Quantitation of aflatoxins in pistachios and groundnuts using HPLC-FLD method

Fatma Hepsa, Ozgur Golgeb,*, Bulent Kabakb,

a Ministry of Food, Agriculture and Livestock, General Directorate of Food and Control, Food Control Laboratory, Adana, Turkey
b Hitit University, Faculty of Engineering, Department of Food Engineering, Cevre Yolu, TR-19030 Corum, Turkey

ABSTRACT

In this study, a sensitive high performance liquid chromatography coupled to a fluorescence detector (HPLC-FLD) method after post-column derivatisation was applied for the presence of aflatoxins (AFs) in 151 pistachios and 151 groundnuts from Turkey. Samples were collected between January 2010 and December 2012 in two provinces of Turkey and checked for AFs levels. Quantification limits were 0.11, 0.11, 0.12 and 0.14 μg kg⁻¹ for aflatoxin B₁ (AFB₁), aflatoxin B₂ (AFB₂), aflatoxin G₁ (AFG₁) and aflatoxin G₂ (AFG₂), respectively. AFs were present in 14.6% of pistachios (22/151) and 19.2% of groundnuts (29/151) at total AFs levels ranging from 0.26 to 385 μg kg⁻¹ and from 0.16 to 60.9 μg kg⁻¹, respectively. Seven pistachio samples were above the European maximum tolerable limit (MTL) of 8 μg kg⁻¹ for AFB₁, while total AFs concentration exceeded the MTL of 10 μg kg⁻¹ in eight pistachio samples. For groundnuts, eight and six samples exceeded MTL of 2 and 4 μg kg⁻¹ for AFB₁ and total AFs, respectively.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Aflatoxins (AFs) are major class of mycotoxins produced mainly by two species of Aspergillus, Aspergillus flavus and Aspergillus parasiticus. The four main naturally produced AFs are known as aflatoxin B₁ (AFB₁), aflatoxin B₂ (AFB₂), aflatoxin G₁ (AFG₁) and aflatoxin G₂ (AFG₂). The “B” and “G” refer to the blue and green fluorescent colours produced by these compounds under UV light on thin-layer chromatography plates, while the subscript numbers 1 and 2 indicate major and minor compounds, respectively (Sweeney & Dobson, 1998). A. flavus is more adapted to the aerial parts of plants (leaves, flowers, and fruit) and produces only B AFs, while A. parasiticus produces both B and G AFs and is well adapted to a soil environment (EFSA, 2004).

AFs have been associated with several toxic effects in animal and human health including carcinogenic, mutagenic, teratogenic and immunosuppressive activity (Eaton & Gallagher, 1994). AFB₁ is the most potent genotoxic and carcinogenic AFs and amongst the most commonly found in agricultural products (Sweeney & Dobson, 1998). Furthermore, the International Agency for Research on Cancer (IARC) acknowledges that there is sufficient evidence in humans for the carcinogenicity of naturally occurring AFB₁ and mixtures of AFs (IARC, 1993, pp. 489–521), with a role in the aetiology of liver cancer, notably among subjects who are carriers of hepatitis B virus surface antigens (IARC, 2002, pp. 171–300).

AFs are most likely to contaminate maize and maize products, cottonseed, spices, figs and other dried fruits, groundnuts and tree nuts such as Brazil nuts, pecans, pistachio nuts and walnuts. Among the tree nuts, pistachios are most prone to AFs contamination. The pistachio tree (Pistacia vera L.), belonging to the family of Anacardiaceae, is native to western Asia and Asia Minor, from Syria to Caucasus and Afghanistan. Archeological records of early human pistachio consumption in Turkey date back to as early as 7000 B.C. (Drehel, 2012). Turkey is the third producer of pistachios in the world with a production of 128 000 metric tonnes in 2011, followed by Iran and United States (FAO, 2011). Pistachio kernels are often eaten as snacks, roasted and salted, and are also used in ice cream and confections such as baklava, helva, lokum and chocolate.

The groundnut (Arachis hypogaea L.), which belongs to the family Fabaceae is also a very popular fruit throughout the world due to its high nutrition value and taste. China is the largest producer of groundnut in the world with 16.1 million metric tonnes (about 44.7% of world production), followed by India (19.3%), Nigeria (8.2%)...
and United States (4.6%) (FAO, 2011). Turkey is the leading producer of groundnut in Europe and associated countries with almost 80,000 metric tonnes. The groundnut is used not only as raw, roasted and salted but also for a variety of applications including groundnut oil, groundnut butter, groundnut flour as well as many other uses. Groundnuts have also been reported to be sensitive to AFs contamination in the field conditions before harvest, during post-harvest drying and curing, and in storage and transportation.

