# Determination of aflatoxins and ochratoxin A levels in nuts and dried fruits in Turkey with evaluation of the estimated daily intake

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**Summary**

A total of 140 samples, including nuts and dried fruits sold in the province of Istanbul, Turkey, were examined for the presence of aflatoxins and ochratoxin A (OTA) by high-performance liquid chromatography (HPLC), which were quantitatively determined and estimated daily intakes (*EDI*) were calculated. It was found that aflatoxins load was higher in nuts and OTA load was higher in dried fruits. The highest level of aflatoxins was found in a packaged roasted hazelnut sample (8.043 $\mu g \cdot kg^{-1}$). The maximum OTA level was detected in an unpackaged raisin sample (0.505 $\mu g \cdot kg^{-1}$). The highest *EDI* values of aflatoxins were seen in hazelnut samples and were determined as 0.025 $ng \cdot kg^{-1}$ body weight (BW) per day. *EDI* of OTA was similar between the groups (0.000–0.001 $ng \cdot kg^{-1}$ BW per day). As a result, the contents detected for both toxins in the products examined were below the legal limits, except for one hazelnut sample (aflatoxin $B_1$: 7.645 $\mu g \cdot kg^{-1}$). The *EDI* values were below the limit values set by the European Food Safety Authority. However, since it is possible for these values to rise to risky levels, the risk of contamination should be minimized by taking the necessary precautions.

**Keywords**

dried fruits; nuts; aflatoxins; ochratoxin A; high-performance liquid chromatography; estimated daily intake

Mycotoxins are known as secondary metabolites produced by some filamentous fungi, including *Aspergillus, Fusarium, Penicillium* and *Alternaria* genera. Since their presence in food is a serious threat to human health, it is crucial to investigate their presence in the food production chain [1].

Nuts, dried fruits and many agricultural products are contaminated with various mycotoxins in the pre-harvest and post-harvest periods. Food and Agriculture Organization (FAO) stated that 25 % of global agricultural products are contaminated by mycotoxins every year [2]. However, this estimate is thought to be lagging behind with a 60–80 % contamination rate obtained in a study conducted in 2019. These levels can probably be explained by considering the higher sensitivity of newly developed analytical methods and/or the impact of climate change [3]. The incidence of mycotoxins varies depending on various factors such as the composition of the food, climatic conditions, agricultural practices, storage conditions or season [1].

Aflatoxins and ochratoxin A (OTA) are considered to be the two most common mycotoxins in food contamination. While aflatoxins mostly cause contamination in the field, OTA is associated with contamination during drying and storage [4]. Among the 18 different aflatoxin species, aflatoxin $B_1$ ($AFB_1$), aflatoxin $B_2$ ($AFB_2$), aflatoxin $G_1$ ($AFG_1$) and aflatoxin $G_2$ ($AFG_2$) are considered the most important [5]. $AFB_1$ is the most

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common aflatoxin with proven human carcinogenity, which is frequently present in many types of foodstuffs worldwide. Aflatoxins show toxicity, carcinogenic and mutagenic activity in the order $AFB_1 > AFG_1 > AFB_2 > AFG_2$ [6]. Ochratoxins were first identified and characterized from fungal cultures, with three derivatives designated as OTA, OTB and OTC [7]. Ochratoxin A is the most important regarding the harmful effects on humans and animals. OTA has been shown to have nephrotoxic, hepatotoxic, neurotoxic, carcinogenic, teratogenic and immunotoxic effects [8].

Dried fruits are a rich source of antioxidants, vitamins, minerals and fibre. Nuts also contain these ingredients, but they are additionally an important source of fatty acids. However, nuts and dried fruits are highly susceptible to mycotoxin contamination. Contamination can occur in the field, during harvest, transport or storage [9, 10]. Rapid Alert System for Food and Feed (RASFF) stated that 89 % of aflatoxins came from hazelnuts and similar products, most of which consisted of pistachios, pumpkin seeds and mixed nuts [11]. This institution explained that there has recently been a significant increase in mycotoxins reporting, which was 4 % for the fruit and vegetable category in 2003, reaching 30 % in 2012 [9, 12].

Preventing the contamination of foods with mycotoxins and other residues at all stages along the food production chain will contribute to the growth of healthy generations in terms of public health. In addition, it is of great economic importance to prevent contamination in products with high exports, such as nuts and dried fruits [13, 14]. The European Commission (EC) has set maximum limits for mycotoxins in food and feed. While the limits set by the EC for $AFB_1$ in nuts and dried fruits vary between 2–8 $\mu g \cdot kg^{-1}$, for total aflatoxins (TAF), i.e. $AFB_1$, $AFB_2$, $AFG_1$, and $AFG_2$, it is between 4–10 $\mu g \cdot kg^{-1}$. For OTA, this range is recommended as 5–10 $\mu g \cdot kg^{-1}$ [15].

This study aimed to determine the amounts of aflatoxins and OTA in nuts and dried fruits consumed in Istanbul and to evaluate the estimated daily intakes of aflatoxins and OTA for the population due to the consumption of these products.

## MATERIALS AND METHODS

### Sampling

The study material consisted of a total of 140 samples, including nuts (60 hazelnuts and 40 peanuts) and dried fruits (20 dried figs and 20 raisins) offered for sale in Istanbul, Turkey. Samples were randomly collected from various regions between September and November 2020 as packaged products from markets and unpackaged products from bazaars and various retail markets in Istanbul. Samples collected from bazaars and retail markets in clear plastic packages were of at least 500 g, and the samples taken from the market in their original packaging were of at least 300 g. The products bought from the bazaars and retail markets were previously kept in 10, 15 or 20 kg packages in the storage areas before being put up for sale. The samples brought to the laboratory were labelled and stored at cooled conditions (4 ± 1 °C) until further analysis for a maximum 15 days.

