



Original Article

## Carcinogenic Mycotoxins in Common Foods: ELISA-Based Measurement of Aflatoxins and Ochratoxins

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### ABSTRACT

Mycotoxins are secondary metabolites produced by certain species of fungi that pose significant risks to human health. Aflatoxins and ochratoxins are among the most important mycotoxin analytes in food and are classified as carcinogenic. Their presence highlights the urgent need for stronger food safety surveillance systems in Iraq and underscores the elevated risks of foodborne diseases in Middle Eastern countries. This study aimed to assess the contamination with aflatoxin and ochratoxin in eleven different types of foods, including chips, cookies, spices, nuts, tea, coffee, cereals, starch, spaghetti, seeds and dried fruits, randomly collected from markets and traditional bazaars in Sulaimani City, Iraq, using the Enzyme-Linked Immunosorbent Assay (ELISA) method. The results revealed that while most levels were within acceptable limits, some samples had concerningly high levels. Specifically, six out of eighty-eight samples tested positive for aflatoxins, and fifteen out of eighty-six for ochratoxin, with some exceeding the European Commission's regulatory limits. The results showed that cinnamon, tea and coffee were highly contaminated with aflatoxin at concentrations ranging from 37.188 to 146.961 µg/Kg. Some samples of chips, spices, walnuts, peanuts, tea, coffee, cereals, and dried food were highly contaminated with ochratoxin at concentrations ranging from 11.099 to 202.25 µg/Kg. These findings present immediate public health concerns for Sulaimani's population, as the contaminated products are among the commonly consumed foods that could contribute to chronic mycotoxin exposure. It also highlights the urgent need for preventive measures to reduce the health risks associated with these carcinogenic substances in the local food supply chain.



### 1. Introduction

Mycotoxins are secondary metabolites of microscopic fungi that have toxic effects on humans, animals, plants, and microorganisms. The metabolism of some fungi, mainly belonging to

*Aspergillus*, *Penicillium* and *Fusarium* spp. Major mycotoxins that have been associated with poisoning in humans include aflatoxin, ochratoxin, cyclopiazonic acid, citreoviridin, fumonisin, 3-nitropropionic acid, certain trichothecenes, and zearalenone (Peraica & Domijan, 2001; Baydar et al.,

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2005; Kollia et al., 2014; Awuchi et al., 2022; Mafe & Dietrich, 2024).

Mycotoxins, such as aflatoxins and ochratoxins, are classes of common carcinogenic mycotoxins. Aflatoxin and ochratoxin contamination naturally occur in many foods, including cereals, tree nuts, oilseeds, spices, tea, coffee, cocoa, fruits, milk and animal products, and their contamination arises during pre-harvest, harvest, processing of food, or storage stages due to improper environmental and storage conditions (Mannaa & Kim, 2017; Li et al., 2021; Gurikar et al., 2023; Balan et al., 2024). Human exposure occurs directly through the consumption or inhalation of contaminated food or indirectly via animal products containing aflatoxin or ochratoxin (Kumar et al., 2017; Li et al., 2021; Gurikar et al., 2023).

Aflatoxins are among the highly toxic mycotoxins produced mainly by *Aspergillus* spp., in particular *A. flavus* and *A. parasiticus*. The four most important aflatoxins (AF) are aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2) (Okechukwu et al., 2024). Aflatoxin B1 is the most potent mycotoxin (AFB1 > AFG1 > AFB2 > AFG2) and also the most lethal naturally occurring hepatocyte carcinogen (Chandra, 2021). Due to its serious effects, the International Agency for Research on Cancer (IARC) classified AFB1 as a Group I carcinogen for humans (Kumar et al., 2017). Aflatoxin B1 was first discovered in England in the 1960s following the outbreak of the poultry disease Turkey X due to contamination of peanut meal feed with *A. flavus* (Hamilton, 1971). Subsequently, other outbreaks of aflatoxin contamination were documented worldwide, particularly in developing countries, leading to acute illnesses and deaths in animals and humans (Lewis et al., 2005; Reddy & Raghavender, 2007).

After entering the body, the aflatoxins are absorbed into the bloodstream via the mucous membranes. They are distributed in various tissues, and the liver. Aflatoxins (especially AFB1) are mainly metabolized by the cytochrome P450 of the liver to

highly reactive aflatoxin-8,9-epoxide or hydroxylated to less harmful aflatoxins, including aflatoxin M1 and aflatoxin Q1 (Bbosa et al., 2013). Aflatoxin 8,9-epoxide binds to proteins, causing acute toxicity (acute aflatoxicosis). The symptoms of acute aflatoxicosis are liver failure, jaundice, nausea, vomiting, coma, and possibly death (at high doses). In addition, prolonged exposure to AFB1 has been found to cause chronic aflatoxicosis and serious complications such as hepatocellular carcinoma, neurotoxicity, respiratory toxicity, cardiovascular toxicity, reproductive toxicity, endocrine toxicity, renal toxicity, immunotoxicity and growth retardation (Lizárraga-Paulín et al., 2011; Bbosa et al., 2013; Kumar et al., 2017; Omotayo et al., 2019; Balan et al., 2024).