The European Union has set maximum levels for certain contaminants in foodstuffs according to Commission Regulation (EC) No 1881/2006 (European Commission, 2006a), which has been amended and replaced with new regulations to revise legal limits and consumption or use as ingredient in foodstuffs. However, the MTL of 2 μg kg⁻¹ for pistachios and apricot kernels, intended for direct human consumption, was taken and transported to the laboratory in an insulated container. According to EC 401/2006, the aggregate sample of dried figs, groundnuts and nuts at retail stage shall be about 300 g (European Commission, 2006b). All samples were ground with Waring blender to produce a homogeneous particle size and stored in a glass container in a refrigerator until analysis.

2.4. Standard solution preparation

Stock solution of aflatoxin standard mix was diluted with methanol to obtain concentration of 10 ng ml⁻¹ for AFB₁, AFB₂, AFG₁, and AFG₂. From this intermediate solution, a series of working standards from 0.1, 0.2, 0.4, 1.2, 2, 4 and 5 ng ml⁻¹ were prepared freshly in LC mobile phase consisting of water–acetonitrile–methanol (6/2/3, v/v/v).

2.5. Extraction and clean-up

The AOAC Official Method 999.31 (Trucksses et al., 1991) was used to detect AFs in pistachio and groundnut samples. This method involves methanol–water extraction, IAC cleanup and liquid chromatography coupled with fluorescence detector after post column derivatisation. Briefly, 25 g of finely-ground subsample was blended with 125 ml methanol–water (87.5:37.5, v/v) and 5 g NaCl using a Waring blender at high speed for 2 min, and filtered using a prefolded filter paper. A 15 ml aliquot of filtrate was diluted with 30 ml water, shaken vigorously and filtered through glass microfiber filter (Whatman GF/A, 125 mm, England). Then, a 15 ml of diluted filtrate was passed through an AfflPrep attached onto a vacuum manifold at a flow rate of about 2–3 ml per min. The column was washed twice with 10 ml of ultrapure water and dried with air. AFs bound to the specific antibody were eluted by passing 1 ml methanol through the column and collected in HPLC vials. The eluate was then diluted with 1 ml of ultrapure water and stored at 4–8 °C until to the HPLC analysis.

2.6. Chromatographic separations

A mixture of potassium bromide (120 mg l⁻¹)–nitric acid (350 μl l⁻¹) and water–acetonitrile–methanol (6/2/3, v/v/v) was isocratically delivered at 1 ml min⁻¹. The column temperature was maintained at 25 °C. The injection volume into HPLC system for both standard and sample was 100 μl. The fluorescence detector was set to an excitation and emission wavelengths of 360 and 430 nm, respectively. The retention times were around 7, 8, 9 and 11 min for AFG₂, AFG₁, AFB₂ and AFB₁, respectively.

Linearity, sensitivity, recovery and accuracy (precision and trueness) were determined to evaluate the performance of analytical method used for AFs. To assess linearity, seven-point calibration curves were constructed over the concentration range of 0.1–5 ng ml⁻¹ for each AFs. Linear regression lines were plotted using the peak area versus the analyte concentration. The linearity was determined by linear regression analysis and expressed as coefficient of determination ($R^2$).

The limit of detection (LOD) and limit of quantification (LOQ) of the analytical method were calculated according to EURACHEM Guide based on data of recovery experiment (EURACHEM, 1998). Blank groundnut samples were spiked with 0.1 μg kg⁻¹ for each analyte and measured in 10 independent replicates. The LODs and LOQs were calculated using the following relations:

$$\text{LOD} = X + 3s,$$
LOQ = X + 10s,

in which, “X” is the mean concentration of fortified sample blank values, and “s” is the sample standard deviation.

