DISCUSSION

Aflatoxins are secondary metabolites of the common foodborne fungi *A. flavus* and *A. parasiticus*, which colonize crops in tropical and subtropical regions worldwide. These fungi can also produce aflatoxin during storage, transportation, and food processing. Aflatoxin contamination primarily occurs in cereals, spices, peanuts, tree nuts (almonds, pistachios, hazelnuts, pecans, and Brazil nuts), and milk. Aflatoxin B_1; the most toxic aflatoxin, is the most potent naturally occurring chemical liver carcinogen known.(10)

The risk of liver cancer in individuals exposed to chronic hepatitis B virus (HBV) infection and aflatoxin may be up to 30 times greater than the risk in individuals exposed to HBV or aflatoxin alone. Acute aflatoxicosis, causing severe gastrointestinal symptoms and often death, results from high aflatoxin doses. In recent years, hundreds of aflatoxicosis cases in Africa have resulted from consumption of contaminated maize. Aflatoxin exposure may also be associated with stunting in children and immune suppression.(74)

Recently, aflatoxin exposure has been associated with liver cirrhosis; aflatoxin and HBV exposure may synergize to substantially increase cirrhosis risk. Currently, over 5 billion people worldwide are at risk of chronic exposure to aflatoxin in food. Maize is one of the main sources of human exposure to aflatoxin, because it is highly consumed worldwide and unfortunately is also one of the most susceptible crops to aflatoxin contamination.(113)

Our results aimed to assessing the possible effect of gamma irradiation at a dose level of 4 KGy, 6 KGy, and 8 KGy on the reduction of aflatoxin B_1 level in cereal grains including maize, wheat, and rice and the impact on nutritive values.

Distribution of aflatoxin B_1 in cereal samples (maize, wheat, and rice) was observed in terms of acceptable limit for aflatoxin B_1 according to Food and Agriculture Organization (FAO).(87)

I. Levels of aflatoxin B_1 in cereals before irradiation.

From the data presented in Figure 5.1, it is evident that out of 20 samples of maize about 75% (15 samples) showed AFB_1 concentration greater than 40 ppb (part per billion), about 15% (3 samples) showed AFB_1 concentration in the range between 31 and 40 ppb, 10% (2 samples) in the range between 21 and 30 ppb, and none showed AFB_1 concentration less than or equal to 20 ppb.

It was found that out of 20 samples of wheat 50% (10 samples) showed AFB_1 concentration more than 40 ppb, 30% (6 samples) showed AFB_1 concentration between 31 and 40 ppb, about 20% (4 samples) showed AFB_1 concentration between 21 and 30 ppb and none showed concentration less than or equal to 20 ppb.

In rice samples it was found that none showed AFB_1 concentration more than 40 ppb, 25% (5 samples) showed AFB_1 concentration between 31 and 40 ppb, 60% (12 samples) showed AFB_1 concentration between 21 and 30 ppb, and 15% (3 samples) showed aflatoxin concentration less than or equal to 20 ppb.

Accordingly, these results indicate that, aflatoxin B_1 levels in the collected samples of maize, wheat, and rice are exceeding the acceptable level (5ppb) defined by the FAO.[87]

II. Effect of gamma radiation on the reduction of aflatoxin B_1 in cereal grains including maize, wheat, and rice.

Tables 5.1&5.2, indicate that before gamma irradiation the order of AFB_1 levels in the three grains is maize > wheat > rice and gamma irradiation reduced the toxin significantly with dose levels 8 KGy >6 KGy > 4 KGy. ANOVA test revealed that there is a direct proportionality between the gamma ray dose levels and AFB_1 reduction percents.

