

Aflatoxin B₁ contamination of wheat flour, coffee, and pistachios consumed in Northern Palestine

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Abstract

The objective of the present work was to investigate the levels of aflatoxin B₁ (AFB₁) in some commonly consumed food items (wheat flour, roasted coffee beans, and pistachios) at the markets of Palestine. The samples were collected from the cities in the Northern West Bank (Nablus, Tulkarm, and Jenin). The pistachio and coffee samples were collected from the main toasters in these cities, while the flour samples were collected from the major bakeries in these cities. A total of 90 samples were collected, 30 from each city. The samples were analysed by a direct quantitative competitive enzyme-linked immunosorbent assay (ELISA) to determine the amounts of AFB₁. AFB₁ contamination was detected in all of the collected samples. The percentage of samples exceeding the permissible limit prescribed by the European Commission (EC) Regulation of Maximum Residual Levels (MRL) was 33.3%. AFB₁ contamination levels in the flour samples ranged between 0.4 - 2 µg/kg, with a mean of 0.75 µg/kg. All of the tested samples were below the EC-MRL level of 12 µg/kg. The amount of AFB₁ in the roasted coffee beans ranged between 12 - 49 µg/kg, with a mean of 25.03 µg/kg. All of the tested samples exceeded the EC-MRL level of 5 µg/kg. The pistachios had a contamination level between 0.7- 2.20 µg/kg, with a mean of 1.28 µg/kg, and none exceeded the EC-MRL level of 8 µg/kg for direct human consumption. No differences were observed between the targeted areas ($p > 0.05$). The results obtained on the contamination levels of AFB₁ indicated a serious public health issues. Further improvement of food handling and storage conditions must be exercised.

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Introduction

Good quality food must be free from food contaminants such as fungi, viruses, parasites, and bacteria, in addition to chemical pollutants and radiation (Rather *et al.*, 2017). Wheat flour, coffee, and pistachios are consumed in huge amounts in Palestine (Essid, 2012). However, there is little information regarding the contamination of these food products. Different contaminants reached the foods during cultivation, harvest, importation, and storage. These foodstuffs cannot be grown in Palestine, and are therefore imported from other countries. Many environmental factors, including temperature and water activity (a_w), contribute in stimulating fungal growth of various species in crop commodities with some of which could be mycotoxigenic (Pitt *et al.*, 2013). Environmental conditions also play an important role in the

production of mycotoxins from fungi that are dispersed in the natural environment. The production of mycotoxins depends on a wide range of factors including the fungal species potential, substrate composition, aeration, moisture content, temperature, and storage conditions. Stress factors such as a shortage of water, insect infestation, and attacks by other pests can also enhance mycotoxin production (Sanchis, 2004). According to FAO statistics, 25% of food and agricultural commodities are contaminated with mycotoxins (Eskola *et al.*, 2019).

Among the most prevalent mycotoxins are aflatoxins which frequently contaminate foods, grains, and various animal products (Agag, 2004). Fungal species producing aflatoxins are *Aspergillus flavus* and *A. parasiticus*. They can contaminate the grains at any point pre-harvest right until consumption (Cotty and Jaime-Garcia, 2007). Aflatoxin analogues such as AFB₁, AFB₂, AFG₁,

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and AFG₂ can cause health problems for humans and animals (Bhat *et al.*, 2010; Mahmoudi and Norian, 2015). AFB₁ is the most prevalent in foods, and considered the most hazardous threat to health and food safety. Its negative effects on human and animal health are attributed to its highly carcinogenic and hepatic toxicity (Reddy *et al.*, 2010).

Aflatoxins in wheat flour is a common occurrence (Halt, 1994). Coffee beans have also been found to be contaminated with *Aspergillus*, *Penicillium*, and *Fusarium* spp. (García-Moraleja *et al.*, 2015), with AFB₁ as the major mycotoxin detected. The occurrence of AFB₁ in pistachio nuts has also been studied in various countries. The levels of aflatoxins have been recorded as 20, 10, and 3.78 µg/kg for nuts that were marketed in Mexico, Japan (WHO, 2008), and Turkey (Fernane *et al.*, 2010), respectively.

