AIM OF THE WORK

This study aims to assess the possible effect of gamma irradiation on the reduction of aflatoxin B_1 in some cereal grains and the impact on nutritive values.

MATERIALS AND METHODS

▪ Study setting

- Samples are collected from Alexandria markets, Egypt.
- All samples except for amino acids profile analyses were analyzed in the Central Laboratory Unit, The Laboratory of Professor Doctor Mohammed El Amin at High Institute of Public Health (HIPH), Alexandria, University.
- Amino acids profile analyses were performed in the Central Laboratory Unit at National Institute of Oceanography and Fisheriest, Alexandria.
- The irradiation of samples carried out at the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Egypt.

▪ Study design

One group pre-post intervention design was conducted on 60 samples divided equally among the three different chosen cereals, wheat (*Triticum aestivum* L.), maize (*Zea mays*), and polished rice (Oryza sativa L).

▪ Sampling

The minimal required sample units was calculated using STATA II software, precision= 10%, α=0.05, and was found to be 60 units, using a power of 80% to detect the average reduction at aflatoxin B_1 level at a group of cereals after radiation was found to be 76% according to Aziz NH et al.[105]

The samples were randomly collected from the local markets and wholesale markets in Alexandria city during June, July, and August, 2013.

Each sample unit was one kilogram, each kilogram was subdivided into equal four sub-groups. The first sub-group was used as a control subgroup. The second sub-group was subjected to a gamma radiation dose of Cobalt-60 of 4 KGy. The third sub-group was subjected to a gamma radiation dose of Cobalt- 60 of 6 KGy. The forth sub-group was subjected to a gamma radiation dose of Cobalt-60 of 8 KGy. All the sub-groups were stored in plastic bags at 4^{o} C until analysis.

All samples were subjected to chemical analysis to ensure their quality and safety for human consumption.

▪ Irradiation

Maize, wheat, and rice seeds (250 g) sub-groups were sealed in polyethylene plastic bags (0.1mm thickness) and irradiated at room temperature (25^{o} C) under ambient atmosphere. The irradiation of samples carried out by using ^{60}Co gamma ray (Indian irradiation unit) installed at the National Center for Radiation Research and Technology

(NCRRT), Atomic Energy Authority, Egypt. All samples were exposed to a dose rate of 0.07 Gy/sec at the time of the experiment.

The calibration of the gamma-ray source, the irradiation with variable radiation doses were carried out according to the international guidelines for radiation protection[106] by professional personnel in the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt.

▪ Methods

➢ Chemical Analysis.

All cereal sub-samples (control and irradiated samples) were transferred to the central laboratory of High Institute of Public Health (HIPH) Alexandria University for the following analysis:-

I. Aflatoxin B_1 determination by High Performance Liquid Chromatography (HPLC). [107]

In the HPLC manipulation there are four main steps: a) Extraction of the analyte (Aflatoxin B_1) from samples, b) Clean-up (or purification), c) Preparation of aflatoxin B_1 standard and d) The chromatographic analysis.

I. A. Extraction.

1. Principles:

The purpose of this step is to dissolve the analyte quantitatively in the solvent, with as few little additional compounds as possible in order to avoid interferences.

2. Chemicals and reagents:

All reagents are HPLC grade e.g. Water, Acetonitrile and Methanol.

3. Procedure:

i. 50g of each ground sample was placed into ultraturrax and then 100 ml of 60% acetonitrile/water (v/v) was added.

ii. The mixture was stirred for 2 min. at high speed.

iii. The extract was filtered through a Whatman No 3 filter paper and then through a microfibre filter.

I. B. Clean-up by immune-affinity chromatography .

1. Principles:

This step consists in removal of the substances, which may interfere with the detection of the analyte.

2. Procedure:

i. 2 ml of the final extract, corresponding to 1 g of the original material was diluted with 48 ml of phosphate buffered saline (PBS, pH 7.4) to give a solvent concentration of 2.5% or less (in order to protect the antibodies in immunoaffinity columns).

ii. The mixture was allowed to pass through a column contains monoclonal antibodies to aflatoxin bound to a solid support. By passing the diluted extract through the column any aflatoxin exists in the sample will bound to the antibody within the column.

iii. The column was then washed with 20 ml of PBS.

iv. The elution of aflatoxin was done with 1.5 ml of methanol and 1.5 ml of pure water (it is a complete denaturation of monoclonal antibodies with the subsequent release of toxin into the solution).

I. C. Standard preparation.

1. Aflatoxin B_1 standard:

Aflatoxin B_1 standard was purchased as crystals from Sigma chemical company (St. Louis Missori, USA).

2. Procedure For creating calibration curve:

Aflatoxin B_1 was used. It is a certified, precisely quantified methanol solution of 250ng/ml AFB_1, For working 100 ng/ml standard was prepared and 8 calibration points (triplicate of each concentration) were settled up for the calibration curve at 0.05, 0.1, 1, 5, 10, 20, 50, and 100 ng mL^{-1} per injection to estimate the linearity of the instrument. Aflatoxin B_1 separated after 2.5- 2.7 minutes (Appendix 4).

I. D. HPLC Chromatography.

Determination of aflatoxin B_1 in cereal grains extracts was carried out by isocratic reversed phase liquid chromatography on (HPLC, Shimadzue), Figure (4-1).

HPLC conditions:

i. 100 ìl of the each extract was injected in triplicate using C18 ODS (Octa Dodecyl Sulphate) reversed phase column (250x4.6 mm, 5 ìm), Japan heated to 40°C. Some aflatoxin B_1 chromatograms are illustrated in (Appendices 5,6).

ii. Mobile phase was water: methanol solution (60:40, v/v).

iii. Flow rate 1 ml/min.

iv. Ultra-violet detection of aflatoxin B_1 at wavelength of 265 nm in (SPD-6AV UV-Vis detector, Shimadzu).

Figure 4.1: High Performance Liquid Chromatography

II. Proximate analysis.

II. A. Determination of moisture content by method 44-15A of American Association of Cereal Chemists International (AACCI), one-stage procedure.[108]

Procedures:

i. Dried pans with lids were weighed accurately.
ii. About 3 g of each sample was placed in the pan and weighed.
iii. Samples were placed in a forced draft oven at 130°C for 1 h, without metal covers, to allow water loss.
iv. Samples were removed from oven, covers were realigned to close, cool, and store in desiccator until weighing.
v. Percentage moisture (wt/wt) was calculated as described below.

$$\text{Moisture \%} = (B - C) / A \times 100 \qquad (4\text{-}1)$$

Where: A = Weight of the Sample.

B = Weight of the pan and sample prior to drying.

C = Weight of the pan and sample after drying.

II. B. Determination of fat content on dry basis by Soxhlet method. [108]

1. Principles:

Fat is extracted, semi-continuously, with an organic solvent. Solvent is heated and volatilized, then condensed above the sample. Solvent drips onto the sample and soaks it to extract the fat. At 15–20 min intervals, the solvent is siphoned to the heating flask, to start the process again. Fat content is measured by weight loss of sample or weight of fat removed.

2. Supplies:

- 3 Aluminum weighing pans, pre-dried in 70°C vacuum oven for 24h.
- Cellulose extraction thimbles, pre-dried in70°C vacuum oven for 24h.
- Desiccator.
- Glass boiling beads.
- Glass wool, pre-dried in 70°C vacuum oven for 24 h.
- Mortar and pestle.

3. Equipments:

- Analytical balance.
- Soxhlet extractor, with glassware.
- Vacuum oven.

4. Chemicals:

Chemical	Cas Number
Petroleum ether	8032-32-4

5. Procedures:

i. About 30 g of each sample was slightly ground with mortar and pestle (excessive grinding lead to greater loss of fat in mortar).

ii. Three pre-dried cellulose extraction thimbles for each sample were removed from the desiccator.

iii. The thimbles were labeled then weighed accurately on an analytical balance.

iv. About 2–3 g of each sample was placed in a thimble and then the thimbles were reweighed.

v. A small plug of dried glass wool was placed in each thimble then the thimbles were reweighed.

vi. The samples were placed in a Soxhlet extractor.

vii. About 350 ml of petroleum ether was added in the flask, several glass boiling beads were added, and were extracted for 6 h. or longer.

viii. Thimbles were removed from the Soxhlet extractor using tongs, air dried overnight in a hood, then dried in a vacuum oven at 70ºC for 24h.

ix. Dried samples were cooled in a dessicator then re-weighed.

x. Correction for moisture content of each sample was done.

6. Calculations:

$$\% \text{ (Fat + moisture)} = (A-B / C-D) \times 100 \quad (4\text{-}2)$$

Where,

A= Initial weight of sample + Thimble + Glass wool.

B= Final weight of sample + Thimble + Glass wool.

C= Weight of wet sample + Thimble.

D= Weight of Thimble.

$$\% \text{ Fat on dry basis (wt/wt)} = (\% \text{ Fat} + \% \text{ Moisture}) - (\% \text{ Moisture}) \quad (4\text{-}3)$$

II. C. Determination of protein content on dry basis by Kjeldahl nitrogen method.[108]

1. Principles:

Kjeldahl method is based on nitrogen determination. The protein content can then be calculated assuming a ratio of protein to nitrogen for the specific

food being analyzed. Kjeldahl procedure can be basically divided into three parts: digestion, distillation, and titration.

In the digestion step, organic nitrogen is converted to an ammonium in the presence of a catalyst at approximately 370°C. **In the distillation step**, the digested sample is made alkaline with NaOH and the nitrogen is distilled off as NH_3. This NH_3 is "trapped" in a boric acid solution. **In the titration step**, the amount of ammonia nitrogen in this solution is quantified with a standard HCl solution. A reagent blank is carried through the analysis and the volume of HCl titrant required for this blank is subtracted from each determination.

2. Chemicals:

Chemical	Cas Number
Boric acid (H_3BO_3)	10043-35-3
Bromocresol green	76-60-8
Ethanol, 95%	64-17-5
Hydrochloric acid, conc.(HCl)	7647-01-0
Methyl red	493-52-7
Sodium hydroxide (NaOH)	1310-73-2
Sulfuric acid, conc. (H_2SO_4)	7664-93-9
Potassium sulfate(K_2SO_4)	7778-80-5
Cupric sulfate	7758-98-7
Titanium dioxide (TiO_2)	13463-67-7
Tris hydroxymethylaminomethane	77-86-1

3. Reagents:

- Sulfuric Acid (concentrated, N-Free).
- Catalyst/Salt Mixture (digestion tablets).

  Contain potassium sulfate, cupric sulfate, and titanium dioxide.
- Sodium Hydroxide Solution, 50%, w/v, NaOH in deionized distilled (dd) water.

2000 g sodium hydroxide (NaOH) pellets was dissolved in about 3.5 L dd (double distilled water). The solution was cooled, (dd) Water was added to make up to 4.0 L.

- Boric Acid Solution:

In a 4-L flask, 160 g boric acid was dissolved in 2 L boiled, and still very hot, dd water. The solution was mixed and then an additional 1.5 L of boiled, hot dd water was added. The solution was cooled to room temperature under tap water.40 ml of bromocresol green solution (100 mg bromocresol green/100 ml ethanol) and 28 ml of methyl red solution (100 mg methyl red/100 ml ethanol) was added. The solution was diluted to 4 L with water and mixed carefully. 25 ml of the boric acid solution was transferred to a receiver flask and a digested blank (a digested catalyst/salt/acid mixture) was distilled. The solution was titrated with 0.1 N NaOH solution until grey color was obtained. The amount of NaOH solution necessary to adjust the boric acid solution in the 4-L flask was calculated with the formula:

ml 0.1 N NaOH =ml titer x 4000ml / 25ml (4-4)

The calculated amount of 0.1 N NaOH solution was added to the boric acid solution. The solution was mixed well. The adjustment results were verified by distilling a new blank sample. The adjusted solution was placed into a bottle.

- Tris hydroxymethyaminomethane (THAM) Solution – (0.01 N).

2g of THAM was placed in a crucible. The crucible was placed in a drying oven at 95°C overnight.The crucible was let to cool in a desiccator. In a 1-L volumetric flask, 1.2114 g of dried THAM was dissolved in distilled water, the volumetric flask was diluted to volume.

- Standardized HCl solution.

3.33 ml conc. HCl was diluted to 4 L with dd water. The titrator was filled with the HCl solution to be standardized. Using a volumetric, dispense 10 ml aliquots of the Tris hydroxymethylaminomethane (THAM) solution was added into three Erlenmeyer flasks (50 ml). 3–5 drops indicator (3 parts 0.1% bromocresol green in ethanol to 1 part of 0.2% methyl red in ethanol) was added to each flask and swirl. Each solution was titrated with the HCl solution to a light pink endpoint. The acid volume used was recorded and the normality was calculated as described below,

Normality =ml THAM x N THAM /Average acid volume (AAV)

=20ml x0.01 N /AAV (4-5)

3. Supplies:

- Erlenmeyer flasks, 250 ml.
- Weighing paper.

4. Equipment:

- Analytical balance.
- Kjeldahl digestion and distillation system.

Procedures:

a) Digestion:

- Digestion block was turned on and heated to appropriate temperature.
- Approximately 0.1 g of each cereal was accurately weighed. The weight was recorded. Samples were placed in digestion tube. Two more samples was Repeated for each cereal.
- One catalyst tablet and appropriate volume (e.g., 7 ml) of concentrated sulfuric acid was added to each tube with cereal. Duplicate blanks: one catalyst tablet, volume of sulfuric acid used in the sample, and weigh paper were prepared.
- Rack of digestion tubes was placed on digestion block. Digestion block was covered with exhaust system turned on.
- Samples were left to digest until digestion was completed. The samples should be clear (but neon green), with no charred material remaining.
- Samples were taken from the digestion block and allowed to cool with the exhaust system still turned on.
- The digest of each sample was carefully diluted with an appropriate volume of dd water.

b) Distillation:

- Appropriate volume of boric acid solution was dispensed into the receiving flask.
- Receiving flask was placed on distillation system. The tube coming from the distillation of the sample was submerged in the boric acid solution.
- A set volume of NaOH solution was delivered to the tube and a steam generator was distilled the sample for a set period of time, 5min.

c) Titration:

Solution in the receiving flask was titrated with standardized HCl solution.