### Chemicals and reagents

The standards of aflatoxins Mix 4 solution in acetonitrile (Supelco, Bellefonte, Pennsylvania, USA) and OTA solution in acetonitrile (10 $\mu g \cdot ml^{-1}$, Supelco) were used. Immunoaffinity columns (IAC) for aflatoxins and OTA were obtained from Romer Labs (Newark, Delaware, USA). High-performance liquid chromatography (HPLC)-grade acetonitrile and methanol were obtained from IsoLab (Eschau, Germany). HPLC-grade acetic acid was obtained from Sigma Aldrich (St. Louis, Missouri, USA). Deionized water Millipore Direct-Q 3 UV Ultrapure (Type 1) from Merck (Darmstadt, Germany) was used in the study. All other chemicals and reagents were of high purity.

### Sample extraction and Immunoaffinity cleanup

AOAC methods 999.07, 991.31, 2000.03 [16–18] and VICAM OchraTest (Waters, Milford, Massachusetts, USA) were applied with some modifications to determine the amount of aflatoxins and OTA in nuts and dried fruits.

For aflatoxins extraction, approximately 50 g of a ground sample of nuts or dried fruits was mixed with 5 g of NaCl and 100 ml of ultrapure water (60 ml for dried fruits) in a Waring blender (Waring Commercial, Torrington, Connecticut, USA) at high speed for 1 min. Then, 150 ml of methanol (240 ml for dried fruits) was added to the mixture and blended for at least 2 min at high speed. The extracts were filtered through Whatman qualitative filter paper-Grade 4 (Sigma Aldrich) and 5 ml of the filtrate was taken and passed through an IAC column attached to a vacuum manifold at a speed of 3 $ml \cdot min^{-1}$ by adding 45 ml of phosphate-buffer solution (PBS). In the preparation of PBS, 0.2 g KCl, 0.2 g $KH_2PO_4$, 1.16 g $Na_2HPO_4$ and 8 g NaCl were dissolved in 0.9 l ultrapure water. After this solution was completely dissolved by magnetic stirrer,

the pH was adjusted to 7.4 using 0.1 mol·l$^{-1}$ HCl or 0.1 mol·l$^{-1}$ NaOH. Afterwards, the solution was completed to 1 liter with ultrapure water. Aflatoxins were eluted from the IAC column with 1 ml of pure methanol. Then, 1 ml of ultrapure water was passed through the column and the total amount of eluate was collected in a vial as 2 ml.

In the OTA extraction process, approximately 50 g of ground sample from nuts or dried fruits was mixed with 80 ml of ultrapure water (60 ml for dried fruits) and 120 ml of acetonitrile (140 ml methanol for dried fruits) in a Waring blender at high speed for 3 min. A volume of 10 ml of the filtrate was taken and 40 ml of PBS was added. Then, 25 ml of the diluted filtrate was passed through the IAC column attached to a vacuum manifold at a speed of 3 ml·min$^{-1}$. OTA was extracted from the IAC column by washing with 1 ml of pure methanol. Then, 1 ml of ultrapure water was passed through IAC and the total amount of eluate was collected in a vial as 2 ml.

### Analysis for aflatoxins and ochratoxin A

The HPLC system was from Shimadzu (Kyoto, Japan), with a fluorescence detector (FLD) RF-20A (Shimadzu). For aflatoxins analysis, a reversed-phase inertsil ODS-3 column (4.6 mm × 250 mm, 5 $\mu$m; GL Sciences, Tokyo, Japan) was used with a mobile phase of acetonitrile, methanol and water (1.5 : 3 : 6, v/v/v,), at a flow rate of 1 ml·min$^{-1}$. The wavelengths of the detector were set at excitation (365 nm) and emission (460 nm). The post-column UV derivatization system for aflatoxins was placed between the column and the fluorescence detector. Derivatization of $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$ was achieved by wrapping Teflon tubing (20 m long, 0.5 mm diameter) on a 60 cm long UV-A lamp (Sylvania, Budapest, Hungary). The whole system was wrapped with aluminum foil. Then, the system was connected between the HPLC column and the fluorescence detector [19].

For OTA analysis, the fluorescence detector was set at 333 nm for excitation and 443 nm for emission, and the mobile phase was acetonitrile, water and acetic acid (47 : 51 : 2, v/v/v), at a flow rate of 1 ml·min$^{-1}$.

### Method validation

Linearity was determined by analysing in triplicate $AFB_1$, $AFB_2$, $AFG_1$, $AFG_2$ and OTA standards that were prepared at 6 concentrations (0.4, 1.0, 2.0, 5.0, 10.0 and 20.0 ng·ml$^{-1}$ for aflatoxins and 0.4, 1.0, 2.0, 4.0, 8.0 and 20.0 ng·ml$^{-1}$ for OTA). The values of the coefficient of determination ($R^2$) for all analytes were found above 0.99. Recovery values were determined by spiking aflatoxins to a blind sample that was previously found to be free of aflatoxins at the concentration level of 1 ng·ml$^{-1}$ for each type of aflatoxins and by spiking OTA to a blind sample that was found to be free of OTA, at the concentration level of 0.5 ng·ml$^{-1}$ for OTA, and this process was repeated ten times. The limit of detection (*LOD*) and limit of quantification (*LOQ*) were estimated based on signal-to-noise (*S*/*N*) ratio of 3 and 10, respectively. The values of *LOD*, *LOQ*, calibration curve, $R^2$, recovery, and *RSD* for aflatoxins and OTA are presented in Tab. 1. In this study, the Food Analysis Performance Assessment Scheme (FAPAS) for mycotoxins in dried figs quality control material (FAPAS QC material data sheet T04367QC) [20] was used to check the accuracy and performance of the method. The recovery for $AFB_1$, $AFB_2$, $AFG_1$, $AFG_2$ and for OTA were found as 98 %, 101 %, 99 %, 98 % and 100 %, respectively.

