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Aflatoxin levels in wheat samples consumed in some regions of Turkey

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Abstract

Aflatoxins (AFs), the secondary metabolites produced by species of *Aspergilli*, specifically *Aspergillus flavus* and *Aspergillus parasiticus*, have harmful effects on humans, animals, and crops that result in illnesses and economic losses. Wheat that is susceptible to these fungi infections through its growth, harvest, transport, and storage, is the most important staple food in Turkey. Therefore, this study has been undertaken to determine the AFB_1, AFB_2, AFG_1, AFG_2 levels by HPLC in forty-one wheat samples grown and consumed in some regions of Turkey. The concentrations of total AFs in the wheat samples were determined to be ranging from 10.4 to 643.5 ng/kg. Fiftynine percent of the samples were found to be positive for total AFs. The percentage of positive samples for AFB_1, AFB_2, AFG_1, and AFG_2 were 42, 12, 37, and 12%, respectively. Although the detected levels are under the permitted levels for AFs in cereals, these amounts should be considered in regard to overall daily exposure to mycotoxins.

Keywords: Aflatoxin; Wheat; HPLC; Turkey

1. Introduction

Aflatoxins (AFs) are toxic secondary metabolites produced by species of *Aspergilli*, especially *Aspergillus flavus* and *Aspergillus parasiticus*. These fungi can grow on certain foods and feeds under favorable conditions of temperature and humidity and generate AFs before and/or during harvest, handling, shipment and storage (Bushby & Wogan, 1979; Bushby & Wogan, 1984; Peraica, Radic, Lucic, & Pavlovic, 1999). The four major naturally occurred AFs are known as aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2). AFs have been shown to be potent carcinogens, mutagens and teratogens (Hendrickse, 1997; Kotsonis, Burdock, & Flamm, 2001; Peraica et al., 1999). AFB_1, the most toxic compound in this series, has been found to be one of the most potent carcinogens occurring naturally and it was classified as Group I human carcinogen by the International Agency for Research on Cancer (IARC) in 1987 (Bushby & Wogan, 1979; IARC, 1987). The primary target organ for toxicity and carcinogenicity is liver in human and animals and the toxicity is known to be mediated by metabolism in the liver by cytochrome P450 system to the highly reactive AFB_1-8, 9-epoxide (Garner & Martin, 1979; Peraica et al., 1999; Williams et al., 2004). The metabolic effects of AFs include: inhibition of DNA, RNA and protein synthesis; reduction in miscellaneous enzyme activities; depression of glucose metabolism; inhibition of lipid synthesis, including that of phospholipids, free fatty acids, triglycerides and cholesterol and its esters; and depression of clothing factor synthesis (Bushby & Wogan, 1981). The increased risk of hepatocarcinoma is caused by deletion mutations in the P53 tumor-suppressing gene and by activation of dominant oncogenes (Dragan & Pitot, 1993).

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On the other hand, AFM_1 and AFM_2 are the hydroxylated metabolites of AFB_1 and AFB_2 formed by cytochrome P450 1A2 and may be found in milk and milk products obtained from livestock that have ingested contaminated feed. AFM_1 and AFM_2 have lower toxicity than the parent compound, but significant because of the widespread consumption of milk and milk products especially by infants (Creppy, 2002; Hendrickse, 1997; Kotsonis et al., 2001).

The occurrence of AFs in food has been recognized as potential threat to human health, either caused by direct contamination via grains and grain products or by "carry over" of mycotoxins and their metabolites in animal tissues, milk and meat after intake of contaminated feedstuffs (Bushby & Wogan, 1984; Kotsonis et al., 2001). There exist a great number of reports that suggest intoxication of humans by the consumption of AF-contaminated agricultural products (Hendrickse et al., 1982; Krishuamachari, Bhat, Nagarajan, & Tihak, 1975; Ngindu, Kenya, & Ocheng, 1982; Williams et al., 2004). Epidemiological studies have shown that AF exposure is associated with increased risk of hepatocellular carcinoma, particularly in combination with hepatit B virus (IARC, 1993; IARC, 2002). The potency of AFs appears to be significantly enhanced in individuals with hepatitis B infection (Hermann & Walker, 1999). Additionally, the food contamination by AFs causes serious economic losses. Due to their frequent occurrence and their severe toxicity, guidelines and tolerance levels of AFs have been set in several countries including Turkey.