5. Data and Calculations:

The percent nitrogen and the percent protein were calculated for each sample, then the average values was determined. The percent protein results on a dry weight basis (dwb) were reported. The actual moisture contents that previously determined were used.

% N= Normality of HCl x (corrected acid volume/ wt of sample) x (14 /mol) x100 **(4-6)**

Normality is in mol/1000 mL.

Corrected acid vol. = (ml std. acid for sample) − (ml std. for blank).

% Protein = % N X Protein Factor* **(4-7)**

Protein factor*:

For maize 6.25, for wheat 5.83, and for rice 5.95.

II. D. Determination of ash content on dry basis.[109]

i. The dried material in the pan left after the determination of moisture was ignited with the flame of a burner till charred.

ii. The pans were transferred into a muffle furnace maintained at 550 – 600ºC and the ignition was continued till grey ash was obtained.

iii. The pans were cooled in a dessicator and weighed.

**According to FAO / WHO 1973.*[110]*

iv. The process was repeated of heating, cooling and weighing at half hour interval till the difference in weight in two consecutive weighings was less than 1mg.

Calculations:

Total ash on dry basis percent = $\{(W_2 - W) / (W_1 - W)\}$ X 100 **(4-8)**

Where,

W_1 = Weight in gm of the dish + material before ashing.

W_2 = Weight in gm of the dish with the ash.

W = Weight in gm of empty dish.

II. E. Determination of crude fiber.[110]

1. Principle:

The definitions of crude fiber states that it is the residue after treating with boiling 0.255 N sulfuric acid and 0.313 N sodium hydroxide.

2. Chemicals:

Chemical	Cas Number
Sulfuric acid, conc. (H_2SO_4)	7664-93-9
Sodium hydroxide (NaOH)	1310-73-2

3. Reagents:

- Reagent alcohol (95%).
- Diethyl ether.

- Hydrochloric acid (1%).

  Dilute 10ml conc HCl with water to 1L.
- Light petroleum ether boiling range 40-60ºC.
- 0.255 M sulfuric acid solution.

  1.25g conc H_2SO_4 in 100ml solution.
- 0.313 M sodium hydroxide solution.

  1.25g NaOH in 100ml solution.

4. Procedures:

i. Samples were ground to pass through 1mm sieve.

ii. 2.7-3g of each sample was weighed.

iii. An empty flask was weighed for blank.

iv. Samples were transferred to an extraction apparatus and extracted with light petroleum ether.

v. Setting and decanting 3 times.

vi. Sample was air dried and transferred to1000 ml conical flask.

vii. Anti-foaming agent was added.

viii. 200 ml of boiled 0.255 N sulfuric acid solution was added to each sample and boiled vigorously for 30 min.

ix. The samples were filtered using Bchner funnel and were washed with 3 X 30 ml boiling water.

x. The residue of each sample was taken in a 1000ml conical flask, anti foaming agent and 200 ml of boiled 0.313 M sodium hydroxide were added.

xi. The flasks were boiled vigorously for 30 min and maintained at constant volume.

xii. The flasks were filtered and the residues were washed with 3 X 30 ml boiling water then with 1% HCl and finally boiling water until free from acid.

xiii. The flasks were washed twice with alcohol and three times with ether.

xiv. The residues were transferred to ashless filter paper.

xv. The residues were dried for 2 h in an oven at 130 ± 2ºC.

xvi. Mass loss was determined after ashing at 350 ± 25ºC in a muffle.

5. Calculations:

The content of crude fiber in cereal samples were calculated in mass percent relative to the content of dry mass in the sample.

[Weight of the residue after ashing / wt of dry sample] X 100 (4-9)

II. F. Calculations for carbohydrate content.[111]

% Carbohydrate =100 - [ash% + moisture% + fat%+ protein%+ crude fiber%] **(4-10)**

II.G. Calculations for cereal calories.[110]

Food standard committee recommended that; for carbohydrate 3.75 Kcal/g, expressed as monosaccharide, for protein 4 Kcal/g, and for fat 9 Kcal/g, calories in Kcal/g calculated by the following equation.

Calories ={fat% x (9)+ carbohydrate% x (3.75) + protein% x (4)} (4-11)

III. Preparation of methyl esters by sodium methoxide method followed by determination of fatty acids profile by Gas Liquid Chromatography (GLC).[108]

1. Principles:

Triacylglycerols and phospholipids are saponified and the fatty acids liberated are esterified to form fatty acid methyl esters (FAMEs) so that the volatility is increased. In the sodium methoxide method, sodium methoxide is used as a catalyst to interesterify fatty acid.

2. Chemicals:

Chemical	Cas Number
Hexane	110-54-3
Methanol	67-56-1
Sodium chloride	7647-14-5
Sodium hydroxide	1310-73-2
Anhydrous sodium sulfate	7757-82-6
Sodium methoxide	124-41-4

3. Reagents:

- Sodium methoxide 0.5N.

  2g of sodium hydroxide was dissolved in 100ml methanol.

4. Preparation of fatty acid methyl ester:

i. 100 mg (± 5 mg) oil (previously separated) from each cereal sample was transferred and weighed using a Pasteur pipette into a vial or small bottle with a tight sealing cap.

ii. 5 ml of hexane was added to each vial and the oil was dissolved in hexane using vortex.

iii. 250 µl of sodium methoxide reagent was added in each vial, the vials were capped tightly, and vortexed for 1 min., pausing every 10 sec. to allow the vortex to collapse.

iv. 5 ml of saturated NaCl solution was added to each vial, the vials were capped, and shaked vigorously for 15 seconds.

v. The vials were let to stand for 10 min. The hexane layer was removed from each vial and transferred carefully to vials containing small amounts of Na_2SO_4.

vi. The hexane phase containing the methyl esters in each vial was allowed to be in contact with Na_2SO_4 for at least 15 min prior to analysis.

vii. The hexane phases were transferred to small bottles for subsequent GC analysis. (Hexane solution can be stored in the freezer).

5. Standard preparation:

100 mg of Supelco GLC Reference Standard FAME Mix dissolved in 10 ml hexane.

6. Injection of standards and samples into GC:

i. The syringe was rinsed three times with hexane, and three times with the reference standard mixture.

ii. 3 µl of standard solution was injected and the syringe was removed from the injection port, then start button was pressed.

iii. The syringe was rinsed again three times with solvent, and three times with the sample solution.

iv. 3 µl of sample solution was injected, the syringe was removed from injection port then start button was pressed.

v. Rinse syringe again three times with solvent.

7. Gas liquid chromatography condition:

- **Instrument:** Gas chromatograph (Hewlett Packard 6890)
- **Detector:** Flame ionization detector (FID)
- **Capillary column:** HP-5 (5% diphenyl, 95% dimethyl polysiloxane).
- **Length:** 30 m
- **ID (internal diameter) :**0.32 mm
- **Film thickness:** 0.25 µm
- **Carrier gas:** Nitrogen
- **Sample injection:** 3 µl
- **Split ratio:** 50:1
- **Flow rate**:1 ml/min (measured at room temperature)
- **Injector temperature:** 220°C
- **Detector temperature:** 250°C

- **Temperature program:**

  - Initial oven temperature 100°C.
  - Initial time 2 min.
  - Rate 5°C/min.
  - Final temperature 230°C.
  - Final time 10 min.

Figure 4.2: Gas Liquid Chromatography

8. Calculations:

i. Retention times and relative peak areas were reported for the information to identify the peaks in the chromatogram.

ii. The retention times for peaks in the chromatogram from the FAME reference standard mixture (Appendix 7) were used to identify the peaks in the chromatograms for each oil sample analyzed.

iii. The percentage (%) of each fatty acid in each cereal sample chromatogram was calculated.

IV. Determination of amino acids profile by amino acid analyzer.

1. Principle:

Amino acid analysis was performed by the method of Anjum et al., (2005)[112] which depends on hydrolysis of protein and analysis of amino acids using amino acid analyzer.

2. Chemicals:

Chemical	Cas Number
HCl	7647-01-0
Sodium citrate	6132-04-3

3. Procedure:

i. 13 mg of each sample was incubated with 3 ml of 6N HCl, under nitrogen purging, in a sealed tube at 110°C for 24 hours.

ii. Samples were dried by evaporation using rotary evaporator.

iii. 3 ml of sodium citrate buffer (0.2N, pH 4.25) was added.

iv. The mixture was centrifuged at 2500 rpm for 15 minutes and the supernatant was collected and stored at 4°C in vials for analysis.

4. Conditions:

Samples were injected in amino acid analyzer model (Dionex ICS-3000, USA), Serial No: 58319.

i. **Injection Volume**: 25 µL.

ii. **Column**: AminoPac PA10 analytical and guard columns.

iii. **Column temperature**: 30 °C.

iv. **Operating Backpressure**: < 3,000 psi.

v. **Eluent**: **E1**: Deionized water (18.2 megohm water) **E2**: 250 mM NaOH **E3**: 1 M Sodium acetate.

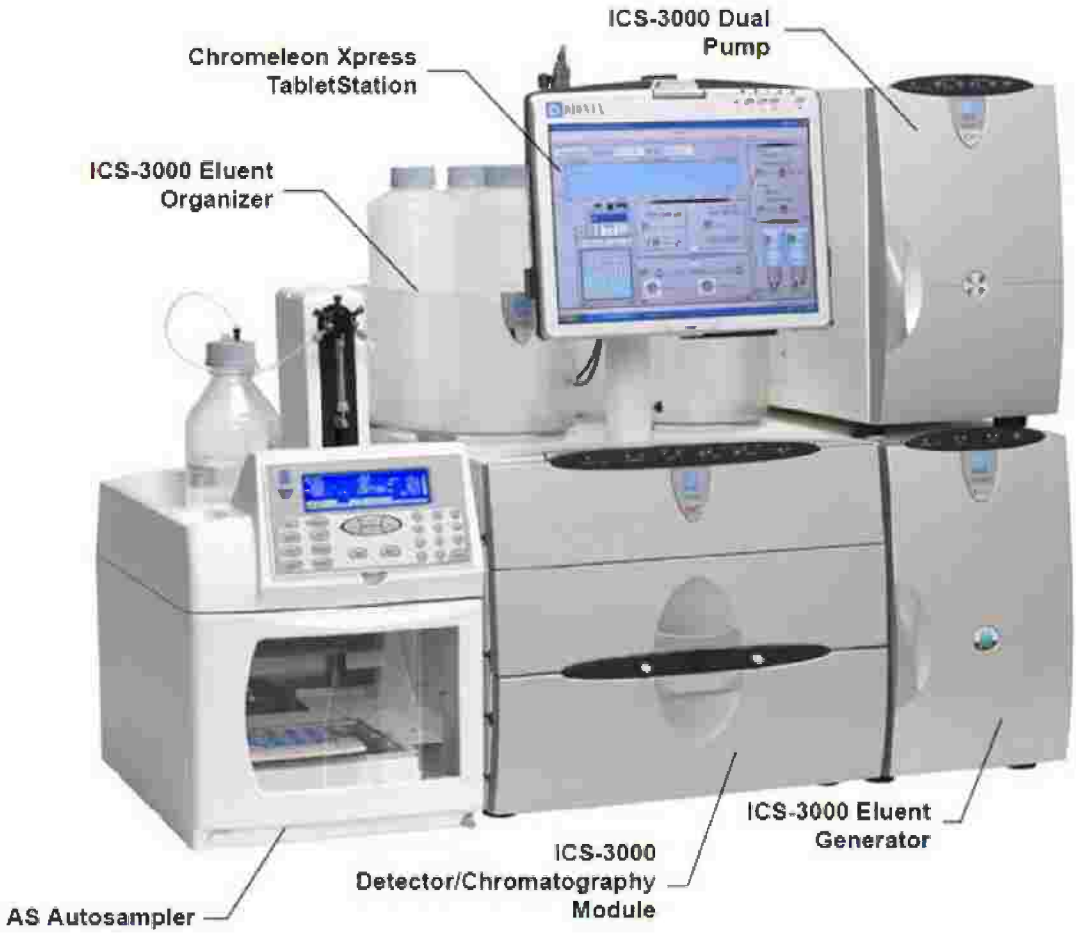

Figure 4.3: Amino acid analyzer

➢ Statistical analysis.

Data analyzed in triplicate, fed to the computer and analyzed using IBM SPSS software package version 20.0. Quantitative data were described using mean and standard deviation. The given graphs were constructed using Microsoft excel software. Comparison between more than two population were analyzed using F-test (ANOVA) and Post Hoc test (LSD). Significance of the obtained results was judged at the 5% level.

RESULTS

In this work three types of seeds were employed, namely, Maize, Wheat and Rice. Twenty samples of each type were used each of one kilogram, to study the effects of gamma irradiation on the aflatoxin B_1 levels and the impact on nutritive values.

I. Aflatoxin B_1 levels before exposure to Gamma radiation.

The distribution of aflatoxin B_1 in cereal samples (maize, wheat, and rice) before exposure to different doses of gamma radiation are illustrated in (Figures 5.1&5.2).

(Figure 5.1), illustrates the levels of aflatoxin B_1 in maize, wheat, and rice before gamma-rays exposure.