As indicated in (Figure 5.3), for **maize** samples, the initial level of aflatoxin B_1 before gamma irradiation is 50.38 ±14.46 ng/g and it reduced to 20.02±6.70 ng/g after irradiation (60.26 reduction percent) at a dose level of 8 KGy which is still above the legal limit which is 5 ng/g. For **wheat** samples the initial level of aflatoxin B_1 before irradiation was 37.61±6.85ng/g and reduced to 11.55±3.41 ng/g (69.29 reduction percent) after irradiation at the same dose level which is also still above the legal limit which is 5 ng/g and for **rice** the initial level of aflatoxin B_1 before irradiation was 27.49±7.32 ng/g it reduced to 9.71±2.43 ng/g (64.68 reduction percent) after irradiation at a dose level of 8 KGy and it is still above the legal limit which is 5 ng/g.

This means that the percentages of reduction in the levels of aflatoxins were greater in wheat than in rice and maize, respectively, using 8 kGy.

On the other hand the reduction percents at 6 KGy are 32.39%, 43.84%, and 56.38% for maize, wheat, and rice respectively, and at 4 KGy the reduction percents are 15.54%, 22.25%, and 27.46% for maize, wheat, and rice, respectively.

From the afford-said data, we concluded that the reduction percentages in aflatoxin B_1 in the maize, wheat and rice samples are directly proportional to the exposed radiation doses (dose dependent manner) with the highest reduction percent on using the 8 KGy gamma radiation, (Figure 5.4). Also the reduction percents are different with different cereal samples.

These results are in agreement with the data reported by Aziz and Youssef (2002)[114], who revealed that treatment of food and agricultural products with gamma irradiation at a dose level of 5 KGy destroy 44-48% of aflatoxin B_1 whereas application of 10 KGy dose resulted in reduction of aflatoxin B_1 by 82-88%, i.e., our results is nearly consistent with this study in that by increasing the radiation dose the percent of reduction of aflatoxin B_1 increases.

According to Ghanem et al., (2008)[115], at a dose level of 10 KGy the % reduction of aflatoxin B_1 degradation reached 81.1% for corn (maize), 87.8% for rice, and for feed samples aflatoxin reduction levels reached 31, 72, and 84% in corn at 4, 6, and 10 KGy, respectively.

According to Ghanem et al., (2008)[115] study, AFB_1 degradation is correlated negatively with oil content in irradiated samples and this is consistent with our study in that aflatoxin B_1 reduction level in maize is lower than wheat and rice as maize contains more oil content.

Also according to Ghanem et al., (2008)[115], there was a positive correlation between the increase in doses of gamma irradiation and the levels of break down of AFB_1 exist in the commodity and the maximum acheivable level of AFB_1 break down varried between tested products and this consistent with our study.

III. Effect of gamma radiation on nutritive values of cereals including maize, wheat, and rice.

Treatment of food with ionizing energy is a well-known method aimed at improving the safety of a wide range of food by reducing or eliminating foodborne pathogens. The results of our work indicated that gamma radiation doesn't affect the nutritive values in cereal samples which is in agreement with other investigators.[106,116]

However, there is statistical significance difference in the levels of carbohydrates and protein between different groups, pre-and post-radiation; Table 5.3, at 4 KGy, 6 KGy, and 8 KGy in maize samples as revealed by ANOVA test.

According to Tables 5.4 and 5.5 although gamma radiation doesn't affect the nutritive values in wheat and rice samples there is statistical significance difference in the levels of fat (fat %) between different groups pre-and post-radiation at 4 KGy, 6 KGy, and 8 KGy in wheat samples as revealed by ANOVA test.

IV. Effect of gamma irradiation on fatty acids composition of the cereal grains.

IV.1 Maize:

After irradiation, maize samples showed some variations in fatty acid methyl esters profile, where a great change was observed in mono-unsaturated fatty acid (oleic acid, C18:1) which is statistical significant at ($p < 0.001$). According to Table 5.6, some other saturated and poly-unsaturated fatty acid methyl esters were not affected much at the chosen doses of gamma irradiations.

Table 5.7, showed that there was a little increase in the saturated fatty acid palmitic acid (C16:0) by 5.14%, 4.97%, and 0.51% at gamma irradiation dose levels of 8, 6, and 4 KGy, respectively and there is a highly significant reduction ($P<0.001$) in the mono-unsaturated fatty acid, oleic acid (C18:1) by 47.32%, 35.94%, and 26.94% at gamma irradiation dose levels of 8, 6, and 4 KGy, respectively.