Turkey has been encountered the AF contamination problem in different foods exported and/or consumed in the country since 1967 (Camlibel, 1995). Wheat that is susceptible to these fungi infections through its growth, harvest, transport and storage, is the most staple food in our country. Turkey is one of the major wheat-producing countries in the world. The average daily consumption of wheat and wheat products by an average Turkish person is about two times as high as 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 (Koksal, 1990). Although there are some studies on the levels of mycotoxins in different foods consumed and produced in Turkey, the limited studies exist on the concentrations of AFs in cereal and cereal products (Aycicek, Yarsan, Sarimehmetoglu, & Cakmak, 2002; Duru & Sahin, 1979; Gokmen & Acar, 2000; Nizam & Oguz, 2003; Omurtag & Beyoglu, 2003; Omurtag & Yazıcıoglu, 2000; Omurtag, Atak, Yurdun, & Ersoy, 1998; Simsek & Bozkurt, 1992). Therefore, this study has been undertaken to determine AFB_1, AFB_2, AFG_1, and AFG_2 levels in order to provide some information on AF levels in wheat seeds grown and consumed in some regions of Turkey.

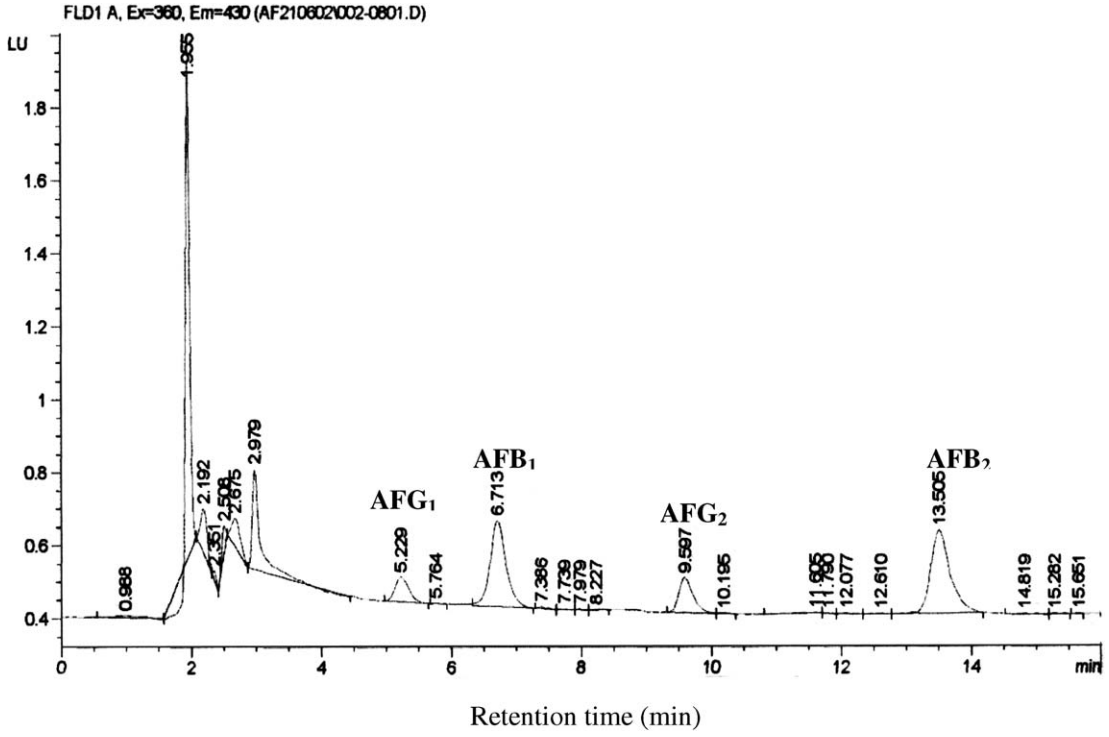

Fig. 1. HPLC chromatogram of AF standards. The amount of each AF standard applied was 20 pg.