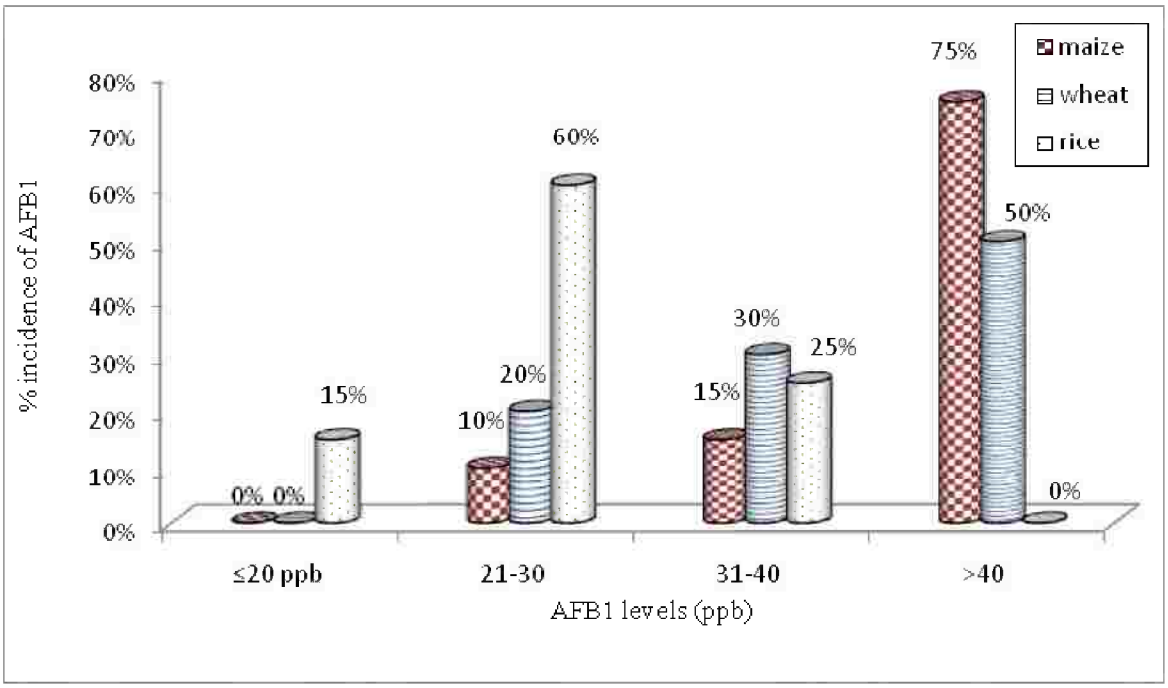

Figure 5.1: Distribution of aflatoxin B_1 in maize, wheat, and rice pre-irradiation.

(Figure 5.2), represents a graphical comparison between aflatoxin B_1 levels in maize, wheat, and rice and the legal upper limit of aflatoxin B_1.

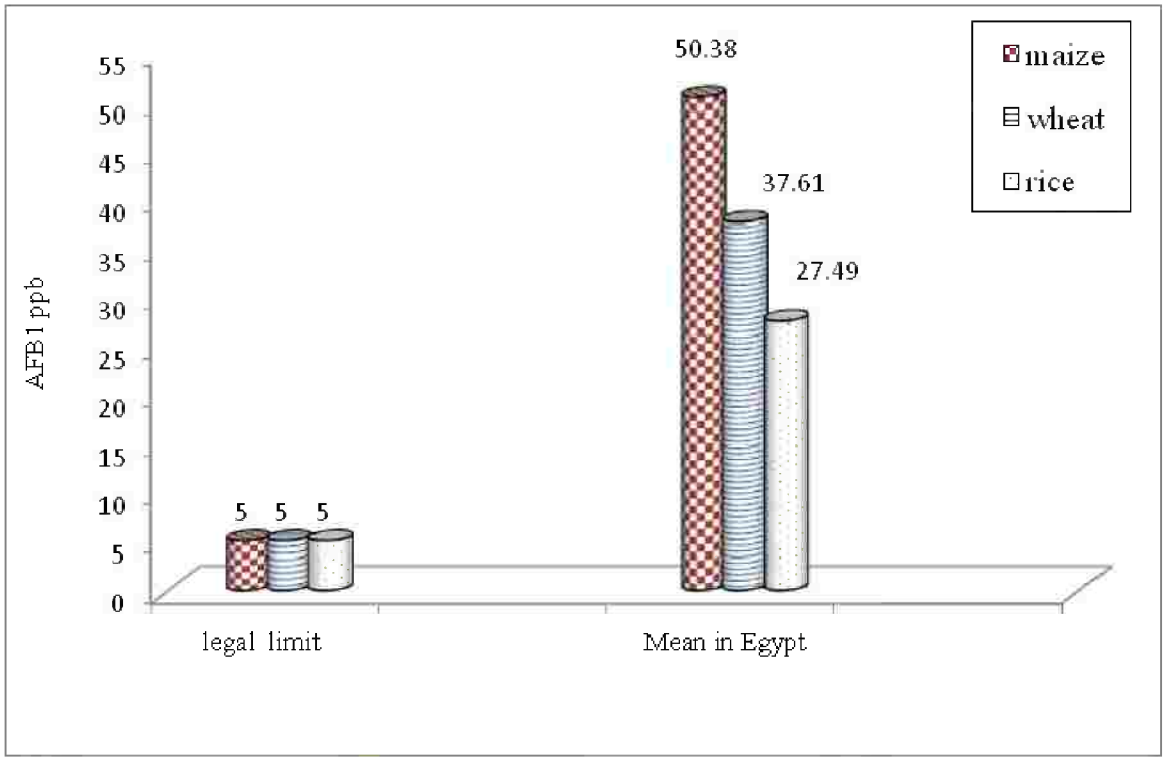

Figure 5.2: Aflatoxin B_1 legal limit and aflatoxin B_1 in Egypt.

a. Aflatoxin B_1 levels after exposure to different doses of Gamma radiation.

Table 5.1, describes the comparison between the alfatoxin B_1 levels in the different seeds samples before and after exposure to 4, 6, and 8 KGy gamma radiation.

Table 5.1 Comparison between different groups pre- and post- radiation according to aflatoxin B_1 levels (ng/g) in maize wheat and rice.

Cereal types	Aflatoxin B_1 Pre-radiation ng/g	Aflatoxin B_1 Post-radiation ng/g			F	p
	0 KGy	4 KGy	6 KGy	8 KGy		
Maize	$50.38^{a}\pm 14.46$	$42.55^{b}\pm 13.04$	$34.06^{c}\pm 12.08$	$20.02^{d}\pm 6.70$	23.702^{*}	$<0.001^{*}$
Wheat	$37.61^{a} \pm 6.85$	$29.24^{b} \pm 6.34$	$21.12^{c} \pm 6.54$	$11.55^{d} \pm 3.41$	70.182^{*}	$<0.001^{*}$
Rice	$27.49^{a} \pm 7.32$	$19.94^{b} \pm 7.87$	$11.99^{c} \pm 7.34$	$9.71^{c} \pm 2.43$	18.834^{*}	$<0.001^{*}$

Different superscripts are significant at $p\leq 0.05$
F: F test (ANOVA)
*: Statistically significant at $p \leq 0.05$
Results expressed as mean ± standard deviation of triplicate observations for each sample

The graphical presentation of the data of Table 5.1, are illustrated in (Figure 5.3).

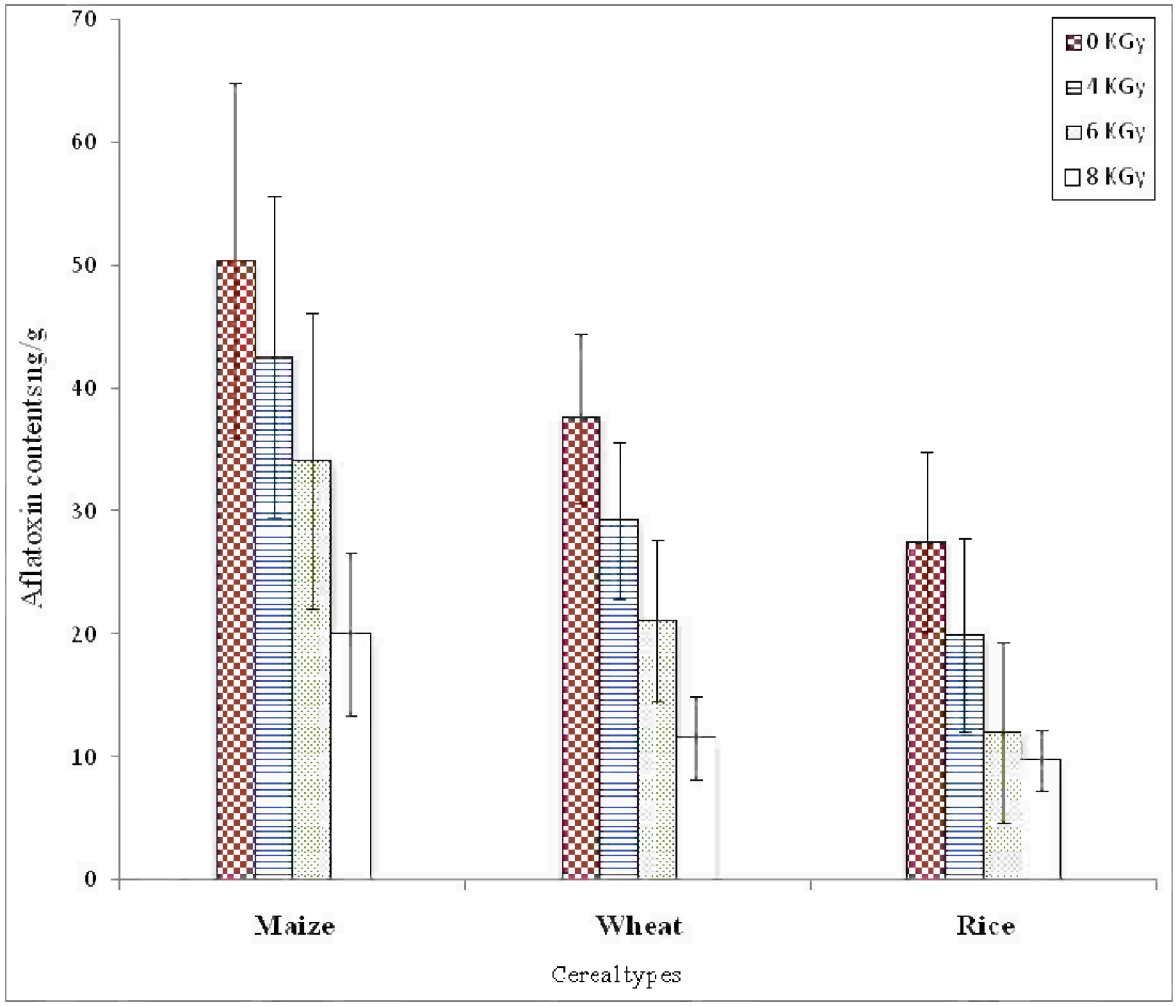

Figure 5.3: Aflatoxin B_1 levels in maize, wheat, and rice with increasing gamma irradiation doses.

Table 5.2, illustrates the reduction percents of aflatoxin B_1 in the different samples after exposure to different radiation doses namely; 4 KGy, 6 KGy, and 8 KGy, and its graphical representation is described in (Figure 5.4).

Table 5.2 Mean %reduction of aflatoxin B_1 by different radiation doses at 4 KGy, 6 KGy, 8 KGy in maize, wheat, and rice.

Cereal types	% Reduction of aflatoxin B_1 Post radiation		
	4 KGy	6 KGy	8 KGy
Maize	15.54	32.39	60.26
Wheat	22.25	43.84	69.29
Rice	27.46	56.38	64.68

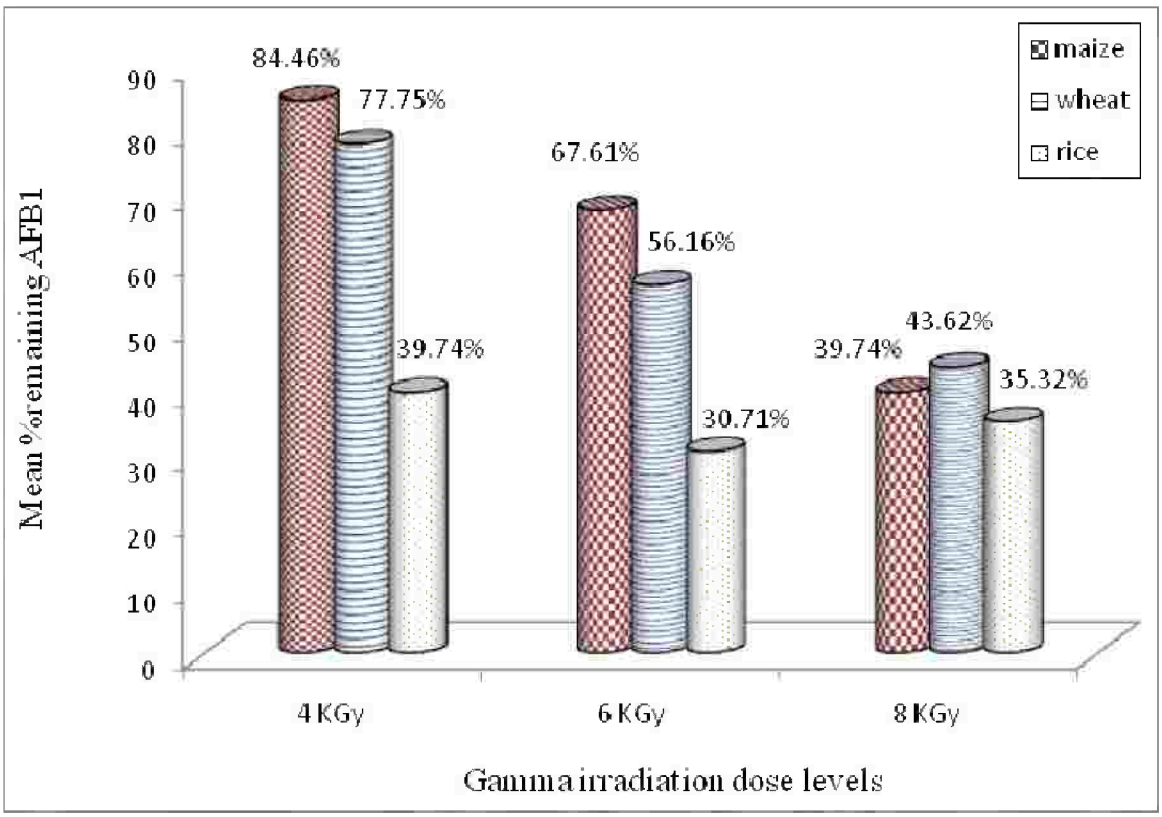

Figure 5.4: %Remaining of aflatoxin B_1 at different gamma irradiation doses in maize, wheat, and rice.