On the other hand, certain studies were conducted and concluded that gamma irradiation interact with fat molecules causing dehydration, polymerization, decarboxylation, and oxidative reactions leading to lipid oxidation.[117,118]

In a recent study Stefanova et al.,(2011)[119], the food chemistry researchers studied the effect of gamma-ray irradiation on the fatty acid profile of beef meat. Three beef meat samples were studied; non irradiated beef meat (control), irradiated beef meat at 5 increasing doses of irradiation (test), and a commercially available beef sample irradiated at an unknown dose. The effects of gamma-ray irradiation on the fatty acid profile (saturated fatty acids and unsaturated fatty acids) in the triglyceride composition of beef meat was studied using nuclear magnetic resonance (NMR) spectroscopy and gas chromatography (GC). Based on the NMR analysis, researchers found that there was a significant increase in the total amount of saturated fatty acids and a decrease in the amount of poly-unsaturated fatty acids of the triglyceride composition of the irradiated samples as opposed to the non irradiated beef sample. The researchers concluded that the decrease in poly-unsaturated fatty acids may be due to the fact that fat-containing foods are sensitive to irradiation. These evident changes in the fatty acid composition shown by proton NMR spectroscopy were also confirmed using GC. The commercially available beef sample confirmed to be exposed to irradiation treatment by both NMR and GC techniques .Therefore, researchers concluded that the unkown beef sample serves as evidence of the potential and reliability of NMR technique for identifying irradiated meat products.

The previous study is consistent with our research results in that there is an increase in saturated fatty acid (palmitic acid, C16:0) and a decrease in unsaturated fatty acid (oleic acid, C18:1) in maize samples.

IV.2 Wheat:

Wheat samples exhibit some variations in fatty acid methyl esters profile, Table 5.8, a great changes were observed in mono-unsaturated fatty acid (oleic acid, C18:1) which is statistical significant at ($p < 0.001$) and poly-unsaturated fatty acid (linolenic acid, C18:3) which is also statistical significant at ($p < 0.001$). Other saturated and unsaturated fatty acid methyl esters were not affected much at the chosen doses of gamma irradiations.

The decrease in the mono-unsaturated fatty acid oleic acid (C18:0) is 60.45%, 46.70%, and 33.10% at gamma irradiation dose level of 8, 6, and 4 KGy, respectively, Table 5.9. However there is a highly significant reduction in the poly-unsaturated fatty acid Linolenic acid (C18:3) by 83.33%, 65.27%, and 36.83% at gamma irradiation dose level of 8, 6, and 4 KGy, respectively. Also, Table 5.9 revealed that by increasing the radiation dose from 4 to 8 KGy the % of reduction increases with the highest reduction occured at 8 KGy.

IV.3 Rice:

After irradiation, rice samples exhibited some variations in fatty acid methyl esters profile, a great changes were observed in mono-unsaturated fatty acid (oleic acid, C18:1) which is statistical significant at ($p < 0.001$) and poly-unsaturated fatty acid C18:3 which is also statistical significant at ($p < 0.001$) other saturated and unsaturated fatty acid methyl esters were not affected much at the chosen doses of gamma irradiations. The decrease in the mono-unsaturated fatty acid oleic acid (C18:0) is 41.15%, 29.64%, and 18.61%, Table 5.11, is observed at gamma irradiation dose levels of 8, 6, and 4 KGy, respectively, and there is a highly significant reduction in the poly-unsaturated fatty acid linolenic acid (C18:3) which is completely disappeared at gamma irradiation dose level of 8 KGy, while at 6 KGy it exhibited reduction at a level of 75.32% and at 4 KGy it reduced by 51.95%. Also this Table revealed that by increasing the radiation dose the % of reduction increases with the highest reduction at 8 KGy.

