Mycotoxins in food from Jordan: Preliminary survey

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1. Introduction

Mycotoxins are secondary metabolites produced by fungi when they grow on agricultural products before or after harvest or during transportation or storage. The major mycotoxin producing fungi are species of Aspergillus, Fusarium and Penicillium (Kumar, Basu, & Rajendran, 2008). Currently, more than 400 mycotoxins are identified in the world, but the most important groups of mycotoxins that are of major health concern for humans and animals, and occur quite often in food: aflatoxins, ochratoxin A, trichothecenes (deoxynivalenol, nivalenol), zearalenone and fumonisins. T-2 toxin is also found in a variety of grains but its occurrence, to date, is less frequent than the preceding five mycotoxins (Bennett & Klich, 2006; Jordan, 2009; Plants & Chemicals, 2009). Mycotoxins can occur both in temperate and tropical regions of the world, depending on the species of fungi. Major food commodities affected are cereals, nuts, dried fruit, coffee, cocoa, spices, oil seeds, dried peas and beans and fruit (Turner et al., 2009). While a number of surveys and monitoring programmes have been carried out in several countries attempting to obtain a general pattern of mycotoxins contamination in food (Ghali, Hmaissia-Khlifa, Ghorbel, Maaroufi, & Hedili, 2008; Manova & Mladenova, 2009; Moreno et al., 2009; Muller & Schwadorf, 1993; Muthomi, Ndung’u, Gathumbi, Mutitui, & Wagacha, 2008; Villa & Markaki, 2009; Zaied et al., 2009; Zinedine et al., 2006), there is no available information on the occurrence of mycotoxins in food consumed by the Jordanian population. This study was undertaken to determine the presence of mycotoxins in foods from Jordan. For this purpose, a total of 108 samples of different groups of foods widely consumed by the Jordanian population were collected during 2008–2009 years. Samples were analyzed for contamination with aflatoxins, ochratoxin A, deoxynivalenol, fumonisins, zearalenone and T-2 toxin by direct competitive enzyme-linked immunosorbent assay (ELISA). The predominant mycotoxin was ochratoxin A with a mean level of 4.17 μg kg⁻¹ in 25% of analyzed samples. Furthermore, aflatoxins, deoxynivalenol and fumonisins were detected with a contamination frequency of 3%, 4% and 2%, respectively. The present report is the first one ever carried out on the occurrence of mycotoxins in food consumed by the Jordanian population. © 2010 Elsevier Ltd. All rights reserved.
information about the occurrence of mycotoxins in foods consumed in Jordan. Jordanian population consumes great amounts of cereals (e.g. wheat, barley and rice), nuts (e.g. walnuts and peanuts) and coffee. Actually, large amounts of cereals, nuts and coffee commercialized in Jordan are imported and little is known about eventual mycotoxin contamination. Furthermore, Jordan is considered a favourable climatic, geographic and social conditions for the growth of fungi and their toxins. Therefore, the objective of present study was to determine the incidence of aflatoxins, ochratoxin A, deoxynivalenol, fumonisins, zearalenone and T-2 toxin in foods consumed in Jordan, using ELISA.

2. Materials and methods

2.1. Samples

A total of 108 samples were randomly collected from different supermarkets and small shops in Jordan, during September 2008 and March 2009. The selected commodity groups were: cereals including wheat, barley and rice, legumes as bean, chickpea and fababean, green coffee and nuts grouped as peanut, walnut, pistachio and hazelnut, and sunflower and sesame seeds. Samples were stored in plastic bags at 5 °C until the analysis.

2.2. Method and analysis

The method used in this study was enzyme-linked immunosorbent assay (ELISA). Commercial ELISA kits were purchased from Romer Labs Singapore Pte Ltd.: AgraQuant™ total aflatoxin (COKAQ1000), AgraQuant™ ochratoxin (COKAQ2000), AgraQuant™ fumonisins (COKAQ3000), AgraQuant™ deoxynivalenol (COKAQ4000), AgraQuant™ zearalenone (COKAQ5000) and AgraQuant™ T-2 toxin (COKAQ6000). According to manufacturer’s description, the detection limits for aflatoxins, ochratoxin A, fumonisins, deoxynivalenol, zearalenone and T-2 toxin were 3, 1.9, 200, 200, 20 and 35 μg kg⁻¹, and the quantification limits were 4, 2, 250, 250, 40 and 75 μg kg⁻¹, respectively.