II. Effect of gamma irradiation on nutritive values of maize wheat and rice samples.

The effects of exposure to different gamma radiation doses on nutritive valus of the considered seeds samples are given in Tables (5.3-5.5) and Figures (5.5-5.11), respectively.

Table 5.3: Comparison between the effects of different irradiation doses on nutritive values as regard maize.

Nutritive values of maize	Nutritive values (%) Pre-radiation	Nutritive values (%) Post-radiation			F	p
	0 KGy	4 KGy	6 KGy	8 KGy		
Ash	$1.76^{a} \pm 0.13$	$1.79^{a} \pm 0.07$	$1.78^{a} \pm 0.08$	$1.76^{a} \pm 0.15$	0.357	0.784
Moisture	$12.11^{a} \pm 1.03$	$12.15^{a} \pm 0.49$	$11.94^{ab} \pm 0.82$	$11.56^{b} \pm 0.58$	2.549	0.062
Fat	$5.55^{a} \pm 0.45$	$5.59^{a} \pm 0.43$	$5.62^{a} \pm 0.50$	$5.51^{a} \pm 0.25$	0.260	0.854
Protein	$10.15^{a} \pm 0.57$	$10.72^{b} \pm 0.59$	$10.57^{b} \pm 0.63$	$10.47^{ab} \pm 0.67$	3.028^{*}	0.035^{*}
Crude fiber	$2.40^{a} \pm 0.28$	$2.42^{a} \pm 0.16$	$2.47^{a} \pm 0.29$	$2.45^{a} \pm 0.26$	0.342	0.795
Carbohydrate	$68.32^{a} \pm 1.22$	$67.34^{b} \pm 1.02$	$67.63^{bc} \pm 0.80$	$68.26^{ac} \pm 1.09$	4.256^{*}	0.008^{*}
Calories	$346.74^{a} \pm 4.46$	$345.67^{a} \pm 2.70$	$346.40^{a} \pm 4.78$	$347.42^{a} \pm 2.86$	0.727	0.539

Different superscripts are significant at $p \leq 0.05$
F: F test (ANOVA)
*: Statistically significant at $p \leq 0.05$
Results expressed as mean ± standard deviation of triplicate observations for each sample

Table 5.4: Comparison between the effects of different irradiation doses on nutritive values as regard wheat.

Nutritive values of wheat	Nutritive values (%) Pre-radiation	Nutritive values (%) Post radiation			F	p
	0 KGy	4 KGy	6 KGy	8 KGy		
Ash	$1.70^{a} \pm 0.04$	$1.76^{a} \pm 0.10$	$1.73^{a} \pm 0.14$	$1.73^{a} \pm 0.13$	0.726	0.540
Moisture	$12.44^{a} \pm 0.35$	$12.19^{b} \pm 0.50$	$12.37^{b} \pm 1.34$	$11.58^{b} \pm 0.72$	4.226^{*}	0.008^{*}
Fat	$1.79^{a} \pm 0.07$	$1.47^{b} \pm 0.08$	$1.42^{cd} \pm 0.04$	$1.45^{bd} \pm 0.05$	135.870^{*}	$<0.001^{*}$
Protein	$12.05^{a} \pm 0.94$	$12.59^{a} \pm 0.94$	$12.45^{a} \pm 0.97$	$12.25^{a} \pm 1.03$	1.096	0.357
Crude fiber	$2.66^{ab} \pm 0.10$	$2.65^{ab} \pm 0.16$	$2.61^{a} \pm 0.15$	$2.72^{b} \pm 0.17$	2.113	0.106
Carbohydrate	$69.36^{a} \pm 1.03$	$69.35^{a} \pm 1.13$	$69.42^{a} \pm 1.46$	$68.37^{b} \pm 0.77$	2.664	0.054
Calories	$324.36^{a} \pm 1.46$	$323.61^{a} \pm 2.05$	$325.37^{a} \pm 3.71$	$318.29^{b} \pm 1.57$	2.208	0.094

Different superscripts are significant at $p \leq 0.05$
F: F test (ANOVA)
*: Statistically significant at $p \leq 0.05$
Results expressed as mean ± standard deviation of triplicate observations for each sample

Table 5.5: Comparison between the effects of different irradiation doses on nutritive values as regard rice.

Nutritive values of Rice	Nutritive values (%) 0 KGy	Nutritive values (%) Post radiation 4 KGy	6 KGy	8 KGy	F	p
Ash	$1.11^{a} \pm 0.11$	$1.18^{a} \pm 0.21$	$1.14^{a} \pm 0.19$	$1.13^{a} \pm 0.16$	0.592	0.622
Moisture	$12.88^{a} \pm 1.61$	$12.83^{a} \pm 1.59$	$12.90^{a} \pm 0.99$	$12.55^{a} \pm 1.17$	0.283	0.837
Fat	$0.88^{a} \pm 0.07$	$0.84^{a} \pm 0.11$	$0.87^{a} \pm 0.07$	$0.89^{a} \pm 0.09$	1.017	0.390
Protein	$6.48^{a} \pm 0.37$	$6.62^{a} \pm 0.39$	$6.57^{a} \pm 0.38$	$6.40^{a} \pm 0.44$	1.156	0.332
Crude fiber	$1.17^{a} \pm 0.09$	$1.27^{b} \pm 0.07$	$1.26^{bc} \pm 0.08$	$1.27^{bc} \pm 0.24$	2.482	0.067
Carbohydrate	$77.47^{a} \pm 1.61$	$77.26^{a} \pm 1.74$	$77.27^{a} \pm 1.12$	$77.76^{a} \pm 1.18$	0.531	0.662
Calories	$324.32^{a} \pm 5.90$	$323.76^{a} \pm 5.92$	$323.83^{a} \pm 3.75$	$323.92^{a} \pm 7.69$	0.329	0.805

Different superscripts are significant at $p \leq 0.05$
F: F test (ANOVA)
*: Statistically significant at $p \leq 0.05$
Results expressed as mean ± standard deviation of triplicate observations for each sample

III.1 Effects of gamma radiation on ash contents:

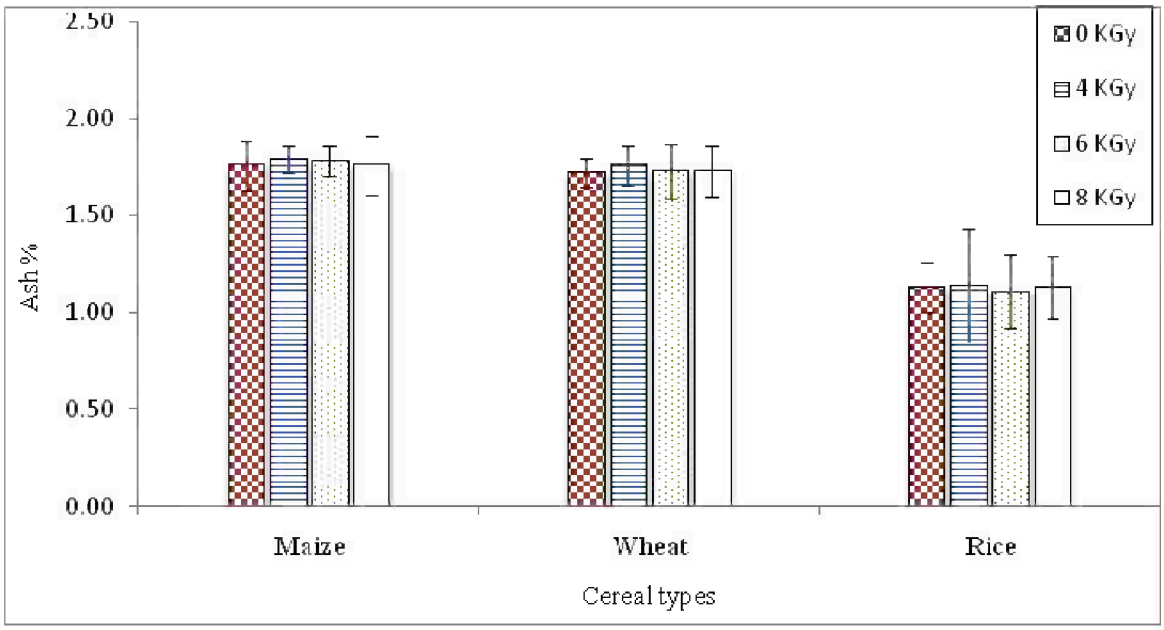

Figure 5.5: Comparison between the effects of different gamma irradiation doses on ash % as regard maize, wheat, and rice.

III.2 Effects of gamma radiation on fat contents:

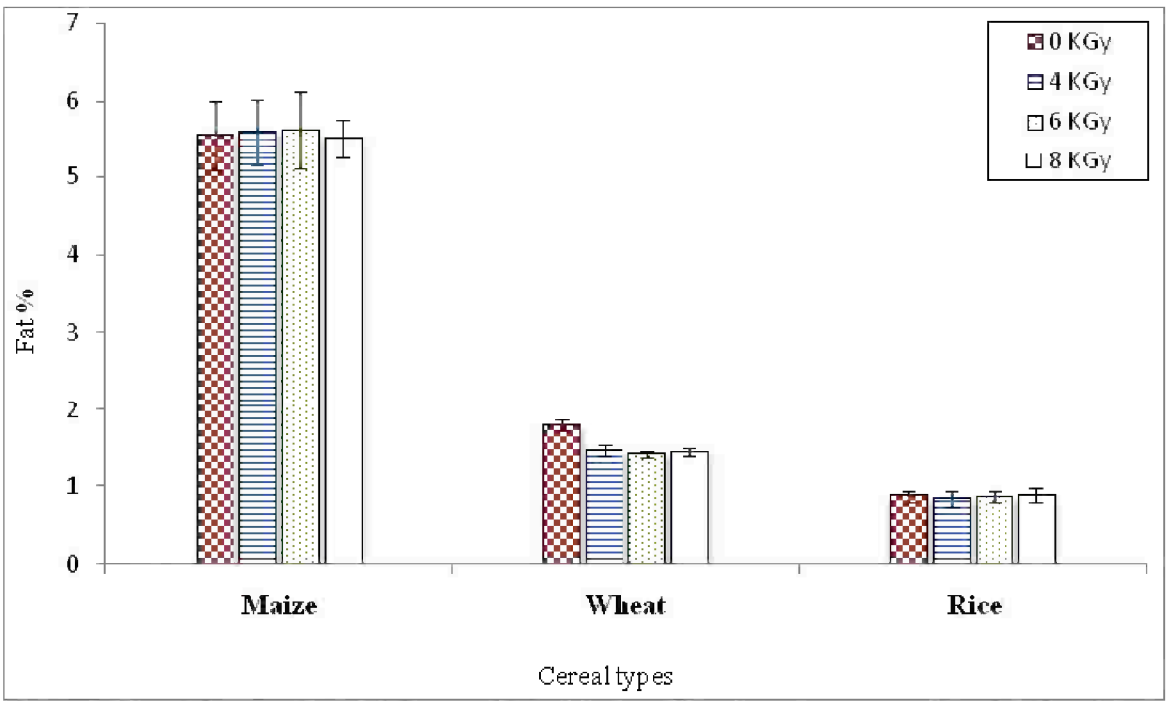

Figure 5.6: Comparison between the effects of different gamma irradiation doses on fat% as regard maize, wheat, and rice.