After aflatoxin, ochratoxin is the most toxic and most widespread mycotoxin in the world (Ringot et al., 2006). It was first discovered in 1965 by van der Merwe and colleagues in South Africa and isolated from a culture of *Aspergillus ochraceus* (Van der Merwe et al., 1965; Altafini et al., 2021; Banahene et al., 2024). Various fungi of the genera *Aspergillus* and *Penicillium* mainly produce ochratoxin. Among ochratoxin subtypes, ochratoxin A (OTA) is the most toxic, followed by analogues such as ochratoxin B (OTB) and ochratoxin C (OTC). The International Agency for Research on Cancer (IARC) has classified it as a Group 2B carcinogen for humans (Altafini et al., 2021; Banahene et al., 2024). After ingestion, OTA is absorbed into the bloodstream via the digestive system, where it binds to albumin. It is distributed to various tissues, such as the kidney and liver, and can be stored in skeletal muscle, adipocytes, and the brain. OTA can be metabolized by both phase I and phase II enzymes. In phase I, OTA and hydroxylated metabolites are formed by cytochrome P450 enzymes. While in phase II, it can be conjugated mainly in the liver with glutathione or glucuronic acid. Although their excretion is slow and inefficient, ochratoxins can be excreted via urine and faeces (Kószegi & Poór, 2016). To date, several toxic effects of ochratoxin, especially OTA, have been identified, namely nephrotoxicity, hepatotoxicity, genotoxicity,

immunotoxicity and neurotoxicity (Altafini et al., 2021; Banahene et al., 2024).

Despite the well-documented global prevalence of mycotoxin contamination in food products, significant knowledge gaps exist regarding mycotoxin contamination in the Arab world, particularly in Iraq's Kurdistan Region. Middle Eastern countries, including Iraq, face heightened risks of foodborne illness due to limited disease surveillance and prevention systems, yet systematic surveillance data for mycotoxin contamination in retail food products remains scarce. While previous research has documented mycotoxin contamination in poultry feed systems in Sulaimani Governorate, a comprehensive assessment of mycotoxin levels in commonly consumed human food products from local markets has not been conducted. (Abdolmaleki et al., 2021; Jallow et al., 2021; Shavakhi et al., 2023). The selection of Sulaimani City also addresses a critical geographic research gap, as most mycotoxin surveillance studies in the Middle East have focused on other regions, leaving the Kurdistan Region of Iraq underrepresented in the scientific literature. This study, therefore, provides essential baseline data that can inform local food safety policies and contribute to the broader understanding of mycotoxin contamination patterns in this understudied region.

## 2. Material and Methods

### 2.1 Study area and the sample collection

Sulaimani City is strategically located in the northeastern Kurdistan Region of Iraq. The city experiences a climate characterized by sweltering, arid summers with temperatures reaching up to 38.8°C and very cold winters with temperatures dropping to -0.2°C, with average relative humidity about 65.7% (Hassan, 2025). The region's high temperature and humidity levels significantly enhance fungal growth rates and mycotoxin production in food products (Gutiérrez Pozo et al., 2024).

Food samples were collected from different supermarkets and food vendors in Sulaimani City, Iraq. A total of one hundred seventy-four food samples, including chips, cookies, spices, nuts, tea, coffee, cereals, starch, spaghetti, seeds and dried fruits. Samples were collected using two different methods. For bulk samples, the items were thoroughly mixed, and a 25 g sample was randomly collected and stored in a sterile container, but for the packaged samples, one package was purchased from each sample. Then the samples have been stored at a temperature of -20°C until the time of analysis. Eighty-eight samples were analysed for aflatoxin, and eighty-six samples were analyzed for ochratoxin.

### 2.2 Sample preparation and extraction

The samples to be analyzed were ground into fine particles (the size of fine instant coffee) so that at least 75 % of them fell through a 20-mesh sieve. The samples were stored in a refrigerator until analysis. For preparing the sample to detect aflatoxin, the solution of 5 g of ground sample in 25 mL of 70% methanol was shaken vigorously for three minutes. The sample for ochratoxin detection was done by mixing 10 g of ground sample in 40 mL of 50% methanol, shaken vigorously for five minutes. The extracts were filtered through Cytiva Whatman™ filter paper with a fineness of 5 µm, and the filtrates were collected as samples for assay analysis (Horv'ath et al., 2022; Sirhan et al., 2023).

### 2.3 Test procedure

The Veratox quantitative assay kit (Neogen, Lansing, MI, USA) is used for aflatoxin and ochratoxin detection in the following foods: Chips, cookies, spices, nuts, tea, coffee, cereals, starch, spaghetti, sunflower seeds, and dried fruits. All reagents were warmed to room temperature (18-30°C) for two hours before use. A red-marked mixing well was taken for each sample to be tested, as well as 4 red-marked wells for the controls. They were placed in the well holder. The same number of antibody-coated wells was used for the assay. One end of the strip was marked "1 and 2", and the strips

were placed in the well holder with the marked end to the left. Each reagent was mixed by swirling before use. 100  $\mu\text{L}$  of the conjugate from the blue-marked bottle was added to each red-marked mixing well. Using a new pipette tip, 100  $\mu\text{L}$  of each of the controls and samples was added to the red-labelled mixing wells (Batrinou et al., 2020; Sirhan et al., 2023). Each plate contained a negative control sample to check for non-specific binding to validate the overall assay results. The positive controls were included to ensure that the samples contained known concentrations of target proteins from which the standard curve was obtained. The blank sample control was provided as a comparison mechanism for assurance that the assay continues and to provide accurate results.