2. Materials and methods

2.1. Chemicals and reagents

Methanol and acetonitrile were HPLC grade and purchased from Riedel (Poole, Dorset, UK). Trifluoroacetic acid (TFA) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). All the other chemicals and solvents were purchased from Merck (Darmstadt, Germany). For sample clean up, immunoaffinity columns were from Vicam (Watertown, Ma, USA).

2.2. Standards

Standards for AFB_1, AFB_2, AFG_1, and AFG_2 were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Stock solutions and standards were prepared and assayed according to AOAC Method 971.22 (AOAC International, 1995a).

2.3. Sample collection and preparation for analysis

Forty-one samples of wheat that were harvested in the summer of 2002–2003, obtained from Turkish Grain Board Office (TGBO), the largest wheat collection, distribution and storage organization, street bazaars and markets in a minimum weight of 200 g in the autumn period of the same years. The grains were grounded and stored in plastic bags at −20 °C until the analysis.

For each sub-sample, 25 g of the ground wheat was weighed into an acid washed pyrex flask at room temperature and extracted with 100 ml methanol:water (80:20, v/v) containing 5 g of sodium chloride by blending vigorously for 1 min in Waring Blender at high speed. The extract was filtered through fluted filter paper (24 cm, Vicam, Watertown, MA, USA) and 10 ml of the filtrate was diluted into 50 ml with deionized water and mixed vigorously. The mixture was passed through microfibre filter paper (11 cm, Vicam, Watertown, MA, USA) and 20 ml of filtrate (equivalent to 1 g wheat sample) was loaded on the immunoaffinity column (Aflatest, Vicam, Watertown, MA, USA). The column was washed two times with 10 ml of deionized water and the AFs were eluted by passing 1 ml of HPLC grade methanol through column at a rate of 1–2 drop/second into acid washed HPLC vials. The eluate was evaporated to dryness under a flow of nitrogen at room temperature. The dry residues were dissolved in 200 μl *n*-hexane. TFA (50 μl) was added and incubated for 5 min at room temperature for derivatization. Phosphate