III.3 Effects of gamma radiation on protein contents:

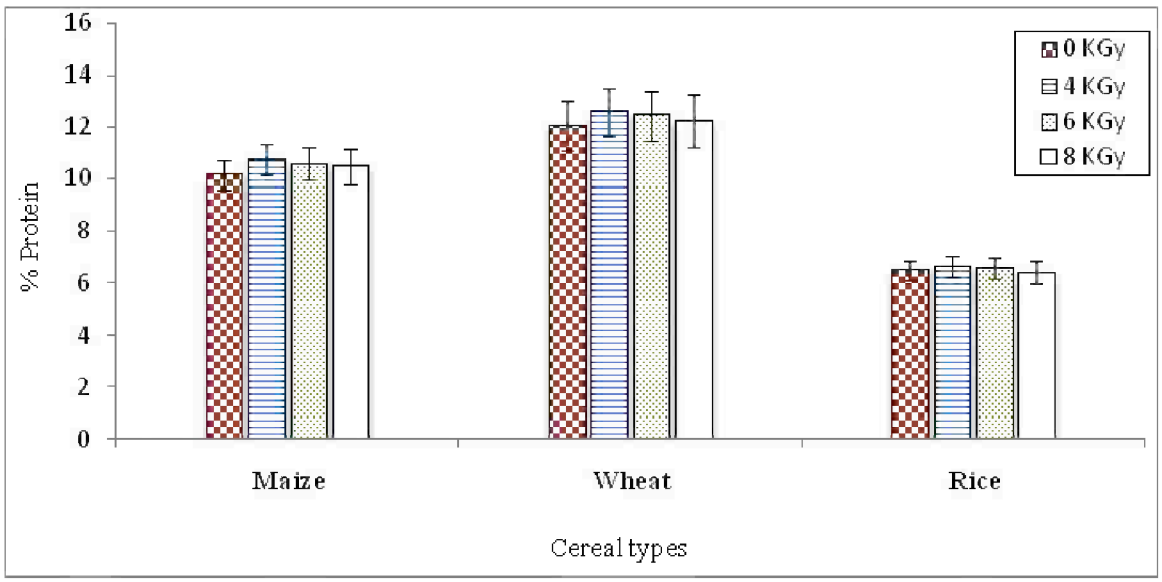

Figure 5.7: **Comparison between the effects of different irradiation doses on protein % as regard maize, wheat, and rice.**

III.4 Effects of gamma radiation on carbohydrates contents:

a) Insoluble carbohydrates:

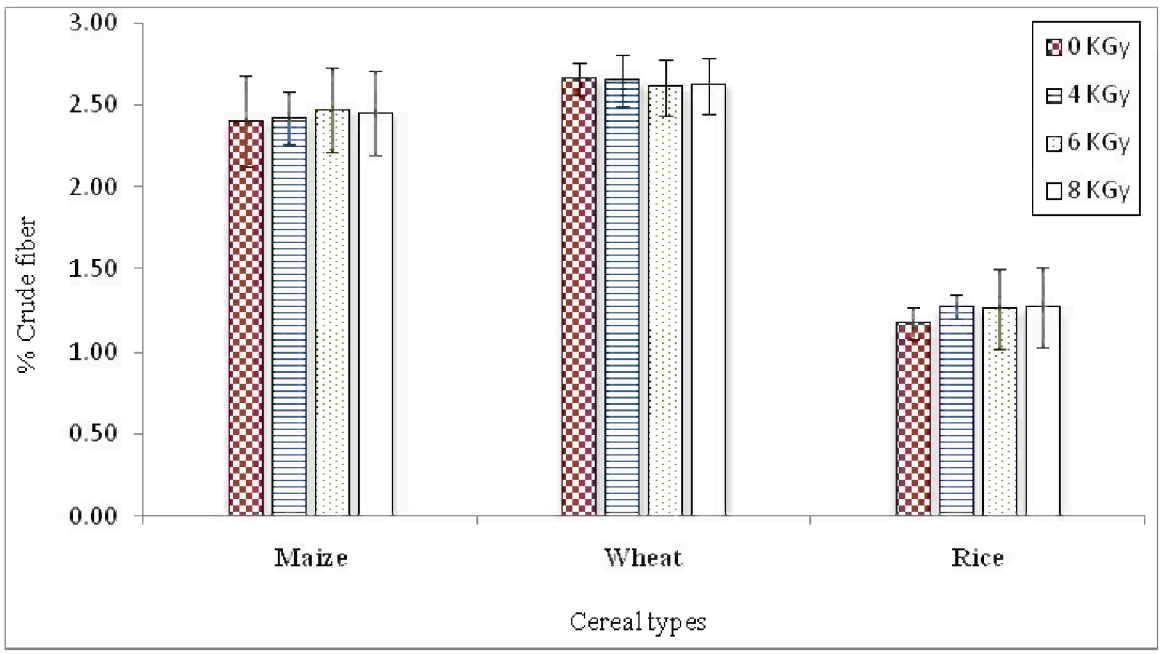

Figure 5.8: **Comparison between the effects of different irradiation doses on crude fiber % as regard maize, wheat, and rice.**

b) Soluble carbohydrates:

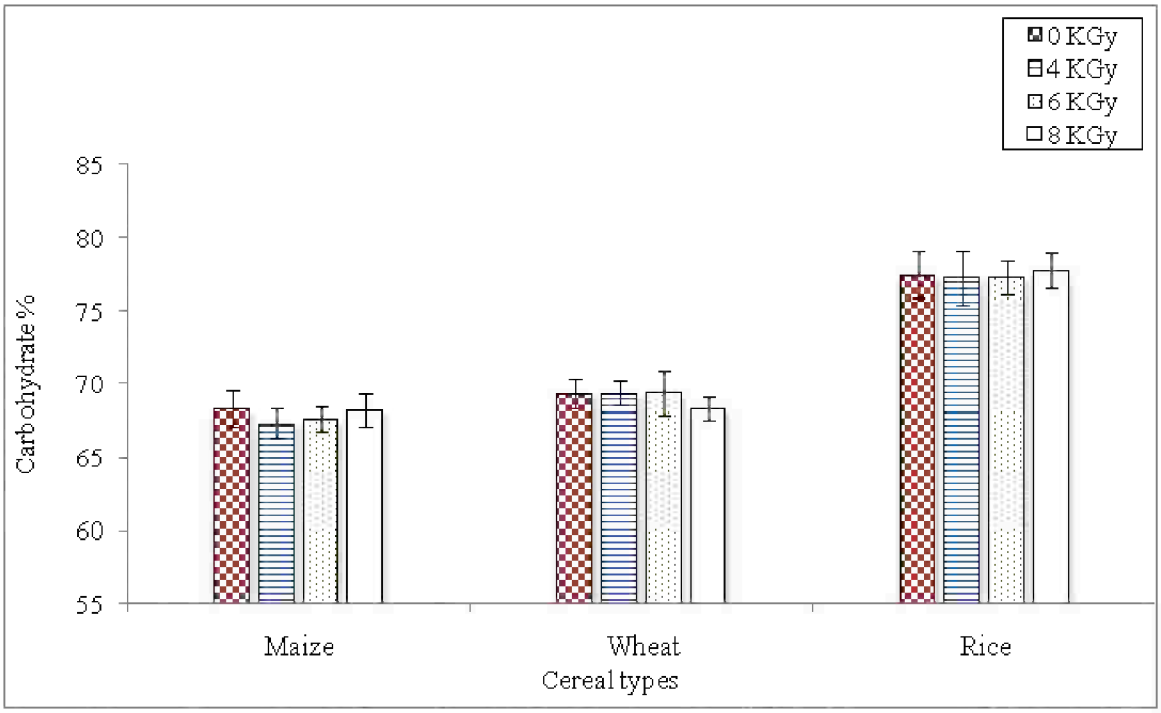

Figure 5.9: **Comparison between the effects of different irradiation doses on carbohydrates % as regard maize, wheat, and rice.**

III.5 Effects of gamma radiation on moisture contents:

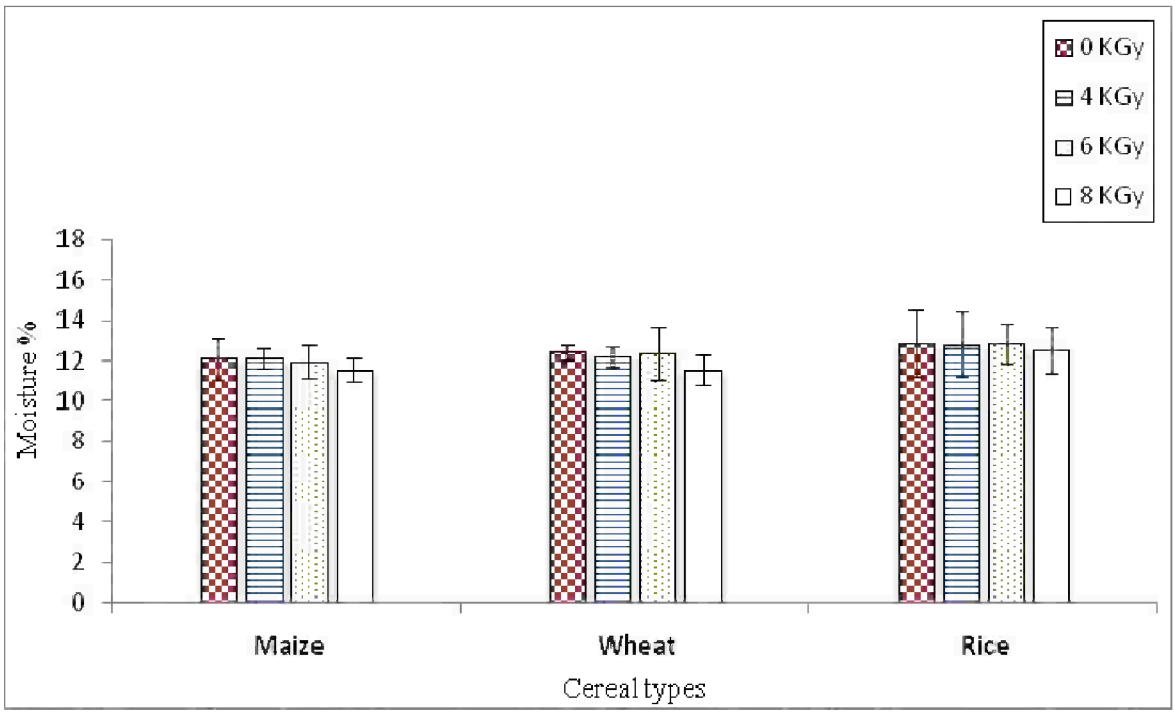

Figure 5.10: **Comparison between the effects of different irradiation doses on mositure % as regard maize, wheat, and rice.**

III.6 Effects of gamma radiation on cereals energy contents:

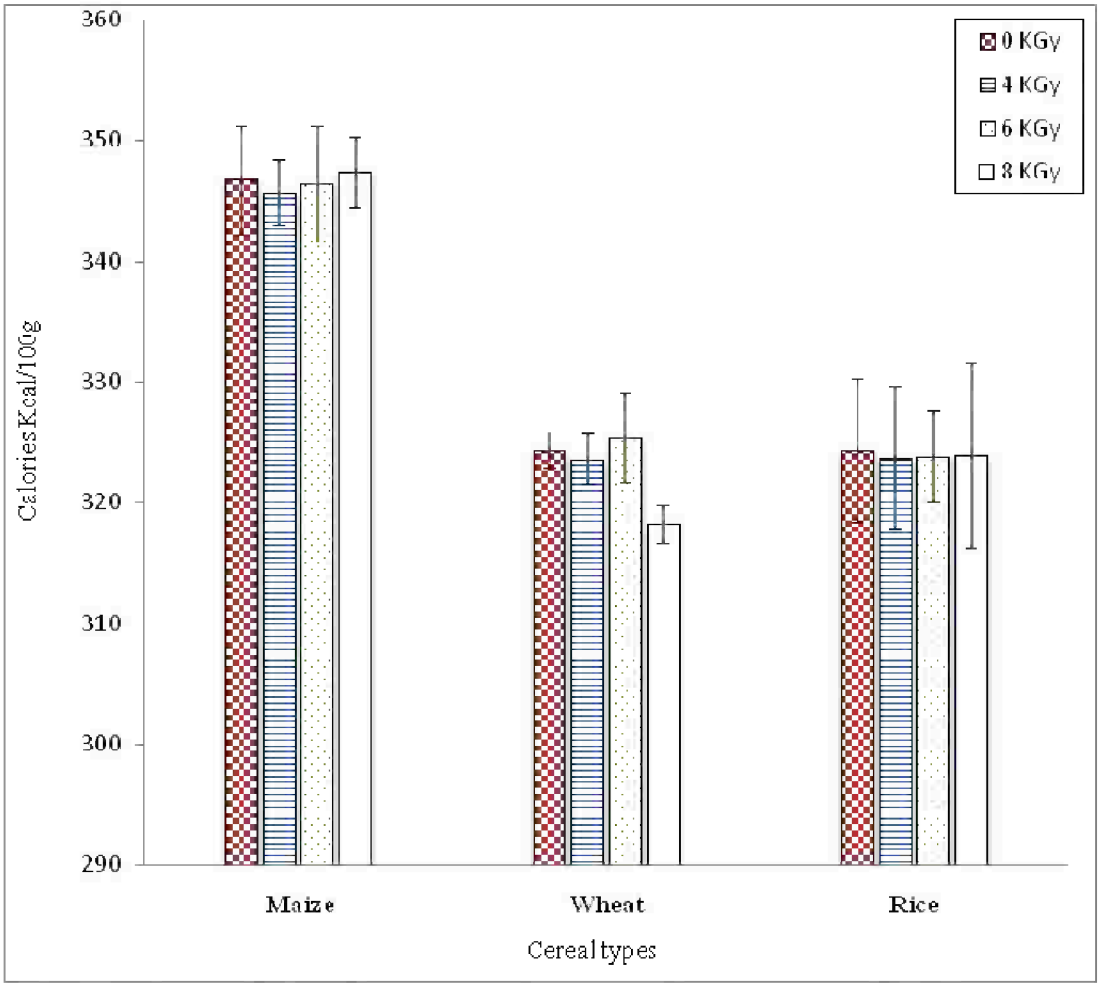

Figure 5.11: **Comparison between the effects of different irradiation doses on Calories as regard maize, wheat, and rice.**

III. Fatty acids profile in seeds pre- and post-gamma ray radiation.

IV.1 Maize:

Table 5.6, represents the fatty acid percents pre- and post-exposure to gamma radiation in maize.

Table 5.6: Comparison between the effects of different irradiation doses on fatty acid methyl esters profile as regard maize.

Fatty acids of maize	Fatty acid % Pre-radiation	Fatty acid % Post-radiation			F	p
	0 KGy	4 KGy	6 KGy	8 KGy		
C8 (Caproicacid)	$4.27^{a} \pm 0.16$	$4.28^{a} \pm 0.16$	$4.28^{a} \pm 0.16$	$4.28^{a} \pm 0.16$	0.023	0.995
C10 (Caprylic acid)	$7.27^{a} \pm 1.23$	$7.28^{a} \pm 1.23$	$7.27^{a} \pm 1.23$	$7.27^{a} \pm 1.24$	0.0	1.000
C14 (Myristic acid)	$0.609^{a} \pm 0.045$	$0.607^{a} \pm 0.41$	$0.607^{a} \pm 0.41$	$0.612^{a} \pm 0.42$	0.063	0.979
C16:0 (Palmitic acid)	$11.68^{a} \pm 0.45$	$11.74^{a} \pm 0.55$	$12.26^{a} \pm 1.01$	12.28 ± 1.06	3.195^{*}	0.028^{*}
C18:0 (Stearic acid)	$4.72^{a} \pm 0.62$	$4.62^{a} \pm 0.53$	$4.47^{a} \pm 0.42$	$4.65^{a} \pm 0.57$	0.778	0.510
C18:1 (Oleic acid)	$34.11^{a} \pm 2.0$	$24.92^{b} \pm 0.76$	$21.85^{c} \pm 0.09$	$17.97^{d} \pm 0.23$	813.432^{*}	$<0.001^{*}$
C18:2 (Linoleic acid)	$50.64^{a} \pm 3.73$	$50.10^{a} \pm 4.83$	$50.57^{a} \pm 4.76$	$50.66^{a} \pm 4.0$	0.074	0.974

The graphical representation of Table 5.6, is illustrated in (Figure.5.12).