Using a 12-channel pipette, the liquid was mixed in the wells by pipetting up and down three times. 100  $\mu\text{L}$  was transferred to the antibody-coated wells. The mixing wells marked in red were discarded. The timer was set to 2 minutes for aflatoxin and 10 minutes for ochratoxin. The wells were mixed during the first 10-20 s at room temperature by sliding the microplate holder back and forth on a flat surface. Care was taken to ensure that the reagents did not splash out of the wells. The contents of the antibody well were shaken out, and the wells were filled with distilled water and emptied. This step was repeated five times. The wells were turned upside down and tapped out on a paper towel until the remaining water was removed. Some volume of the substrate from the green-marked bottle was poured into the green-marked reagent boat. Using the new tips of the 12-channel pipette, 100  $\mu\text{L}$  of substrate was pipetted into the wells. The timer was set for 3 minutes for aflatoxin and 10 minutes for ochratoxin, and the wells were mixed by sliding them back and forth on a flat surface for the first 10-20 seconds. The remaining substrate was discarded, and the reagent boat was rinsed with water. Red stop solution from the red-marked bottle was poured into the red-marked reagent boat. The excess substrate was ejected from the 12-channel pipette, the tips were filled, and 100  $\mu\text{L}$  of red stop solution was pipetted

and dropped into each well. It was mixed by sliding back and forth on a flat surface. The bottom of the microwells was wiped with a dry cloth and read in a microwell reader with a 650-nm filter. Air bubbles were eliminated because they could interfere with the analysis results. Results were read within 20 minutes after the addition of a red stop.

#### 2.4 Data Collection and Measurements

Statistical analysis was performed using SPSS 22.0 software was used to analyze the data, which were presented as mean (M) with standard deviation (SD).

### 3. Results

In this study, eleven different types of consumed foods were analyzed using an enzymatic immunosorbent assay to detect aflatoxin and ochratoxin contamination. Tables 1 and 2 show the aflatoxin and ochratoxin contamination levels in the food samples analyzed. All samples were contaminated with mycotoxins, except for the chip samples, which did not contain aflatoxins, and the spaghetti samples, which did not contain ochratoxins.

The most contaminated samples were spice (cinnamon), tea, and coffee, which were contaminated with aflatoxin, while the chips, spices, walnut, peanut, tea, coffee, chick pea, and dried food samples contained ochratoxin above the acceptable limits. Of the three coffee samples, two had aflatoxin levels greater than 10  $\mu\text{g}/\text{kg}$ ; the other sample contained aflatoxin at about 4.361  $\mu\text{g}/\text{kg}$ . Some samples of cookies, spices, nuts, cereals, and starch had aflatoxin levels between 0.1 and 10  $\mu\text{g}/\text{kg}$ . While one sample of each type of spaghetti, seeds, and dried food was contaminated with aflatoxin levels between 0.1 and 10  $\mu\text{g}/\text{kg}$ . Thus, most of the food samples tested in this study had aflatoxin levels below 10  $\mu\text{g}/\text{kg}$ , which is within the acceptable limits (above 10  $\mu\text{g}/\text{kg}$ ). According to the results, aflatoxin levels in tea, coffee, and spice (cinnamon) were higher (above 10  $\mu\text{g}/\text{kg}$ ), i.e., significantly above the acceptable limits. In the European Union, maximum

permitted levels of mycotoxins in foodstuffs are laid down under Commission Regulation (EC) No 1881/2006 and Commission Regulation (EC) No 1152/2009 (European Commission, 2006; European Commission, 2009).

Some samples of chips, cookies, spices, nuts, cereals, and seeds tested had ochratoxin concentrations ranging from 0.1 to 10 µg/kg.

Two samples of each type of chips, spices, nuts, and tea had ochratoxin concentration values ranging from 10.1 to 29.9 µg/kg. One sample of each type of spices, nuts and tea had an ochratoxin level between

30-49.9 µg/kg. Ochratoxin levels in some samples of the spices, tea, cereals, and dried fruits were above 50 µg/kg. According to the results, ochratoxin levels in chips, spices, walnut, peanut, tea, coffee, cereals, and dried food were higher (above 10 µg/kg), i.e., significantly above the acceptable limits (Commission Regulation (EU) No 420/2011 of 29 April 2011 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs Text with EEA relevance (European Commission, 2006; European Commission, 2009).

**Table 1.** Representation of the range of aflatoxin contamination in the tested food samples in the concentration values µg/Kg or parts per billion.

Food Samples	Number of tested samples	Aflatoxin content (total µg/kg)					Min.	Max.	Mean	SD
		ND or<0.1	0.1-10	10.1-29.9	30-49.9	>50				
Chips	14	14	0	0	0	0	0.000	0.000	0.000	0.000
Cookies	10	7	3	0	0	0	0.112	0.278	0.060	0.102
Spices	6	2	1	2*	0	1*	1.155	84.91	17.96	33.28
Nut	14	12	2	0	0	0	0.007	0.496	0.055	0.144
Tea	4	0	0	0	1*	3*	37.18	69.979	54.584	13.70
Coffee	3	0	1	1*	0	1*	4.361	146.6	53.78	80.78
Cereals	25	21	4	0	0	0	0.004	1.662	0.094	0.334
Starch	5	3	2	0	0	0	0.170	1.767	0.388	0.774
Spaghetti	4	3	1	0	0	0	0.000	0.177	0.044	0.088
Seeds	2	1	1	0	0	0	0.069	2.618	1.343	1.802
Dried fruit	1	0	1	0	0	0	6.445	6.445	6.445	0.000

\* Exceeding the maximum limit of the Commission of the European Communities. ND, Not detectable; min., minimum; max., maximum; SD, standard deviation.

#### 4. Discussion

A human will be exposed to mycotoxin through food. The detection of mycotoxin contaminants and health risks in commonly consumed foods and feed is essential to improve human health. Monitoring mycotoxins depends on accurate and reliable analytical methods. However, there is no universal method for detecting and quantifying mycotoxins in feed or food because mycotoxins vary widely chemically, as do the matrices in which they are found (Rahmani et al., 2009; Turner et al., 2009).