Nevertheless, there is no information on AFB₁ contamination of commonly consumed food items in Palestine. Therefore, the objective of the present work was to investigate AFB₁ contamination of wheat flour, coffee beans, and pistachios obtained from Palestinian markets. Thus far, this is the first attempt to determine mycotoxin contamination in Palestinian foods.

Materials and methods

Samples

Samples (flour, roasted coffee beans, and pistachios) were collected from the main toasters and bakeries located in the cities of Nablus, Tulkarm, and Jenin (North Palestine). A total of 90 samples were collected, 30 from each city, and 10 samples of each item per city. Approximately, 40 g of samples were placed in tight vials, and transported to the Food Analysis Laboratory, Faculty of Agriculture and Veterinary Medicine, An-Najah National University, Palestine for analyses. The samples were stored in a cool place while protected from light.

Sample preparation

The samples were prepared following the manufacturer's instructions for RIDASCREEN® AFB₁ 30/15 (RIDASCREEN®, R-Biopharm AG, Darmstadt, Germany), a competitive enzyme immunoassay. Briefly, a representative sample was ground and mixed homogeneously prior to extraction. The samples were manually shaken vigorously for 3 min, and centrifuged for 10 min at 3,500 g at room temperature. Next, 0.5 mL of the obtained clear supernatant was diluted with 0.5 mL of deionised distilled water. Then, 0.5 µL of the

diluted supernatant was used per well in the test.

Test principle and procedure

RIDASCREEN® AFB₁ 30/15 (RIDASCREEN®, R-Biopharm AG, Darmstadt, Germany) (Art. No. R1211) is a direct quantitative competitive enzyme-linked immunosorbent assay (ELISA). It was used to determine the amount of AFB₁ in the tested samples (Leszczyńska *et al.*, 2001). The test was based on the antigen-antibody reaction, as the microtiter wells are coated with anti-AFB₁ antibodies. The analysis was based on the principle of competition, where the free aflatoxin and aflatoxin enzyme conjugate will compete for the aflatoxin antibody binding sites in a process known as the competitive enzyme immunoassay. At the same time, they were immobilised to capture the antibodies which were linked to the anti-AFB₁ antibodies. Afterwards, a washing step was performed, aiming to remove any unbound enzyme conjugation. Chromogen was then added to the wells and the bound enzyme conjugate converted the chromogen into a blue product. At the end of the reaction, a stop solution was used, and it changed the colour from blue to yellow.

For the quantitative detection of AFB₁ in the sample extracts, a 50 µL of 0, 5, 10, 20, and 50 µg of standard or prepared samples were added into separate wells. A 50 µL of the conjugate was then added to each well. Afterwards, 50 µL of antibody was added to each well and mixed gently by shaking the plate manually, and then incubated for 30 min at room temperature. After the incubation, the liquid was discarded, and the microwell holder was tapped vigorously, upside down, against an absorbent paper for three times to ensure complete removal of liquid from the wells. All of the wells were filled with 250 µL wash buffer, and discarded again for three times. After washing, 100 µL of substrate/chromogen was added to each well, mixed gently by shaking the plate manually, and then incubated for 15 min at room temperature. Finally, 100 µL of stop solution was added to each well, mixed gently by shaking the plate manually, and the absorbance was measured at 450 nm.

The measurement was performed photometrically at 450 nm. The absorbance was inversely proportional to the AFB₁ concentration in the samples. The specificity of the test for AFB₁ was 100%, and the detection limit of the kit was 1 µg/kg.