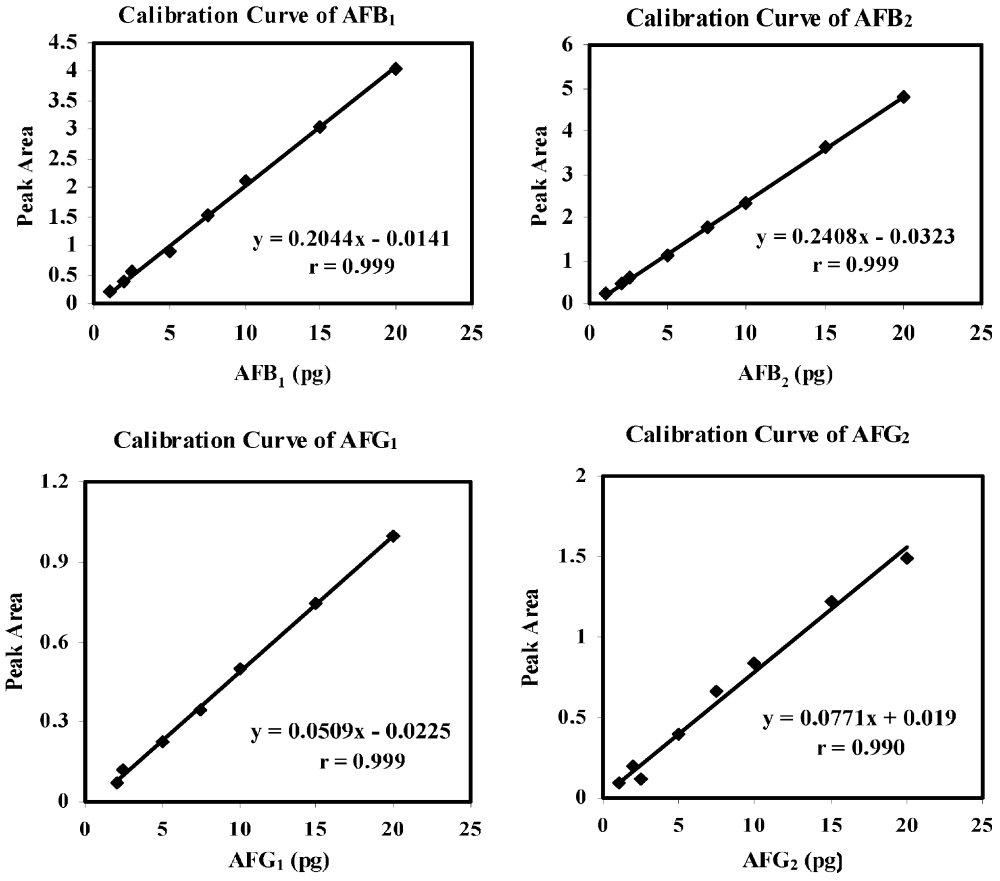

Fig. 2. The calibration curves of AFB_1, AFB_2, AFG_1 and AFG_2 standards.

buffer (950 μl) (pH 6.5): ACN (9:1; v/v) was added and the derivatized sample was left for 10 min in order to let the layers to be separated. The upper *n*-hexane layer was discarded. The derivatized sample (100 μl) was injected onto HPLC. The standards and samples were protected from direct light during all procedures (AOAC International, 1995b).

2.4. Analysis of AFB_1, AFB_2, AFG_1 and AFG_2 by HPLC

Determination of AFB_1, AFB_2, AFG_1 and AFG_2 levels in standards and the derivatized samples were carried out by HPLC equipped with an auto sampler (Hewlett Packard (HP) Agilent 1100 Series, Vienna, Austria) using a fluorescence detector (excitation at 360 nm, emission at 430 nm). A Spherisorb S5ODS2 column (3.8 mm i.d. and length 25 cm, 5 μm particle size, Waters, Milford, MA, USA) was used. The mobile phase was deionized water:acetonitrile:methanol (62:16:22, v/v) and the flow rate was 1 ml/ min. The injection volume was 100 μl. The retention times for the AFs were 5.2 min for AFG_1, 6.7 min for AFB_1, 9.6 min for AFG_2 and 13.5 min for AFB_2. An HPLC chromatogram of AFs is shown in Fig. 1. Recovery studies were performed on blank samples of wheat spiked with levels of 10 μg/kg of AFB_1, AFB_2, AFG_1

Table 1
The levels of AF in wheat samples obtained from different regions of Turkey