## RESULTS AND DISCUSSION

Fig. 1 and Fig. 2 show the chromatogram of the aflatoxin standard and roasted hazelnut sample naturally contaminated with aflatoxins. Fig. 3 and Fig. 4 show the chromatogram of OTA standard and raisin sample naturally contaminated with OTA, respectively. As seen from the chromatograms, aflatoxins and OTA are well separated.

**Tab. 1.** Validation of aflatoxins and ochratoxin A determination by HPLC analysis.

| Mycotoxin | *LOD* [$\mu$g·kg$^{-1}$] | *LOQ* [$\mu$g·kg$^{-1}$] | Calibration curve | $R^2$ | Recovery [%] | *RSD* [%] |
|---|---|---|---|---|---|---|
| Aflatoxin $B_1$ | 0.033 | 0.111 | $y = 4832.5x + 131.64$ | 0.9997 | 105.7 | 1.1 |
| Aflatoxin $B_2$ | 0.021 | 0.070 | $y = 9597.8x - 176.7$ | 1 | 103.4 | 0.7 |
| Aflatoxin $G_1$ | 0.011 | 0.038 | $y = 5235.5x + 297.68$ | 0.9999 | 106.1 | 0.4 |
| Aflatoxin $G_2$ | 0.014 | 0.047 | $y = 8944.8x - 97.983$ | 1 | 103.6 | 0.5 |
| Ochratoxin A | 0.090 | 0.030 | $y = 2547.1x - 0.3617$ | 1 | 95.4 | 0.6 |

*LOD* – limit of detection, *LOQ* – limit of quantification, $R^2$ – coefficient of determination, *RSD* – relative standard deviation.

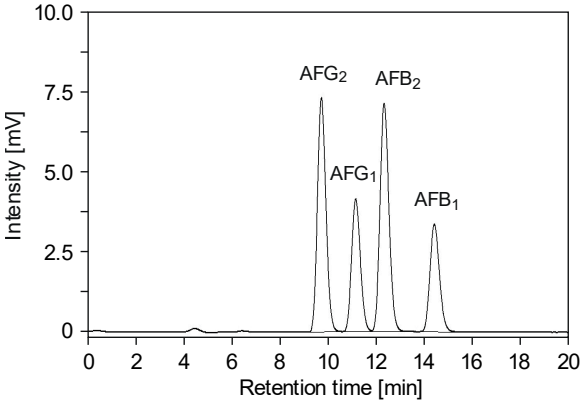


**Fig. 1.** Chromatogram of standards of aflatoxins.

$AFB_1$ – aflatoxin $B_1$ (0.02 $\mu g \cdot ml^{-1}$), $AFB_2$ – aflatoxin $B_2$ (0.02 $\mu g \cdot ml^{-1}$), $AFG_1$ – aflatoxin $G_1$ (0.02 $\mu g \cdot ml^{-1}$), $AFG_2$ – aflatoxin $G_2$ (0.02 $\mu g \cdot ml^{-1}$).

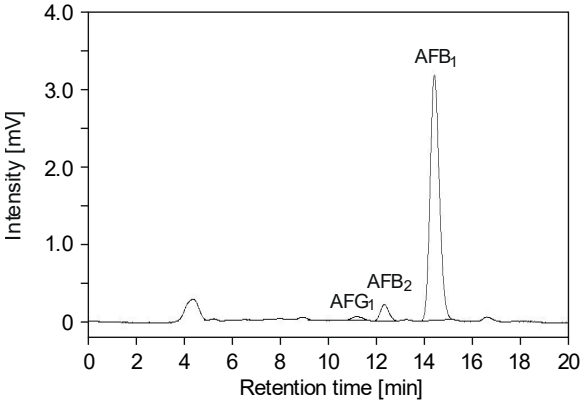


**Fig. 2.** Chromatogram of roasted hazelnut sample naturally contaminated with aflatoxins.

$AFB_1$ – aflatoxin $B_1$ (7.645 $\mu g \cdot kg^{-1}$), $AFB_2$ – aflatoxin $B_2$ (0.289 $\mu g \cdot kg^{-1}$), $AFG_1$ – aflatoxin $G_1$ (0.109 $\mu g \cdot kg^{-1}$).

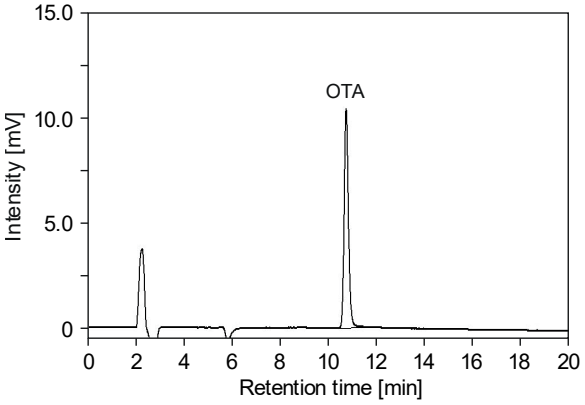


**Fig. 3.** Chromatogram of the standard of ochratoxin A.

OTA – ochratoxin A (0.02 $\mu g \cdot ml^{-1}$).

