Incidence and exposure assessment of aflatoxins and ochratoxin A in Egyptian wheat

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ABSTRACT

Aflatoxins and ochratoxin A occur frequently in grains and are associated with carcinogenic, and nephrotoxic properties. Therefore, the aim of this study was to determine the level of aflatoxins and ochratoxin A contamination in wheat samples obtained from different Egyptian governorates and to assess the effect of gamma irradiation on AFB1 in spiked wheat samples, as well as to evaluate the estimated daily intake and hazard index. Thirty-six wheat grain samples purchased from different sale points were analyzed by High-Performance Liquid Chromatography. Data revealed that 33.33 % of the wheat grain samples were contaminated by aflatoxin B1, whereas only 16.66 % of the wheat samples were above the maximum limits (2 μg/kg) set by the European Commission. Ochratoxin A was only detected in two wheat grain samples, and the results were considered below the maximum limit (5 μg/kg) set by the European Commission. On studying the effect of gamma irradiation on wheat samples spiked by aflatoxin B1 (20 μg/kg), results revealed that aflatoxin B1 was reduced to 1.22 and 0.94 μg/kg for samples gamma-irradiated at a dose of 10 and 20 KGY respectively. Estimated daily intake of ochratoxin A in wheat samples was found to be higher than that of the tolerable daily intake; however, hazard index values were below one, thus demonstrating no threat to human health.

1. Introduction

Wheat (Triticum sp.), is one of the most important food grains and cereal sources worldwide and is chiefly used for the manufacture of flour, bread, biscuits, cookies, noodles, and many other products [1]. Wheat is the only grain capable of producing strong and cohesive dough, which are qualities essential for a wide range of food products [2]. In Egypt, consumption per capita of bread products ranks among the highest in the world. Unfortunately, wheat grains are prone to fungal infection by many toxigenic fungal species that produce mycotoxins [3]. Mycotoxins that contaminate wheat grains may include aflatoxins (AFs), ochratoxin A (OTA), and Fusarium toxins [4].

Mycotoxins are considered a significant issue in the situation of food safety, due to their acute and chronic toxic effects on animals and humans [5]. Thus, most countries established and enforced maximum regulatory limits for mycotoxins in response to food safety threats [6]. Prolonged exposure to mycotoxins, mainly in low-income countries, even in low quantities, may cause immune disorders as well as liver damage and cancer [7].

Aflatoxins are mainly produced by Aspergillus flavidus and Aspergillus parasiticus [8]. Among the known AFs, aflatoxins B1 (AFB1) is considered as the most potent one that is associated with both toxicity and carcinogenicity in humans and animals [9] and has been classified as a Group 1 human carcinogen by the International Agency for Research on Cancer [10]. On the other hand, ochratoxin A is produced by Penicillium and Aspergillus species [11], and possesses nephrotoxic, teratogenic, hepatotoxic, and immunosuppressive properties and is classified as a class 2B human carcinogen by the International Agency for Research on Cancer [10,12].

As human exposure to these mycotoxins occurs primarily through the consumption of wheat grain and their products, thus, it is important to emphasize on the procedures that may decrease these mycotoxins. An effective physical method for food preservation is the treatment with ionizing radiation [13], which is becoming more frequently used in the sterilization of a wide variety of foods. Gamma irradiation is established on the capability of high-energy photons generated by radiation of 60Co to prompt chemical changes of the target molecule by causing its degradation or remodeling [14]. In a study on naturally contaminated maize samples, Markov et al. [15] reported that gamma irradiation at a 10 KGY-dose reduced AFB1 by 94.5 %. Meanwhile, Patil et al. [16] indicated that AFB1 was reduced by 20–43% in artificially spiked peanuts using gamma irradiation (5–9 KGY). On the other hand, other studies showed conflicting results depending on the food matrix and the experimental setup [17,18]. Regardless of much public dispute...
on the safety of irradiated foods, ionization radiation is becoming more frequently used in the sterilization of a wide variety of foods [19,20]. Studies examining the impact of gamma irradiation on the nutritional quality of food concluded that gamma irradiation has no effects on the nutritional quality of food and has no adverse effect on human and animal health [21,22].

