Total Aflatoxin, Aflatoxin B_1 and Ochratoxin A Residues in Wheat Flour Produced in Kurdistan Province, Iran

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ABSTRACT

Background and objectives: Contamination of food products with mycotoxins is a public health problem. The International Agency for Research on Cancer has identified mycotoxins as hepatotoxic and carcinogenic agents to humans (Group 1). The Kurdistan Province is the ninth largest producer of wheat in Iran. We aimed to determine the level of contamination with total aflatoxin (TAF), aflatoxin B_1 (AFB_1) and ochratoxin A (OTA) in 66 wheat samples randomly selected from 11 wheat flour factories in spring and summer.

Methods: The level of toxins was measured by microtiter plate enzyme-linked immunosorbent assay (ELISA) using a microtitre plate ELISA reader and total AF, AFB_1 and OTA commercial kits.

Results: Overall, the level of TAF and AFB in 16.67% of the samples exceeded the maximum tolerable limit set by the Institute of Standard and Industrial Research of Iran (ISIRI). However, the level of OTA contamination did not exceed the maximum tolerable limit set by the ISIRI. In addition, the level of TAF, AFB_1 and OTA exceeded the maximum tolerable limit set by the EU in 68.18, 90.91 and 36.36% of the samples, respectively. The level of contamination with these mycotoxins differed significantly in spring and summer ($P<0.05$).

Conclusion: The level of mycotoxin contamination in wheat samples produced in the Kurdistan Province is alarmingly high and appropriate measures should be taken to eliminate the causes of this issue.

KEYWORDS: Aflatoxin, Aflatoxin B_1, Ochratoxin A, Wheat, ELISA.

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INTRODUCTION

Humans are exposed to toxins through consumption of foodstuffs contaminated with fungi and fungal byproducts. Since it is difficult to prevent growth of fungi in food, such exposures could not be easily avoided. When Good Agricultural Practices are not adopted during cultivation and storage of wheat grains, filamentous fungi such as *Furasarium*, *Penicillium* and *Aspergillus* may develop and produce secondary metabolites called mycotoxins (1, 2). Aflatoxin (AF) is a naturally occurring carcinogenic substance, which is extremely toxic to humans (3). According to the International Agency for Research on Cancer, they are hepatotoxic and carcinogenic agents to humans (Group 1) and capable of inducing liver cancer and cirrhosis as well as reducing individuals' immune resistance, causing outbreaks of hepatitis B (4). Kurdistan Province is a mountainous region located in west of Iran with cold climate, although some areas may have a warmer climate. According to the official survey of Statistical Centre of Iran, Kurdistan Province is the ninth largest producer of wheat in Iran (5). Nevertheless, some problems such as traditional agriculture practices, improper storage and distribution systems result in wheat and wheat flour fungal contamination in the province. According to the Institute of Standards and Industrial Research of Iran (ISIRI), the maximum tolerable limit for total AF (TAF), AFB_1 and ochratoxin A (OTA) in wheat flour is 15, 5 and 5 μg/Kg , respectively (6). However, the EU Commission (EC) has set the maximum tolerable limit for TAF and AFB_1 in cereals and all products derived from cereals at 4 and 2 μg/Kg, respectively. In addition, the maximum tolerable limit for OTA in all products derived from cereals is 3 μg/Kg (6).

MATERIAL AND METHODS

This study was performed on 66 samples randomly collected from 11 wheat flour manufactures in the Kurdistan Province, Iran. Sampling was carried out in spring (33 samples) and summer (33 samples) to determine the effect of season on the level AF and OTA contamination. The sampling was carried out at mid-month from 14-Kg whole-wheat flour bags.

All samples were stored in plastic bags and kept in refrigerator at 4-6 °C. Microtitre plate ELISA reader (Bio-Tek Inst, ELX 800, USA) and total AF, AFB_1 and OTA measurement kits (Agrastrip, Romer Labs, Singapore Pte Ltd.) were used. The Agra Quant® ELISA AF (Art. No: COKAQ1000), Agra Quant® ELISA AFB_1 (Art. No: COKAQ8000), Agra Quant® ELISA Ochratoxin A (Art. No: COKAQ2000) kits were obtained from Romero Labs for quantitative analysis of AF residues in cereals and feedstuffs. The functional properties of the kits used in the study are shown in table 1. After extraction of mycotoxins, all well plates were placed in an ELISA microplate reader (Bio-Tek Inst, ELX800, USA) with a 450 nm filter. Estimated amount of mycotoxins in each sample was calculated by extrapolation from the standard curve.