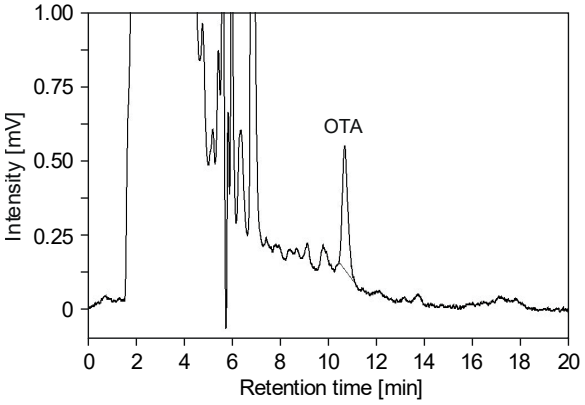


**Fig. 4.** Chromatogram of a raisin sample naturally contaminated with ochratoxin A.

OTA – ochratoxin A (0.505 $\mu g \cdot kg^{-1}$).

#### Validation

In order to make this evaluation, linearity data consisting of linearity range (0.4–20 $\mu g \cdot l^{-1}$), linearity equation and $R^2$, as well as *LOD*, *LOQ*, and recovery were analysed (Tab. 1). The results obtained were judged as acceptable. For aflatoxins, they were $R^2 > 0.99$ and recovery 70–110 % for a toxin content of 1–10 $\mu g \cdot kg^{-1}$. For ochratoxin A, they were $R^2 > 0.99$ and recovery 50–120 % for a toxin content of < 1 $\mu g \cdot kg^{-1}$ [21–23]. In addition, *LOD* and *LOQ* values were considered sufficiently low for the detection of aflatoxins and OTA in each matrix (*LOD* of 0.011–0.033 $\mu g \cdot kg^{-1}$ and *LOQ* of 0.038-0.111 $\mu g \cdot kg^{-1}$ for aflatoxins, *LOD* of 0.090 $\mu g \cdot kg^{-1}$ and *LOQ* of 0.030 $\mu g \cdot kg^{-1}$ for OTA). The FAPAS quality control material assigned value for $AFB_1$, $AFB_2$, $AFG_1$, $AFG_2$ and for OTA were 3.97 $\mu g \cdot kg^{-1}$, 2.29 $\mu g \cdot kg^{-1}$, 3.14 $\mu g \cdot kg^{-1}$, 1.65 $\mu g \cdot kg^{-1}$ and 4.75 $\mu g \cdot kg^{-1}$. Recovery was determined as 105.7 %, 103.4 %, 106.1 %, 103.6 % and 95.4 % for $AFB_1$, $AFB_2$, $AFG_1$, $AFG_2$ and OTA, respectively. Relative standard deviation (*RSD*) was in the range of 0.4–1.1 % for aflatoxins and 0.6 % for OTA.

#### Aflatoxins in nuts and dried fruits

Aflatoxins $AFB_1$, $AFB_2$, $AFG_1$, $AFG_2$ and OTA were analysed by HPLC in 140 samples. The determined contents of these toxins in food products are shown in Tab. 2. Aflatoxins were detected in all 60 hazelnut samples with the mean value of 0.351 $\mu g \cdot kg^{-1}$. Among hazelnut varieties, the highest mean value in case of $AFB_1$ and total aflatoxins was found in roasted hazelnut products (0.475 $\mu g \cdot kg^{-1}$ and 0.600 $\mu g \cdot kg^{-1}$, respectively). In addition, a sample of roasted hazelnuts was

found to exceed the maximum limit for $AFB_1$ of 5 $\mu g \cdot kg^{-1}$ set by EU (7.645 $\mu g \cdot kg^{-1}$). In hazelnut varieties, the highest contamination rate was in case of $AFB_1$ (98.3 %), followed by $AFG_1$ (85 %) and AFG2 (40–65 %). On the other hand, $AFB_2$ was found in 2 hazelnut samples, one raw and one roasted, but it was not found in shell hazelnuts. Fig. 2 shows an HPLC-FLD chromatogram of a roasted hazelnut sample containing 7.645 $\mu g \cdot kg^{-1}$ AFB1, 0.289 $\mu g \cdot kg^{-1}$ $AFB_2$ and 0.109 $\mu g \cdot kg^{-1}$ $AFG_1$. In a study conducted by Kabak [14] with 170 hazelnut samples collected from various cities in Turkey, the aflatoxins contamination rates were found to be between 1.7 % and 12.0 %, which was lower than in our present study (5–100 %). In the same study, the mean contaminations were reported to be between 0.513 $\mu g \cdot kg^{-1}$ and 4.81 $\mu g \cdot kg^{-1}$, which was higher than our present study (0.053–0.600 $\mu g \cdot kg^{-1}$). Similar to our present study, it was determined that the products were contaminated with $AFB_1$ at the highest level in the study [14]. In another study [24] carried out in the province of Trabzon, Turkey, it was found that 98.7 % of the hazelnut samples collected from exporting companies were contaminated with aflatoxins. The maximum values of the $AFB_1$ and total aflatoxins range were higher than in our present study (0.02–69.14 $\mu g \cdot kg^{-1}$ and 0.07–78.98 $\mu g \cdot kg^{-1}$, respectively). In addition to the similarities, the differences seen between the studies may be associated with the number of samples, the physical condition of the samples studied (with shell, without shell, roasted, raw), the cities where the products were collected, climatic differences, the year and season (spring, summer, autumn, winter) when the products were collected and dried, the sales places where the products were collected (market, nuts shop, exporter company), storage duration or the reference values (*LOD* or *LOQ*) taken for acceptance of the presence of contamination as a result of the analysis, being different from each other. According to the report published by The Rapid Alert System for Food and Feed (RASFF) [25], the most frequently reported mycotoxin in foods, especially in nuts, was aflatoxin. One-hundred-four notifications were made regarding aflatoxins in foods from Turkey as the country of origin. Fifty-five of these notifications regarded nuts and, in terms of aflatoxins reporting in nuts, Turkey ranked third after USA and Argentina [25].