Studies considering food contamination and dietary exposure to mycotoxins are predominantly rare in the Middle East [23]. Therefore, the aim of this study was to determine the level of AFs and OTA contamination in wheat samples obtained from different Egyptian governorates and to assess the effect of gamma radiation on AFB1 in spiked wheat samples, as well as to estimate the possible threat of these mycotoxins on public health according to estimated daily intake and hazard index.

2. Materials and methods

2.1. Reagents

AFB1 and OTA standards were purchased from Sigma-Aldrich Chemical Company (Saint Louis, MO 63103, USA). Methanol and acetonitrile HPLC grade were purchased from Merck (Darmstadt, Germany). Filter papers (Whatman No. 4) were purchased from Whatman (Cytiva, Marlborough, MA 01752, USA). Pure water was obtained from a Milli-Q system (Millipore, Billerica, MA 01821, USA).

2.2. Preparation of standard solution

Aflatoxin B1 was dissolved in acetonitrile at a concentration of 1 mg/mL, whereas OTA was dissolved in methanol at a concentration of 1 mg/mL. Appropriate dilutions of the stock solutions were made for the preparation of a standard curve, and spiking of wheat samples. All the solutions were stored at −20°C in amber glass vials in the dark and were brought to room temperature before use. Based on the average of three experiments, the LOD and LOQ values were determined according to the following equations for the calibration method.

\[
\text{LOD} = 3 \times (\sigma/s)
\]

\[
\text{LOQ} = 10 \times (\sigma/s)
\]

Where \(\sigma\) is the standard deviation of the response or standard deviation of y-intercepts; \(s\) is the slope of the calibration curve.

2.3. Sample collection

A total of 36 wheat samples were purchased from different retailers in four Egyptian governorates (Alexandria, Cairo, El-Fayoum, and Giza) during November and December 2016. Sampling of wheat was done according to the guidelines provided by the European Commission and the International Organization for Standardization [24,25]. Wheat samples were stored under refrigeration conditions (< 10°C) in polythene bags until analysis [26].

2.4. Aflatoxin analysis

Ground wheat samples (50 g) were mixed with NaCl (10 g) and methanol: water (80:20) (200 mL) in a blender jar and blended at high speed for 1 min. The extract was passed through a fluted filter paper. The filtered extract (10 mL) was diluted with purified water (20 mL) and mixed well. The diluted extract was filtered through a glass microfilter. The diluted filtered extract (2.0 mL) was completely passed through the immunoaffinity column (AflaTest, Vicam, Milford, MA 01757, USA) at a rate of about 1 drop/second. The column was washed with purified water (1.0 mL) at a rate of 1–2 drops/seconds twice. The eluate was eluted using HPLC grade methanol (1.5 mL) and then analyzed by HPLC.

2.5. Ochratoxin A analysis

Ground wheat samples (50 g) were mixed with acetonitrile: water (60:40) (100 mL) in a blender jar and blended at high speed for 1 min. The extract was passed through a fluted filter paper. The filtered extract (10 mL) was diluted with Phosphate Buffer Saline (PBS) (40 mL) and mixed well. The diluted extract was filtered through a glass microfiber filter. The diluted filtered extract (10 mL) was completely passed through the immunoaffinity column (OchraTes, Vicam, Milford, MA 01757, USA) at a rate of about 1 drop/second. After which PBS (10 mL) was passed at a rate of 1–2 drops/seconds, then purified water (10 mL) was passed at a rate of 1–2 drops/seconds. The eluate was eluted using HPLC grade methanol (1.5 mL), then purified water (1.5 mL) was added, vortexed, and analyzed by HPLC.

2.6. Effect of gamma irradiation on aflatoxin B1

Gamma irradiation was carried out in the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt. Wheat samples (50 g) free from AFB1 were ground, spiked with AFB1 at a concentration of 2, 10, and 20 μg/kg, packaged in polyethylene bags and gamma-irradiated in the dose of 5, 10, and 20 KGY at room temperature (25°C). The irradiation was performed using a cobalt-60 γ-source irradiator with a dose rate of ~ 2.77 KGY/h. After the gamma irradiation of spiked wheat samples, AFB1 was extracted as mentioned previously, and analyzed by HPLC.

