Iranian J Publ Health, Vol. 41, No.9, Sep 2012, pp.42-47

Original Article

Aflatoxin Contamination in Wheat Flour Samples from Golestan Province, Northeast of Iran

N Taheri [1], S Semnani [1], G Roshandel [1], M Namjoo [2], H Keshavarzian [3], AG Chogan [3], F Ghasemi Kebria [1],*H Joshaghani [1]

1. *Golestan Research center of Gastroenterology and Hepatology, Golestan University of Medical Sciences;. ShahidNabavi Polyclinics, Gorgan, Iran*
2. *Faculty of Paramedicine, Golestan University of Medical Sciences, Gorgan, Iran*
3. *Deputy of Drug and Food, Golestan University of Medical Sciences, Gorgan, Iran*

***Corresponding Author:** Tel: +981712340835 Email: HR_joshghani@yahoo.com

(Received 18 Jan 2012; accepted 27 Jul 2012)

Abstract

Background: Due to the high toxicity of aflatoxin and its effects on public health, determination of aflatoxin level in Wheat flour samples in the Golestan province, north of Iran was investigated. To examine the effect of seasonal changes, summer and winter sampling was performed with standard sampling methods.

Methods: A total of 200 flour samples were collected from 25 factories. HPLC method with immunoaffinity chromatography was used to measure aflatoxin types (G2, G1, B2 and B1). Statistical analysis was performed by the Pearson correlation test, One-way ANOVA and multivariate regression analysis.

Results: Mean total aflatoxin levels of samples were 0.82 and 1.99 ng/g in summer and winter, respectively. Aflatoxin B1 levels were detected in 3.1%, 7.4% over permissible limits by worldwide regulations in samples collected in summer and winter, respectively. Aflatoxins in winter were higher than summer. The highest frequency of aflatoxin contamination in winter was B2 (98%) and in summer G1 (51%). The relationship between humidity and rate of aflatoxin B1 and total aflatoxin was significant in winter. Results of multivariate regression were showed the strongest relationship with humidity and aflatoxin level. Despite the contamination of flour samples, there was no contamination higher than the standard limit of Iran Standard Institute. But it was significantly higher than similar studies from other regions.

Conclusions: Therefore, with regard to negative impacts of aflatoxin on health, aflatoxin contamination should be considered in future programs. Decrease of aflatoxin contamination may be made practical through reducing wheat storage duration and controlling humidity.

Keywords: Aflatoxin, Wheat flour, Iran

Introduction

Humans are exposed to toxins by consuming foods contaminated with products of fungal growth throughout life time. Since prevention of fungal growth in foods is difficult, such exposures are not easy to be avoided. Foodborne illness caused by microorganisms is a large and growing public health problem.

Fungi have been found to contaminate a wide variety of important agricultural products worldwide. Fungal metabolites have toxic, mutagenic and teratogenic effects (1, 2). Mycotoxins have received considerable attention due to their significance in agricultural loss and human health. Amongst the

mycotoxins that are known to cause human diseases, aflatoxins have been studied most (2).

Aflatoxins are a group of mycotoxins produced by strains of *Aspergillus*, especially *A. flavus* and *A. parasiticus*. Aflatoxin has been found as contaminats in agricultural and food products especially in cereals and cereal products (3, 4).

In numerous studies, wheat and wheat flour contamination has been considered. Caldas in 2002 analyzed 366 food samples including cereals, peanuts and rice, and reported that 19.6% of the samples were contaminated with aflatoxins (5). Aflatoxin B1 (AFB1) is the most potent hepatocarcinogen known in mammals and is classified by the International Agency of Research on Cancer (IARC) as Group 1 carcinogen (6). Aflatoxin B1 was detected in 8.8% of 352 cereal samples at concentration below 26ng/g (7). Halt detect AFB1 in a mean level of 16.3 ng/g in wheat samples (8).

Iran also conducted research on aflatoxin. 63.7% and 2.54% of the samples were contaminated with *Aspergillus*, and aflatoxin respectively (9).

Due to the toxicity of aflatoxin, its presence in flour and flour products and its effect in public health, this study was conducted to determine the level of aflatoxin and factors affecting aflatoxin formation in wheat flour in Golestan Province.

Materials and Methods

This cross-sectional study was conducted in 2010 in Golestan Province, northern Iran. According to the Institute Standard and Industrial Research of Iran No: 2836 (sampling of agricultural products), a total of 200 samples obtained from 25 wheat flour manufactures in the province. To determine the seasonal effects on aflatoxin contamination, sampling was conducted in two stages, the warm season (mid-summer) (100 samples), and the cold season (mid-winter) (100 samples). To determine the effect of environmental factors in aflatoxin formation, characteristics of silos including temperature, humidity, and storage duration of wheat and flour were recorded. Storage duration of wheat was defined as the time between delivery of wheat to silos and the time of flour production. Storage duration of flour was defined as the time between flour production and the time of sampling.

All samples were stored in plastic bags, taken to the laboratory of biochemistry of the Golestan Medical University and kept in the refrigerator for maximum three months after sampling. HPLC (High-performance liquid chromatography), and immunoaffinity chromatography were used for aflatoxin detection.