Sample no.	Region	Source	AFB_1 (ng/kg)	AFB_2 (ng/kg)	AFG_1 (ng/kg)	AFG_2 (ng/kg)	Total AFs (ng/kg)
1	A	TGBO	N.D.	N.D.	N.D.	N.D.	N.D.
2	A	TGBO	N.D.	N.D.	N.D.	N.D.	N.D.
3	A	TGBO	N.D.	N.D.	N.D.	N.D.	N.D.
4	A	TGBO	81.6	N.D.	214.2	27.2	323.0
5	A	TGBO	N.D.	N.D.	N.D.	N.D.	N.D.
6	A	TGBO	N.D.	N.D.	N.D.	N.D.	N.D.
7	A	TGBO	10.4	N.D.	N.D.	N.D.	10.4
8	A	TGBO	135.9	N.D.	446.1	61.5	643.5
9	B	TGBO	N.D.	N.D.	N.D.	N.D.	N.D.
10	B	TGBO	N.D.	N.D.	N.D.	N.D.	N.D.
11	B	TGBO	N.D.	N.D.	N.D.	N.D.	N.D.
12	B	TGBO	70.4	N.D.	N.D.	N.D.	70.4
13	B	TGBO	20.3	34.5	N.D.	N.D.	54.8
14	B	TGBO	N.D.	26.2	N.D.	N.D.	26.2
15	B	TGBO	123.2	N.D.	354.3	128.7	606.2
16	B	Bazaar	31.9	N.D.	30.5	N.D.	62.5
17	B	Bazaar	39.3	N.D.	23.2	N.D.	62.5
18	B	Bazaar	31.3	N.D.	31.9	N.D.	63.2
19	B	Market	35.1	N.D.	N.D.	N.D.	35.1
20	B	Market	N.D.	N.D.	N.D.	N.D.	N.D
21	B	Market	N.D.	N.D.	21.3	N.D.	21.3
22	B	Market	N.D.	N.D.	N.D.	N.D.	N.D
23	B	Market	N.D	N.D.	24.5	N.D.	24.5
24	B	Market	N.D.	N.D.	N.D.	N.D.	N.D
25	B	Market	15.0	N.D	N.D.	N.D.	15.0
26	B	Market	N.D.	36.4	N.D.	N.D.	36.4
27	B	Market	N.D.	N.D.	N.D.	N.D.	N.D
28	B	Market	N.D.	N.D.	N.D.	N.D.	N.D
29	B	Market	N.D.	N.D.	N.D.	N.D.	N.D
30	C	TGBO	N.D.	N.D.	N.D.	N.D.	N.D
31	C	TGBO	30.6	N.D	31.0	N.D.	61.7
32	C	TGBO	N.D.	N.D.	25.7	N.D.	25.7
33	C	TGBO	144.2	N.D.	414.9	58.4	617.5
34	C	TGBO	N.D.	12.9	21.0	N.D.	33.9
35	C	TGBO	144.2	N.D.	414.9	58.4	617.5
36	C	TGBO	30.8	N.D	N.D.	N.D.	30.8
37	D	TGBO	31.3	N.D	25.0	N.D.	56.3
38	D	TGBO	N.D.	N.D.	N.D.	N.D.	N.D
39	D	TGBO	101.5	N.D.	350.0	N.D.	451.4
40	D	TGBO	N.D.	N.D.	N.D.	N.D.	N.D
41	D	TGBO	N.D	34.7	N.D.	N.D.	34.7

AFs: Aflatoxins; AFB_1: aflatoxin B_1; AFB_2: aflatoxin B_2; AFG_1: aflatoxin G_1; AFG_2: aflatoxin G_2; N.D.: non-detectable (<10 ng/kg for AF B_1, B_2, G_2; <20 ng/kg for AFG_1); TGBO: Turkish Grain Board Office; A: Marmara and Aegean regions (West of Turkey) ($n = 8$); B: Central Anatolia Region ($n = 21$); C: Mediterranean and Southern Anatolia Regions (South of Turkey) ($n = 7$); D: Eastern Anatolia and Black Sea Regions (North and East of Turkey) ($n = 5$).

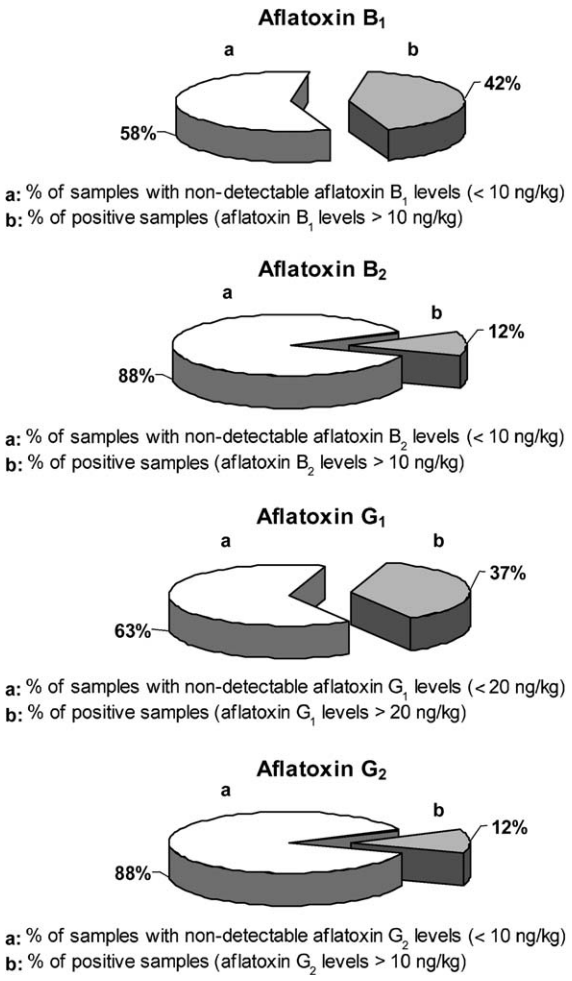

Fig. 3. Percentage of samples with non-detectable aflatoxin levels and of positive samples in all regions.

