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Investigation of aflatoxins levels in commercial dried figs from western Turkey

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Abstract

The aim of the present work was to investigate aflatoxins in dried figs from Izmir, Turkey. A total of 1,973 samples of dried figs were obtained from retail shops and public outlets from 2014 to 2018. Aflatoxin contamination was determined by high-performance liquid chromatography coupled with fluorescence detection (HPLC-FLD) following clean-up by immunoaffinity (IAC). The obtained results were evaluated against the maximum limit set by the Turkish Regulation and European Commission for total aflatoxins (AF_S) and aflatoxin B_1 (AFB_1). The number of samples exceeding the legal limit was 40 according to the Commission Regulation (EU) No 1058/2012 for the limit of aflatoxin B_1 (6 µg/kg), and 42 samples exceeded the legal limit for the total aflatoxin content (10 µg/kg). Thirty-four and forty-two samples of dried figs exceeded the legal limit for AFB_1 and AF_S (8 and 10 µg/kg, respectively) based on the Turkish Food Codex.

Introduction

Mycotoxins, a large group of low molecular weight compounds (below 1,000 Da), are secondary metabolites generated by several filamentous moulds such as *Aspergillus, Fusarium, Penicillium,* and *Alternaria*. The presence of mycotoxins in food products is a grave threat to human health. Therefore, the investigation of their occurrence in food systems is of extreme importance (Heshmati *et al.,* 2017). These moulds are capable of growing when the temperature, relative humidity, and product moisture are favourable (Iamanaka *et al.,* 2007). More than 400 mycotoxins have been identified; aflatoxins, fumonisins, ochratoxins, zearalenone, and trichothecenes, and are regulated internationally because of their adverse effects on human and animal health (Njumbe Ediage *et al.,* 2015). *Aspergillus flavus* and *A. parasiticus* are well-known aflatoxin-producing species (Peromingo *et al.,* 2016). Thus far, 20 analogues of aflatoxin have been reported, notable among which is aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), and aflatoxin G_2 (AFG_2) (Figure 1), which are the most widespread contaminants of foods and feeds. Among them, AFB_1 is the most carcinogenic (Group 1 carcinogen) based on the International Agency for Research on Cancer on (IARC, 1993). Since 2008, the Committee on Food Additives of Joint FAO/WHO (JECFA) has evaluated mycotoxins as potential human carcinogens and has urged that dietary exposure should be reduced to the lowest practicable levels to reduce the potential risk as much as possible (WHO, 2008). Currently, too many distinct regulations are applied to food and feed production chains all over the world. Hence, there is an increasing need for technology to reduce and monitor aflatoxin contamination in production, handling, storage, processing, and packaging processes (Prietto *et al.,* 2015). The European Commission declared the maximum residue level (MRLs) of AFB_1 to be 6 µg/kg and those of total aflatoxin (AF_S) to be 10 µg/kg in dried fruit and processed products in Commission Regulation (EU) No 1058/2012. The Turkish Food Codex (TFC, 2011) accepted and used the same regulation for aflatoxin contamination (Bircan and Koç, 2012). The limits of AFB_1 and AF_S are 5 and 10 µg/kg, respectively, in various foods in Turkey (TFC, 2011).

To analyse mycotoxins in foods, several sample preparation strategies have been developed, including on-line solid-phase extraction (on-line SPE), matrix solid-phase dispersion, dispersive liquid-liquid microextraction, accelerated solvent extraction, and immunoaffinity columns (Sanzo *et al.,* 2018).

Dried fruits have high nutritional value in the human diet. However, these products also provide a suitable medium for mould growth and further mycotoxin contamination (Heshmati *et al.,* 2017). Dried figs are one of the most popular commercial dried fruits in the world, and they contain high value nutritional components and minerals (Benalia *et al.,* 2016). Dried figs are one of the main traditional and commercial products of the Aegean region in Turkey (Karbancıoglu-Güler and Heperkan, 2009). Figs can be consumed fresh, from cans or as a dried fruit and fig paste. Since Turkey is the largest exporting country of figs, supplying 52%