Immunological methods are preferable because they can reduce the time and cost of analyzing these naturally occurring toxins. In addition, the ELISA technique is very sensitive and specific and can be automated for the analysis of numerous samples (Abouzied et al., 1991; Peraica & Domijan, 2001; Okuma et al., 2018). In the present study, the total content of aflatoxins and ochratoxins in the samples was determined by ELISA to obtain a rapid overview of the content of mycotoxins.

**Table 2.** Representation of the range of ochratoxin contamination in the tested food samples in the concentration values µg/Kg or parts per billion.

Food Samples	Number of tested samples	Ochratoxin content (total µg/kg)					Min.	Max.	Mean	SD
		ND or<0.1	0.1-10	10.1-29.9	30-49.9	>50				
Chips	10	3	5	2*	0	0	0.026	21.554	4.751	7.144
Cookies	9	5	4	0	0	0	0.074	3.89	0.689	1.259
Spices	13	1	8	2*	1*	1*	1.054	202.25	23.34	54.980
Nut	13	2	8	2*	1*	0	0.05	40.05	7.168	12.870
Tea	4	0	0	2*	1*	1*	18.1	66.694	36.465	21.745
Coffee	2	0	2	0	0	0	0.406	2.235	1.320	1.293
Cereals	24	13	10	0	0	1*	0.117	78.40	4.587	15.890
Starch	4	3	1	0	0	0	0.00	9.148	2.287	4.574
Spaghetti	2	2	0	0	0	0	0.00	0.000	0.000	0.000
Seeds	3	1	2	0	0	0	0.674	0.948	0.540	0.487
Dried fruit	2	0	1	0	0	1*	1.95	60.920	31.417	41.722

\* Exceeding the maximum limit of the Commission of the European Communities. ND, Not detectable; min., minimum; max, maximum; SD, standard deviation.

According to European regulations on mycotoxins in food, the limit value for total mycotoxin content ranges from 0.1 to 10 µg/kg (European Commission, 2006; European Commission, 2009). In this study, spices, tea, and coffee had aflatoxin levels above 10 µg/kg; the other samples had levels below the acceptable level. The highest mean value of (54.58 µg/kg) for aflatoxin was found for tea, (53.78 µg/kg) for coffee and (17.96 µg/kg) for spices, while the samples of spaghetti tested had the lowest mean value of (0.04 µg/kg). In contrast, in Pakistan, a study (Lutfullah & Hussain, 2012) reported average levels of 4.6 and 10.4 µg/kg for the samples of cereal samples (rice and maize) analysed for aflatoxins, respectively. This result contradicts findings from multiple research groups who have documented significant aflatoxin contamination in corn and related products (Baydar et al., 2005; Karlovsky et al., 2016). In the present study, the ochratoxin contamination levels in chips, spices, nuts, tea, cereals, and dried food exceeded the acceptable limit. The highest mean value of (23.34 µg/kg) for ochratoxin was found for spices, (36.465 µg/kg) for tea, (31.417 µg/kg) for dried fruit, while

the samples of seeds tested had the lowest mean value of (0.54 µg/kg).

Similar to the current study, aflatoxin contamination in bakery and cookie products has been reported in different regions. For example, an Iranian study focused on the contamination of traditional cookies with AFB1 in 40 cookie samples. Although all samples were contaminated with AFB1 at levels ranging from 1.64 to 3.95 µg/kg, the aflatoxin level did not reach the above acceptable limit (Jafarbeigi et al., 2025). This finding aligns with current research, as 3 out of 10 cookie samples contained aflatoxin, with concentrations ranging from 0.112 to 0.278 µg/kg. Moreover, a study by Pakistani researchers reported that 87.5% of locally baked peanut cookies were contaminated with AFB1 at levels below 20 µg/kg, while 12.5% contained high aflatoxin levels above 20 µg/kg (Mujeeb et al., 2022). Additionally, our analysis revealed that the chip samples were free of aflatoxin contamination, which contrasts with the results of an Iraqi study that isolated the primary producer of aflatoxin (*Aspergillus* spp.) in potato chips (AL-Ameri and Ramadan, 2020). Recently, Evuti and colleagues (2023) also reported the primary producer of

aflatoxin (*Aspergillus* spp.) and aflatoxin contamination as one of the major mycotoxins in potato chips in Nigeria.

In this study, when extending the examination to other samples (cereals, starch, and spaghetti), none of them exceeded the EU regulatory limits for aflatoxin level. Similarly, the study of Akter and colleagues in Bangladesh reported aflatoxin contamination in 73.03% of cereals and cereal products, but all samples had aflatoxin levels below the acceptable limit (10 µg/kg) (Akter et al., 2025). In our investigation, aflatoxin levels in sesame, flax, and fenugreek seeds were all below 10 µg/kg. These sesame results do not agree with those reported by Heshmati et al. (2021) in their study of Iranian sesame samples, tahini, and tahini halva, where AFB1 ranged from 0.21 to 12.35 µg/kg.