2.7. HPLC system

HPLC system used was an Agilent 1200 series system (Agilent, Berks, UK) with a fluorescence detector (FLD G1321A), an autosampler (ALS G1329A), FC/ALS therm (G1330B), Degasser (G1379B), Bin Bump (G1312A) and a C18 (Phenomenex, Luna 5 μm, 150 × 4.6 mm) column joined to a pre-column (security guard, 4 × 3-mm cartridge, Phenomenex Luna). The mobile phase for AFB1 determination was water: acetonitrile: methanol (3:1:1, v/v/v). The mobile phase for OTA determination was acetonitrile: water: acetic acid (99:99:2, v/v/v).

2.8. Estimation of daily intake and hazard index

The estimated daily intake (EDI) of AFs and OTA were calculated as follows:

\[
\text{EDI} = \frac{C \times ADC}{BW}
\]

Where C is the concentration of mycotoxins in wheat (μg/kg); ADC is the average daily consumption of wheat (g/person/day), and BW is the body weight (kg). Based on WHO, [27], adults in the Middle East have an average daily consumption of 327.3 g/person/day for wheat. The bodyweight of an average adult was set to 70 kg.

The Hazard Index (HI) of AFB1 and OTA were calculated as follows:

\[
\text{HI} = \sum_{i=0}^{n} \left( \frac{\text{EDI}_i}{\text{TD}_{50}} \right) \times 50,000
\]

EDI was divided by TD_{50}, which was then divided by a safety factor of 50,000. TD_{50} is the dose (μg/kg/body weight/day) required to induce tumors in half of the tested animals that would have remained tumor-free at a zero dose as described by Taghizadeh et al. [28], Echodu et al. [29], and Kortei et al. [30].

2.9. Statistical analysis

All values were expressed as mean (95 % CI). Statistical analysis of the data was carried out using Microsoft Excel 2010 statistical program. A one-way analysis of variance (ANOVA) was performed, in which
A total of 36 samples of wheat grains were analyzed for AFs and OTA using HPLC. The various wheat grain samples were collected from four governorates (samples were collected from three districts from each governorate). Wheat grain samples analyzed were contaminated with variable concentrations of AFs, whereas, OTA was the least detected. Data in Table 1 revealed that AFB1 was not detected in any of the samples were contaminated by AFB1, whereas only 16.66 % of the wheat samples were above 30 μg/kg.

Similar observations were reported by Toteja et al. [33] who noticed that 16 % of the wheat samples were contaminated by AFB1 at a concentration of 30 μg/kg. On the other hand, in Lebanon, 71.8 % of the wheat samples were contaminated by AFB1, among which 29.4 % exceeded the European Commission [34]. In a study on stored wheat, AFB1 was detected in four wheat samples at levels above the European Commission regulatory limit of 2 ppb in grains for human consumption [35]. In a recent study, Shi et al. [36] found that wheat samples grown under low heat unit climate conditions were not contaminated by AFB1.

AFB2 was detected in 75 % of the wheat grain samples, whereas wheat grain samples obtained from the following districts; Al-Manshieh (Alexandria), Al-Maadi (Cairo), and Etsa (El-Fayoum) were highly contaminated by AFG1. Atfih districts respectively (Table 1). These concentrations were considered below the AFB1 maximum limits (2 μg/kg) set by both the European Commission [31] and the Egyptian Organization for Standardization and Quality Control [32]. Meanwhile, in Giza governorate, AFB1 was detected in high concentrations recording 41.38 and 30.41 μg/kg respectively, followed by both Al-Omraniyah and Atfih districts respectively (Table 1). These concentrations were considered below the AFB1 maximum limits (2 μg/kg) set by both the European Commission [31] and the Egyptian Organization for Standardization and Quality Control [32], thus these wheat samples could be considered unfit for human consumption. Results also indicated that 33.33 % of the wheat grain samples were contaminated by AFB1, whereas only 16.66 % of the wheat samples were above 30 μg/kg.