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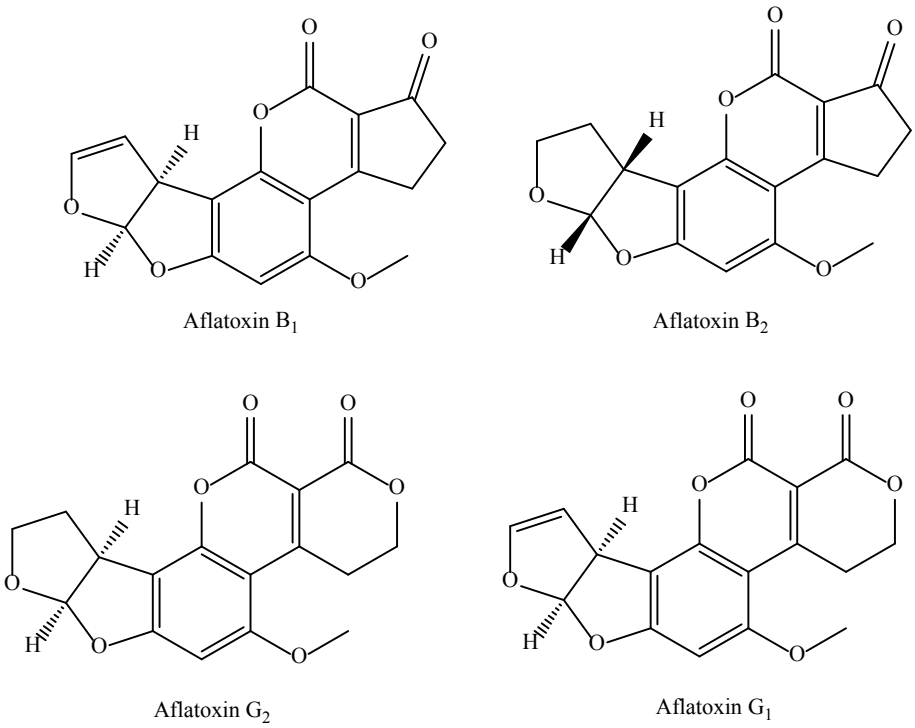

Figure 1. The chemical structure of aflatoxins.

of the world's dried fig exports, it occasionally faces export problems due to contamination by AFs (Heperkan *et al.,* 2012). Fig composition and the climate conditions of the growing area are viable conditions for mould contamination, which is the major contributor to reduced quality. Particularly, aflatoxin-producing *Aspergillus* species are the most important problem in dried figs and related products. AFB_1, AFB_2, AFG_1, and AFG_2 have been detected in samples contaminated with *A. parasiticus*. AFB_1 and AFB_2 were detected in *A. flavus*-contaminated samples (Şenyuva *et al.,* 2007). Temperatures between 27 and 38°C, 0.99 water activity (a_w), and high relative humidity (85%) are excellent conditions for the development of *Aspergillus*. Attention has been focused on blue / green fluorescence on the surface of dried figs and its correlation with aflatoxin contamination, which led to the use of UV light to screen figs. This process has been the standard practice for quality control in Turkey for many years and has helped to reduce the overall levels of aflatoxin contamination in figs marketed for human consumption (Şenyuva *et al.,* 2008). Aflatoxins cause both significant health concerns and economic losses (Bircan and Koç, 2012). Aflatoxin contamination is commonly confirmed via the nailing method (Durmuş *et al.,* 2015). In this method, a special knurled needle is used to penetrate fig samples to fix aflatoxin residues, and then, the needle is placed under a UV lamp. However, it is important to determine the contamination level in this kind of product due to the adverse effects of aflatoxins on human health.

The aims of the present work were therefore to determine the AF_S levels in dried figs consumed domestically in the Aegean region of Turkey by high performance liquid chromatography coupled with fluorescence detection (HPLC-FLD) and the potential impact of the aflatoxin exposure limits of Turkey and the European Union.

Materials and methods

Samples

A total of 1,973 dried fig samples were obtained (≈1,000 g per sample) during the period of 2014 - 2018 from retail shops and public outlets in the Aegean region of Turkey. The samples were brought to the laboratory and stored at 4°C until further analysis. Each sample was mixed in a blender (Robot Coupe R23, Jackson, MI, USA), and 50 g was taken for analysis.

Chemicals, reagents, and standards

The aflatoxin mixture standard (catalogue No. 46304-U) was purchased from Supelco® (Bellefonte, PA, USA) as 1 µg of AFB_1 and AFG_1, 0.3 µg of AFB_2 and AFG_2, in 1 mL of methanol. The main stock solution concentration was 260 ng/mL in methanol, and stored in the dark at -18°C. The stock solution was regenerated every three months. Working calibration standard solutions were prepared daily from the stock solution in MeOH:H_2O in ranges of 0.2 - 8.0 ng/mL for AFB_1 and AFG_1, and 0.06 - 2.4 ng/mL for AFB_2

and AFG2. Aflatest™ immunoaffinity columns (IAC) were purchased from VICAM (Watertown, MA, USA). Analytical grade chemicals were used in all experiments.

