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$_1$ 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.
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 *Furassarium, 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), AFB1 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 total AF (TAF) in cereals and all products derived from cereals at 4 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, AFB1 and OTA measurement kits (Agrastrip, Romer Labs, Singapore Pte Ltd.) were used. The Agra Quant® ELISA AF (Art. No: COKAQ1000), Agra Quant® ELISA AFB1 (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(%) |
|-----------|---------------------------|-----------------------------|-----------------------------|-------------------|-----------------|-------|
| OTA       | 1.9                       | 2                           | 2-40                        | 0,2,5,20,40       | -               | -     |
| AFB1      | 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.
As shown in table 3, the amount of AFB1 in 9.09% of the samples was lower than the limit of detection and allowed limit set by the EC (2 μg/Kg). In addition, the level of AFB1 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 be 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, AFB1 and OTA in samples collected in spring and summer (Table 4). The level of TAF, AFB1 and OTA in both seasons was within the tolerable limit set by the ISIRI. However, only the level of OAT in spring was lower than the tolerable limit set by the EC.

**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 AFB1 and AFG1, respectively. Zinedine et al. reported that the mean level of TAF and AFB1 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 OTA.
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 AFB1 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 AFB2 (98%) and AFG1 (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). Biliukeni 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.

REFERENCES
1. Gregori R, Meriggi P, Pietri A, Formenti S, Baccarini G, Battilani P. Dynamics of fungi and related mycotoxins during cereal storage in silo bags. Food Control. 2013; 30(1): 280-7.
2. Mylona K, Sulyok M, Magan N. Relationship between environmental factors, dry matter loss and mycotoxin levels in stored wheat and maize infected with Fusarium species. Food Additives & Contaminants: Part A. 2012; 29(7): 1118-28. doi: 10.1080/19440049.2012.672340.
3. Songsermsakul P, Razzaqi-Fazeli E. A review of recent trends in applications of liquid chromatography-mass spectrometry for determination of mycotoxins. Journal of Liquid Chromatography & Related Technologies. 2008; 31(11-12): 1641-86. DOI: 10.1080/10826070802126395.
4. World Health Organisation (WHO). International Agency for Research on Cancer. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. IARC Press, Lyon, France. 2002; 82: 193-210.
5. Institute of Standards and Industrial Research of Iran (ISIRI). Wheat flour: Specification and test methods. 2010; No.103. Available from www.isiri.org.
6. Commission E. Commission Regulation (EC) No 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs. Brussels: European Commission. 2001.
7. Institute of Standards and Industrial Research of Iran (ISIRI). Sampling methods to official control of mycotoxin levels. Food and agriculture products. 2009. Available from www.isiri.org.
8. Institute of Standards and Industrial Research of Iran (ISIRI). Food and Feed Mycotoxins-Maximum tolerated level. NO:5925. 2002. Available from www.isiri.org.
9. MacDonald S, Wilson P, Barnes K, Damant A, Massey R, Morby E, Shepherd MJ. Ochratoxin A in dried vine fruit: method development and survey. Food Additives & Contaminants. 1999; 16(6): 253-260. DOI: 10.1080/026520399284019.
10. Oveis M-R, Jannat B, Sadeghi N, Haji mahmoodi M, Nikzad A. Presence of aflatoxin M 1 in milk and infant milk products in Tehran, Iran. Food Control. 2007; 18(10): 1216-8.
11. Fallah AA. Aflatoxin M 1 contamination in dairy products marketed in Iran during winter and summer. Food control. 2010; 21(11): 1478-81. DOI: 10.1016/j.foodcont.2010.04.017.
12. Rahimi E, Mohammadhosseini Anari M, Alimoradi M, Rezaei P, Arab M, Goudarzi M. Aflatoxin M1 in pasteurized milk and white cheese in Ahvaz, Iran. Global Veterinaria. 2012; 9(4): 384-7.