RESULTS

The results showed that all samples were contaminated with TAF. In addition, 68.18% of the wheat flour samples contained 11.83 ± 3.29 μg/Kg TAF (4.01-19.65 μg/Kg), which exceeds the maximum tolerable limit set by the EC (Table 2).

Moreover, the amount of TAF present in 16.67% of the samples exceeded the maximum tolerable limit for TAF set by the ISIRI (15 μg/Kg) (8).

Table 1- The functional properties of the kits used in the study

Mycotoxin	Limit of detection (μg/Kg)	Limit of quantitation (μg/Kg)	Range of quantitation(μg/Kg)	Standards (μg/Kg)	Percent recovery	CV(%)
OT	1.9	2	2-40	0,2,5,20,40	-	-
AFB_1	2	2	2-50	0,2,5,20,50	87-103	≤10%
TAF	3	3	4-40	0,4,10,20,40	-	-

All data were analyzed in SPSS (version 21) using independent t-test and ANOVA. To evaluate differences between means, Tukey's test was used.

Table 2- TAF levels in wheat flour samples tested in the study

TAF(µg/kg)	Number of positive samples	Mean (µg/kg) ± standard error	Percentage
≤4 [a]	21	3.3±-0.3	31.82
4-15	34	9/11±1.5	51.51
>15 [b]	11	17/17±2.1	16.67

a: The maximum tolerable limit of TAF according to EC regulations
b: The maximum tolerable limit of TAF according to ISIRI regulations

Table 3- Total AFB_1 levels in wheat flour samples tested in the study

AFB1 (µg/kg)	Number of positive samples	Mean (µg/kg) ± standard error	Percentage
≤2 [a]	6	6	9.09
2-5	49	3.3±0.87	74.24
>5[b]	11	5.28±1.23	16.67

a: The maximum tolerable limit of AFB_1 according to EC regulations
b: The maximum tolerable limit of AFB_1 according to ISIRI regulations

As shown in table 3, the amount of AFB_1 in 9.09% of the samples was lower that the limit of detection and allowed limit set by the EC (2 µg/Kg). In addition, the level of AFB_1 in 16.67% of the samples was higher than the maximum tolerable limit set by the ISIRI (5 µg/Kg).

OTA is found predominantly in cereal grains, cereal products, legumes, oilseed, coffee beans and feedstuff (9).

In our study, the level of OTA was determined to 3.17 ± 0.18 µg/Kg. While all samples were contaminated with OTA, the level of OTA in 63.63% of the samples was lower than the tolerable limit set by the EC (3 µg/Kg).However, level of OTA in none of the samples exceeded the tolerable limit set by the ISIRI (5 µg/kg).

Mycotoxin levels in spring and summer

There were statistically significant differences between the level of TAF, AFB_1 and OTA in samples collected in spring and summer (Table 4).

The level of TAF, AFB_1 and OTA in both seasons was within the tolerable limit set by the ISRI. However, only the level of OAT in spring was lower than the tolerable limit set by the EC.

Table 4- Mean level of mycotoxins in the samples collected in spring and summer

Mycotoxin	Season	Number of samples	Mean level (µg/Kg)	Standard deviation
TAF	Spring	33	11.01 ^{A*}	4.24
	Summer	33	10.83^{B}	2.90
AFB1	Spring	33	3.65^{A*}	2.28
	Summer	33	3.12^{B}	2.11
OTA	Spring	33	2.08^{A*}	1.08
	Summer	33	3.77^{B}	1.54

* Statistically significantly difference (P< 0.05)

