

AFLATOXIN AND OCHRATOXIN IN VARIOUS TYPES OF COMMONLY CONSUMED RETAIL GROUND SAMPLES IN ANKARA, TURKEY

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Abstract: To detect aflatoxin (AF) or ochratoxin A (OTA) contamination, 25 retail ground samples of 12 different types of seed-, pulses-, and cereal-flours and starches were randomly collected from markets and traditional bazaars in Ankara, Turkey. The levels of AF in the retail ground samples were determined by high performance liquid chromatography (HPLC) and ranged from 0.03–3.16 ppb. The percentage of contaminated samples for aflatoxin B₁, B₂, G₁, and G₂ were 64, 60, 72, and 76%, respectively. The determination of OTA level was performed by enzyme-linked immunosorbent assay (ELISA), and they were ranged between 0.27–4.07 ppb (n=24). However, the screened mycotoxin levels in the samples were under the permission limits of Turkey, the daily intake of these products corresponds to at least 50 % of daily diet in our country. Routine measurements of the toxin levels in foods and feeds should be carried out to prevent their harmful effects on health.

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INTRODUCTION

A variety of moulds routinely infect the world's cereal crops. Mycotoxins are the secondary metabolites produced by fungi in foods and feeds, on ingestion, which can result in the illness or death of animals and humans. A number of mycotoxins and their effects on human and livestock health have been identified [1, 2, 6, 18, 21]. These natural food contaminants are found in different kinds of food, spices, and beverages, such as cereals, beans, dried fruits, coffee, cocoa, wine, beer, spice, juice, and milk [6, 7, 12, 18, 19, 20, 27]. Mycotoxins such as aflatoxins (AFs) and ochratoxin A (OTA) can grow easily according to the unsuitable conditions of growth, harvest, transport, and storage [1, 4, 9, 28, 31, 33].

Aflatoxins can contaminate agricultural commodities including corn, wheat, rice, peanuts, and many other crops

[3, 31]. AFs, each of which is a group of closely related mycotoxins, may be produced by 3 species of *Aspergillus*-*A. flavus*, *A. parasiticus*, and the rare *A. nomius*-which contaminate plants and plant products. *A. flavus* produces only the B type of aflatoxin, while the other 2 species produce both aflatoxins B and G [10]. The severity of acute and chronic toxicity, which reflects the role played by epoxidation of the 8,9-double bond, is AFB₁ > AFG₁ > AFB₂ > AFG₂ [21] and these 4 compounds are distinguished by the colour of their fluorescence under long-wave ultraviolet illumination [6, 21]. AFB₁ is the most potent hepatocarcinogen known for mammals, and the risk assessment of AFB₁ is very well established [6, 10]. On the other hand, OTA is produced by *Penicillium verrucosum* and by *A. ochraceus*, together with a low percentage of isolates of the closely related *A. niger*. These 3 groups of species differ in their ecological niches,

in the commodities they affect, and in the frequency of occurrence in different geographical regions. OTA was found more frequently and at high average concentrations in human plasma samples obtained from people living in the regions where a fatal human kidney disease (Balkan Endemic Nephropathy, BEN) occurs. The disease is known to be associated with an increased incidence of tumours of the upper urinary tract [7, 10, 14, 26]. Nevertheless, similar results have been found in some other European countries where BEN has not been observed. This could be due to the difference of the analytical methods and eating habits [10]. Because of its resistance to technological processes, OTA can also be found in cereal and meat derivatives [7, 26].

Turkey has encountered the AF contamination problem in different foods exported and/or consumed in the country since 1967 [8]. Since 2002, in cereal products, the permitted limit for AFB₁ and total AFs (B₁+B₂+G₁+G₂) in our country are 2 ppb and 4 ppb, respectively. The limit for OTA is 3 ppb in all types of cereal products [23]. Although there are some studies on the levels of mycotoxins in different foods produced and consumed in Turkey, limited data exists on the concentrations of aflatoxins in cereal and cereal products [13, 24, 25, 30]. Therefore, this study has been performed to investigate ochratoxin A and aflatoxin B₁, B₂, G₁, and G₂ contaminations of randomly selected retail ground samples.