Aflatoxin extraction and clean-up

A high-speed blender (Robot Coupe R23, Jackson, MI, USA), Waring blender (Waring 8011S, Torrington, CT), and Sartorius analytical balance (model ED323S-CW) were used for the extraction of aflatoxins. A 50 g test portion was weighed from each homogenised sample and mixed with 250 mL extraction solution (MeOH:H_2O (3:2)) and 4 g of NaCl for 3 min in a Waring blender (Waring 8011S, Torrington, CT) until homogeneous. The slurry was filtered through filter paper (Whatman no. 4). Next, 10 mL of phosphate buffer saline (PBS) solution was added to 5 mL filtrate. The solution was loaded on an Aflatest ™ IAC at 1 - 2 drops/min, followed by washing with 20 mL distilled water. The aflatoxins were eluted with 1 mL of HPLC grade methanol, and then 1 mL of HPLC grade water using at 1 - 2 drops/s. Finally, 100 µL of extracted samples were injected to HPLC.

Aflatoxin determination by HPLC

Chromatographic analyses were performed via high-performance liquid chromatography coupled with fluorescence detection (HPLC-FLD). HPLC analyses were carried out using a HPLC 1200 (Agilent, Santa Clara, USA) system coupled with post-column derivatisation using a cobra cell (100 µA; R-Biopharm Rhone Ltd, Glasgow, UK); detection was performed by a fluorescence detector (excitation 362 nm, emission 455 nm) with a C_{18} column (Agilent, Santa Clara, USA) (25 cm, 4.6 mm, 5 µm). The mobile phase was water:acetonitrile:methanol (6:2:3), 132 mg potassium bromide, and 385 µL 5 M nitric acid. The temperature of the column was adjusted to 40°C with a flow rate of 1 mL/min. Next, 100 µL of extracted samples were injected. A calibration curve (0.52 - 20.8 ng/mL) was established for each aflatoxin.

Method validation

The HPLC method for the determination of each AFs in dried figs was validated in terms of linearity, limit of detection (LOD), limit of quantification (LOQ) and recovery. To assess linearity, calibration curves were constructed by using the peak area of analytes against concentrations. Linear regression analysis was performed for the determination of the linearity. Models having coefficient of determination (R^2) value of >0.99 for each AFs was accepted. Data analysis were performed by using Microsoft Excel Software (ver. 2017).

Results and discussion

Method performance

The linearity of the relative response was evaluated by injecting seven standard calibration solutions. The signal-to-noise approach, which is defined as the levels that result in signal-to-noise ratios of 3 and 10, was used to calculate the limit of detection (LOD) and limit for quantification (LOQ). The analytical response and the chromatographic noise were used as measurement conditions, and they were adjusted by using a blank sample and the aflatoxin calibration curve (0.52 - 20.8 ng/mL) (Table 1) (Pietri *et al.,* 2012). The HPLC measurement method was based on Directive 2006/401/EC, and the results were evaluated against the Commission Regulation (EU, 2012). The chromatogram of an aflatoxin-contaminated sample is shown in Figure 2. A known blank of dried fig sample (six replicates) was used to determine the recovery percentages. The average recoveries were between 94.05 and 100.42% (Table 2), with satisfactory relative standard deviations (RSDs), completely fulfilling the performance criteria of Regulation 401/2006 of the European Communities (EU, 2006). The proficiency test of the National Food Reference Laboratory (UGRL) was performed to assess the accuracy of the method. The analytical scope of AFB_1, AFB_2, AFG_1, and AFG_2 was properly identified in this analysis method; the *z* scores were -1.2, -1.4, -0.6, -0.3, respectively, which are acceptable values ($z < 2$).

Table 1. Linearity range, limit of detection (LOD), and limit of quantification (LOQ) for aflatoxins.

Analyte	Linearity range (µg/kg)	Linear regression equation	R^2	LOD (µg/kg)	LOQ (µg/kg)
AFB_1	0.20 - 8.0	*y = 7.59x – 0.41*	0.9999	0.46	0.59
AFB_2	0.06 - 2.4	*y = 4.66x – 0.91*	0.9997	0.13	0.14
AFG_1	0.20 - 8.0	*y = 7.99x – 0.36*	0.9998	0.43	0.50
AFG_2	0.06 - 2.4	*y = 3.99x – 0.72*	0.9997	0.13	0.15

Aflatoxin occurrence in samples

In the present work, a total of 1,973 dried fig samples were analysed for aflatoxins. During the period of 2014 - 2018, around 437, 390, 387, 467, and 292 of these samples were tested. Table 3 shows a summary of the aflatoxins found in dried figs. Two hundred-seventy (13.68%) dried fig samples contained detectable residues at or under the maximum level set by the Turkish Food Codex. The mean value of AFs was 8.50 µg/kg and the overall mean level of AFB_1 was 5.70 µg/kg in dried fig samples.