In the present, aflatoxin in all nut and dried fruit samples remained below the EU regulatory limit, but 4 out of 15 nut and dried fruit samples reached the highest level of ochratoxin. Similarly, the occurrence of aflatoxin and OTA in 140 samples of nuts and dried fruits collected from Istanbul, Turkey, was investigated. The results showed that 93.6% of the sample was contaminated with aflatoxin, with the highest occurrence in hazelnuts (8.043 µg/kg). For OTA, 10% of the sample were contaminated with OTA levels below EU regulatory limits (Uğur et al., 2023). Moreover, in this study, 3 out of 6 spice samples and 4 out of 13 samples were contaminated with aflatoxin and ochratoxin above the permissible limit of these toxins, respectively. Similarly, Iranian researchers investigated these mycotoxin contaminations in 80 spice samples, and it was reported that 40 spice samples were contaminated with aflatoxin and 48 samples with OTA, with the highest contamination in red pepper exceeding the permitted levels of aflatoxin and OTA (Zareshahrabadi et al., 2020). Furthermore, in Lebanon, the contamination of AFB1 and OTA was reported in spices, with AFB1 (mean 0.97 µg/kg) and OTA (mean 38.8 µg/kg), and low levels of these mycotoxins were detected in nuts (mean 0.4 µg/kg) and herbs (mean 0.3 µg/kg) (Daou et al., 2023). On

the other hand, in Indonesia, a study reported that 92% of spice samples were contaminated with ochratoxin at an average value of 6.18 µg/kg, which is below the EU regulatory limit (Widiyanti & Maryam, 2023).

In the current study, the chip samples were free from aflatoxin contamination; however, ochratoxin was detected in 2 out of 10 samples. This finding is supported by previous research (AL-Ameri & Ramadan, 2020), which similarly reported ochratoxin contamination and isolated the ochratoxin-producing fungi, particularly *Aspergillus* and *Penicillium* spp., in potato chips. The low detectable ochratoxins in cookies, coffee, starch, spaghetti, and seeds were reported in the present study. Previously, in a Turkish study, it was confirmed that despite the occurrence of OTA in 24 breakfast cereals, the levels are very low and well within the permissible level for ochratoxin (Kabak, 2009). In contrast to the current study, a group of Iranian researchers showed that instant and roasted coffees may have potential health risks from OTA because the OTA levels reached around 26.6 µg/kg (Yazdanfar et al., 2022). Another Iranian study evaluated the occurrence of OTA in wheat flour used for popular Iranian breads, and low amounts of OTA were detected (Saber-Hasanabadi et al., 2023). Their findings are consistent with the current results for cookies and spaghetti, which are produced from flour. In Pakistan, it is confirmed that maize grains are vulnerable to OTA contamination, and in this study, 22% of maize samples exceeded the permissible limits, and OTA levels reached up to 53.9 µg/kg (Gillani et al., 2022). In contrast, our results on starch showed very low ochratoxin levels.

Most of the results of previous studies showed a high frequency of mycotoxin contamination in other food products such as sunflower, oil seeds, fruits, vegetables, and corn (Jimenez et al., 1991; Thirumala-Devi et al., 2002; Saha et al., 2007; Ali et al., 2017; Nan et al., 2022). However, in the present study, high frequencies of aflatoxin and ochratoxin were found in tea, coffee and spices. This could be because only a few teas, coffee, and spice samples

were taken for this study. It is possible that the results would have been different if more and different types of tea, coffee and spices had been sampled. Contamination with fungi could occur at different stages of tea and coffee processing due to the warm and humid climate that favors fungal development (Swelim et al., 1994; Turner et al., 2009; Sedova et al., 2018; El-Sayed et al., 2022). The results obtained in this study are also in agreement with Malir and colleagues (Malir et al., 2014), who reported extremely high ochratoxin concentrations in black tea from the Czech Republic. A study in Spain revealed tea contamination with very high levels of aflatoxins, ochratoxin A, and other mycotoxins (Santos et al., 2009). The contamination levels identified in this study present several immediate and long-term public health concerns for Sulaimani's population because large doses of aflatoxins and ochratoxin can lead to acute poisoning and can be life threatening through damaging multiple organs in the human body (Kumar et al., 2017; Kumar et al., 2020).

## 5. Conclusion

This study provides an assessment of mycotoxin contamination in retail food products from Sulaimani City, revealing significant contamination levels in commonly consumed foods that pose immediate public health concerns. The detection of aflatoxins and ochratoxins exceeding European Commission regulatory limits in various food categories necessitates urgent, coordinated action across multiple sectors.

The results of this study should be helpful to food regulatory agencies and launch awareness programs about mycotoxin risks, proper food storage practices, and identification of contaminated products. This study strengthens pre-import testing for mycotoxins in imported food products, especially from regions with known contamination issues. It is also helpful to

establish and enforce proper storage requirements for food retailers, including temperature, humidity, and pest control standards, to prevent post-retail contamination. To this end, the findings of this study will help in the development of comprehensive monitoring systems for mycotoxin contamination in food products, invest in laboratory capacity building for mycotoxin testing, establish regional testing centers, and create rapid detection systems at border checkpoints and major markets.

## Conflict of interest

The authors declare that the research was conducted without a conflict of interest.

## CRedit authorship contribution statement

**Narmeen Ahmad:** conceptualization, funding acquisition, student supervision, methodology, sample selection, data collection, data analysis, data interpretation, fully contributed to drafting, reviewing, and revising the manuscript at all stages. **Ali Arif:** statistical analysis, data interpretation, and conceptualization, fully wrote the manuscript, and fully contributed the revised copies for the reviewers. **Shanya Sadiq:** funding acquisition, study design, sample collection, and data collection. **Eşref Çelik:** student supervision and reviewing the manuscript. **Mohammed Mahmood:** reviewing the manuscript. **Kani Radha, Darya Ahmad, and Jinan Hasan:** sample preparation and data collection. All authors approved the final version of the manuscript.

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## References

Abdolmaleki, K., Khedri, S., Alizadeh, L., Javanmardi, F., Oliveira, C. A., Khaneghah, A. M., (2021): The

mycotoxins in edible oils: An overview of prevalence, concentration, toxicity, detection and decontamination techniques. *Trends in Food Science & Technology*, 115, pp. 500–511.