The recovery was calculated by the analysis of six representative samples spiked with AFB1, AFB2, AFG1 and AFG2 at two concentration levels of 0.5 and 3 μg kg\(^{-1}\). The observed signal was plotted against the actual concentration. The measured concentration was determined using the obtained calibration curves and the recovery value was calculated by the following equation:

\[
\% \text{ recovery} = 100 \times \frac{\text{measured concentration for spiked sample}}{\text{spiked(added) concentration}}
\]

The accuracy refers to a combination of precision and trueness. The precision of the method in terms of repeatability was evaluated by six-replicated analysis of spiked samples at two concentrations (0.5 and 3 μg kg\(^{-1}\)) of the analyte in the sample on the same day. The precision was calculated as the relative standard deviation (RSD) of replicate results. The trueness, in terms of bias (a measurement of systematic error) was calculated according to the following equation:

\[
\text{Bias (\%)} = \left( \frac{|X_i - X_e|}{X_e} \right) \times 100,
\]

where “X\(_i\)” is the expected value and “X\(_e\)” is measured value.

3. Results and discussion

3.1. Method performance

The analytical method was validated in terms of linearity, sensitivity, recovery and accuracy (precision and trueness) prior to the analysis of samples. Fig. 1A shows an HPLC-FLD chromatogram of AFs standards solution (1.2 μg AFB1 \(1^{-1}\), 1.2 μg AFB2 \(1^{-1}\), 1.2 μg AFG1 \(1^{-1}\) and 1.2 μg AFG2 \(1^{-1}\)). The linear range, linear regression equation and coefficient of determination (R\(^2\)) for each analyte are given in Table 1. The calibration curves revealed good linearity for all analytes in related concentration ranges, with coefficient of determination greater than 0.999.

The LODs and LOQs of the global method, the results of recovery and intra-day precision of the analytical method are summarised in Table 2. The LODs and LOQs were ranged from 0.10 to 0.11 μg kg\(^{-1}\) and 0.11–0.14 μg kg\(^{-1}\) for target analytes, respectively. These values are much smaller than to be enforced limits by European Commission.

The recovery values, ranging between 87.8 and 97.5%, are in good agreement with the Commission Regulation (EC) No 401/2006 (European Commission, 2006b) performance criteria for the EU maximum tolerable limit of 10 μg kg\(^{-1}\) for total AFs. In 129 out of 151 pistachio samples (85.4%), none of the AFs was detected. The total AFs concentration for the positive samples ranged between 0.26 and 385 μg kg\(^{-1}\). AFB1 was detected in 22 pistachio samples (14.6%) at levels ranging from 0.26 to 368 μg kg\(^{-1}\), with a mean level of 31.2 μg kg\(^{-1}\). In seven pistachio samples, AFB1 was found at a level above the EU MTL of 8 μg kg\(^{-1}\), while eight samples exceeded the MTL of 10 μg kg\(^{-1}\) for total AFs. Other AFs occurred less frequently, as expected, with AFB2 being the most abundant, positive in 11.3% (17/151) of pistachio samples. The AFB2 contamination levels varied from 0.11 to 16.7 μg kg\(^{-1}\). Aflatoxin G1 was found in 3 out of 151 pistachio samples (2%), with maximum concentration of 8.72 μg kg\(^{-1}\), while AFG2 was detected in only one pistachio sample (0.7%) at a level of 0.42 μg kg\(^{-1}\). HPLC-FLD chromatograms of naturally contaminated pistachio with AFB1 (20.9 μg kg\(^{-1}\)) and AFB2 (1.9 μg kg\(^{-1}\)), and AFB2-free pistachio are shown in Fig. 1B and C, respectively.

These results confirm the previous observation by Ulca, Evcimen, and Senyuva (2010), who reported 19%, 37% and 36% of pistachio samples commercialised in Turkey tested in 2007, 2008 and 2009, respectively, containing AFB1 at levels above 2 μg kg\(^{-1}\); and AFG2. The 50 sample sets contained excessively high levels ranging from 10 to 477 μg kg\(^{-1}\). In another study, AFB1 was detected in 8 out of 65 pistachio nuts from Turkey (11%) at levels ranging from 0.48 to 36.8 μg kg\(^{-1}\) (Basaran & Ozcan, 2009). However, 60.8% of pistachio nuts collected during the years 2008–2009 from Turkey, was contaminated with AFB1 up to levels of 7.72 μg kg\(^{-1}\) (Set & Erkmen, 2010). Moreover, our previous study showed that the addition of pistachio nuts as a flavouring agent in the manufacturing of traditional Turkish sweet (helva) resulted in the increasing a risk of aflatoxin contamination. While AFB1 was not detected in any of the plain helva and helva with cacao, eight of 34 helva with pistachio nuts (23.5%) contained AFB1, with a mean concentration of 7.5 μg kg\(^{-1}\) (Var, Kabak, & Gök, 2007).