MATERIALS AND METHODS

Samples. Retail ground samples (n=25) of 12 different types of seed-, pulses-, and cereal-flours (wheat, barley, oat, rye, vetch, corn, rice, lentil, soy) and starches (wheat, corn, and rice) were randomly collected from supermarkets and traditional bazaars in Ankara, Turkey in the period 2002–2003. The samples were stored in plastic bags at –20°C until the analysis. These samples were screened for OTA content, and each AF level such as B₁, B₂, G₁, G₂ were determined.

Aflatoxin analysis by HPLC. Aflatoxin standards (B₁, B₂, G₁, G₂) and trifluoroacetic acid (TFA) were purchased from Sigma Chemical Co (St Louis, MO, USA). HPLC grade methanol (MeOH) and acetonitrile (ACN) were obtained from Riedel (Poole, Dorset, UK). The other chemicals and solvents were obtained from Merck (Darmstadt, Germany). The immunoaffinity columns and filter papers were from Vicam (Watertown, MA, USA).

Each AF standard was prepared in a final concentration of 0.25 pg/ml in ACN: toluene (90:10, v/v). The concentrations of aflatoxins were checked by UV absorption spectrophotometer (Shimadzu UV 160, Tokyo, Japan) as described in the AOAC official method for aflatoxin analysis [11]. Each reference standard was used to prepare the working standards and the calibration curve.

AF B₁, B₂, G₁, G₂ were eluted from the samples by using immunoaffinity columns, and quantified with HPLC. The AFs were extracted from each 50 g ground

sample with a solvent mixture of ACN: water (60:40, v/v) by blending vigorously for 1 minute in a blender at high speed. The extract was filtered through fluted filter paper, and the filtrate was diluted and mixed with deionized water (10:40, v/v). The mixture was applied to an immunoaffinity column (AflaOchratest, Vicam) containing antibodies specific for AFs B₁, B₂, G₁, G₂ and ochratoxin A. The aflatoxins were eluted with MeOH into acid washed vials. The eluate was evaporated to dryness under a gentle flow of nitrogen at room temperature. The dry residues were derivatized with TFA using an adapted method [11].

Determination of aflatoxin levels in the sample extracts was performed by isocratic reversed-phase liquid chromatography, using a Spherisorb S50DS2 column (4.6 mm × 25 cm, 5µm) and with a fluorescence detector and computing integrator (HP, Agilent 1100 series, Vienna, Austria); excitation and emission wavelengths were 360 nm and 430 nm, respectively. The mobile phase was water: ACN: MeOH mixture (62:16:22, v/v/v), and the flow rate was 1.0 ml/min.

The concentration of each aflatoxin in the samples was separately calculated by using the individual calibration curves obtained from the peak heights of each aflatoxin. Standards and samples were analyzed in duplicate. Aflatoxin level in each retail ground sample was expressed as ppb. Since the detection limit for HPLC method was 0.03 ppb, the samples with aflatoxin level below this concentration were considered as non-detectable.

Ochratoxin A analysis by ELISA. The analytical grade chemicals were purchased from the following suppliers: sodium hydrogen carbonate (NaHCO₃) and hydrochloric acid (HCl) from Merck (Darmstadt, Germany), and dichloromethane (DCM) from Carlo Erba Reagenti (Milan, Italy). OTA levels were determined by a commercially available ochratoxin A ELISA kit (Ridascreen®, R-Biopharm AG, Darmstadt, Germany).

In order to determine ochratoxin A by ELISA, according to the instructions of the manufacturer, 1 N HCl solution was added on each ground sample (2 g) and shaken, then the sample mixture was extracted with DCM. Following the centrifugation (3500 g, 4°C, 15 minutes), DCM phase was collected and mixed with equal volume of 0.13 M NaHCO₃ buffer (pH 8.1). The removed aqueous phase was diluted by the NaHCO₃ solution (pH 8.1), and used for the ochratoxin A ELISA kit.

The optical density was measured at 450 nm by using ELISA 96-well microplate reader (Sunrise, GmbH, Tecan, Austria). OTA contamination in each retail ground sample was expressed as ppb. According to the manufacturer's description, the detection limit for OTA by ELISA was 0.025 ppb.

RESULTS

In this study, 25 retail ground samples were analyzed for the levels of OTA by ELISA, and each AF by HPLC.