- Abouzied, M., Azcona, J., Braselton, W., and Pestka, J., (1991): Immunochemical assessment of mycotoxins in 1989 grain foods: evidence for deoxynivalenol (vomitoxin) contamination. *Applied and Environmental Microbiology*, 57(3), pp.672–677.
- AL-Ameri, H. A., & Ramadan, N. A., (2020): Isolation and identification of fungi contaminating potato chips intended for children's consumption and assessing their toxins. *Al-Mukhtar Journal of Science*, 35, pp.273-83.
- Ali, M. D., AL-Musawi, M. L., Fouad, F. A., Kalif, A. H., Abdulla, K. G., Kanaan, H. M., and Hassan, Z. A. A., (2017): Comparison of mycotoxin contamination levels of local and imported corn in Iraq. *American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS)*, 28(1), pp.181–186.
- Altafini, A., Roncada, P., Guerrini, A., Minkoumba Sonfack, G., Fedrizzi, G., and Caprai, E., (2021): Occurrence of ochratoxin A in different types of cheese offered for sale in Italy. *Toxins*, 13(8), p.540.
- Akter, T., Shamsi, S., Reza, S., Nowar, A., Al Noman, M. A., Hassan, M. T., and Alim, S. R., (2025): Fungi isolation, identification and detection of aflatoxins in selected cereals and cereal products. *Bioresearch Communications*, 11(1), pp.1633-1642.
- Awuchi, C. G., Ondari, E. N., Nwozo, S., Odongo, G. A., Eseoghene, I. J., Twinomuhwezi, H., Ogbonna, C. U., Upadhyay, A. K., Adeleye, A. O., and Okpala, C. O. R. (2022): Mycotoxins' toxicological mechanisms involving humans, livestock and their associated health concerns: A Review. *Toxins*, 14(3), pp.167.
- Balan, B., Dhaulaniya, A. S., Kumar, M., Kumar, M., and Kumar, P., (2024): Aflatoxins in food: prevalence, health effects, and emerging trends in its mitigation—an updated review. *Food Safety and Health*, 2(1), pp.39–71.
- Banahene, J. C. M., Ofosu, I. W., Odai, B. T., Lutterodt, H. E., Agyemang, P. A., and Ellis, W. O., (2024): Ochratoxin A in food commodities: a review of occurrence, toxicity, and management strategies. *Heliyon*, 10(20), p.e39313.
- Batrinou, A., Houhoula, D., & Papageorgiou, E., (2020): Rapid detection of mycotoxins on foods and beverages with enzyme-linked immunosorbent assay. *Quality Assurance and Safety of Crops & Foods*, 12(1), pp.40–49.
- Baydar, T., Engin, A. B., Girgin, G., Aydin, S., and Sahin, G., (2005): Aflatoxin and ochratoxin in various types of commonly consumed retail ground samples in Ankara, Turkey. *Annals of Agricultural and Environmental Medicine*, 12(2), pp.193–197.
- Bbosa, G. S., Kitya, D., Lubega, A., Ogwal-Okeng, J., Anokbonggo, W. W., and Kyegombe, D. B., (2013): Review of the biological and health effects of aflatoxins on body organs and body systems. *Aflatoxins—Recent Advances and Future Prospects*, 12, pp.239–265.
- Chandra, P., (2021): Aflatoxins: food safety, human health hazards and their prevention. In Lukman, B. A., (Ed.), *Aflatoxins: Occurrence, Detoxification, Determination and Health Risks*. Prentice IntechOpen, London. DOI: 10.5772/intechopen.96647
- Daou, R., Hoteit, M., Bookari, K., Joubrane, K., Khabbaz, L. R., Ismail, A., Maroun, R. G., & Khoury, A. E., (2023): Public health risk associated with the co-occurrence of aflatoxin B<sub>1</sub> and ochratoxin A in spices, herbs, and nuts in Lebanon. *Frontiers in Public Health*, 10, Article 1072727
- El-Sayed, R. A., Jebur, A. B., Kang, W., and El-Demerdash, F. M., (2022): An overview on the major mycotoxins in food products: characteristics, toxicity, and analysis. *Journal of Future Foods*, 2(2), pp.91–102.