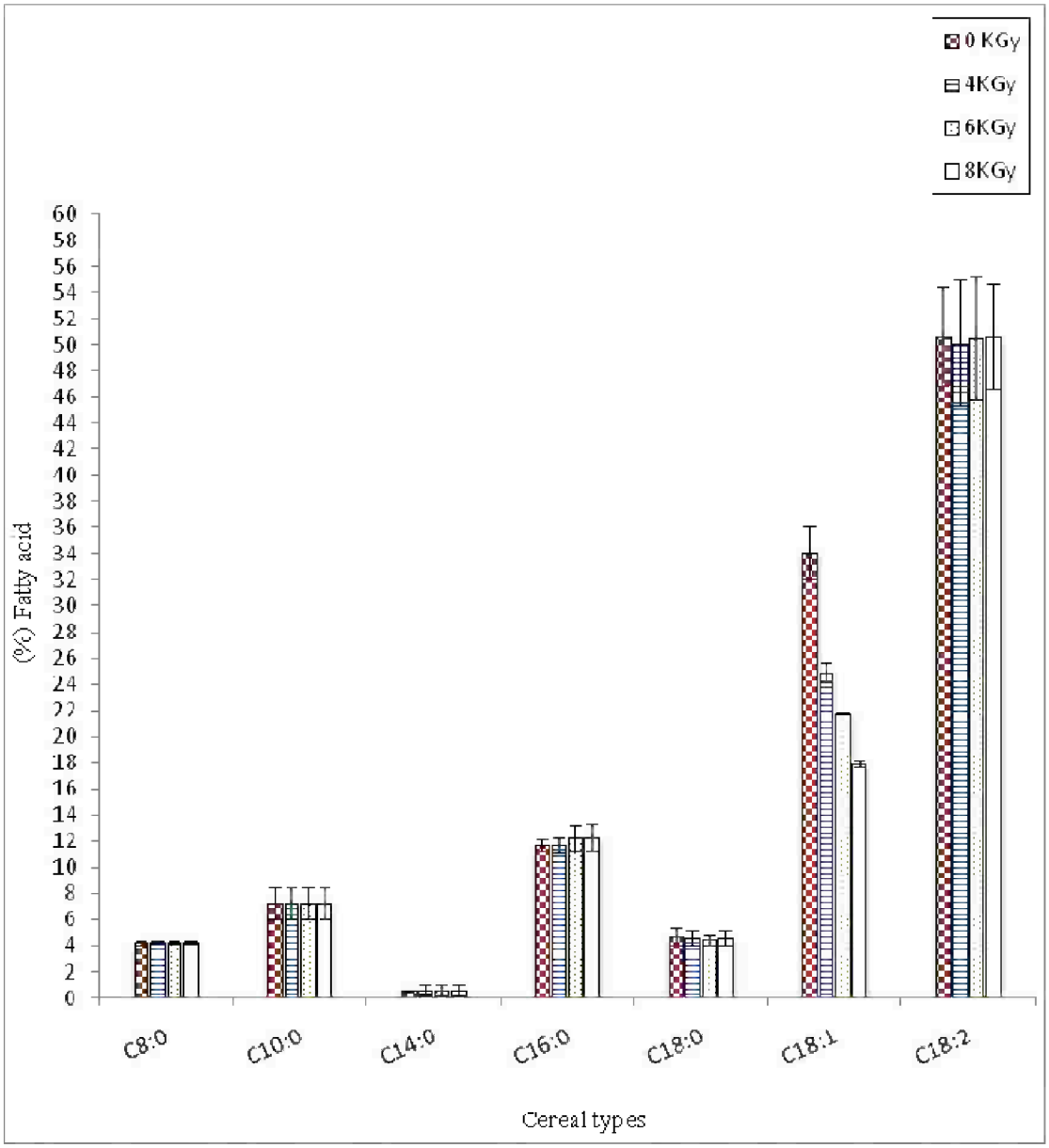

Figure 5.12: The effects of different irradiation doses on fatty acid methyl ester profiles as regard maize.

C8:0 (Caproic acid), C10:0 (Caprylic acid), C14:0 (Myristic acid), C16:0 (Palmitic acid) C18:0(Stearic acid) C18:1 (Oleic acid) C18:2 (Linoleic acid)

The changes in C16:0 saturated fatty acid (Palmitic acid), and C18:1 mono-unsaturated fatty acid (oleic acid), as a result of gamma radiation, is described in Table 5.7.

Table 5.7: Effects of different γ-irradiation doses on palmitic acid (C16:0) and oleic acid (C18:1) in maize.

Fatty acid changes in maize	Post radiation		
	4 KGy	6 KGy	8 KGy
Increase of palmitic acid (C16:0)	0.51%	4.97%	5.14%
Reduction of oleic (C18:1)	26.94%	35.94%	47.32%

IV.2 Wheat:

Table (5.8), represents the fatty acid percents pre- and post-exposure to gamma radiation in Wheat.

Table 5.8: Comparison between the effects of different irradiation doses on fatty acid methyl esters profile as regard wheat.

Fatty acids of wheat	Fatty acid % Pre-radiation	Fatty acid % Post radiation			F	p
	0 KGy	4 KGy	6 KGy	8 KGy		
C16:0 (Palmitic acid)	$13.66^{a} \pm 0.34$	$13.62^{a} \pm 0.28$	$13.68^{a} \pm 0.29$	$13.72^{a} \pm 0.33$	0.367	0.777
C18:0 (Stearic acid)	$1.50^{a} \pm 0.05$	$1.51^{a} \pm 0.04$	$1.51^{a} \pm 0.04$	$1.52^{a} \pm 0.04$	0.751	0.525
C18:1 (Oleic acid)	$26.83^{a} \pm 0.37$	$17.95^{b} \pm 0.29$	$14.30^{c} \pm 0.27$	$10.61^{d} \pm 0.31$	9967.779*	<0.001*
C18:2 (Linoleic acid)	$50.57^{a} \pm 2.44$	$50.51^{a} \pm 2.42$	$49.95^{a} \pm 3.54$	49.98 ± 3.39	0.242	0.867
C18:3 (Linolenic)	$10.02^{a} \pm 0.50$	$6.33^{b} \pm 0.36$	$3.48^{c} \pm 0.14$	$1.67^{d} \pm 0.11$	2588.487*	<0.001*

Different superscripts are significant at $p \leq 0.05$
F: F test (ANOVA)
*: Statistically significant at $p \leq 0.05$
Each value is a mean ± standard deviation (SD) of a triplicate analysis

The graphical representation of Table 5.8, is illustrated in (Figure 5.13).

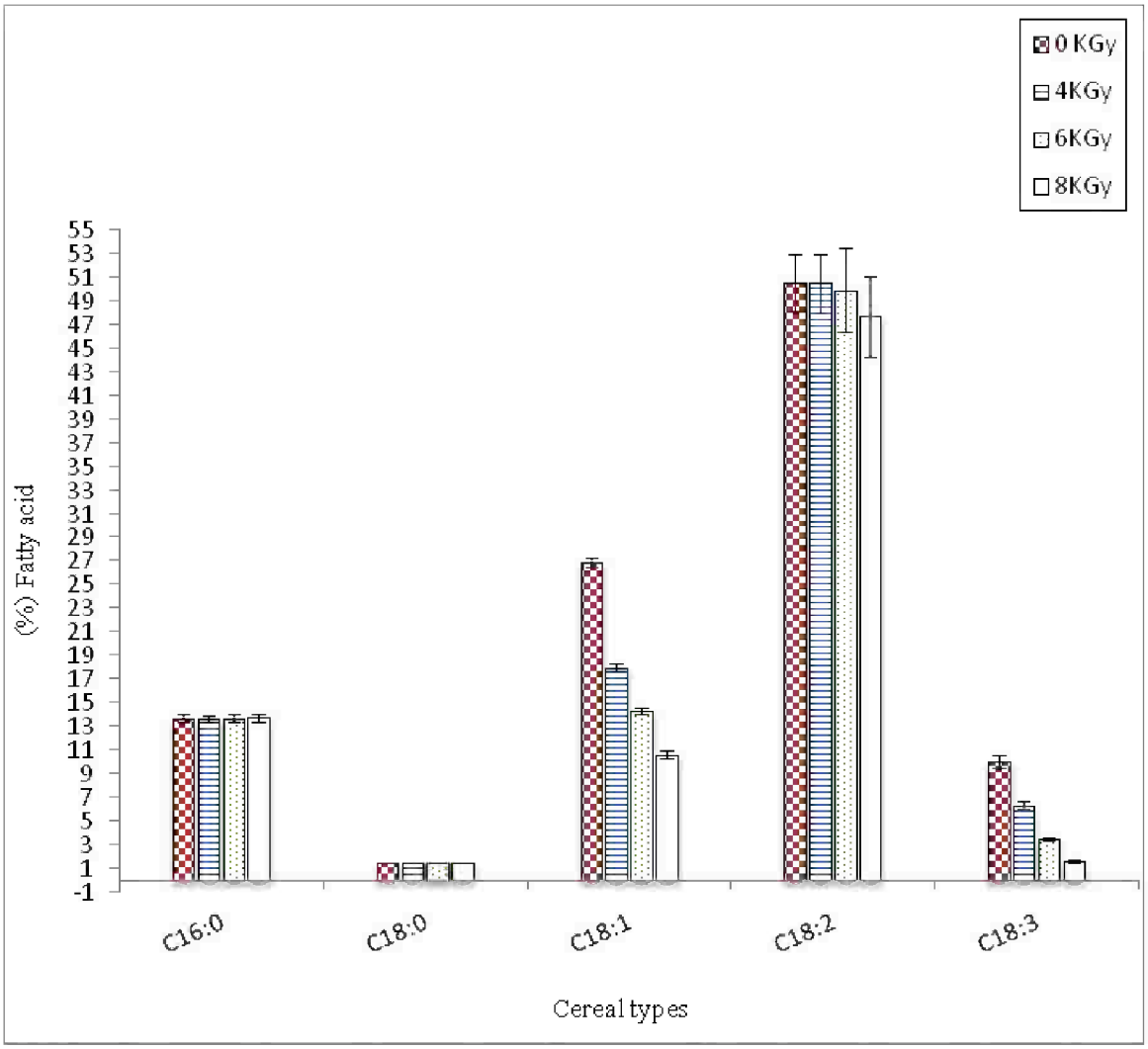

Figure 5.13: The effects of different irradiation doses on fatty acid methyl ester profiles as regard wheat.

C16:0 (Palmitic acid) C18:0 (Stearic acid) C18:1 (Oleic acid) C18:2 (Linoleic acid)

The changes in C18:1 mono-unsaturated fatty acid (oleic acid), and C18:3 poly-unsaturated fatty acid (linolenic acid), as a result of gamma radiation, is described in Table 5.9.

Table 5.9: Effects of different γ-irradiation doses on oleic acid (C18:0) and Linolenic acid (C18:3) in wheat.

Fatty acid	Mean % reduction Post radiation		
	4 KGy	6 KGy	8 KGy
Oleic acid (C18:1)	33.10	46.70	60.45
Linolenic acid (C18:3)	36.83	65.27	83.33

IV.3 Rice:

Table 5.10, represents the fatty acid percents pre- and post-exposure to gamma radiation in rice.

Table 5.10: Comparison between the effects of different irradiation doses on fatty acid methyl esters profile as regard rice.

Fatty acid types	Fatty acid % Pre-radiation	Fatty acid % Post radiation			F	p
	0 KGy	4 KGy	6 KGy	8 KGy		
C14:0 (Myristic acid)	$1.15^{a} \pm 0.04$	$1.16^{a} \pm 0.04$	$1.17^{a} \pm 0.04$	$1.19^{a} \pm 0.10$	2.086	0.109
C16:0 (Palmitic acid)	$17.69^{a} \pm 0.30$	$17.70^{a} \pm 0.30$	$17.71^{a} \pm 0.30$	$17.71^{a} \pm 0.29$	0.011	0.998
C18:0 (Stearic acid)	$2.70^{a} \pm 0.26$	$2.71^{a} \pm 0.26$	$2.71^{a} \pm 0.26$	$2.72^{a} \pm 0.26$	0.028	0.994
C18:1 (Oleic)	$48.04^{a} \pm 1.16$	$39.10^{b} \pm 0.21$	$33.80^{c} \pm 0.35$	$28.27^{d} \pm 0.35$	3450.973^{*}	$<0.001^{*}$
C18:2 (Linoleic acid)	$39.23^{a} \pm 0.30$	$39.57^{b} \pm 0.33$	$39.57^{b} \pm 0.35$	$39.59^{b} \pm 0.29$	6.075^{*}	0.001^{*}
C18:3 (Linolenic acid)	$0.77^{a} \pm 0.05$	$0.37^{b} \pm 0.03$	$0.19^{c} \pm 0.03$	ND	1331.873^{*}	$<0.001^{*}$

Different superscripts are significant at $p \leq 0.05$
F: F test (ANOVA)
*: Statistically significant
Each value is a mean ± standard deviation (SD) of a triplicate analysis

The graphical representation of Table 5.9, is illustrated in (Figure 5.14).