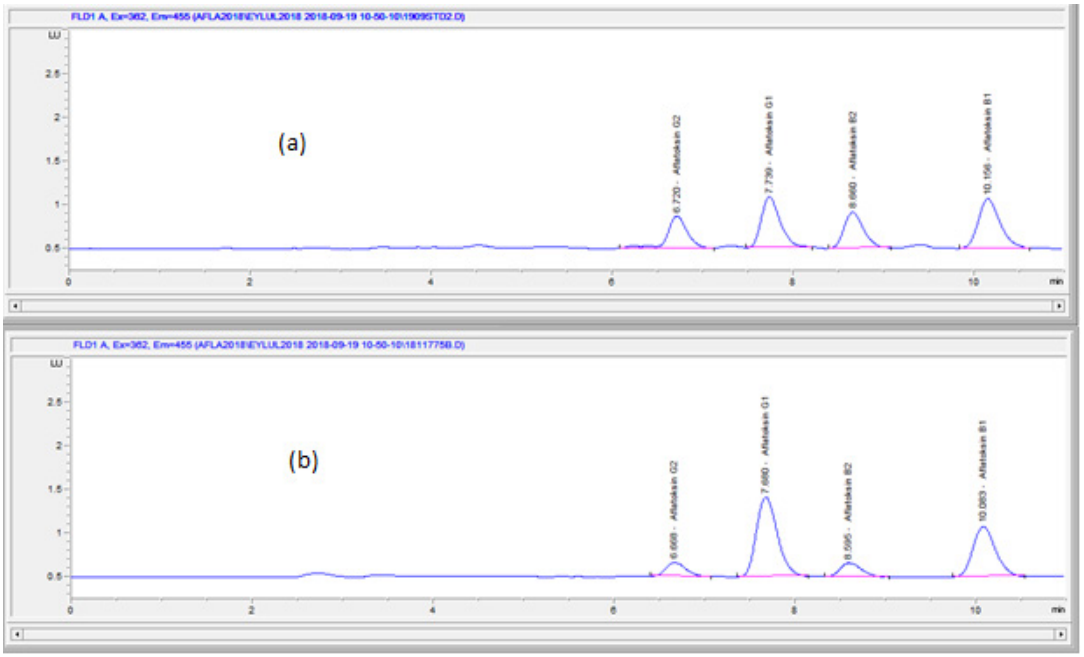

Figure 2. HPLC-FLD chromatograms, (a) standard solution of aflatoxin (AFG_2: 0.48 ng; AFG_1: 1.60 ng; AFB_2: 0.48 ng; AFB_1: 1.60 ng), and (b) naturally contaminated dried figs (AFG_2: 0.30 ng; AFG_1: 2.36 ng; AFB_2: 0.28 ng; AFB_1: 1.79 ng). Injection volume = 100 µL.

Table 2. Recovery data for aflatoxins in dried figs.

Analyte	Level of spiking (µg/kg)	Recovery (%)	Intra-day repeatability, %RSD	Inter-day repeatability, %RSD
AFB_1	0.40	100.42	5.11	4.06
	3.20	99.18	0.33	0.34
AFB_2	0.12	97.74	4.29	2.82
	0.96	99.43	0.78	0.23
AFG_1	0.40	94.05	3.14	3.90
	3.20	99.54	1.24	0.29
AFG_2	0.12	99.32	2.64	2.71
	0.96	99.39	1.62	0.52

AF_S were detected in 42 dried fig samples (2.13%) in accordance with the European Commission Directive limit, and the level of AFB_1 was higher than the accepted maximum limit in 40 samples. AFB_1 contamination ranged from 0.59 to 69.92 µg/kg, and AF_S contamination ranged from 0.14 to 132.37 µg/kg. AFB_1 contamination was detected over the maximum regulatory limit (8 µg/kg) in 1.72% of samples, and one sample exceeded the maximum limit (AFB_1) according to the Turkish Food Codex (TFC, 2011) in the 2015 samples. Eleven dried fig samples contained AFB_1 levels above the maximum, in the range of 8.03 - 69.92 µg/kg, in the 2018 samples. No AF_S contamination was found in the 2015 samples, while levels over the maximum approved level were detected in 5, 15, 10, and 12 samples from the 2014, 2016, 2017, and 2018 samples, respectively, based on the Turkish Food Codex limits. Aflatoxin contamination was not detected in 84.14% of all the dried fig samples. However, the percent of contamination detected increased yearly using the maximum level standards. Using the European Commission maximum level (1058/2012) for dried figs of 6 µg/kg for AFB_1, values exceeding the limit were identified in samples from all years. Based on those standards, the highest number of samples exceeding the maximum levels was in those from 2017.