3. Results and discussion

| Governorates | Districts | Mycotoxin concentration (μg/kg) |
|-------------|-----------|---------------------------------|
| Alexandria  | Al-Manshieh | AFB1: < LOD, AFB2: < LOD, AFG1: 16.29, AFG2: < LOD, OTA: < LOD |
|             | Abu-Qir    | AFB1: < LOD, AFB2: 1.47, AFG1: (1.15 – 1.79), AFG2: < LOD, OTA: < LOD |
| Cairo       | Helwan     | AFB1: 0.19, AFB2: 0.09, AFG1: (0.08 – 0.10), AFG2: < LOD, OTA: < LOD |
|             | Al-Maadi   | AFB1: 0.13, AFB2: (0.05 – 0.21), AFG1: < LOD, AFG2: 4.16, OTA: < LOD |
|             | Al-Shuruq  | AFB1: 0.09, AFB2: (0.05 – 0.13), AFG1: 4.10, AFG2: < LOD, OTA: < LOD |
|             | Giza       | Al-Omraniyah: 35.21, AFB1: < LOD, AFB2: 1.94, AFG1: (1.21 – 2.65), AFG2: < LOD, OTA: < LOD |
|             | Dokki      | AFB1: 0.12, AFB2: (0.12 – 0.32), AFG1: 2.44, AFG2: (1.65 – 3.23), OTA: < LOD |
|             | Atfih      | AFB1: 49.79, AFB2: 2.96, AFG1: (37.41 – 62.17), AFG2: (2.10 – 3.82), OTA: < LOD |
|             | LOD        | 0.04, 0.12, 0.30 |
|             | LOQ        | 0.10, 0.40, 0.36 |

Results are expressed as mean (95 % CI). 95 % CI: 95 % Confidence interval of the mean. LOD: Limit of detection; LOQ: Limit of quantification. Statistical analysis was carried out for censored numbers (< LOD) given zero. Within each column, means showed significant difference at P < 0.05 (LSD = 4.97). Within each raw means showed no significant difference at P > 0.05.
AFB1 in toxicity. Data in Table 1 revealed that wheat grain samples obtained from Egypt were highly contaminated, and they all exceeded the maximum limit (4 μg/kg) set by the European Commission [31] and Egyptian Organization for Standardization and Quality Control [32]. This increase in total AFs could be due to the increase in AFG1 concentration.

Aflatoxins represent a public health problem due to their high toxicity, and because they remain partially stable during their industrial procedure [48,49]. On the other hand, during milling of wheat, mycotoxins are reallocated in the bran, thus minimizing mycotoxin concentration in fraction used for human consumption, as bran is only used for animal feed [50,51]. However, in recent years the human consumption of wheat bran has increased [32]. Therefore, mycotoxin concentration in wheat bran intended for human consumption must be reduced. Also, the co-occurrence of both nephrotoxic OTA and genotoxic-carcinogenic AFs in the wheat samples analyzed in this study may further increase the health risks. Therefore, there is a great need to detoxify cereal grains before human consumption to decrease mycotoxin concentration, as well as to decrease the health risks.

3.1. Effect of gamma irradiation

The effects of different radiation doses on wheat samples spiked with different concentrations of AFB1 are shown in Table 2. Data clearly showed that gamma irradiation had an impact on the reduction of AFB1, whereas higher reductions (2.35, 1.93 μg/kg) were recorded for spiked samples by AFB1 (10 μg/kg) radiated at a dose of 10 and 20 KGY respectively. Results also showed that by increasing the concentration of AFB1 in spiked samples, the reduction percentage increased. It was noticed that by increasing the radiation dose the reduction of AFB1 increased. Our results are considered similar to Mohamed et al. [53] who found that gamma radiation at a dose of 8 KGY reduced AFB1 in wheat samples by 69.29 %. In a similar study by Ghanem et al. [54], the authors reported that AFB1 was reduced by 85 and 69 % in unpeeled and peeled pistachio respectively. Recently, gamma irradiation (10KGY-10 min) treatments on AFB1 in spiked hazelnut were investigated, whereas AFB1 was reduced by 47 % [55]. These results indicate that there are several factors that influence the success of gamma irradiation; these include the type of mycotoxins, the concentration of mycotoxins, the radiation dose, and the presence of other compounds or matrix components [56].