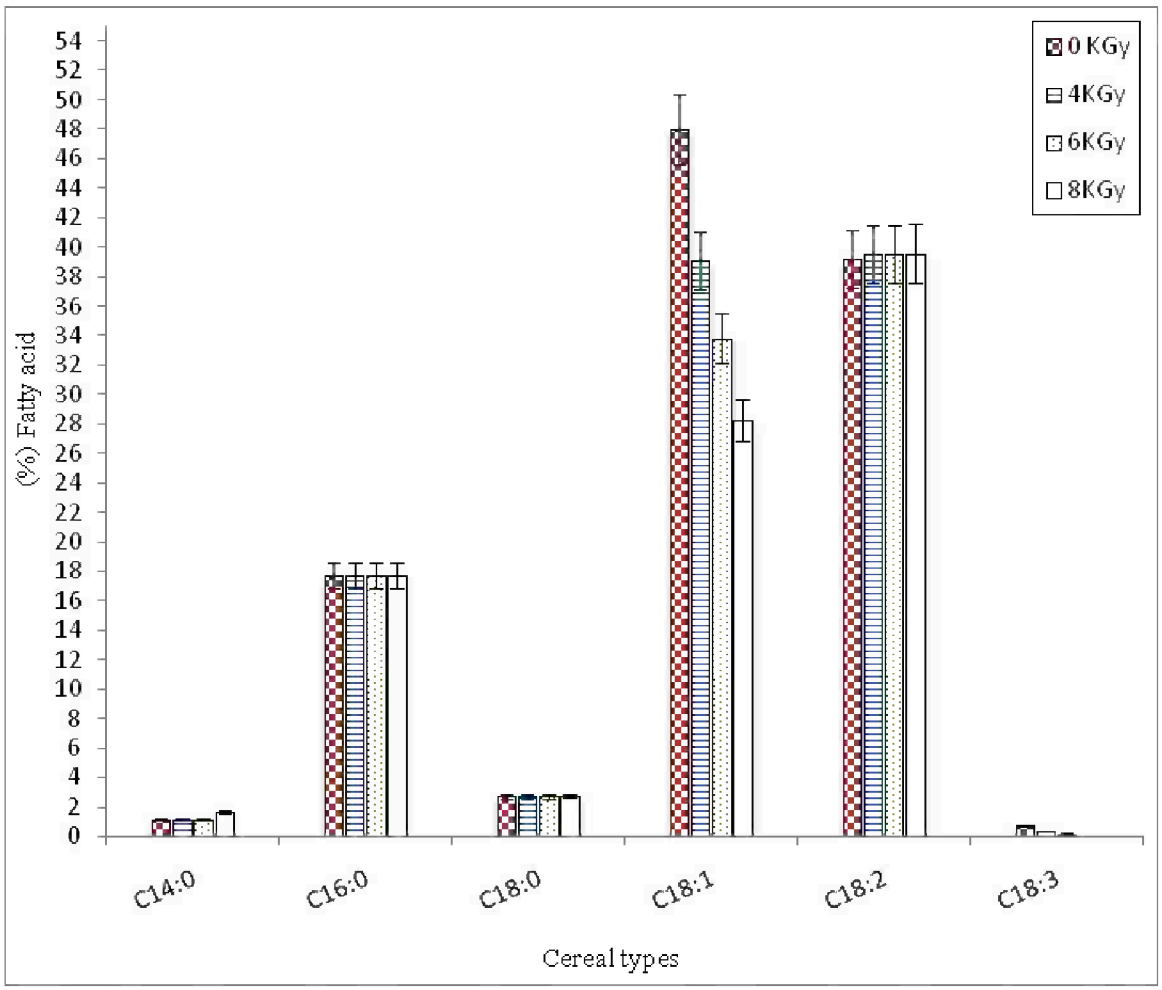

Figure 5.14: Comparison between the effects of different irradiation doses on fatty acid methyl ester profiles as regard rice.

C14:0 (Myristic acid) C16:0 (Palmitic acid) C18:0 (Stearic acid) C18:1(Oleic acid) C18:2 (Linoleic acid) C18:3 (Linolenic)

The changes in C18:1 mono-unsaturated fatty acid (oleic acid), and C18:3 poly-unsaturated fatty acid (linolenic acid), as a result of gamma radiation, is described in Table 5.11.

Table 5.11: Effects of different γ-irradiation doses on oleic acid (C18:0) and linolenic acid (C18:3) in rice.

Fatty acid changes	Mean % reduction Post radiation		
	4 KGy	6 KGy	8 KGy
Oleic acid (C18:1)	18.61%	29.64%	41.15%
Linolenic (C18:3)	51.95%	75.32%	ND

IV. Effects of gamma irradiation on the amino acids profile pre- and post-irradiation procedure.

V.1 Maize:

Table 5.12, and (Figure 5.15), illustrate the amino acid contents in mg/g protein and the graphical presentation, respectively.

Table 5.12: Comparison between the different groups according to amino acid (mg/g protein) as regard maize.

Amino acids of maize	Amino acids ng/g protein Pre-radiation	Amino acids ng/g protein Post-radiation			F	p
	0KGy	4 KGy	6 KGy	8 KGy		
Tryptophan	$46.38^{a} \pm 0.47$	$46.16^{a} \pm 0.60$	$46.50^{a} \pm 0.60$	$46.43^{a} \pm 0.60$	1.317	0.275
Threomine	$38.36^{a} \pm 0.39$	$33.37^{b} \pm 0.34$	$29.52^{c} \pm 0.29$	$25.64^{d} \pm 0.95$	1888.94*	<0.001*
Isoleucine	$31.20^{a} \pm 0.60$	$31.58^{ab} \pm 0.74$	$31.12^{a} \pm 0.77$	$31.01^{a} \pm 0.77$	2.375	0.077
Leucine	$68.03^{a} \pm 0.52$	$73.87^{b} \pm 0.63$	$76.56^{c} \pm 0.28$	$74.12^{d} \pm 3.86$	1647.336*	<0.001*
Lysine	$20.72^{a} \pm 0.33$	$20.59^{a} \pm 0.72$	$20.49^{a} \pm 0.72$	$20.41^{a} \pm 0.86$	0.756	0.522
Valine	$41.27^{a} \pm 0.57$	$40.99^{ab} \pm 0.62$	$40.98^{abc} \pm 0.48$	$40.92^{bc} \pm 0.52$	1.630	0.189
Phenylalanine	$49.19^{a} \pm 0.39$	$46.53^{b} \pm 0.62$	$44.96^{c} \pm 0.70$	$42.60^{d} \pm 0.28$	552.875*	<0.001*
Methionine	$17.09^{a} \pm 0.78$	$17.20^{a} \pm 0.78$	$17.50^{a} \pm 0.91$	$17.49^{a} \pm 0.83$	1.281	0.287
Cysteine	$22.16^{a} \pm 0.31$	$22.30^{a} \pm 0.49$	$22.30^{a} \pm 0.71$	$22.20^{a} \pm 0.56$	0.342	0.795
Arginine	$46.09^{a} \pm 0.40$	$46.38^{a} \pm 0.63$	$46.28^{a} \pm 0.57$	$46.31^{a} \pm 0.56$	1.045	0.378
Tyrosine	$49.15^{a} \pm 0.37$	$49.28^{a} \pm 0.48$	$49.45^{ab} \pm 0.56$	$49.68^{b} \pm 0.53$	4.276*	0.008*
Histidine	$43.47^{a} \pm 0.39$	$43.31^{a} \pm 0.29$	$43.17^{b} \pm 0.25$	$43.25^{b} \pm 0.26$	3.431*	0.021*

Different superscripts are significant at $p \leq 0.05$
F: F test (ANOVA)
Statistically significant at $p \leq 0.05$
Each value is a mean ± standard deviation (SD) of a triplicate analysis

The changes in leucine, phenyl alanine, and threomine amino acids, as a result of gamma radiation, is described in Table 5.13.

Table 5.13: Changes in amino acid of maize as a result of gamma irradiation.

Amino acids changes	Post radiation		
	4 KGy	6 KGy	8 KGy
Increase of leucine	8.58%	12.54%	8.95%
Reduction of phenylalanine	5.41%	8.60%	13.40%
Reduction of threomine	13.03%	23.04%	33.15%

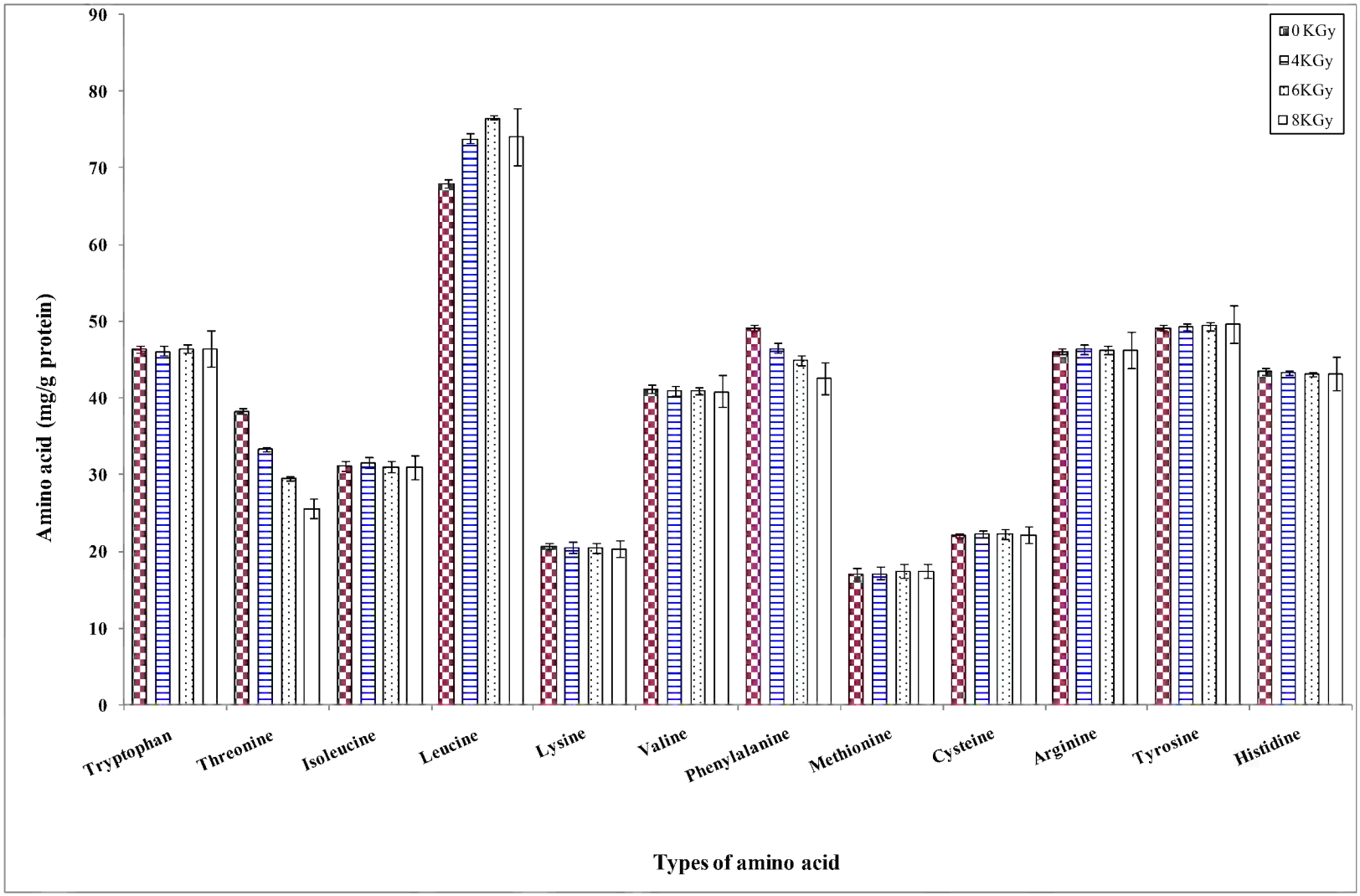

Figure 5.15: The effects of different irradiation doses on amino acids profile as regard maize.

V.2 Wheat:

Table 5.14, and (Figure 5.16), illustrate the amino acid contents in mg/g protein and the graphical presentation, respectively.

Table 5.14: Comparison between the different groups according to amino acid (mg/g protein) as regard wheat.

Amino acids of wheat	Amino acids in mg/g protein Pre-radiation	Amino acids in mg/g protein Post-radiation			F	p
	0 KGy	4 KGy	6 KGy	8 KGy		
Tryptophan	$24.84^{a} \pm 0.38$	$21.70^{b} \pm 0.52$	$21.35^{c} \pm 0.55$	$21.26^{c} \pm 0.61$	218.576^{*}	$<0.001^{*}$
Threonine	$22.83^{a} \pm 0.34$	$22.75^{ab} \pm 0.48$	$23.37^{c} \pm 0.46$	$23.31^{c} \pm 0.43$	10.716^{*}	$<0.001^{*}$
Isoleucine	$27.91^{a} \pm 0.32$	$26.71^{b} \pm 0.50$	$26.64^{c} \pm 0.54$	$25.58^{c} \pm 0.57$	99.106^{*}	$<0.001^{*}$
Leucine	$54.27^{a} \pm 0.65$	$55.81^{b} \pm 0.36$	$58.05^{c} \pm 0.68$	$57.97^{c} \pm 0.75$	169.121^{*}	$<0.001^{*}$
Lysine	$24.51^{a} \pm 0.35$	$20.48^{b} \pm 0.24$	$20.26^{b} \pm 0.60$	$20.19^{b} \pm 0.70$	340.859^{*}	$<0.001^{*}$
Valine	$22.96^{a} \pm 0.66$	$23.48^{b} \pm 0.19$	$22.88^{c} \pm 0.56$	$23.81^{bc} \pm 0.57$	12.631^{*}	$<0.001^{*}$
Phenylalanine	$48.16^{a} \pm 0.45$	$46.72^{b} \pm 0.43$	$45.63^{c} \pm 0.54$	$45.49^{c} \pm 0.52$	128.724^{*}	$<0.001^{*}$
Methionine	$15.86^{a} \pm 0.28$	$15.56^{b} \pm 0.48$	$14.93^{c} \pm 0.47$	$14.69^{c} \pm 0.58$	27.380^{*}	$<0.001^{*}$
Cysteine	$26.88^{a} \pm 0.34$	$26.72^{ab} \pm 0.49$	$26.68^{ab} \pm 0.52$	$26.58^{b} \pm 0.47$	1.482	0.226
Arginine	$47.80^{a} \pm 0.31$	$45.73^{b} \pm 1.09$	$45.79^{b} \pm 0.86$	$45.50^{b} \pm 0.92$	31.778^{*}	$<0.001^{*}$
Tyrosine	$42.84^{a} \pm 0.30$	$43.36^{b} \pm 0.42$	$43.30^{b} \pm 0.68$	$42.92^{a} \pm 0.62$	5.005^{*}	0.003^{*}
Histidine	$41.41^{a} \pm 0.36$	$41.16^{a} \pm 0.39$	$40.77^{b} \pm 0.59$	$40.64^{b} \pm 0.64$	9.629^{*}	$<0.001^{*}$
Proline	$39.90^{a} \pm 0.46$	$39.92^{a} \pm 1.64$	$39.83^{a} \pm 1.59$	$39.74^{a} \pm 1.57$	0.062	0.980
Glycine	$28.30^{a} \pm 0.32$	$28.40^{ab} \pm 0.51$	$28.05^{ac} \pm 0.50$	$27.99^{cd} \pm 0.53$	3.533^{*}	0.019^{*}

Different superscripts are significant at $p \leq 0.05$
F: F test (ANOVA)
*: Statistically significant at $p \leq 0.05$
Each value is a mean ± standard deviation (SD) of a triplicate analysis

The changes in tryptophan, isoleucine, leucine, phenyl alanine, and arginine amino acids, as a result of gamma radiation, is described in Table 5.15.