The main world producers of pistachio are Iran, United States, Turkey, China and Syria. Pistachio nuts originating mainly from Iran have been found in many cases to be contaminated with excessive levels of AFB1 and total AFs. In Iran, AFB1 were detected in 95% of pistachios with a mean level of 215 μg kg\(^{-1}\) (Pour, Rasti, Zighamian, & Garmakhanli, 2010). In another survey of 10 068 samples of Iranian pistachios, AFB1 was found in 36.7% of samples with an average level of 5.9 μg kg\(^{-1}\) and 11.8% of pistachios exceeded the maximum limit of 5 μg kg\(^{-1}\) set by national legislations (Cheraghali et al., 2007). In a recent study, no AFs were detected in pistachios originating from United States, Turkey and Spain, whereas 25% of prepacked pistachios and 67% of bulk pistachios from Iran contained AFs but within acceptable limits (Ariño et al., 2009).

It is surprising that no much data on the levels of AFs in pistachios from the United States and exposure assessment of the US population to AFs from the consumption of pistachios have yet been reported. However, a total of 133 notifications on AFs in pistachios originating from the US between the years 2002–2012, of which 61 were border rejection (45.9%), 51 information (38.3%) and 21 alert notifications (15.8%). The AFB1 and total AFs levels in pistachios from the US found up to 1613 and 1727 μg kg\(^{-1}\) respectively (http://webgate.ec.europa.eu/rasff-window/portal).

Our data also showed that AFB1 was found at a level above the EU limit of 8 μg kg\(^{-1}\) in seven pistachio samples, while eight samples exceeded the maximum tolerable limit of 10 μg kg\(^{-1}\) for total AFs. During the years 2002–2012, The Rapid Alert System for Food and Feed (RASFF) reported a total of 5988 notifications in the category “nuts, nut products and seeds” on AFs, of which 2649 (44.2%) concerned pistachio nuts. The number of notifications as regards pistachios has drastically reduced in 2012 in comparison
with last 10 years (Fig. 1). The 28 notifications on AFs in pistachio nuts were mainly from Iran (71.4%) up to levels of 711 μg kg⁻¹ and 823 μg kg⁻¹ for AFB₁ and total AFs, followed by Turkey (28.6%). The frequency of Rapid Alerts presents a serious socio-economic threat for consumers and producers.

The main mycotoxin hazard associated with pistachios preharvest is AFs that are produced by fungi belonging to the genus *Aspergillus* in the growing fruit. The pistachio is a semidry stone fruit consisting of a single kernel enclosed in a thin, bonny shell, which is surrounded by the hull. The main problem with pistachios
is the “early split” formed preharvest. The shell partially splits to varying extents at least a month before maturity and harvest (Codex Alimentarius Commission, 2002). The hull covering the shell usually remains intact, protecting the kernels from invasion by moulds and insects (Boutrif, 1998). Normally, the hull does not rupture when the shell splits in the immature pistachio fruit. However, in a small percentage of the pistachios the shell and the still adhering hull splits together. The hull rupture, often referred to as “early splitting” is a very important for infection with the aflatoxin producing fungi A. flavus/A. parasiticus (Codex Alimentarius Commission, 2002).

Infection of tree nuts with aflatoxicogenic fungi probably occurs most often in the field before and/or during harvest while the kernels are still moist. During the maturation of pistachio nuts, several fungi can colonise and cause decay of the fruits. These fungi include A. flavus, A. parasiticus, Aspergillus ochraceus, Aspergillus niger, Alternaria alternata, Cladosporium herbarum, Stemphylium botryosum, Botryosphaeria dothidea, Fusarium spp. and Pestalotiopsis spp. (Michailides, Morgan, & Doster, 1995). Moreover, pistachio nuts with ruptured hulls can be infected with insects, especially the larvae of navel orangeworm (NOW, Amyloides transitalia). Nuts infested with navel orangeworm are more likely to be moldy and contaminated with AFs than are noninfested nuts (Doster & Michailides, 1999). It has been reported that pistachio kernels infested by the NOW had substantially more infections by A. niger, A. flavus or A. parasiticus, and A. ochraceus or Aspergillus melleus and had 84% of all aflatoxin detected (Doster & Michailides, 1994).