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CONFLICT OF INTEREST
The authors have no conflict of interest to declare.

13. Vagrf R, Mahmoudi R. Occurrence of Aflatoxin M1 in raw and pasteurized milk produced in west region of Iran (during summer and winter). International Food Research Journal. 2013;20(3): 1421-1425.
14. Mahmoudi R, Golchin A, Hosseinzadeh N, Ghajar beygi P. Aflatoxin M1 and B1 contaminations in products of animal origin in Iran. J Qazvin Univ Med Sci. 2014;18(4):49-59.
15. Zinedine A, Juan C, Soriano J, Molto J, Idrissi L, Manes J. Limited survey for the occurrence of aflatoxins in cereals and poultry feeds from Rabat, Morocco. International Journal of Food Microbiology. 2007;115(1):124-7.
16. Abdullah N, Nawawi A, Othman I. Survey of fungal counts and natural occurrence of aflatoxins in Malaysian starch-based foods. Mycopathologia. 1998;143(1): 53-8.
17. Giray B, Girgin G, Engin AB, Aydin S, Sahin G. Aflatoxin levels in wheat samples consumed in some regions of Turkey. Food control. 2007;18(1): 23-9.
18. Aydin A, Günsen U, Demirel S. Total aflatoxin, aflatoxin B1 and ochratoxin A levels in Turkish wheat flour. Journal of Food and Drug Analysis. 2008;16(2): 48-53.
19. Taheri N, Sennani S, Roshandel G, Namjoo M, Keshavarzian H, Chogan A, et al. Aflatoxin contamination in wheat flour samples from Golestan Province, Northeast of Iran. Iranian journal of public health. 2012; 41(9): 42-47.
20. Azizi G, Khoushevis S, Hashemi S. Aflatoxin M1 level in pasteurized and sterilized milk of Babol city, Tehran University Medical Journal TUMS Publications. 2008; 65(13): 20-4.
21. Halil M. Aspergillus flavus and aflatoxin B1 in flour production. European journal of epidemiology. 1994; 10(5): 555-8.
22. Ayalew A, Fahrman H, Lepshcy J, Beck R, Abate D. Natural occurrence of mycotoxins in staple cereals from Ethiopia. Mycopathologia. 2006; 162(1): 57-63.
23. Behfar A, Nazari Z, HEYDARI R. Identification and Determination of Ochratoxin a Concentration in Wheat Flour of Flour Factories in the Ahvaz city Using HPLC. Jundishapur Scientific Medical Journal. 2013; 12(2): 217-227.
24. Yazdanpanah H, Miraglia M, Calfapietra F, Brera C. Natural occurrence of aflatoxins and ochratoxin a in corn and barley from mazandaran and golestan in north provinces of IR Iran. Mycotoxin research. 2001; 17(1): 21-30. DOI:10.1007/BF02946114.
25. Mahmoudi M, Aryaee P, Ghanbari M, Ansari H, Nourafcan H, editors. The determination of aflatoxin and ochratoxin of flour and wheat in northern Iran. International Conference on Environment, Agriculture and Food Sciences (ICEAFS’2012). 2012; 5-8.
26. Muscarella M, Palermo C, Rotunno T, Quaranta V, D’Antini P. Survey of Ochratoxin A in cereals from Puglia and Basilicata. Veterinary research communications. 2004; 28(Suppl 1): 229-32.
27. Baliukoniene V, Bakutis B, Stankevicius H. Mycological and mycotoxicological evaluation of grain. Annals of Agricultural and Environmental Medicine. 2003; 10(2): 223-7.
28. Basilico J. Mycotoxinas in food: PhD thesis. National University of Litoral, Santa Fe, Argentina; 1995.
29. Obrian G, Georgianna D, Wilkinson J, Yu J, Abbas H, Bhatnagar D, et al. The effect of elevated temperature on gene transcription and aflatoxin biosynthesis. Mycologia. 2007; 99(2): 232-9.