Aflatoxins were detected in 39 out of 40 peanut samples (97.5 %), and the contents ranged from 0.026 $\mu g \cdot kg^{-1}$ to 0.886 $\mu g \cdot kg^{-1}$. The most common types of aflatoxins were $AFB_1$ and $AFG_1$, with contents ranging from 0.040 $\mu g \cdot kg^{-1}$ to 0.185 $\mu g \cdot kg^{-1}$ and from 0.030 $\mu g \cdot kg^{-1}$ to 0.240 $\mu g \cdot kg^{-1}$, respectively. While $AFB_1$ was found at higher rates in shell peanuts (90 % vs 95 %), $AFB_2$, $AFG_1$ and $AFG_2$ were found at higher rates in peanuts without shell (5 %, 90 % and 25 % vs 10 %, 95 % and 40 %, respectively). On the other hand, it was observed that the highest content was found for $AFG_2$ (0.270 $\mu g \cdot kg^{-1}$) in peanuts with shell, while $AFB_2$ (0.514 $\mu g \cdot kg^{-1}$) was found at the highest content in roasted peanuts. Hepsag et al. [26] reported that 29 of 151 peanut samples (19.2 %) were contaminated with aflatoxins at 0.16–60.9 $\mu g \cdot kg^{-1}$. Similar to our present study, the rate of $AFB_1$ was found to be higher than those of other aflatoxin types. In another study [27] conducted with 73 peanut samples collected from five cities in Turkey, it was determined that 17.8 % (13 samples) of the samples were contaminated with aflatoxins at 0.7–98.0 $\mu g \cdot kg^{-1}$. In addition, in that study, it was observed that the products were contaminated at the highest level with $AFB_1$ [27]. In a study carried out in Pakistan [13], the aflatoxins contamination rate of peanuts was 70 % and the content range was found to be from *LOD* to 21.34 $\mu g \cdot kg^{-1}$. In the comparisons we made with national and international studies [13, 26, 27], it was determined that while the aflatoxin contamination rates in peanuts were lower than in our present study, the contamination contents were higher. In addition to many reasons, it is thought that the difference in contamination rates between studies may be due to the use of HPLC-FLD after purificaion on IAC column. This is an analytical method with high selectivity and sensitivity, which allowed aflatoxins to be monitored even at low content levels in our present study [13, 14, 24, 26].

Aflatoxins were also determined in dried figs and raisins with 32 of 40 samples (80 %) being positive for aflatoxins. The highest aflatoxins occurrence was observed in raisins with contamination level, mean and content range of 85 %, 0.203 $\mu g \cdot kg^{-1}$ and 0.074–0.389 $\mu g \cdot kg^{-1}$, respectively. When compared with the results of the study conducted by Yilmaz [28] in Sakarya province, Turkey, the aflatoxins contamination rate of raisins was higher than in figs, as in our present research. However, the mean value for raisins was lower than for figs, which was different from our present study (51 % vs 64 % and 1.78 $\mu g \cdot kg^{-1}$ vs 0.40 $\mu g \cdot kg^{-1}$). A study carried out in Morocco [29] reported that raisin and dried fig samples were contaminated in 20 % and 30 % cases, respectively, and the mean content values were 10.7 $\mu g \cdot kg^{-1}$ and 8.70 $\mu g \cdot kg^{-1}$, respectively. According to the report of RASFF [25], in which aflatoxin contamination notifications in fruits and vegetables