In a study conducted by Sabahat, et al., (2013)[120], small change in fatty acid methyl esters profile was detected in total saturated and poly-unsaturated fatty acids, while a significant ($p<0.05$) change in percentage concentration of mono-unsaturated fatty acid was observed. This study is consistent with our results, and this reduction may be attributed to the breakdown of the double bonds at a high dose of irradiation or with hidden artifacts.

According to the previous study Sabahat, et al., (2013)[(120)] the effect of gamma irradiation dose levels on palmitic (16:0), stearic (18:0), linoleic (18:2), linolenic (18:3) and arachidic (20:0) acids was statistically non significant whereas, oleic acid showed significant change in concentration. In our study there is no statistical significant difference on the percentage of palmitic (16:0), stearic (18:0), linoleic (18:2) but there is a statistical significance difference in the percents of concentration of mono-unsaturated fatty acid (oleic acid, C18:1) and poly-unsaturated fatty acid (linolenic acid C18:3) at $P < 0.001$.

V. Effect of gamma irradiation on amino acids composition of protein in cereals.

The amino acids are very important to human health. Of the twenty standard amino acids known, eight are called essential amino acids because the human body cannot synthesize them from other compounds at the level needed for normal growth, so they must be obtained from food. The amino group ($-NH_2$) is the most radiosensitive portion of the amino acids.[(121)]

V.1 Maize:

After irradiation, maize samples exhibit some variations in amino acids profile, a great changes were observed in threomine, phenyl alanine and leucine the three amino acids have statistical significant difference at ($p < 0.001$) other amino acids were not affected much at the chosen doses of gamma irradiations.

From Table 5.13, it was found that leucine increased by increasing the radiation doses while phenyl alanine exhibit decrease. Leucine increased by 8.95%, 12.54%, and 8.58% at 8, 6, and 4 KGy, respectively, while phenyl alanine decreased by 13.40%, 8.60%, 5.41% at the same radiation doses and threomine decreased by 33.15%, 23.04% and 13.03% at the same radiation doses. Other amino acids didn’t affected and this in agreement with WHO report (1977)[(122)], that mention that, no change in the nutritional quality of irradiated cereals even at higher dose(10 KGy). The observed increase in amino acid (leucine) content with the exposure to ionizing radiation is in agreement with the data of Satter *et al*., (1990) [(123)].

Siddhuraju *et al*., (2002)[(121)], reported that amino acids, e.g. phenyl-alanine, leucine and arginine, were increased with increasing gamma dose from 0.5 kGy to 5 kGy whereas methionine, threonine, valine, isoleucine were decreased at higher dose (5 kGy) in wheat, maize, mug bean and chickpea.

V.2 Wheat:

According to Table 5.14, there is a statistical significance difference in some amino acids including tryptophan, isoleucine, leucine, phenylalanine, arginine, and lysine. Tryptophan, isoleucine, phenylalanine, arginine, and lysine decreased whereas leucine increased.

Table 5.15, represents that, at the higher radiation dose (8KGy) the percents of decrease of tryptophan, isoleucine, phenylalanine, arginine, and lysine are 14.41%, 8.35%, 5.54%, 4.81%, and 17.62%, respectively, whereas leucine increased by 6.82% at the same radiation dose, at a dose level of (6 KGy) the percents of decrease of tryptophan, isoleucine, phenylalanine, arginine, and lysine are 14.05%, 4.55%, 5.25%, 4.21%, and 17.33%, respectively, whereas leucine increased by 6.97% and at a dose level of 4 KGy the percents of decrease of tryptophan, isoleucine, phenylalanine, arginine, and lysine are 12.64%, 4.30%, 2.99%, 4.33%, 16.44%, respectively, whereas leucine increased by 2.84%. Joseph *et al*., (2005)[(124)] reported that, with the exception of tyrosine (which increased significantly), the amino acids in cowpea (acidic, basic, polar and non-polar amino acids) were decreased significantly with the increase of gamma radiation compared to their respective control. So the reduction in tryptophan, isoleucine, phenylalanine, arginine, and lysine is consistence with Joseph *et al.,*(2005)[(124)].

V.3 Rice:

According to Table 5.16, there is a slight change in the amino acids profile of rice, leucine increased whereas phenylalanine decreased.