It is well known that climate influences contamination of various crops, in part due to direct effect on the aflatoxin-producing fungi. The contamination process is frequently broken down into two phases with the first phase occurring on the developing crop and the second phase affecting the crop after maturation. Rain and temperature influence the phases differently with dry hot conditions favouring the phases differently with dry hot conditions favouring the former and warm, wet conditions favouring the latter (Cotty & Jaime-Garcia, 2007). Turkey's pistachios are produced mainly in Gaziantep, Şanlıurfa, Siirt and Kahramanmaraş provinces (82%) in the southeast region of Anatolia (TUIK, 2011). The climate of southeastern region of Anatolia is semi-arid continental, with hot and dry long summers and cold and often snowy winters. The average air temperature in the region is between 8 and 31 °C during March through August, while the average annual precipitation varies from 468 to 738 mm (Turkish State Meteorological Service, http://www.mgm.gov.tr).

Climate influences not only the quantity of aflatoxicogenic fungi but also the types of aflatoxin-producers present (Cotty & Jaime-Garcia, 2007). The frequency of B aflatoxins contamination (14.6%) higher than G aflatoxins (2%) in pistachios analysed in this survey can be explained by both occurrence and invasion of pistachios by A. flavus rather than A. parasiticus. In similar, Arriño et al. (2009) reported an incidence of B group AFs in 19% of pistachios, while aflatoxin G series were not detected in any pistachio sample from Iran, USA, Turkey and Spain. In Spain, fungal contamination due to Aspergillus section Nigri, A. flavus and Penicillium spp. in pistachios were also reported (Fernane, Cano-Sancho, Sanchis, Marín, & Ramos, 2010).

3.3. Occurrence of AFs in groundnuts

AFs were found in 29 of 151 groundnut samples (19.2%) with levels ranging from 0.16 to 60.9 µg kg⁻¹ (Table 3). AFB₁ was present in all AFs-positive groundnut samples with maximum concentration of 49.9 µg kg⁻¹. AFB₃ was simultaneously present in 21 (13.9%) groundnut samples. AFB₃ contamination varied from 0.11 to 11.0 µg kg⁻¹. AFB₁ was found in only one groundnut sample (0.7%) at a level of 0.59 µg kg⁻¹, while no AFB₂ was detected in groundnuts above the LOQ of 0.14 µg kg⁻¹. Fig. 1D shows an HPLC chromatogram of naturally contaminated peanut with AFB₁ (0.49 µg kg⁻¹).

For groundnuts, eight and six samples exceeded the European maximum tolerable limits of 2 and 4 µg kg⁻¹ for AFB₁ and total AFs, respectively. The notifications of RASFF in groundnuts originating from Turkey have been reported for the first time in 1998 and there were only 16 notifications up until April 2013. However, the RASFF received a total of 2077 notifications regarding the occurrence of AFs in groundnuts and derived products during the years 2002–2012. Most of these notifications were originating from China (737 notifications, 35.5%), Argentina (386 notifications, 18.6%), Egypt (161 notifications, 7.8%), Brazil (142 notifications, 6.8%), India (119 notifications, 5.7%), USA (104 notifications, 5%) and South Africa (93 notifications, 4.5%).

Groundnuts are mainly cultivated in Osmaniye, Adana and Mersin provinces (91%) in the Mediterranean Region of Turkey, but are widely consumed throughout the country. It has a typical Mediterranean climate, with hot and dry summers and windy winters. In Cukurova, groundnut is planted either in the months of April to mid-May or during June as second crop. The crop is harvested at optimum maturity during early September–November. The average air temperature in the region is 17.5–28.5 °C during April through August and November temperatures can drop below 15 °C. The average yearly precipitation in the region is 616 mm (Turkish State Meteorological Service, http://www.mgm.gov.tr). The susceptibility of groundnuts growing in Cukurova areas to infection with Aspergillus has been reported (Gursoy & Bicici, 2006) and AFs have been detected in several investigations. Both incidence and levels of AFs in groundnuts detected in the current study are lower than previous report from Turkey by Gurses (2006), who found that 38.9% of groundnut samples (7/18) were contaminated with AFB₁ between 2006–2010.
2006). More recently, however, Basaran and Ozcan (2009) detected AFB$_1$ in 16.4% of groundnuts at levels ranging from 0.22 to 33.4 $\mu$g kg$^{-1}$. The main goal for aflatoxin prevention in storage is to prevent fungal development on the groundnuts due to condensation or leaks in warehouse. It is known that stock piling of groundnuts can cause heat built-up and moisture accumulation with resultant mould growth and aflatoxin contamination (Kabak, Var, & Dobson, 2006). In groundnuts, the minimum moisture content for $A. flavus$ growth is 8–10% at around 82% relative humidity, and aflatoxin production on groundnuts is optimum at between 15 and 35% moisture content (Bracket, 1989).