The concentrations of AFB₁, AFB₂, AFG₁, and AFG₂, and OTA detected in all samples are given in Table 1.

The retention times for the AFs were: 8.2 min for B₁, 16.7 min for B₂, 6.2 min for G₁ and 11.7 min for G₂. A sample HPLC chromatogram of each aflatoxin is shown in Figure 1. The recoveries were carried out in duplicate on blank wheat samples, spiked with aflatoxin B₁, B₂, G₁ and G₂ at a concentration of 0.2 ppb for each. The average recoveries were 53.95% for B₁, 51.33% for B₂, 89.60% for G₁ and 47.63% for G₂.

DISCUSSION

Mycotoxins are highly toxic secondary products of the metabolism of some fungi mainly belonging to *Aspergillus*, *Penicillium*, and *Fusarium* spp. Monitoring of mycotoxins depend on precise and reliable analytical methods. However, there is no universal method for detection and quantification of mycotoxins in feeds or foods because the mycotoxins are chemically quite diverse, as are the matrices in which they occur. While common analytical methods employ different separation/detection techniques, such as ELISA, HPLC or thin layer chromatography, all procedures require a suitable sample extraction step [15, 32]. The immunological based methods are preferable because they may reduce the time and expense required for analyses of these naturally occurring toxicants [29]. Additionally, the ELISA technique is highly sensitive, specific and can be automated to analyze numerous samples. In the present study, first of all, total aflatoxins of the samples were determined by HPLC. In the meantime, the same samples were applied to the ELISA in order to get a quick idea of the OTA levels.

A total of 25 samples (22 flour and 3 starch) were analyzed for total aflatoxin and ochratoxin A by ELISA. Twenty-four samples contained OTA ranged from 0.31–4.07 ppb, as shown in Table 1. Of the 25 samples, 3 samples (12%) were found to be contaminated with ochratoxin, which surpassed the levels established as guideline levels (3 ppb) in Turkey. 21 samples had OTA contamination below 1 ppb. According to the Turkish regulations, ochratoxin A limits were set at 3 ppb in cereal products (including all types). The detection limit for OTA by ELISA was also below the tolerance levels that have been under discussion for several years within the European Union [27].

64% of the samples were found to contain AFB₁ with the levels between 0.03–1.61 ppb. These amounts are considerably below the levels (2 ppb) established in Turkey as guideline levels for cereal-based feeds. AFB₂ was determined in 15 samples (60%) with the levels ranging from 0.03–0.18 ppb. The concentrations of AFG₁ were found to range from 0.03–2.79 ppb in 72 % of the samples. AFG₂ was detected in 19 of the samples (76%) with the levels between 0.03–0.15 ppb. The levels of total AFs (B₁+B₂+G₁+G₂) of all samples were determined to range between 0.03–3.16 ppb. As shown in Table 1,

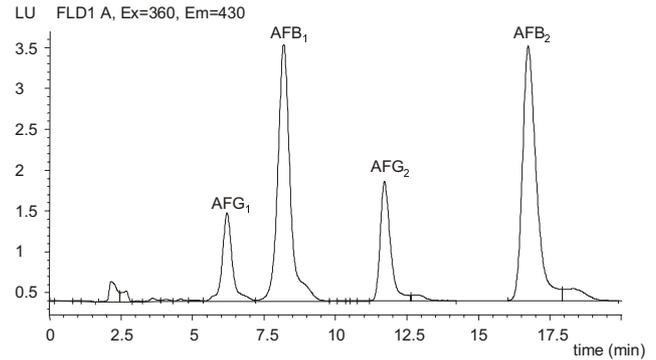


Figure 1. An HPLC chromatogram of aflatoxin standards B₁, B₂, G₁, and G₂.

Table 1. Aflatoxin (AFB₁, AFB₂, AFG₁, AFG₂; ppb) and ochratoxin A (OTA; ppb) levels in retail ground samples (n=25) obtained by HPLC and ELISA, respectively.