- European Commission, (2006): Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union, L 364, pp. 5–24.
- European Commission, (2009): Commission Regulation (EC) No 1152/2009 of 27 November 2009 imposing special conditions governing the import of certain foodstuffs from certain third countries due to contamination risk by aflatoxins and repealing Decision 2006/504/EC. Official Journal of the European Union, L 313, pp.40–49.
- Evuti, M. M., Galadima, M., Makun, H. A., Badmos, F. O., Unekwuajo, A. B., Friday, A., and Hamidu, A., (2023): Health implication of mycotoxigenic fungi and mycotoxins analysed from sun dried yam and potato chips sold in Minna Niger State, Nigeria. *International Journal of Pathogen Research*, 13(1), pp.16-31.
- Gillani, S. W. U. H. S., Sadeq, Y., Imran, M., Raza, H. M. F., Ghani, A., Anwar, S., and Hussain, S. (2022): Determination and detoxification of aflatoxin and ochratoxin in maize from different regions of Pakistan. *Environmental Monitoring and Assessment*, 194(9), p.613.
- Gurikar, C., Shivaprasad, D., Sabillón, L., Gowda, N. N., and Siliveru, K., (2023): Impact of mycotoxins and their metabolites associated with food grains. *Grain & Oil Science and Technology*, 6(1), pp.1–9.
- Gutiérrez-Pozo, M., Verheecke-Vaessen, C., Kourmpetli, S., Terry, L. A., & Medina, Á., (2024): Effect of temperature, relative humidity, and incubation time on the mycotoxin production by *Fusarium* spp. responsible for dry rot in potato tubers. *Toxins*, 16(10), p. 414.
- Hamilton, P. B., (1971): A natural and extremely severe occurrence of aflatoxicosis in laying hens. *Poultry Science*, 50(6), pp.1880–1882.
- Hassan, N. J., (2025): Climate change and trend analysis of temperature: The case of Sulaymaniah Region, Iraq. *Journal of Babylon Center for Humanities Studies*, 15(1), pp.63–87.
- Heshmati, A., Khorshidi, M., and Khaneghah, A. M., (2021): The prevalence and risk assessment of aflatoxin in sesame-based products. *Italian Journal of Food Science/Rivista Italiana di Scienza degli Alimenti*, 33.
- Horváth, E., Pusztahelyi, T., Adácsi, C., Tanyi, E., and Pócsi, I., (2022): Optimization and validation of ELISA for aflatoxin B<sub>1</sub> detection in fermented forages and feeds. *Scientifica*, p. 6059880.
- Jallow, A., Xie, H., Tang, X., Qi, Z., and Li, P., (2021): Worldwide aflatoxin contamination of agricultural products and foods: From occurrence to control. *Comprehensive Reviews in Food Science and Food Safety*, 20(3), pp. 2332–2381.
- Jafarbeigi, Z., Sadeghi, E., Abdolmaleki, K., Soltani, M., Dousti, S., Mir, S., Fattahi, N., Rezvani Ghalhari, M., and Moradinazar, M., (2025). Aflatoxin B<sub>1</sub> measurement in traditional Kermanshah cookies and risk assessment in dietary exposure. *Journal of Food Quality and Hazards Control*, 12(2), pp.84–93.
- Jimenez, M., Mateo, R., Querol, A., Huerta, T., and Hernandez, E., (1991): Mycotoxins and mycotoxigenic moulds in nuts and sunflower seeds for human consumption. *Mycopathologia*, 115, pp.121–127.
- Kabak, B., (2009): Ochratoxin A in cereal-derived products in Turkey: occurrence and exposure assessment. *Food and Chemical Toxicology*, 47(2), pp.348-352.
- Karlovsky, P., Suman, M., Berthiller, F., De Meester, J., Eisenbrand, G., Perrin, I., Oswald, I. P., Speijers, G., Chiodini, A., and Recker, T., (2016): Impact of food processing and detoxification treatments on mycotoxin contamination. *Mycotoxin Research*, 32, pp.179–205.
- Kollia, E., Kanapitsas, A., and Markaki, P., (2014): Occurrence of aflatoxin B<sub>1</sub> and ochratoxin A in dried vine fruits from Greek market. *Food Additives & Contaminants: Part B*, 7(1), pp.11–16.

- Kőszegi, T., and Poór, M., (2016): Ochratoxin A: molecular interactions, mechanisms of toxicity and prevention at the molecular level. *Toxins*, 8(4), p.111.
- Kumar, P., Mahato, D. K., Kamle, M., Mohanta, T. K., and Kang, S. G., (2017): Aflatoxins: a global concern for food safety, human health and their management. *Frontiers in Microbiology*, 7, p.2170.
- Kumar, P., Mahato, D. K., Sharma, B., Borah, R., Haque, S., Mahmud, M. M. C., Shah, A. K., Rawal, D., Bora, H., and Bui, S., (2020): Ochratoxins in food and feed: Occurrence and its impact on human health and management strategies. *Toxicon*, 187, pp. 151–162.
- Lewis, L., Onsongo, M., Njapau, H., Schurz-Rogers, H., Lubber, G., Kieszak, S., Nyamongo, J., Backer, L., Dahiye, A. M., and Misore, A., (2005): Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya. *Environmental Health Perspectives*, 113(12), pp.1763–1767.
- Li, X., Ma, W., Ma, Z., Zhang, Q., and Li, H., (2021): The occurrence and contamination level of ochratoxin A in plant and animal-derived food commodities. *Molecules*, 26(22), p.6928.
- Lizárraga-Paulín, E. G., Moreno-Martínez, E., and Miranda-Castro, S. P., (2011): Aflatoxins and their impact on human and animal health: an emerging problem. *Aflatoxins—Biochemistry and Molecular Biology*, 13, pp.255–262.
- Lutfullah, G., and Hussain, A., (2012): Studies on contamination level of aflatoxins in some cereals and beans of Pakistan. *Food Control*, 23(1), pp.32–36.
- Malir, F., Ostry, V., Pfohl-Leszkowicz, A., Toman, J., Bazin, I., and Roubal, T., (2014): Transfer of ochratoxin A into tea and coffee beverages. *Toxins*, 6(12), pp.3438–3453.
- Mafe, A. N. and Büsselberg, D., (2024): Mycotoxins in food: Cancer risks and strategies for control. *Foods*, 13(21), p. 3502.
- Mannaa, M., and Kim, K. D., (2017): Influence of temperature and water activity on deleterious fungi and mycotoxin production during grain storage. *Mycobiology*, 45(4), pp.240–254.
- Mujeeb, S., Bukhari, F. A., Rashid, N., Mujeeb, I., Asadullah, Hilal, B., Mustafa, M. Z., Ahamd, I., Hameed, T., and Khan, M. A., (2022): A comparative study to determine the nutritive value of national brands and locally baked cookies, detection of aflatoxin B1 and aflatoxigenic fungi in Quetta. *Pak-Euro Journal of Medical and Life Sciences*, 5(2), pp.249–256.
- Nan, M., Xue, H., and Bi, Y., (2022): Contamination, detection and control of mycotoxins in fruits and vegetables. *Toxins*, 14(5), p.309.
- Okechukwu, V. O., Adelusi, O. A., Kappo, A. P., Njobeh, P. B., and Mamo, M. A., (2024): Aflatoxins: occurrence, biosynthesis, mechanism of action and effects, conventional/emerging detection techniques. *Food Chemistry*, 436, p.137775.
- Okuma, T. A., Huynh, T. P., and Hellberg, R. S., (2018): Use of enzyme-linked immunosorbent assay to screen for aflatoxins, ochratoxin A, and deoxynivalenol in dry pet foods. *Mycotoxin Research*, 34, pp.69–75.
- Omotayo, O. P., Omotayo, A. O., Mwanza, M., and Babalola, O. O., (2019): Prevalence of mycotoxins and their consequences on human health. *Toxicological Research*, 35, pp.1–7.
- Peraica, M., and Domijan, A.-M., (2001): Contamination of food with mycotoxins and human health. *Arhiv Za Higijenu Rada I Toksikologiju*, 52(1), pp.23–35.
- Rahmani, A., Jinap, S., and Soleimany, F., (2009): Qualitative and quantitative analysis of mycotoxins. *Comprehensive Reviews in Food Science and Food Safety*, 8(3), pp.202–251.