2.2.1. Samples preparation

Samples preparation and test method were conducted according to the instructions outlined in the Romer Labs ELISA kits (Romer Labs, 2002, 2003, 2007), as described below. All commodity samples except nuts, sunflower and sesame seeds were ground to a fine powder such that over 75% of the material passed through a 20-mesh sieve. Nuts, sunflower and sesame seeds were finely chopped and then used to extract mycotoxins. Subsamples of 20 g were mixed with 100 ml of methanol:water (70:30, v/v) for aflatoxins, ochratoxin A, fumonisins, zearalenone and T-2 toxin and 100 ml of distilled water for deoxynivalenol and shaken in a waring blender at high speed for 3 min. The extract was allowed to settle, and then filtered through a Whatman (Maidstone, UK) filter paper (No. 1). A 5-ml filtrate was diluted at 1:4, 1:10 and 1:20 with distilled water, respectively, for deoxynivalenol, T-2 toxin and fumisin, and at 1:5 with methanol:water (70:30, v/v) for zearalenone. For aflatoxins and ochratoxin A, filtrate was used in ELISA without any further dilution.

2.3. Analysis of mycotoxins in samples by ELISA

According to the manufacturer’s instructions, a sufficient number of microtiter wells were inserted into the microwell holder for all standards and samples to be run in duplicate. Briefly, standard solutions and prepared samples (100 μl) were mixed with 200 μl of conjugate in individual dilution wells, and then 100 μl from each dilution well was transferred to a respective antibody-coated well. After 10 min incubation at room temperature for ochratoxin A, zearalenone and T-2 toxin, and 15 min for aflatoxins, fumonisins and deoxynivalenol, wells were washed five times with 250 μl distilled water. Substrate (100 μl) was added to each well and incubated for 5 min at room temperature. Following the addition of stop solution (100 μl) to each well, the intensity of the resulting yellow color was measured at a wavelength of 450 nm using ELISA 96-well plate reader (Anthos 2010; Anthos Labtecn Instruments, Salzburg, Austria). The absorbance values obtained for standards and the samples were divided by the absorbance value of the first standard (zero standard) and multiplied by 100 (percentage of maximum absorbance). The absorption intensity was found to be inversely proportional to mycotoxin concentration in the samples. The log-log sheets supplied with the kits were used to generate a standard curve and to calculate the concentration of each mycotoxin in the samples.

Prior to analysis of the samples, the ELISA method was validated to ensure data quality. Validation of ELISA was carried out by determination of recoveries and the standard deviations (SD) of uncontaminated samples spiked at level of 20 μg kg⁻¹ for aflatoxins and ochratoxin A, 250 μg kg⁻¹ for deoxynivalenol and fumonisins and 300 μg kg⁻¹ for zearalenone and T-2 toxin. Additionally, the repeatability (RSDr) and reproducibility (RSDr) were also calculated at spiking levels as mentioned above. With regard to repeatability, this parameter was estimated under repetitive conditions at the same day while reproducibility was estimated at time intervals (at four different days of the month). In the present study, the average recoveries with their SDs were 98.7 ± 9.3%, 99.5 ± 9.3%, 100 ± 6.9%, 99.3 ± 3.9%, 107.7 ± 7.7% and 110.9 ± 11.2%, respectively, for aflatoxins, ochratoxin A, deoxynivalenol, fumonisins, zearalenone and T-2 toxin. The repeatability and reproducibility were in the range of 1–2.9% and 3.7–11.1%, respectively for all mycotoxins studied. These performance characteristics of the method used were found within the acceptable ranges indicated in Commission Regulation No. 401/2006 (European Commission Regulation, 2006a) for method of sampling and analysis of official control of mycotoxins. Furthermore, an inter-laboratory proficiency test with internationally recognized Food Analysis Performance Assessment Scheme (FAPAS®) was successfully done.

3. Results and discussion

A total of 108 food samples (63 cereals, 15 nuts, 11 green coffee, 14 legumes, three sunflower and two sesame seeds) were analyzed for mycotoxins. The occurrence of aflatoxins, ochratoxin A, deoxynivalenol, fumonisins, zearalenone and T-2 toxin in the analyzed samples is presented in Table 1. Of the 108 samples analyzed, 26 (24%) were found to be contaminated with mycotoxins. The incidence and levels of mycotoxins varied in samples. The most prevalent was ochratoxin A, followed by deoxynivalenol, aflatoxins and fumonisins. However, zearalenone and T-2 toxin were not detected (less than limit of quantification) in the samples analyzed.