**Tab. 2.** Content of aflatoxins and ochratoxin A in nuts and dried fruits.

| Total number of samples | Contaminant | *LOD* [$\mu g \cdot kg^{-1}$] | Positive samples | | | | |
|---|---|---|---|---|---|---|---|
| | | | Number | [%] | Min. [$\mu g \cdot kg^{-1}$] | Max. [$\mu g \cdot kg^{-1}$] | Mean [$\mu g \cdot kg^{-1}$] |
| **Hazelnuts, with shell** | | | | | | | |
| 20 | $AFB_1$ | 0.033 | 19 | 95 | 0.041 | 0.218 | 0.116 |
| | $AFB_2$ | 0.021 | 0 | 0 | < *LOD* | < *LOD* | < *LOD* |
| | $AFG_1$ | 0.011 | 17 | 85 | 0.033 | 0.079 | 0.053 |
| | $AFG_2$ | 0.014 | 13 | 65 | 0.026 | 0.227 | 0.078 |
| | TAF | NC | 20 | 100 | 0.068 | 0.498 | 0.207 |
| | OTA | 0.090 | 4 | 20 | 0.025 | 0.076 | 0.054 |
| **Hazelnuts, raw** | | | | | | | |
| 20 | $AFB_1$ | 0.033 | 20 | 100 | 0.044 | 0.377 | 0.104 |
| | $AFB_2$ | 0.021 | 1 | 5 | 0.183 | 0.183 | 0.183 |
| | $AFG_1$ | 0.011 | 17 | 85 | 0.019 | 0.179 | 0.071 |
| | $AFG_2$ | 0.014 | 10 | 50 | 0.041 | 0.400 | 0.139 |
| | TAF | NC | 20 | 100 | 0.111 | 0.777 | 0.246 |
| | OTA | 0.090 | 1 | 5 | 0.111 | 0.111 | 0.111 |
| **Hazelnuts, roasted** | | | | | | | |
| 20 | $AFB_1$ | 0.033 | 20 | 100 | 0.036 | 7.645 | 0.475 |
| | $AFB_2$ | 0.021 | 1 | 5 | 0.289 | 0.289 | 0.289 |
| | $AFG_1$ | 0.011 | 17 | 85 | 0.016 | 0.109 | 0.056 |
| | $AFG_2$ | 0.014 | 8 | 40 | 0.041 | 0.285 | 0.155 |
| | TAF | NC | 20 | 100 | 0.052 | 8.043 | 0.600 |
| | OTA | 0.090 | 0 | 0 | ND | ND | ND |
| **Peanuts, with shell** | | | | | | | |
| 20 | $AFB_1$ | 0.033 | 19 | 95 | 0.080 | 0.185 | 0.100 |
| | $AFB_2$ | 0.021 | 1 | 5 | 0.181 | 0.181 | 0.181 |
| | $AFG_1$ | 0.011 | 18 | 90 | 0.030 | 0.240 | 0.076 |
| | $AFG_2$ | 0.014 | 5 | 25 | 0.020 | 0.270 | 0.109 |
| | TAF | NC | 19 | 95 | 0.120 | 0.625 | 0.211 |
| | OTA | 0.090 | 1 | 5 | 0.040 | 0.040 | 0.040 |
| **Peanuts, roasted** | | | | | | | |
| 20 | $AFB_1$ | 0.033 | 18 | 90 | 0.040 | 0.172 | 0.095 |
| | $AFB_2$ | 0.021 | 2 | 10 | 0.245 | 0.514 | 0.379 |
| | $AFG_1$ | 0.011 | 19 | 95 | 0.040 | 0.172 | 0.097 |
| | $AFG_2$ | 0.014 | 8 | 40 | 0.023 | 0.190 | 0.078 |
| | TAF | NC | 20 | 100 | 0.026 | 0.886 | 0.248 |
| | OTA | 0.090 | 1 | 5 | 0.046 | 0.046 | 0.046 |
| **Dried figs** | | | | | | | |
| 20 | $AFB_1$ | 0.033 | 12 | 60 | 0.036 | 0.131 | 0.092 |
| | $AFB_2$ | 0.021 | 1 | 5 | 0.043 | 0.043 | 0.043 |
| | $AFG_1$ | 0.011 | 15 | 75 | 0.016 | 0.220 | 0.066 |
| | $AFG_2$ | 0.014 | 4 | 20 | 0.023 | 0.110 | 0.059 |
| | TAF | NC | 15 | 75 | 0.028 | 0.332 | 0.159 |
| | OTA | 0.090 | 2 | 10 | 0.036 | 0.227 | 0.131 |
| **Raisins** | | | | | | | |
| 20 | $AFB_1$ | 0.033 | 17 | 85 | 0.036 | 0.182 | 0.109 |
| | $AFB_2$ | 0.021 | 1 | 5 | 0.109 | 0.109 | 0.109 |
| | $AFG_1$ | 0.011 | 16 | 80 | 0.035 | 0.182 | 0.069 |
| | $AFG_2$ | 0.014 | 5 | 25 | 0.039 | 0.113 | 0.074 |
| | TAF | NC | 17 | 85 | 0.074 | 0.389 | 0.203 |
| | OTA | 0.090 | 5 | 25 | 0.023 | 0.505 | 0.179 |

$AFB_1$ – aflatoxin $B_1$, $AFB_2$ – aflatoxin $B_2$, $AFG_1$ – aflatoxin $G_1$, $AFG_2$ – aflatoxin $G_2$, TAF – total aflatoxins, OTA – ochratoxin A, *LOD* – limit of detection, ND – not detected, NC – not calculated.

were announced globally, 49 notifications regarding aflatoxins were made for fruits and vegetables originating from Turkey.

When the hazelnut, peanut, dried fig and raisin sample groups were compared in our present study, it was found that the highest contamination with aflatoxins (100 %) appeared in all hazelnut groups and in peanuts without shell. It was observed that the mean contents were the highest in the roasted hazelnut group (0.600 $\mu g \cdot kg^{-1}$). In the literature, when studies with various nuts and dried fruits from Turkey are examined, there are different studies in which figs had higher aflatoxins contamination rates than hazelnuts [14], and peanuts had higher rates than hazelnuts and dried figs [30]. In a study conducted in Pakistan [13], similar to our present study, the aflatoxins contamination rate and mean content value in peanuts were found to be higher than in case of dried figs or raisins.

**Ochratoxin A in nuts and dried fruits**

Occurrence of OTA in 140 samples of nuts and dried fruits collected from Istanbul was investigated and the results are presented in Tab. 2. The findings showed that 14 out of 140 samples (10 %) were positive for OTA and the highest mean level of 0.179 $\mu g \cdot kg^{-1}$ was observed in the raisins samples. However, all of the samples appeared to have levels of OTA below the recommended legal limit (5–10 $\mu g \cdot kg^{-1}$, not specified for peanuts) [15]. Our study determined that the OTA contamination rate for all hazelnut products was 8.3 % (5 samples) and the content ranges were established as 0.025–0.111 $\mu g \cdot kg^{-1}$. In the earlier reports [31], 2 out of 50 (4 %) samples of hazelnuts had amounts of OTA ≥ *LOD*, with a content range of 0.016–0.152 $\mu g \cdot kg^{-1}$ for samples from nut sellers, bazaars and markets in Ankara and Çorum province, Turkey. When compared with our present study, it was found that both the contamination rates and the content ranges were similar. A study conducted in Libya [32] determined that 13.3 % of 15 hazelnut samples were contaminated with OTA and the contents ranged from 1.5 $\mu g \cdot kg^{-1}$ to 2.2 $\mu g \cdot kg^{-1}$. According to our present study, both the contamination rates and the content ranges were relatively higher. OTA, which is chemically stable, is resistant to heat and is not affected much by baking, roasting or usual processing temperatures, so whether the product is raw or roasted does not cause a significant change in the rate and content of the contamination [33]. In our present study, although the frequency of OTA contamination was relatively low in hazelnut samples, the fact that it was not found in roasted varieties did not indicate a similarity with this feature of OTA. However, in subsequent studies it was suggested that increasing the number of products and making a comparison according to the condition of the products before and after roasting would be more useful in understanding the possible degradation of OTA in various temperature conditions.