and AFG_2. The average recoveries were 75.9% for total AFs, 70.9% for AFB_1, 84.4% for AFB_2, 72.8% for AFG_1 and 70.0% for AFG_2. The concentrations of AFs in the samples were calculated by using the calibration curves of peak area prepared for each AF standard separately (Fig. 2). The detection limits were determined as 1 pg for AFB_1, AFB_2, AFG_2, and as 2 pg for AFG_1.

3. Results and Discussion

Forty-one samples of wheat grown and consumed in different regions of Turkey were analyzed for the levels of AFB_1, AFB_2, AFG_1, and AFG_2 by HPLC.

The concentrations of individual AFs and total AFs detected in all samples are given in Table 1. The samples (42%) were found to contain AFB_1 with levels of 10.4–144.2 ng/kg (Fig. 3). These amounts are under the permission limit of 2 μg/kg for AFB_1 accepted by the Ministry of Agriculture in Turkey (Official Journal of Turkish Republic, 2002). AFB_2 were determined in five samples (12%) with levels ranging from 12.9 to 36.4 ng/kg. The concentrations of AFG_1 were found to be ranging from 21.0 to 446.1 ng/kg in 37% of the samples. It was measured AFG_2 in five of samples (12%) with levels between 27.2 and 128.7 ng/kg (Fig. 3). The levels of total AFs were determined to be ranging from 10.4 to 643.5 ng/kg. Although these amounts are under the permitted levels of 4 μg/kg for total AFs in Turkey, it was seen that 59% of the samples were contaminated with AFs. Wheat and wheat products are main source of food for Turkish population and daily intake of these products corresponds to at least 50% of daily diet. Therefore, the AFs contamination detected in wheat samples is considerable problem despite low levels.

On the other hand, the distribution of positive samples and the ranges of AF levels according to the regions are given in Table 2. The percentage of positive samples for total and individual AFs (AFB_1, AFB_2, AFG_1, AFG_2) in Region C (south of Turkey) were determined to be 86%, 57%, 14%, 71%, and 29%, respectively and these values except AFB_2 were higher than the other regions. The production of AFs and the growth of the responsible fungi are dependent upon factors such as temperature, humidity, handling during harvesting and conditions during storage (Smith & Moss, 1985). High moisture levels in the samples and high temperature are favorable for the growth of AF-producing fungi. Optimum conditions are 16–24% moisture at 20–38 °C. However, it is reported that AF production can also

Table 2
Distribution and levels of AF in the wheat samples in some regions of Turkey

Region	No. of samples	AFB_1		AFB_2		AFG_1		AFG_2		Total AF	
		% of positives[a]	Range (ng/kg)	% of positives[a]	Range (ng/kg)	% of positives[a]	Range (ng/kg)	% of positives[a]	Range (ng/kg)	% of positives[a]	Range (ng/kg)
A	8	38	10.4–135.9	0	–	25	214.2–446.1	25	27.2–61.5	38	10.4–643.5
B	21	38	15.0–123.2	14	26.2–36.4	29	21.3–354.3	5	128.7	57	15.0–606.2
C	7	57	30.6–144.2	14	12.9	71	21.0–414.9	29	58.4	86	25.7–617.5
D	5	40	31.3–101.5	20	34.7	40	25.0–350.0	0	–	60	34.7–451.4
All	41	42	10.4–144.2	12	12.9–36.4	37	21.0–446.1	12	27.2–128.7	59	10.4–643.5

A: Marmara and Aegean regions (West of Turkey); B: Central Anatolia Region; C: Mediterranean and Southern Anatolia Regions (South of Turkey); D: Eastern Anatolia and Black Sea Regions (North and East of Turkey); AF: Aflatoxin.