The incidence of ochratoxin A in cereals was 14.6% (7/48) with a mean level of 2.8 μg kg⁻¹ (Table 2). Our findings showed a high incidence of ochratoxin A in wheat (29%) compared with barely (12.5%) and rice (4.3%). The contamination levels in wheat and rice were below the maximum tolerable limit of ochratoxin A set by European Union Commission regulation in raw cereal grains (5 μg kg⁻¹); however, in barley it was higher than this limit (European Commission Regulation, 2006b, 2007). Cereals represent a main food for the Jordanian population; therefore it has a high social, economic and nutritional relevance. Furthermore, they are usually stored in conditions which favour mould growth and mycotoxin production. Worldwide ochratoxin A has been widely detected in cereals and cereals-derived products (Bento, Pena, Lino,
imposed limits for ochratoxin A in coffee, 5.0 µg kg\(^{-1}\). Ochratoxin A, generally have higher levels of ochratoxin A than coffees from Latin America or Asia (Food, 2006). As of April 2005, the European Union imposed limits for ochratoxin A in coffee, 5.0 µg kg\(^{-1}\) for roasted coffee and 10.0 µg kg\(^{-1}\) for instant coffee. Limits for green coffee beans were not imposed, however, a number of European nations have already set maximum tolerable limits for ochratoxin A in green coffee beans; the most stringent is Italy, at 8 µg kg\(^{-1}\) (Bayman & Baker, 2006).

Of the 15 nuts samples analyzed, 47% of samples were contaminated with ochratoxin A at levels varying from 2.75 to 7.42 µg kg\(^{-1}\). All contaminated samples of nuts (one hazelnut, two peanut and four walnuts) were below the maximum tolerable limit of ochratoxin A (10 µg kg\(^{-1}\)) set by European Union Commission regulation (European Commission Regulation, 2006b, 2007). In comparison, surveys carried out in Morocco, reported incidences rate of ochratoxin A in walnuts and peanuts of 35% and 25%, respectively. The averages for ochratoxin A in positive samples of peanut and walnut were 0.68 and 0.11 µg kg\(^{-1}\), respectively (Zinedine et al., 2006). Jordanian population consumes large amounts of nuts directly or as ingredients included in special sweets and cookies prepared during the ‘Ramadan’ fasting month and festival days. Almost all nuts such as pistachio, hazelnut, walnut and peanut consumed in Jordan are imported and little is known about their quality. Consequently, there is an importance to study the presence of mycotoxins, since there is a lack of information in the literature about their occurrence in these products.

Coffee beans, like other crops, can be contaminated by microorganisms during different stages of growing, harvesting, processing, transport and storage. Many studies revealed that the important toxigenic fungal genera (Aspergillus and Penicillium) are natural coffee contaminants, and are present from the field to storage (Food, 2006; Nakajima, Tsubouchi, Miyabe, & Ueno, 1997; Silva, Schwan, Dias, & Wheals, 2000). Of the 11 green coffee samples analyzed, 63% of samples were contaminated with ochratoxin A at levels varying from 2.19 to 6.57 µg kg\(^{-1}\). Presence of ochratoxin A in green coffee bean was reported by several authors in concentration ranging between 0.2 and 360 µg kg\(^{-1}\) (Noomin, Maha-karanchanakul, Nielsen, Frisvad, & Samson, 2008). Levels of ochratoxin A contamination, and probably the species that produce ochratoxin A, vary from region to region. Coffees from Africa generally have higher levels of ochratoxin A than coffees from Latin America or Asia (Food, 2006). Of April 2005, the European Union imposed limits for ochratoxin A in coffee, 5.0 µg kg\(^{-1}\) for roasted coffee and 10.0 µg kg\(^{-1}\) for instant coffee. Limits for green coffee beans were not imposed, however, a number of European nations have already set maximum tolerable limits for ochratoxin A in green coffee beans; the most stringent is Italy, at 8 µg kg\(^{-1}\) (Bayman & Baker, 2006).

Currently, ochratoxin A is receiving increasing attention for its toxic effects on human health and high incidence in a wide range of food commodities. Although the ochratoxin A amounts detected in food from Jordan were relatively low, the levels may accumulate in the body of humans consuming contaminated food. Ochratoxin A is often not rapidly removed from the body and it is frequently found in human blood due to its long elimination half-life (about 35 days in serum), as a consequence of its binding to plasma proteins, its enterohepatic circulation and its reabsorption from urine (Studer-Rohr, Schlatter, & Dietriech, 2000). This makes of ochratoxin A the most detected mycotoxin in human blood all over the world (Pena, Seifrtova, Lino, Silveira, & Solich, 2006). Therefore, public health authorities in Jordan should urgently pay attention to ochratoxin A particularly by monitoring food and feedstuff. At present, Jordan has not a maximum tolerable limits for ochratoxin A in foodstuffs, but there is a tendency of Jordan Food and Drug Administration (JFDA) to adopt the European Union limits for this mycotoxin. JFDA is currently the central regulatory agency involved in food safety control in Jordan.

Aflatoxins were detected in 3% (2/67) of the analyzed samples, with mean level of 9.62 µg kg\(^{-1}\) in walnut and rice. Aflatoxins levels in both samples exceeded the maximum tolerable limits of 4 µg kg\(^{-1}\) for total aflatoxins (B1, B2, G1 and G2) in various food intended for direct human consumption or use as an ingredient in foodstuffs, including rice and walnut (European Commission Regulation, 2006b, 2007). Aflatoxins have been detected in various food commodities from many parts of the world (Ariño et al., 2009; Reddy, Reddy, & Muralidharan, 2009; Zinedine et al., 2006) and are presently considered as one of the most dangerous contaminants of food and feed.