Table 5.15: Changes in amino acids in wheat as a result of gamma irradiation.

Amino acids changes	Post radiation		
	4 KGy	6 KGy	8 KGy
Reductionof tryptophan	12.64%	14.05%	14.41%
Reduction of isoleucine	4.30%	4.55%	8.35%
Increase of leucine	2.84%	6.97%	6.82%
Reductionof phenylalanine	2.99%	5.25%	5.54%
Reduction of arginine	4.33%	4.21%	4.81%
Reduction of lysine	16.44%	17.33%	17.62%

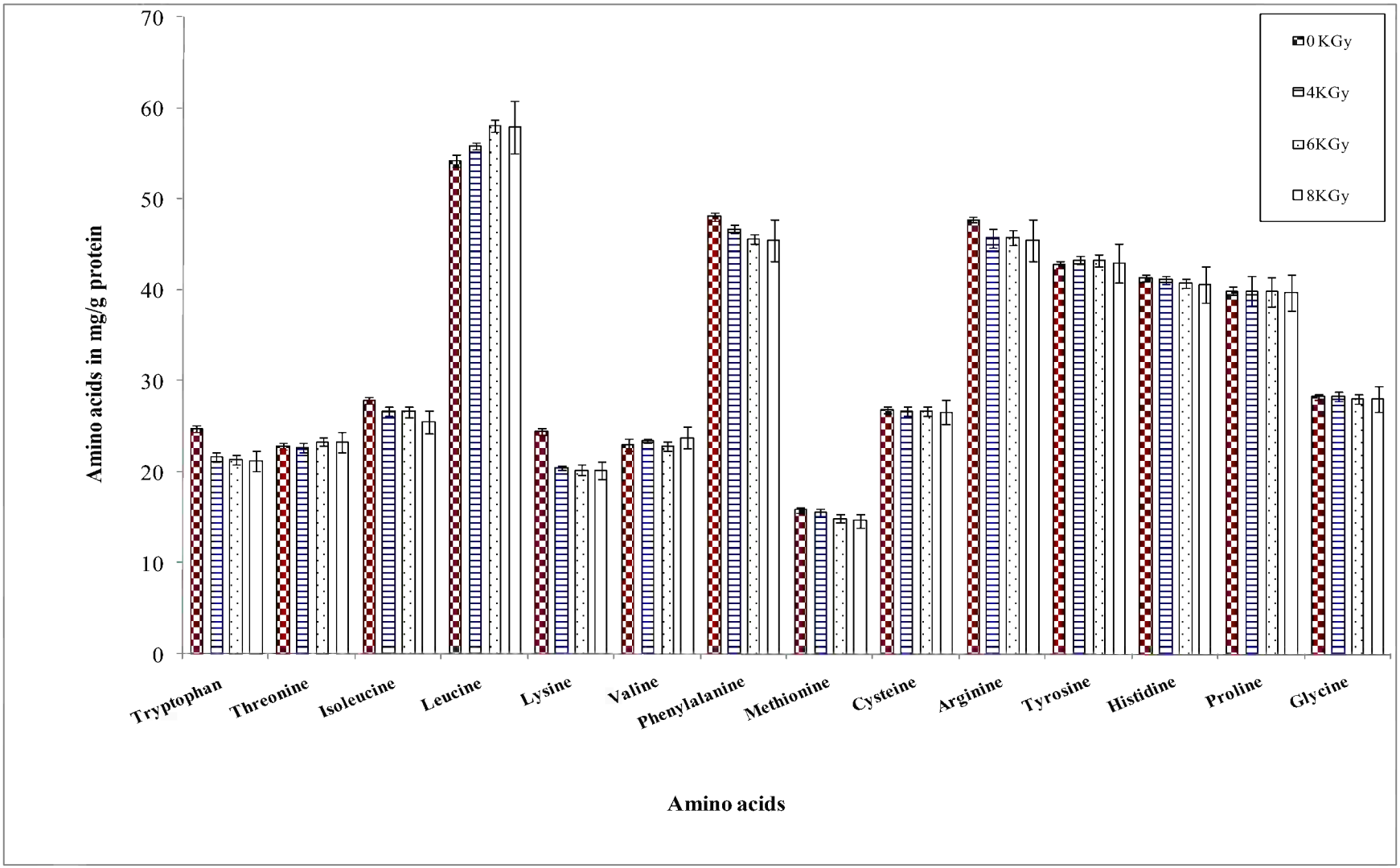

Figure 5.16: The effects of different irradiation doses on amino acids profile as regard wheat

V.3 Rice:

Table 5.16, and (Figure 5.17), illustrate the amino acid contents in mg/g protein and the graphical presentation, respectively.

Table 5.16: Comparison between the different groups according to amino acid (mg/g protein) as regard rice.

Amino acids of rice	Amino acids in mg/g protein Pre-radiation	Amino acids in mg/g protein Post radiation			F	p
	0 KGy	4 KGy	6 KGy	8 KGy		
Tryptophan	$14.03^{a} \pm 0.46$	$13.92^{a} \pm 0.53$	$14.01^{a} \pm 0.45$	$13.80^{a} \pm 0.49$	0.878	0.456
Threonine	$29.02^{a} \pm 0.64$	$28.25^{b} \pm 2.46$	$28.21^{b} \pm 2.48$	$28.25^{b} \pm 2.50$	0.653	0.583
Isoleucine	$25.61^{a} \pm 0.27$	$25.36^{b} \pm 0.26$	$25.33^{b} \pm 0.26$	$25.25^{b} \pm 0.24$	7.305*	<0.001*
Leucine	$58.14^{a} \pm 0.64$	$59.87^{b} \pm 0.59$	$62.11^{c} \pm 0.61$	$61.84^{c} \pm 0.60$	185.246*	<0.001*
Lysine	$43.46^{a} \pm 0.38$	$43.34^{ab} \pm 0.51$	$43.11^{bc} \pm 0.49$	$43.01^{c} \pm 0.54$	3.563*	0.018*
Valine	$41.04^{a} \pm 0.64$	$40.64^{b} \pm 0.64$	$40.94^{ab} \pm 0.55$	$40.82^{ab} \pm 0.62$	1.613	0.193
Phenylalanine	$39.14^{a} \pm 0.32$	$35.32^{b} \pm 0.20$	$34.48^{c} \pm 0.50$	$34.48^{c} \pm 0.45$	669.511*	<0.001*
Methionine	$12.53^{a} \pm 0.25$	$11.86^{b} \pm 0.44$	$11.53^{c} \pm 0.45$	$11.48^{c} \pm 0.42$	29.467	<0.001*
Cysteine	$11.53^{a} \pm 0.25$	$11.33^{b} \pm 0.21$	$11.16^{bc} \pm 0.28$	$11.01^{c} \pm 0.36$	13.066*	<0.001*
Arginine	$49.20^{a} \pm 0.33$	$49.11^{a} \pm 0.43$	$49.02^{a} \pm 0.58$	$48.92^{a} \pm 0.50$	1.314	0.276
Tyrosine	$51.56^{a} \pm 0.24$	$51.73^{b} \pm 0.23$	$51.40^{c} \pm 0.25$	$51.31^{c} \pm 0.26$	11.373*	<0.001*
Histidine	$40.12^{a} \pm 0.51$	$40.68^{a} \pm 2.18$	$40.53^{a} \pm 2.09$	$40.32^{a} \pm 2.14$	0.342	0.795
Proline	$38.34^{a} \pm 0.25$	$38.17^{ab} \pm 0.34$	$38.02^{b} \pm 0.43$	$37.98^{b} \pm 0.48$	3.547*	0.018*
Glycine	$33.24^{a} \pm 0.47$	$33.0^{ab} \pm 0.52$	$32.91^{b} \pm 0.52$	$32.77^{b} \pm 0.52$	3.089*	0.032*

Different superscripts are significant at $p \leq 0.05$
F: F test (ANOVA)
*: Statistically significant at $p \leq 0.05$
Each value is a mean ± standard deviation (SD) of a triplicate analysis

The changes in leucine and phenyl amino acids, as a result of gamma radiation, is described in Table 5.17.

Table 5.17: Changes in amino acids in rice as a result of gamma irradiation.

Amino acids changes	Post radiation		
	4 KGy	6 KGy	8 KGy
Increase of leucine	2.98%	6.83%	6.36%
Reduction of phenylalanine	9.76%	11.91%	11.91%

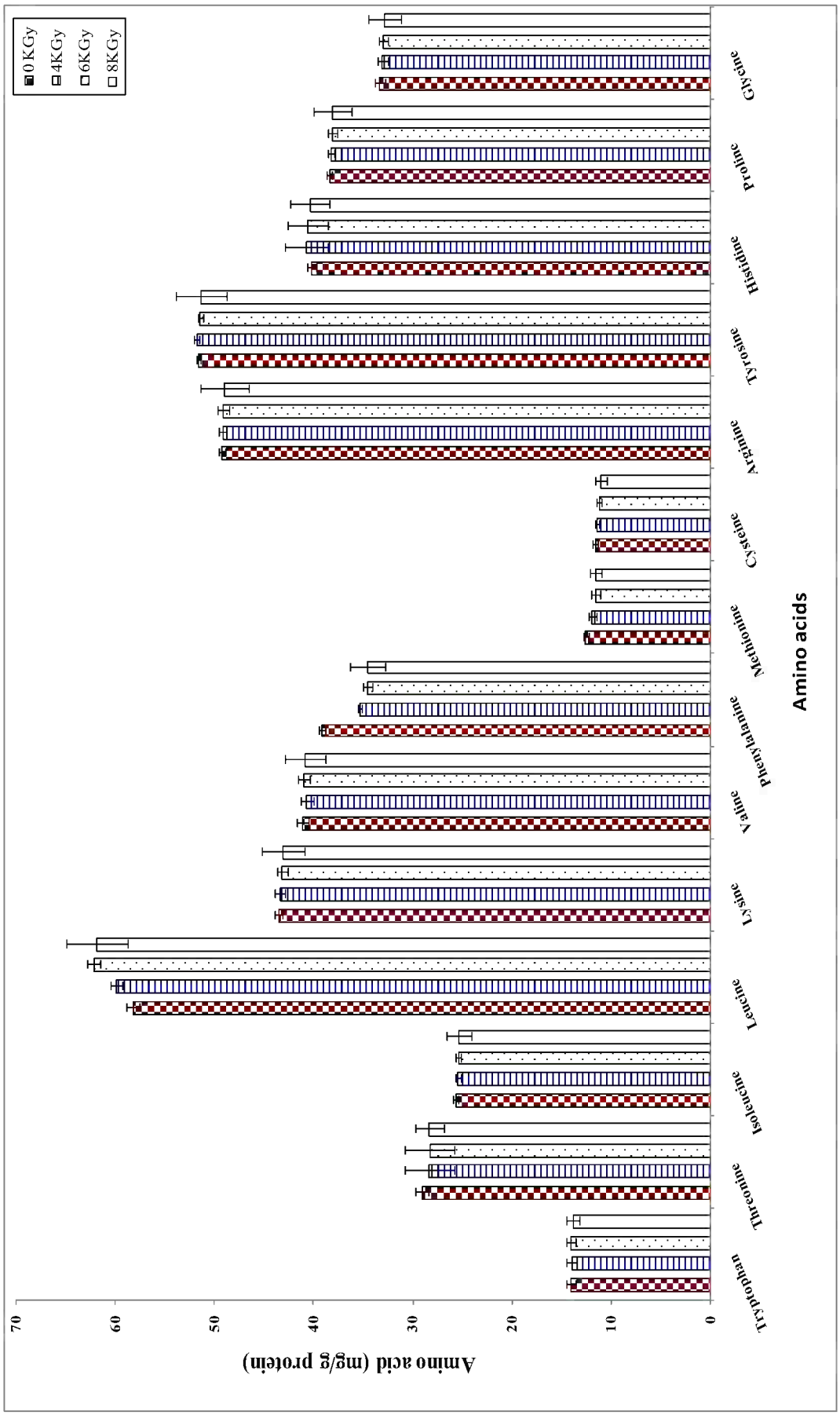

Figure 5.17: The effects of different irradiation doses on amino acids profile as regard rice