Uses of irradiation for aflatoxin decontamination have shown promising results as the gamma rays are suitable due to their deep infiltration through food [57]. On the other hand, gamma irradiation at a dose of up to 10 KGY has been reported to have no adverse effect on human and animal health, and have no effects on the nutritional quality of food as reported by the World Health Organization [58].
3.2. Risk assessment of exposure to AFs and OTA via consumption of wheat

3.2.1. Estimated daily intake

The results of the estimated daily intakes of AFs and OTA using the consumption of wheat grains from the concentrations determined during the experiment (Table 3). Data revealed that the EDI for AFB\(_1\) for wheat samples obtained from the Giza district was very high, whereas the mean and the maximum EDI recorded 132.46 and 232.80 μg/kg body weight/day respectively, thus indicating that wheat samples obtained from Giza governorate are of public health concern. In a previous study in Egypt, Hussain et al. [59] indicated that many of the cereal grains including wheat obtained from Giza governorate were highly contaminated by fungi. Therefore, our results could be due to climate conditions which are one of the most important factors that have a great effect on fungal growth as well as mycotoxin production. On the other hand, Giza governorate was considered one of the highest governorates in temperature averages [60].

Meanwhile, EDI for wheat samples obtained from Cairo governorate recorded 0.49 and 0.88 μg/kg body weight/day respectively for mean and maximum samples and thus was considered low. Although these low concentration might cause a risk to human health, as the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and Scientific Committee on Food (SCF) reports [61,62], revealed that a very low exposure level to aflatoxins at a concentration of 1 ng/kg body weight/day may induce liver cancer, and the “As Low as Reasonably Achievable” approach is recommended [63].

Results also revealed that the EDI for OTA in wheat samples obtained from Cairo governorate were considered high, whereas the mean and the maximum EDI recorded 2.14 and 5.41 μg/kg body weight/day respectively. Meanwhile, EDI for wheat samples obtained from El-Fayoum governorate was also high, although considered lower than those recorded for wheat samples obtained from Cairo governorate. These results were considered higher than the Tolerable Daily Intake (TDI) for OTA (0.005 μg/kg body weight/day) thus indicating the risk to public health. The risks of the increase in EDI for OTA to consumers have been evaluated by the Scientific Committee on Food [64], the Joint FAO/WHO Expert Committee on Food Additives [65–67] as well as the European Food Safety Authority [68].

3.2.2. Hazard index

Results in Table 4 indicated that the recorded hazard index values ranged from \(5.63 \times 10^{-7}\) to \(1.46 \times 10^{-3}\) and from \(9.73 \times 10^{-8}\) to \(1.35 \times 10^{-4}\) for AFB\(_1\) and OTA respectively.
1.46 × 10⁻⁶ for AFB₁ and OTA respectively. Data also clearly showed wheat samples were contaminated with AFB₁, whereas two samples to toxins and ochratoxin A in wheat. About thirty-three percent of the samples obtained from Egypt, thus indicating that consumers are at high risk due to the exposure to AFB₁ and OTA. However, the hazard index values recorded less than one and thus consumers were at no risk.

4. Conclusions

The present study, carried out in Egypt, provided additional information related to the occurrence and exposure assessment of aflatoxins and ochratoxin A in wheat. About thirty-three percent of the wheat samples were contaminated with AFB₁, whereas two samples exceeded the European Commission’s maximum level for AFB₁. Meanwhile, about sixteen percent of the wheat samples were contaminated with OTA, whereas no samples exceeded the European Commission’s maximum level for OTA. Gamma irradiation had an impact on the reduction of AFB₁, whereas higher reductions (2.35, 1.93 µg/kg) were recorded for spiked samples (10 µg/kg) irradiated at a dose of 10 and 20 KGY respectively. The estimated daily intake for both mycotoxins was found to be higher than that of the tolerable daily intake, however, the hazard index values were less than one and therefore the consumption of wheat was found to pose no health risk to consumers.

Authors statement

Ahmed Fouzy: managed and coordinated the responsibility for the research activity, and provided resources for the study; Amal Hathout, Shaaban Abdel-Fattah, and Yehia Abou-Sree: developed and designed the methodology, conducted the research and investigation process, and wrote the original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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