Table 1
Occurrence of mycotoxins in foods from Jordan, as detected by ELISA.

<table>
<thead>
<tr>
<th>Mycotoxins</th>
<th>No. analyzed samples</th>
<th>No. positive samples (%)(^a)</th>
<th>Mean of contamination(^b) (µg kg(^{-1}))</th>
<th>Range of contamination (µg kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ochratoxin A</td>
<td>89</td>
<td>22 (25%)</td>
<td>4.17</td>
<td>2.04–7.42</td>
</tr>
<tr>
<td>Aflatoxins</td>
<td>67</td>
<td>2 (3%)</td>
<td>9.62</td>
<td>5.16–14.08</td>
</tr>
<tr>
<td>Deoxynivalenol</td>
<td>51</td>
<td>2 (4%)</td>
<td>600</td>
<td>250–950</td>
</tr>
<tr>
<td>Fumonisins</td>
<td>55</td>
<td>1 (2%)</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>Zearealenone</td>
<td>50</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-2 toxin</td>
<td>61</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Percentage of contamination.

\(^b\) Mean contamination of positive samples.

Table 2
Ochratoxin A levels in different analyzed samples of food from Jordan, as detected by ELISA.

<table>
<thead>
<tr>
<th>Food commodity</th>
<th>No. analyzed samples</th>
<th>No. positive samples (%)(^a)</th>
<th>Mean of contamination(^b) (µg kg(^{-1}))</th>
<th>Range of contamination (µg kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereals</td>
<td>48</td>
<td>7 (14.6%)</td>
<td>2.8</td>
<td>2.04–5.86</td>
</tr>
<tr>
<td>Wheat</td>
<td>17</td>
<td>5 (29%)</td>
<td>2.26</td>
<td>2.04–2.56</td>
</tr>
<tr>
<td>Barely</td>
<td>8</td>
<td>1 (13%)</td>
<td>5.86</td>
<td></td>
</tr>
<tr>
<td>Rice</td>
<td>23</td>
<td>1 (4%)</td>
<td>2.17</td>
<td></td>
</tr>
<tr>
<td>Nuts</td>
<td>15</td>
<td>7 (47%)</td>
<td>5.64</td>
<td>2.75–7.42</td>
</tr>
<tr>
<td>Green coffee</td>
<td>11</td>
<td>7 (63%)</td>
<td>4.09</td>
<td>2.19–6.57</td>
</tr>
<tr>
<td>Legumes</td>
<td>10</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sesame seeds</td>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sunflower seeds</td>
<td>3</td>
<td>1 (33%)</td>
<td>4.34</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Percentage of contamination.

\(^b\) Mean contamination of positive samples.
Mladenova, 2009; Moreno et al., 2009; Muthoni, Ndung’u, Guthumbi, Mutiti, & Wagacha, 2008; Villa & Markaki, 2009; Zinedine & Mañes, 2009). Aflatoxin B1 and ochratoxin A are among the most frequent observed combinations of mycotoxins in different plant products (Speijers & Speijers, 2004). The co-occurrence of mycotoxins can affect both the level of mycotoxin production and the toxicology of the contaminated grain resulting in additive and synergistic effects. Sedmikova, Reisnerova, Dufkova, Barta, and Zilek (2001) found that ochratoxin A could increase the mutagenicity of aflatoxin B1 in the case of their simultaneous occurrence in the same substrate. Other mycotoxins detected were deoxynivalenol and fumonisins, with incidence rate of 4% for deoxynivalenol and 2% for fumonisins. Two samples of rice and one sample of barley were found contaminated with deoxynivalenol and fumonisins, respectively. However, none of the samples exceeded the European Union maximum tolerable limits for deoxynivalenol and fumonisins in cereals (European Commission Regulation, 2006b, 2007).

4. Conclusion
As far as we know, this is the first report to have shown the contamination of food available in Jordan by mycotoxins. The overall results demonstrate that 24% of food samples (n = 108) from Jordan showed contamination by ochratoxin A, aflatoxins, deoxynivalenol and fumonisins. This could be explained firstly by the climatic conditions especially humidity and temperature of Jordan which are in favour of the fungal growth and toxin production; secondly by the fact that Jordan does not have enough economical sustenance to control the storage conditions and to prevent, also, the fungal contamination in food. Although low levels of these mycotoxins were detected in most samples, accumulation of such mycotoxins is expected to create adverse health problems to human fed on the contaminated food. Accordingly, frequent analytical surveillance program by food control agencies is highly recommended to control the incidence of mycotoxic contamination in Jordan especially in cereals and nuts.

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References