- Reddy, B. N., and Raghavender, C. R., (2007): Outbreaks of aflatoxicoses in India. *African Journal of Food, Agriculture, Nutrition and Development*, 7(5).
- Ringot, D., Chango, A., Schneider, Y.-J., and Larondelle, Y., (2006): Toxicokinetics and toxicodynamics of ochratoxin A, an update. *Chemico-biological Interactions*, 159(1), pp.18-46.
- Saberi-Hasanabadi, P., Shokrzadeh Lamuki, M., Shahsavari, V., and Saeedi, M., (2023): Ochratoxin a (ota) determination in wheat flour samples from different bakeries in Sari, Iran. *Journal of Mazandaran University of Medical Sciences*, 33(225), pp.174-184.
- Saha, D., Acharya, D., Roy, D., Shrestha, D., and Dhar, T. K., (2007): Simultaneous enzyme immunoassay for the screening of aflatoxin B<sub>1</sub> and ochratoxin A in chili samples. *Analytica Chimica Acta*, 584(2), pp.343-349.
- Santos, L., Marín, S., Sanchis, V., and Ramos, A. J., (2009): Screening of mycotoxin multicontamination in medicinal and aromatic herbs sampled in Spain. *Journal of the Science of Food and Agriculture*, 89(10), pp.1802-1807.
- Sedova, I., Kiseleva, M., and Tutelyan, V., (2018): Mycotoxins in tea: occurrence, methods of determination and risk evaluation. *Toxins*, 10(11), p.444.
- Shavakhi, F., Rahmani, A., & Piravi Vanak, Z., (2023): A global systematic review and meta analysis on prevalence of the aflatoxin B<sub>1</sub> contamination in olive oil. *Journal of Food Science and Technology*, 60(4), pp. 1255-1264.
- Sirhan, A., AlRashdan, Y., Najdawi, M., Hassouneh, L. K., Talhouni, A., Abuirmeileh, A., Jarrar, Q., Ayoub, R., and Abdulra'uf, L. B., (2023): Quantification of ochratoxin A in 90 spice and herb samples using the ELISA method. *Journal of Medicine and Life*, 16(9), pp. 1393-1399.
- Swelim, M., Baka, Z., El-Dohlob, S., Hazzaa, M., and El-Sayed, T., (1994): Mycoflora of stored poultry fodder in Egypt and their ability to produce aflatoxins. *Microbiological Research*, 149(4), pp.435-442.
- Thirumala-Devi, K., Mayo, M., Reddy, G., and Reddy, D., (2002): Occurrence of aflatoxins and ochratoxin A in Indian poultry feeds. *Journal of Food Protection*, 65(8), pp.1338-1340.
- Turner, N. W., Subrahmanyam, S., and Piletsky, S. A., (2009): Analytical methods for determination of mycotoxins: a review. *Analytica Chimica Acta*, 632(2), pp.168-180.
- Uğur, H., Omurtag, G. Z., Omurtag-Korkmaz, B. İ., & Yaman, M., (2023): Determination of aflatoxins and ochratoxin A levels in nuts and dried fruits in Turkey with evaluation of the estimated daily intake. *Journal of Food and Nutrition Research*, 62(4), pp. 314-324.
- Van der Merwe, K., Steyn, P., Fourie, L., Scott, D. B., and Theron, J., (1965): Ochratoxin A, a toxic metabolite produced by *Aspergillus ochraceus* Wilh. *Nature*, 205(4976), pp.1112-1113.
- Widiyanti, P. M., & Maryam, R., (2023): Ochratoxin A (OTA) contamination detection by enzyme linked immunosorbent assay (ELISA). In *IOP Conference Series: Earth and Environmental Science*, 1253, Art. 012100.
- Yazdanfar, N., Mahmudiono, T., Fakhri, Y., Mahvi, A. H., Sadighara, P., Mohammadi, A. A., and Yousefi, M., (2022): Concentration of ochratoxin A in coffee products and probabilistic health risk assessment. *Arabian Journal of Chemistry*, 15(12), 104376.
- Zareshahrabadi, Z., Bahmyari, R., Nouraei, H., Khodadadi, H., Mehryar, P., Asadian, F., and Zomorodian, K., (2020): Detection of aflatoxin and ochratoxin A in spices by high performance liquid chromatography. *Journal of Food Quality*, 2020, Article 8858889, pp. 1-8.