[a] Positive samples were those with detectable aflatoxin levels (<10 ng/kg for AFB_1, AFB_2 and AFG_2; <20 ng/kg for AFG_1).

take place at temperatures as low as 7–12 °C (Steyn & Stander, 2000). After harvest, if grain is not dried quickly or during storage remains at a moisture high enough, mycotoxins such as AFs may occur. Since south of Turkey is the warmest region in our country, the conditions during harvest and storage of wheat samples are possibly proper for AF production due to humidity and high temperature. Moreover, the farmers of the region may not be aware of AF problem due to their low education levels and it can not be possible to use suitable harvesting, handling, drying and storage procedures in order to eliminate the AF production and contamination problem. Region B (Central Anatolia) is the major wheat-producing region in Turkey. The wheat samples grown in this region are generally distributed to the other regions, especially to the east of Turkey where the agriculture is very poor due to the geographical properties and climate. Therefore, 51% of the samples analyzed were collected from this region. The percentages of samples with detectable AF levels in this region were found to be 38%, 14%, 29%, 5%, and 57% for AFB_1, AFB_2, AFG_1, AFG_2, and total AF, respectively (Table 2). Samples (38%) grown in Region A (West of Turkey) for total AF were found to have the detectable levels. Although the climate in this region is also proper for the occurrence of AFs due to hot and humid conditions, this ratio is low compared to Region C that has the same climate possibly due to more suitable harvesting, handling, drying and storage conditions applied by more conscious farmers and agriculturists. The growing of wheat is quite low in Region D (East and North of Turkey). Therefore, only a few samples obtained from this region could be analyzed in this study in spite of limited number for representing the region. AFB_1 and AFG_1 were detected in two of five samples; AFB_2 was determined in only one sample, while no AFG_2 was found in the samples (Table 2).

On the other hand, the effects of food-processing techniques on AF contents must be taken into account. Purified AFs are essentially heat stable up to their melting point. Reduction of AF contents in food might also be expected during conventional food-processing techniques, however the percentage of loss in AF content and stability are also found to be variable depending on the products or using technique (Bushby & Wogan, 1984; Goldblatt & Dollear, 1979). No reduction in the amount of added AFB_1 was noted during the baking of whole wheat bread (Bushby & Wogan, 1984). Moreover, AF contamination may occur during the process and also, under practical conditions, feed commodities are often contaminated with more than one mycotoxin, as mold species produce different mycotoxins at the same time. These co-occurring mycotoxins can exert additive effects (Fink-Gremmels, 1999). In the other study of our research group in order to evaluate total AF levels in flour samples by ELISA, one of the 25 samples analyzed was found to be exceeding the permission limit (Baydar, Engin, Aydın, Girgin, & Şahin, 2003).

AF contamination in foods, which causes the economic loss and threats the public health is an important problem encountered from time to time in our country. Although some results were obtained from the studies on the levels of mycotoxins in different foods in Turkey (Aycicek et al., 2002; Gokmen & Acar, 2000; Nizam & Oguz, 2003; Omurtag & Beyoglu, 2003; Omurtag & Yazıcıoglu, 2000; Omurtag et al., 1998), there are limited studies especially on AF concentrations in grain and grain products (Duru et al., 1979; Simsek & Bozkurt, 1992). In these studies, the percentage of contaminated samples was found not to be high. However, it is obvious that the largest screening studies for the levels of AFs in order to be able to evaluate the presence of contamination problem in wheat and wheat products, which are the most staple foods in our country, is necessary.

In conclusion, the results of this study demonstrate that the concentrations of AFs in all analyzed wheat samples were under the permitted levels. However, these amounts should be considered in regard to overall daily exposure to mycotoxins. Mycotoxin contamination should be monitored routinely for food safety. It is so important to establish the permanent controlling and monitoring program from the production until consumption of cereals in order to minimize the contamination problem of AFs. On the other hand, the training programs on this problem should be developed especially for farmers and agriculturists. Using the optimum techniques for harvesting, handling and storage and selection of proper time for harvesting reduce or eliminate this problem for foods and prevent the threat to human health and the risk of great economic loss.

Acknowledgements

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