Table 5.17, shows that, at a radiation dose level of 8 KGy leucine increase by 6.36% whereas phenylalanine decreased by 11.91%, whereas at a radiation dose of 6 KGy leucine increased by 6.83% whereas phenyl alanine decreased by 11.91% and at a radiation dose of 4 KGy leucine increased by 2.98% whereas phenylalanine decreased by 9.79%.

According to Sabahat et al. (2013)[(120)], there was no change in the amino acid composition of the irradiated samples when compared to the control samples (non-irradiated). The radiation exposure resulted in a significant ($p<0.05$) increase in leucine at 4 kGy. Other essential amino acids were non-significantly affected with radiation. The increase in leucine amino acid is in agreement with our results. Similar results have been reported in the literature of WHO, (1994).[(125)]

CONCLUSION

Aflatoxin B_1 is not only a big problem at crops production level, but also it has become a global health issue because of the consequences that the consumption of this toxin generates in animals and human beings. According to our research results all tested samples contain aflatoxin B_1 which exceeds the legal limit by several folds. Efforts to reduce aflatoxin exposure require the commitment of sufficient resources and the collaboration between the agriculture and public health communities as well as local regional, national, and international governments. It is also important to increase disease surveillance, food monitoring, laboratory detection of mycotoxins and public health response capacity in Egypt in order to reduce cancers and decrease the mortality rates in human and animals.

From our research results we conclude that:

1. Maize samples contain the highest level of aflatoxin B_1 (mean level of 50.38 ppb) than wheat (mean level is 37.61%) and rice (mean level 27.4%).

2. Gamma irradiation is a suitable technique that reduces the levels of aflatoxin B_1 in cereal samples without affecting the nutritive values. At **4 KGy** the reduction percents of aflatoxin B_1 were 15.54%, 22.25%, and 27.46% for maize, wheat, and rice, respectively whereas at **6 KGy** the reduction percents of aflatoxin B_1 were 32.39%, 43.84%, and 56.38% for maize, wheat, and rice, respectively and **8 KGy** radiation dose removed 60.26% of the toxin in maize, 64.68% in rice and 69.29% in wheat samples so, we require higher radiation doses to remove the toxin until it reaches the legal limit (5ppb), and this requires more research .

3. By increasing the radiation doses the percents of reduction of aflatoxin B_1 increase.

4. The percents of reduction decrease with increasing the oil content of cereals so maize had the lowest aflatoxin B_1 reduction level after exposure to different gamma irradiation doses.

5. Gamma irradiation affects on the fatty acid profile of cereal grains, **8 KGy** causes reduction in mono-unsaturated fatty acid (oleic acid) by 47.32% in maize, 60.45% in wheat and 41.15% in rice and poly-unsaturated fatty acid (linolenic acid) by 83.33% in wheat and below detection limit in rice, **6 KGy** causes reduction in oleic acid by 35.94%, 46.70%, and 29.64%, for maize, wheat and rice, respectively and for linolenic acid 65.27 and 75.32% for wheat and rice respectively and **4 KGy** causes reduction in oleic acid by 26.94%, 33.10%, and 18.61% for maize, wheat and rice, respectively and for leinolenic acid 36.83% and 51.95% for wheat and rice respectively.

6. Gamma irradiation affects slightly on amino acid profile of cereal grains; 8 KGy causes **leucine** increase by 8.95% in maize, 6. 82% in wheat, and in rice 6.36%, while **phenylalanine** decrease by 13.4% in maize, 5.54% in wheat and 11.9% in rice, 6 KGy causes **leucine** increase by 12.54%, 6.97%, and 6.83% for maize, wheat, and rice, respectively and **phenyl alanine** decreased by 8.60%, 5.25%, and 11.91% for maize, wheat, and rice respectively, and 4 KGy causes **leucine** increase by 8.58%, 2.84%, and 2.98% for maize, wheat, and rice respectively, and **phenyl alanine** decrease by 5.41%, 2.99%, 9.76% for maize, wheat, and rice, respectively.