Statistical analysis

The optical density (OD) values of the standards and samples were normalised to the mean

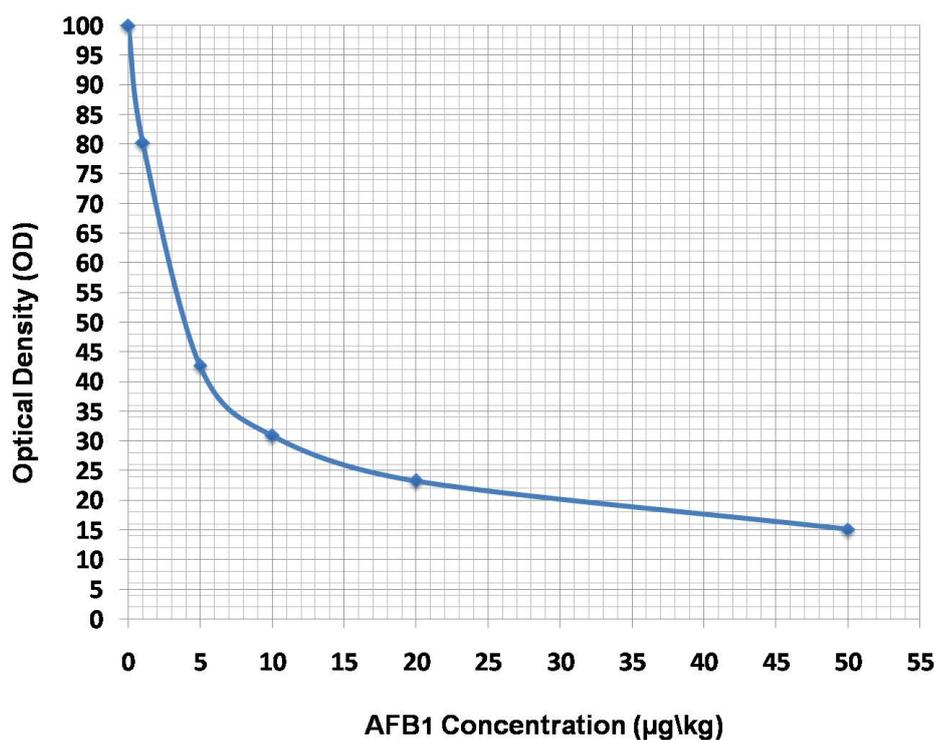


Figure 1. Calibration curve of AFB₁ standards: 0, 5, 10, 20, and 50 µg/kg. The optical density (O.D.) of each standard was normalised to 0 µg/kg standard. The AFB₁ concentration corresponding to the experimental extinction of each sample was obtained directly from the calibration curve.

OD value of the zero standards. The percentages of the absorbance values that were obtained for the standards and samples were plotted on semi-logarithmic graph paper against the concentration of AFB₁ standard in µg/kg (Figure 1). A calibration curve was used to obtain the AFB₁ concentration in the samples. The AFB₁ concentration that corresponded to the extinction of each sample was obtained directly from the calibration curve. Analysis of variance (ANOVA) was used for the comparison of the average of AFB₁ (µg/L) in the different foodstuffs and cities. The results were considered statistically significant at $p < 0.05$.

Results and discussion

Mycotoxins, especially aflatoxins, are widespread in nature and they contaminate cereals, coffee, and other crop commodities. AFB₁ is one of the most important mycotoxins due to its negative health effects as it causes liver cancer and other health-related damages (Pitt *et al.*, 2013). Various validated methods of analysis exist, and the enzyme-linked immunosorbent assay (ELISA) is among the most often used in mycotoxin detection and quantification (van Egmond, 2004). In the present work, a total of 90 samples were analysed using quantitative ELISA for the determination of

AFB₁ contamination in the roasted coffee beans, wheat flour, and pistachios. Overall, AFB₁ was detected above the limit in 33.33% samples. The occurrence and levels of AFB₁ in the three foodstuffs that were collected in Nablus, Tulkarm, and Jenin (North Palestine) markets during April 2019 showed no significant differences between the three cities ($p > 0.05$). The number of samples and AFB₁ concentrations are shown in Table 1.