It was observed that 2 of 40 peanut samples examined in our study were contaminated with OTA and their mean content values were 0.040 $\mu g \cdot kg^{-1}$ and 0.046 $\mu g \cdot kg^{-1}$. There is no published study investigating OTA contamination in peanuts in Turkey. In samples from Portugal, Cunha et al. [10] documented that 25 % (1 sample) of peanut samples had contamination OTA and the content was 5.3 $\mu g \cdot kg^{-1}$. In a different study carried out in Nigeria [34] with 84 peanut samples, the contamination rate and content range were found to be 2.4 % and 1–3 $\mu g \cdot kg^{-1}$. When our research results are compared with studies conducted in other countries, it is seen that there are some different results as well as similarities. Apart from the variability seen between countries, it has been shown that can produce very different results unclear, it should be reformulated due to factors such as the collection of products in months and years with varying degrees of precipitation, the use of different analytical methods and the small or large number of regions from which the samples were collected.

In our present study, two of the dried fig samples (10 %) and 5 of the raisin samples (25 %) were contaminated with OTA. The content ranges were found to be 0.036–0.227 $\mu g \cdot kg^{-1}$ and 0.023–0.505 $\mu g \cdot kg^{-1}$, respectively. In a previous study [31] investigating the OTA contamination of 50 dried fig and 50 raisin samples collected from two different cities in Turkey, it was found that the contamination rates were 14 % and 42 %, respectively, and the content ranges were 0.083–0.192 $\mu g \cdot kg^{-1}$ and 0.268–0.337 $\mu g \cdot kg^{-1}$, respectively. In a study conducted in Iran [35], it was found that 10.4 % of 48 dried fig samples and 44.7 % of 38 raisin samples were contaminated with OTA. Content ranges were 2.3–14.2 $\mu g \cdot kg^{-1}$ and 2.9–18.2 $\mu g \cdot kg^{-1}$, respectively. RASFF [25] reported that OTA is mainly found in fruits and vegetables, especially raisins and dried figs. In the same report, there were 39 notifications about OTA from Turkey.

When the hazelnut, peanut, dried fig and raisin sample groups were compared, the highest contamination with OTA was found in raisin samples (25 %) and the average OTA content was also the highest in the raisin group (0.179 $\mu g \cdot kg^{-1}$). In a study by Külahi and Kabak [31], including

a large sample group consisting of nuts and dried fruits, similar to our research, it was observed that the highest OTA contamination rate and average content (42 % and 0.303 $\mu g \cdot kg^{-1}$, respectively) belonged to raisin samples. In a study conducted with 320 samples of nuts and dried fruits in Pakistan [2], the highest OTA contamination rate was found in apricots (33.3 %), plums (25 %), and grapes (23.5 %), respectively. The highest content was 4.65 $\mu g \cdot kg^{-1}$ determined in a raisin sample.

In addition, we found that a total of 14 products, namely, hazelnuts with shell (4 samples), raw hazelnust (1 sample), peanuts with shell (1 sample), peanut without shell (1 sample), dried figs (2 samples) and raisins (5 samples) were contaminated with both aflatoxins and OTA. Every product contaminated with OTA was also contaminated with aflatoxins.

**Estimated daily intake**

The estimated contents and estimated daily intakes of aflatoxins and OTA at medium bound (MB) estimate, lower bound (LB) estimate and upper bound (UB) estimate levels in nut and dried fruit samples are presented in Tab. 3. Estimated contents of aflatoxins and OTA at LB, MB and UB levels in these products and estimated daily aflatoxins and OTA intakes of adults consuming these products were calculated at LB, MB and UB levels. The weight of the average adult was considered to be 70 kg. Turkish Statistical Institute reported [36] that per capita daily consumption of hazelnuts, peanuts, dried figs and raisins was 4.4 g, 5.6 g, 0.7 g and 0.7 g, respectively. European Food Safety Authority (EFSA) has not determined the tolerable daily intake (*TDI*) value for aflatoxins, only the benchmark dose lower limit (*BMDL*) 170 $ng \cdot kg^{-1}$ body weight (BW) per day was specified for $AFB_1$. In addition, EFSA determined *BMDL* as 14.5 $\mu g \cdot kg^{-1}$ BW per day to characterize neoplastic effects of OTA [37, 38]. The highest estimated daily intakes at UB level of $AFB_1$, total aflatoxins and OTA in nut and dried fruit samples were 0.016 $ng \cdot kg^{-1}$, 0.025 $ng \cdot kg^{-1}$, and 0.001 $ng \cdot kg^{-1}$ BW per day, respectively (Tab. 3). It is thought that the higher estimated intakes of aflatoxins for hazelnuts and peanuts can be explained by the higher consumption rate of these products and their higher contents of aflatoxins. Estimated daily intakes of OTA were found to be close to each other among the sample groups (0.000–0.001 $ng \cdot kg^{-1}$ BW per day). This was