No.	Source	HPLC				ELISA	
		AFB ₁	AFB ₂	AFG ₁	AFG ₂	(B ₁ +B ₂ +G ₁ +G ₂)	OTA
Wheat-based flours							
1	Wheat	0.03	0.04	n.d.	n.d.	0.07	0.78
2	Wheat	n.d.	n.d.	0.03	n.d.	0.03	0.48
3	Wheat	0.08	n.d.	0.03	0.07	0.18	0.38
4	Wheat	0.08	n.d.	0.10	0.08	0.26	0.90
5	Wheat	0.09	0.08	0.08	0.08	0.33	0.78
6	Wheat	0.21	0.03	0.09	0.13	0.46	0.49
7	Wheat	n.d.	n.d.	n.d.	0.03	0.03	2.23
8	Wheat	0.03	0.04	0.06	0.05	0.18	0.36
9	Wheat	0.08	0.03	0.03	0.10	0.24	0.50
10	Wheat	n.d.	n.d.	n.d.	0.04	0.04	0.71
11	Wheat	n.d.	n.d.	0.03	0.05	0.08	0.68
12	Wheat	0.12	n.d.	n.d.	n.d.	0.12	0.70
Other cereal-based flours							
1	Corn	0.03	0.06	0.05	0.10	0.24	0.47
2	Corn	n.d.	0.03	n.d.	0.05	0.08	n.d.
3	Potato	n.d.	0.11	0.03	n.d.	0.14	0.32
4	Lentil	0.57	0.05	0.03	0.06	0.71	0.83
5	Rice	0.71	0.16	n.d.	0.03	0.90	0.27
6	Vetch	n.d.	n.d.	0.03	n.d.	0.03	0.71
7	Soy	0.94	0.18	0.03	n.d.	1.15	0.57
8	Barley	n.d.	0.07	2.79	0.30	3.16	4.07
9	Rye	0.91	0.06	0.04	0.03	1.04	3.69
10	Oat	n.d.	n.d.	0.03	0.03	0.06	3.45
Starches							
1	Wheat	0.03	0.03	0.05	0.15	0.26	0.31
2	Rice	1.61	0.10	0.03	0.04	1.78	0.44
3	Corn	0.15	n.d.	n.d.	0.08	0.23	0.33

n.d. non-detectable

AFG₁ and total AFs (B₁+B₂+G₁+G₂) levels in the barley-flour, were determined to be higher than the other ground samples. According to the European aflatoxin regulations, the AFB₁ and total AF limits were set at 2 and 4 ppb, respectively, in groundnuts, nuts, dried fruit, cereals (including buckwheat) and processed products for human consumption [9]. Although these results are also under the permitted levels of 4 ppb for total AFs (B₁+B₂+G₁+G₂) in Turkey, it seems that there are not any ground samples without contamination of AFs. Ground seed-, pulses-, and cereal-flours and starches are the main sources of foods for Turkish population and the daily intake of these products corresponds to at least 50% of daily diet, especially in the Anatolian region. Therefore, the mycotoxin contamination detected in the samples is a considerable problem despite the low levels.

On the other hand, sweeteners and/or starch are usually produced from starchy materials maize and wheat, etc. It has been shown that aflatoxins were destroyed during starch conversion [3]. In this study, none of the starches containing the tested mycotoxins was above the permission limits.

Cereal is widely grown in Turkey, which is one of the major wheat producing countries in the world. In our country, cereal and its products are crucial foodstuffs for feeding humans and animals. Additionally, the average daily consumption of wheat and wheat products and retail ground products by a Turkish adult is about twice as high as than most Western countries. Daily consumption of these products is estimated to be approximately 400 g and this amount corresponds to almost 50% of daily diet [17].

Any mycotoxin contamination in foods and feeds may cause economic loss and threaten public health, which is an important problem encountered from time to time in our country. Although some results obtained from the studies on the levels of mycotoxins in different type of foods in Turkey [5, 13, 16, 17, 22, 24, 25, 30, 34], the data are limited, especially the levels in grain and grain products [13, 24, 25, 30]. In this study, the results pointed out that the contamination of the aflatoxin and ochratoxin in the retail ground samples in Ankara, Turkey, were under the permitted levels. Nevertheless, overall daily intake of the mycotoxins should be considered for evaluation of their health risk.

CONCLUSION

The contaminations should be monitored routinely for food safety. Overall, the integrated, multidisciplinary research on mycotoxins contaminations has provided the scientific platform on the base decisions regarding acceptable exposures and priorities for interventions to reduce human risk in public health.

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