AFB₁ contamination was detected in all of the wheat flour samples ($n = 30$). The AFB₁ contamination levels ranged from 0.4 - 2 µg/kg. According to the Palestinian Food Standards that follow the European Commission limits, the maximum residue limit in wheat flour is 12 µg/kg (EC, 2006), and all the investigated samples were below this limit. Wheat flour is susceptible to fungal infections during wheat growth, harvesting, transportation, and storage. Environmental conditions play an important role in influencing mycotoxin development. Hassane *et al.* (2017) showed that the growth of toxic fungi producing AFB₁ needs a temperature of 25 - 30°C. High population of *A. flavus* was recorded at 30°C, and high levels of AFB₁ production were detected at 25°C (Hassane *et al.*, 2017). The current study's findings are consistent with a study in Iran that reported AFB₁ at 77% ($n = 100$) in the samples that

Table 1. The concentration of AFB₁ in coffee, wheat flour, and pistachio samples collected from three cities in Northern Palestine.

		N (positive)	Mean ± SEM (µg/kg)	Range	Samples above the limit (%)*
Sample	Flour ^a	30 (30)	0.75 ± 0.37	0.40 - 2.00	0
	Coffee ^b	30 (30)	25.03 ± 2.33	12.0 - 49.00	100
	Pistachio ^a	30 (30)	1.28 ± 0.06	0.70 - 2.20	0
	Total	90 (90)	9.03 ± 1.43	0.40 - 49.00	33.33
City	Nablus ^a	30 (30)	12.26 ± 3.41	0.40 - 75.00	10
	Jenin ^a	30 (30)	6.29 ± 1.52	0.4 - 27.50	10
	Tulkarm ^a	30 (30)	8.51 ± 2.04	0.40 - 32.50	10
	Total	90 (90)	9.02 ± 1.43	0.40 - 75.00	33.33

*Percentage of samples that exceeded the European Commission (EC) Regulation for Maximum Residual Levels (MRL).

^{a,b} Means in different foodstuff and cities followed by different letters were significantly different ($p < 0.05$).

were collected in winter, with a mean of 0.52 µg/kg (Taheri *et al.*, 2012). Another study in Iran also reported a contamination range for AFB₁ of 1.36 - 1.78 µg/kg (Hedayati and Mohammadpour, 2005). In a study by Halt (1994), the mean level of AFB₁ was 11.13 µg/kg. Aydın *et al.* (2008) reported that wheat flour in Turkey was positive for AFB₁ at a concentration of 25.6 µg/kg. In another study, Giray *et al.* (2007) analysed wheat samples in Turkey, and found that the highest AFB₁ level in the samples was 12.2 µg/kg. Despite the level of AFB₁ in the wheat flour samples, it did not exceed the limits set by EC regulation, which are followed by Palestine. Nevertheless, AFB₁ was detected in all of the samples. In Palestine, bread is considered as the most consumed food (Essid, 2012). Wheat flour is the main component of bread, which is a traditional daily intake in almost all meals. A low-level exposure over time can result in chronic toxicity, which can be mistaken for other diseases (Baines *et al.*, 2018). Further studies are therefore required to determine the total of aflatoxins and prevalence of other mycotoxins in wheat and wheat products (flour, bread, etc.).