DISCUSSION

Contamination of food products is a major problem in Iran (10-14). The most staple food in Iran is wheat, which is susceptible to fungal contamination during cultivation, harvest, transport and storage. In our study, the level of TAF was higher than that reported by previous studies in Iran. In our study, 2.54% of the samples were contaminated with AF, while 2.54% and 3.39% of the samples were contaminated with AFB_1 and AFG_1, respectively. Zinedine et al. reported that the mean level of TAF and AFB_1 in wheat flour was 0.07 ng/g and 0.07 ng/g, respectively. They also reported that none of the samples was contaminated with AFB_2, AFG_1 and AFG_2 (15). Abdullah et al. analyzed 83 wheat flour samples in Malaysia (16), and reported that 21.7% of the samples was contaminated with AF. In another study, Giray et al. analyzed 41 wheat samples in Turkey, and reported that 59% of the samples was contaminated with AFB_1 (42%), AFB_2 (12%), AFG_1 (37%) and AFG_2 (12%) (17). Aydin et al. assessed the level of TAF in 100 wheat flour samples, and concluded that 45% of them contained 0.005-14.0 μg/Kg AF. In addition, the level of TAF in 2% of the samples exceeded the maximum tolerable limit in Turkey (4 μg/kg) (18). A study in Iran showed that 3.1% and 7.4% of wheat flour sample were contaminated with AFB_1 in summer and winter, respectively, with levels exceeding the permissible limit (19). In another study, the level AFB_1 was reported to be 0.93 ng/g, while 6.5% of the samples contained AFB_1 levels higher than the maximum limit set by international regulations (20). A study in Croatia reported that the mean level of AFB_1 was 16.3 μg/Kg in 475 wheat grain samples (21). Ayalew et al. detected AFB_1 contamination in 8.8% of 352 cereal samples (wheat, barley, sorghum, and teff grass) (22). Although the highest level of AFB_1 found in our study was lower than that in studies of Giray et al., Halt and Ayalew et al., it was higher than the level reported by studies of Vagef and Mahmoudi in Iran (13, 17, 21, 22).

A study determined the concentration of OTA in wheat flour samples collected from Ahvaz using HPLC method. In the mentioned study, 93.75% of the samples were contaminated with OTA at mean concentration of 0.09 ng/g, which is lower than its maximum tolerable limit in wheat according to the ISIRI and WHO (23). Yazdanpanah et al. found no OTA contamination in 14 barley and nine corn samples (destined for animal feed) collected from the Golestan and Mazandaran provinces in north of Iran (24). However, they found one case of AF and OTA co-contamination. Mahmoudi et al. found that 24.3, 11.4 and 14.3 % of the wheat flour samples from the Mazandaran Province contained AFB_1, TAF and OTA levels exceeding the maximum tolerable limit, respectively (25).

In our study, detection of OTA in 33.3% of wheat flour samples is alarming. Study of Zinedine et al. in Morocco reported that 40% of wheat samples were contaminated with OTA at mean concentration of 1.73 μg/Kg (15). In Ethiopia, Ayalew et al. reported that 23.4% of 107 wheat samples had OTA-contamination, and the highest level of OTA recorded was 66.0 μg/Kg (22). In Italy, Muscarella et al. reported that 95 durum wheat, 15 flour wheat, 80 maize and 85 barley samples were contaminated with OTA (26).

In our study, the level of AFB_1 contamination in 3.1% and 7.4% of samples collected in summer and winter was higher than the international permissible limit, respectively. AF level in winter was higher than in summer, and the highest frequency of AF contamination in winter and summer was related to AFB_2 (98%) and AFG_1 (51%), respectively. The difference between the mean levels of AF in the two seasons may be related to geographical and climatic characteristics as well as temperature and humidity. Agricultural practices, soil characteristics, type of wheat, sampling method, and wheat and flour storage conditions can affect the contamination level in flour (19). Baliukoniene et al. showed that storage conditions have a significant impact on levels of AF contamination (27). In summer, temperature and humidity are higher and more suitable for mycotoxin production. However, we showed that TAF levels in spring were higher than in summer. This could be due to duration of wheat storage and other adverse conditions. Based on the results of our study and other studies, it can be concluded that there is a significant relationship between humidity and the level of TAF (19). Basilico showed that fungal growth and toxin production are influenced by the interaction between fungi, host and environment (28). It is well known that the type of crop, weather pattern, temperature, humidity, water activity, level of oxygen, poor storage condition, insufficient drying and presence of insects or rodents can affect growth of *Aspergillus* species and AF production (29). Unfortunately, most of the wheat produced in the Kurdistan Province is stored in small or tradition silos, and transported at unsuitable conditions.

CONCLUSION

The results of this study show that all wheat flour samples produced in the Kurdistan Province are contaminated with different levels of mycotoxins. We found that the level of contamination is different in spring and summer. Considering the difference between the standard limits for mycotoxins set by the ISRI and EC and the alarming rate of contamination in our samples, it is necessary to review and modify the existing standards in accordance with the international standards.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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