**Tab. 3.** Estimated contents and estimated daily intakes of aflatoxin $B_1$, total aflatoxins and ochratoxin A in nuts and dried fruits.

| Number of samples | Contaminant | Daily consumption [g] | Mean content in food product [$\mu g \cdot kg^{-1}$] | | | Mean daily intake [$ng \cdot kg^{-1}$] | | |
|---|---|---|---|---|---|---|---|---|
| | | | MB | LB | UB | MB | LB | UB |
| **Hazelnuts** | | | | | | | | |
| 60 | $AFB_1$ | 4.4 | 0.216 | 0.202 | 0.252 | 0.014 | 0.013 | 0.016 |
| | TAF | | 0.347 | 0.316 | 0.403 | 0.022 | 0.020 | 0.025 |
| | OTA | | 0.009 | 0.005 | 0.014 | 0.001 | 0.000 | 0.001 |
| **Peanuts** | | | | | | | | |
| 40 | $AFB_1$ | 5.6 | 0.072 | 0.055 | 0.112 | 0.005 | 0.004 | 0.009 |
| | TAF | | 0.219 | 0.186 | 0.278 | 0.018 | 0.015 | 0.022 |
| | OTA | | 0.006 | 0.002 | 0.011 | 0.001 | 0.000 | 0.001 |
| **Dried figs** | | | | | | | | |
| 20 | $AFB_1$ | 0.7 | 0.050 | 0.033 | 0.081 | 0.001 | 0.000 | 0.001 |
| | TAF | | 0.129 | 0.092 | 0.184 | 0.001 | 0.001 | 0.002 |
| | OTA | | 0.017 | 0.013 | 0.021 | 0.000 | 0.000 | 0.000 |
| **Raisins** | | | | | | | | |
| 20 | $AFB_1$ | 0.7 | 0.080 | 0.067 | 0.111 | 0.001 | 0.000 | 0.001 |
| | TAF | | 0.175 | 0.144 | 0.224 | 0.002 | 0.001 | 0.002 |
| | OTA | | 0.048 | 0.044 | 0.052 | 0.001 | 0.000 | 0.001 |

Mean daily intake is expressed per kilogram of body weight.
MB – medium bound estimate (for those with content < *LOD*, half value of *LOD* was used, for those with content < *LOQ*, half value of *LOQ* was used), LB – lower bound estimate (for those with content < *LOD*, zero value was used, for those with content < *LOQ*, *LOD* was used), UB – upper bound estimate (for those with content < *LOD*, *LOD* was used, for those with content < *LOQ*, *LOQ* was used).
*LOD* – limit of detection, *LOQ* – limit of quantification. $AFB_1$ – aflatoxin $B_1$, TAF – total aflatoxins, OTA – ochratoxin A.

because the daily consumption amount was less in dried figs and raisins with higher OTA contents. In a study conducted by KABAK [14] in Turkey on hazelnuts and dried figs, the estimated aflatoxins intake at UB level from these foods was reported as 0.023 ng·kg$^{-1}$ and 0.005 ng·kg$^{-1}$ BW per day, respectively. In a study in Turkey [31] on the estimated intake of OTA at the UB level, the values were found to be 0.058 ng·kg$^{-1}$, 0.013 ng·kg$^{-1}$, and 0.046 ng·kg$^{-1}$ BW per week for hazelnut, dried figs and raisins, respectively. It was observed that these values corresponded to 0.05 %, 0.01 % and 0.04 %, respectively, of the tolerable weekly intake (120 ng·kg$^{-1}$ BW per week) determined by EFSA [39]. In a study conducted in China [40], the highest estimated intake of OTA from dried figs and raisins was observed at 0.0045 ng·kg$^{-1}$ and 0.2510 ng·kg$^{-1}$ BW per day.

## CONCLUSIONS

In addition to the higher frequency of aflatoxins in nut and dried fruit samples, OTA was also found. It was observed that 89.3 % of all samples were contaminated in varying amounts with $AFB_1$, 93.6 % with TAF and 10 % with OTA. While the frequency of $AFB_1$, TAF, and OTA contamination in nuts was 96.0 %, 99.0 %, and 7.0 %, respectively, the amount ranges were found to be 0.036–7.645 $\mu$g·kg$^{-1}$, 0.026–8.043 $\mu$g·kg$^{-1}$, and 0.025–0.111 $\mu$g·kg$^{-1}$, respectively. In dried fruits, the contamination frequencies of $AFB_1$, total aflatoxins and OTA were 72.5 %, 80 % and 17.5 %, respectively, while the content ranges were determined as 0.036–0.182 $\mu$g·kg$^{-1}$, 0.028–0.389 $\mu$g·kg$^{-1}$, and 0.023–0.505 $\mu$g·kg$^{-1}$, respectively. It could be seen that the aflatoxins load was higher in nuts than in dried fruits, and the OTA load was higher in dried fruits than in nuts. The highest estimated daily intakes of $AFB_1$ and total aflatoxins were seen in hazelnut samples and were determined as 0.016 ng·kg$^{-1}$ and 0.025 ng·kg$^{-1}$ BW per day, respectively. The estimated daily intake of OTA was similar between the groups (0.000–0.001 ng·kg$^{-1}$ BW per day). Nuts and dried fruits have a high consumption rate worldwide. However, they are among the foods in the risk group regarding contamination by mycotoxins. Therefore, preventing this contamination is very important for public health. Regarding this, it is important to determine the sources of contamination and take the necessary precautions while paying attention to temperature and humidity at every stage, from seed to table.

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Received 10 March 2023; 1st revised 8 July 2023; accepted 9 August 2023; published online 15 October 2023.