There was a 100% incidence of contamination levels within the roasted coffee beans samples ($n = 30$), which ranged between 12 - 49 µg/kg, with a mean of 25.03 µg/kg (Table 1). All of the analysed samples exceeded the permissible limit established by the European Commission of 5 µg/kg (EC, 2006). Similarly, in Saudi Arabia, Bokhari (2007) reported that AFB₁ levels at 83% ($n = 18$) for the coffee beans ranged between 2.1 - 219 µg/kg. The levels of

AFB₁ in the coffee beans were high, despite the process of roasting the coffee beans. AFB₁ withstands temperatures of up to 260°C, with a loss of toxin concentration by 40 - 55% (Jalili, 2016). The roasting process of coffee beans does not eliminate the aflatoxins because they resist high temperatures. A study in Yemen that was conducted by Humaid *et al.* (2019) showed aflatoxins (AFB₁, AFB₂, AFG₁, and AFG₂) were found at 100% ($n = 25$) in green and roasted coffee beans, at concentrations ranging from 14.69 - 27.17 and 14.25 - 23.23 µg/kg, respectively. In Palestine, coffee is considered as the most popular hot beverage for daily consumption. It is considered to be a symbol of generosity and hospitality. It is usually served on various occasions such as weddings, funerals, festivals, and others. The high level of AFB₁ in roasted coffee beans, with daily consumption, will increase the risk of toxigenic effects. Besides, this high level of AFB₁ could be an indicator of the presence of other mycotoxins, mainly ochratoxins (Bokhari, 2007; Reichert *et al.*, 2018). Further studies are urgently needed to determine other contaminants in the coffee. Coffee cannot be grown in Palestine, so cross-border trade regulation must be enforced during importation to avoid contaminated food products from entering Palestine. The storage at warehouses should also be monitored to minimise fungal growth on this commodity.

AFB₁ was detected in all of the pistachio samples ($n = 30$), and the contamination level ranged from 0.7 - 2.20 µg/kg, with a mean of 1.28 µg/kg (Table 1). The EC-MRL is 8 µg/kg for pistachios

used for direct human consumption, and 15 µg/kg for pistachios that are subjected to further processing (EC, 2006). All of the investigated samples were below the EC-MRL level. The current findings are consistent with another study in Turkey, where AFB₁ was detected at 50.5% ($n = 48/95$) of pistachios, and they ranged from 0.06 to 0.96 µg/kg (Set and Erkmen, 2010). In Iran, Taghizadeh *et al.* (2018) reported that the highest mean of AFB₁ was 4.33 µg/kg, and all of the samples analysed were lower than the EC-MRL level. In Spain, Ariño *et al.* (2009) analysed pistachios that were imported from Iran, Turkey, and USA. Around 50% ($n = 32$) of the Iranian bulk roasted pistachios were contaminated, ranging from 0.14 - 0.29 µg/kg, with mean contamination of 0.12 µg/kg; however, no sample exceeded the EC-MRL level. Besides, the levels of AFB₁ remained unchanged during roasting, indicating that commercial roasting is not a critical step in the control for AFB₁ in pistachios (Ariño *et al.*, 2009).

Studies have found that persistent and long-term exposure leads to immunodeficiency and interference of micronutrient metabolism in children. The high presence of aflatoxins in staple foods resulted from lack of regulation and laws that control food safety, especially during transportation, processing, and storage (Wu *et al.*, 2014). Numerous researches have been conducted to reduce the spread of aflatoxins in foods, but results have shown that it cannot be eliminated because of its resistance to heat. Furthermore, the use of certain substances to treat/reduce/detoxify aflatoxins in foods may create other significant problems (Ahlberg *et al.*, 2019).

Conclusions

The present work demonstrated that AFB₁ was a contaminant for some of the popular foodstuffs in Palestine. This problematic situation should attract greater attention as it concerns public health. Further improvement in food handling and storage conditions must be carried out. The need for an accredited laboratory for regulatory purposes such as assessing and monitoring food contamination is also highly recommended. It is also highly recommended to have integrated efforts among different stakeholders to exercise all the necessary preventive measures. Cross-border trade regulations must also be enforced during the importing process to avoid contaminated food products from entering Palestine. The storage at the warehouses must also be monitored. Institutions and ministries must execute their roles and follow standards and specifications to

determine AFB₁ and other mycotoxins within foodstuffs. They must also establish a valid method of mycotoxin analysis.

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