A study by Kabak (2016) has determined the level of AFs contamination in dried figs. However, the contamination level detected was higher than that presented in the present work. The highest contamination was reported in the high-level range of 117.9 to 471.9 µg/kg in dried figs (Karaca and Nas, 2006). These results show that preventative efforts have been increased. Moreover, the location of the samples might be related to their commercial activities, and producers cannot risk economical losses due to aflatoxin contamination.

In another study, 6% of exported dried fig samples (130 samples) were found to be non-compliant and unfit for human consumption according to

Table 3. AFB_1 and AF_S in dried figs.

Years	Parameter	Sample size / above TFC MRLs	Sample size / above EU MRLs	Range (min-max, µg/kg)	Mean of positive samples (µg/kg)
2014	AFB_1	437/3	437/6	0.83 - 12.15	3.17
	AF_S	437/5	437/5	0.14 - 13.54	4.03
2015	AFB_1	390/1	390/1	0.61 - 8.64	1.73
	AF_S	390/0	390/0	0.20 - 9.37	2.87
2016	AFB_1	387/10	387/12	0.59 - 32.30	6.08
	AF_S	387/15	387/15	0.30 - 56.07	9.15
2017	AFB_1	467/9	467/10	0.63 - 60.76	5.95
	AF_S	467/10	467/10	0.22 - 87.75	7.47
2018	AFB_1	292/11	292/11	0.61 - 69.92	7.24
	AF_S	292/12	292/12	0.28 - 132.37	13.14

European Directive regulations (2 µg/kg for AFB_1 and 4 µg/kg for AF_S). However, this percentage could be decreased to 2.2% by application of the national AF_S limit (10 µ/kg). AF contamination was detected between 0.1 and 28.2 µg/kg in 12.3% of samples, and AFB_1 was observed as the major contaminant in 12.3% of samples at a range of 0.1 - 12.5 µg/kg (Bircan and Koç, 2012). AFB_1 was found to be higher than 2 µg/kg in six samples and the total AF_S level was obtained at or up to 4 µg/kg higher than the European Commission criteria (Kabak, 2016).

Sharman *et al.* (1991) reported AFs contamination in 8.6% of figs in the United Kingdom. However, contamination was not monitored at a detectable level in Germany (Reinhold and Reinhardt, 2011). Forty-nine dried figs were exposed to AF_S contamination at 0.62 µg/kg in Catalonia, Spain (Cano-Sancho *et al.,* 2013). Azaiez *et al.* (2015) analysed AF_S contamination in 28 dried figs sold in Spanish and Tunisian markets (14 samples were from Spain; the others were from outside Spain), and most of the samples in their study were imported from Turkey and Iran. AFG_1 contamination was detected at 3.96 -6.38 µg/kg (European Commission permitted level of 10 µg/kg for AF_S) in 4 samples (EU, 2010). The samples showed varieties of local contamination, but there was no contamination in the Spanish samples.

The dried fig processes and packing conditions can be favourable for fungal contamination. Particularly, *Aspergillus* species can synthesise various toxic and carcinogenic contaminants, such as aflatoxins. For this reason, it is very important that they be detected in figs before they arrive in markets or are exported (Özer *et al.,* 2016). Aflatoxin levels in dried figs may exceed the relatively high levels that could trigger harmful effects in humans. In the present work, AF_S contamination and its level were precisely detected by analytical HPLC-FLD via post-column derivatisation. Forty-three dried fig samples (2.18%) were detected to exceed the maximum level for AF_S according to the Turkish Food Codex standard and 45 (2.28%) according to the European Union directives. However, 84.14% of dried fig samples showed no contamination throughout the four years of this study by Turkish Food Codex.

Conclusion

Non-European countries consume more aflatoxin-containing dried figs than European countries due to the European Union directives. This increased consumption of contaminated figs can cause important labour loss due to the impact on human health in non-European Commission citizens. Periodic monitoring and control of all processes should be performed carefully to avoid aflatoxin contamination. Hence, mould contamination control methods should be developed to detect and prevent mould growth. All reports on aflatoxin contamination suggest that more sensitive, economical, and efficient equipment should be developed to protect against AF_S contamination. In addition, governments must apply district control protocols from production to marketing.

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