### Table 3

Occurrence of AFS in nut samples commercialised in Turkey.

<table>
<thead>
<tr>
<th>Nut</th>
<th>Parameter</th>
<th>AFB$_1$</th>
<th>AFB$_2$</th>
<th>AFG$_1$</th>
<th>AFG$_2$</th>
<th>AFsTOT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pistachio</td>
<td>Positive samples a (%)</td>
<td>22 (14.6)</td>
<td>17 (11.3)</td>
<td>3 (2)</td>
<td>1 (0.7)</td>
<td>22 (14.6)</td>
</tr>
<tr>
<td></td>
<td>No. samples above EU limit (%)</td>
<td>7 (4.6)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8 (5.3)</td>
</tr>
<tr>
<td></td>
<td>Range ($\mu$g kg$^{-1}$)</td>
<td>0.26–368</td>
<td>0.11–16.7</td>
<td>0.79–8.72</td>
<td>0.42</td>
<td>0.26–385</td>
</tr>
<tr>
<td></td>
<td>Mean of positive samples ($\mu$g kg$^{-1}$)</td>
<td>31.2</td>
<td>2.77</td>
<td>3.49</td>
<td>0.42</td>
<td>37.9</td>
</tr>
<tr>
<td></td>
<td>Median value ($\mu$g kg$^{-1}$)</td>
<td>4.55</td>
<td>0.33</td>
<td>0.07</td>
<td>0.00</td>
<td>1.95</td>
</tr>
<tr>
<td></td>
<td>Median value ($\mu$g kg$^{-1}$)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Groundnut</td>
<td>Positive samples a (%)</td>
<td>29 (19.2)</td>
<td>21 (13.9)</td>
<td>1 (0.7)</td>
<td>&lt;LOQ</td>
<td>29 (19.2)</td>
</tr>
<tr>
<td></td>
<td>No. samples above EU limit (%)</td>
<td>8 (5.3)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6 (4)</td>
</tr>
<tr>
<td></td>
<td>Range ($\mu$g kg$^{-1}$)</td>
<td>0.16–49.9</td>
<td>0.11–11.0</td>
<td>0.59</td>
<td>&lt;LOQ</td>
<td>0.16–60.9</td>
</tr>
<tr>
<td></td>
<td>Mean of positive samples ($\mu$g kg$^{-1}$)</td>
<td>4.99</td>
<td>1.43</td>
<td>0.59</td>
<td>&lt;LOQ</td>
<td>6.05</td>
</tr>
<tr>
<td></td>
<td>Median value ($\mu$g kg$^{-1}$)</td>
<td>0.96</td>
<td>0.20</td>
<td>0.00</td>
<td>0.00</td>
<td>1.16</td>
</tr>
<tr>
<td></td>
<td>Median value ($\mu$g kg$^{-1}$)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

AFsTOT: Sum of AFB$_1$, AFB$_2$, AFG$_1$, and AFG$_2$.

a Positive samples: AFS level > LOQ.

4. Conclusions

This study was designed to detect presence and levels of AFSs in pistachios and groundnuts intended for human consumption in Turkey. AFSs are present in 14.6% of pistachio and 19.2% of groundnut samples analysed, but the contamination levels are found up to 385 and 60.9 $\mu$g kg$^{-1}$, respectively. Compared to groundnuts, pistachio samples show a higher distribution of AFS levels. The data reported in this survey could be used to carry out a preliminary risk assessment on AFSs through nuts consumption. Several codes of practice have been developed by Codex Alimentarius for the prevention and reduction of AFSs in nuts and other foods. The producers and processors should be considered the general principles given in the Code, taking into account Good Agricultural Practices (GAP), followed by the implementation of Good Manufacturing Practices (GMP) and Good Storage Practices (GMP) during the handling, processing, storage and distribution of nuts for human consumption.

Acknowledgements

The authors have declared no conflict of interest.

References


European Food Safety Authority (EFSA). (2004). Opinion on the scientific panel on contaminants in the food chain on a request from the commission related to aflatoxin B$_1$ as undesirable substance in animal feed. The EFSA Journal, 39, 1–27.


