values of some antinutritional factors for both raw and processed soy flour demonstrated that the irradiation (5 & 10 KGy) or/and extrusion processing reduced the tannin content, phytic acid and trypsin inhibitor concentration in soybean flour. Villavicencio et al. [43] and Mechi et al. [46] reported that gamma radiation promoted reduction in the tannin contents as the radiation dose increased until a limited dose. The mechanism of gamma irradiation action on tannin has been related to generation of the hydroxyl and superoxide anion radicals [47], but mode of electron beam action on tannins has not been demonstrated [48]. The effect of extrusion on tannin was studied by El-hady and Habiba [49] and they have reported significant reduction in tannin content after extruding legume seeds at different moisture contents. Also, Alonso et al. [50] studied the effects of extrusion and conventional processing methods on protein and antinutritional factors and they found varietal changes in the tannin contents, and extrusion was most effective in reducing tannins than the other processes.

The elimination of phytic acid by  $\gamma$ -irradiation is probably due to chemical degradation of phytate to lower inositol phosphates and inositol, by the action of free radicals, which have lower chelating power, or cleavage of the phytate ring itself [51,52]. Authors suggested that during extrusion inositol hexaphosphate could have been hydrolyzed to lower molecular weight forms resulted in decreasing the phytic content [53,54].

Inactivation of trypsin inhibitor in irradiated samples could be attributed to the destruction of disulphide (-S-S-) groups [55,56]. Abu-Tarboush [57] found reduction of 34.9% on the trypsin inhibitory activity in soybean flour radiated with 10 kGy. The author attributed this reduction to the breakage of the trypsin inhibitory structure with the radiation treatment. Farag et al. [58] observed an increase in the inactivation level with increase in the doses used (41.8%, 56.3%, 62.7% and 72.5% of loss in the trypsin inhibitory activity) for doses of 5, 15, 30 and 60 kGy, respectively.

Thermal treatment of ANFs had been reported to be a valuable process for the inactivation of TIA. The reduction in TIA following extrusion by up to 90% was reported in the literature for other foods particularly mungbean, cowpea and blends with other crops [59].

## Conclusion

In conclusion, the results obtained in this study suggest that irradiation or/and extrusion may be chosen as beneficial methods not only in reducing phytic acid, tannins and trypsin inhibitors as antinutritional factors but also in increasing the total phenolic contents of the raw flour. However, the nutritional attributes i.e. fat, protein, fiber and ash of the soybean flour remained constant after gamma irradiation exposure or/and extrusion processing.

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