Aflatoxins were extracted from 50 g of sample using methanol: water (80:20 v/v). The extract was filtered. 20 ml of filtered solution was diluted, centrifuged, and filtered, then the mixture of the filtered diluted extract (50 ml) was applied to the immunoaffinity column (LCTech, Germany) and allowed to flow at rate of 1 ml per min. Washing with deionoised water was done to remove unbound materials. In the next step, aflatoxins were eluted with 2 ml methanol. Sample was injected into HPLC for quantitative determination of aflatoxin B1, B2, G1, and G2.

Then the samples were passed through affinity columns and extracted samples were measured by HPLC with C18 column, with a mobile phase consisting of water / MeOH / acetonitrile (60:30:15, v /v/ v) and the detector wavelength 440 nm (emission) and 365 nm (excitation).

Only aflatoxin B2 and G2 were fluorescent. Photochemical reactor was used to convert the wavelength of the absorption of aflatoxin B1 and G1 in the wavelength 254 nm for detection with fluorescence detector to derive quantifiable. Aflatoxin assay by HPLC was done after fluorescence detector extraction with photochemical reactor.

Worldwide regulations for mycotoxins in food and feed (total aflatoxin: 4ng/g and aflatoxin B1: 2ng/g) (10) and the regulation of Institute of Standards and Industrial Research of Iran (ISRI) No:6872 (15 ng/g for total aflatoxin, 5 ng/g for aflatoxin B1) (11) were used to determine the permissible limits of aflatoxins in wheat flour.

SPSS-13 and STAT-8 were used for data analysis. Pearson correlation test was used in order to examine the relationship between quantitative variables and for study the relationship between

qualitative and quantitative variables the Student *t*-test was used.

Considering that aflatoxin B1 was the most important and toxic aflatoxin of wheat, multivariate regression analysis was used for study the effect of different variables on this type of aflatoxin.

Variables with *P*-value less than 0.2 in univariate analysis were entered into the multivariate analysis. *P* values less than 0.05 was considered significant.

Results

A total of 200 samples were taken from 25 wheat flour manufactures in Golestan Province (100 in summer 100 in winter). Characteristics of silos at the time of sampling are shown in Table 1.

Results showed that the incidences of aflatoxin in wheat flour in summer and winter were about 99% and 70%, respectively. The levels of total aflatoxin and aflatoxin B1 in all wheat flour samples were within permissible limits by the regulation of Institute of Standards and Industrial Research of Iran (ISRI) No:6872 (15 ng/g for total aflatoxin, 5 ng/g for aflatoxin B1) (11).

Table 1: Storage condition at the time of sampling

Variable	Winter	Summer	*P*-value
Humidity (%)(mean ± SD)	43 (10)	63 (6)	<0.001
Temperature (°C) (mean ± SD)	18.35 (2.95)	28.87(2.2)	<0.001
Storage duration of flour - Day	5	6.5	=0.04
Storage duration of wheat – Day	205	23	<0.01

Mean and proportion of contamination of wheat flour samples with different types of aflatoxins in two seasons are shown in Table 2. Mean (SD) of total aflatoxin in samples in winter and summer were 0.99 (1.96) ng/g and 0.82 (3.05) ng /g, respectively (Table 2).

Table 2: Occurrence of aflatoxins in hot and cold season

Types of aflatoxin	Mean (SD) ng /g			Proportion of contamination (%)	
	Winter	Summer	*P.* value	Winter	Summer
G1	0.55(0.93)	0.11(0.34)	<0.001	85	51
G2	0.59(0.62)	0.13(0.191)	<0.001	70	26
B1	0.53(0.87)	0.5(2.6)	0.9	77	33
B2	0.30(0.71)	0.07(0.32)	0.004	98	37
Total	0.99(1.96)	0.82(3.05)	0.002	99	70

Aflatoxin B1 levels were detected in 3.1%, 7.4% and 5.2% over permissible limits by worldwide regulations (2ng/g) (9) in samples collected in summer, winter and total samples, respectively. These values for total aflatoxins were 3.1%, 12.6% and 7.9% (regarding the permissible limits by worldwide regulations of 4 ng/g), respectively.

The relationships between aflatoxin levels and humidity, storage temperature and storage duration of wheat and flour in two seasons are shown in Table 3. We found significant relationship between the levels of aflatoxin G1, G2, B1, B2 and total aflatoxin with the temperature and humidity of silos in summer (Table 3).

In winter, wheat and flour storage duration and aflatoxin G1 were significantly correlated. Aflatoxin B1 and total aflatoxin was significantly associated with humidity in the winter.

The results of multivariate regression showed that humidity of silos had the strongest relationship with levels of aflatoxin B1 in flour samples (Standardized beta coefficient=0.25; *P*=0.04).

Table 3: relationships between aflatoxin levels and characteristics of silos in two seasons*

Types of aflatoxin		G1	G2	B1	B2	Total
Temperature	summer	0.12 (0.22)	0.21 (0.36)	0.22 (0.02)	0.21 (0.04)	0.21 (0.03)
	winter	0.11 (0.28)	0.10 (0.33)	0.16 (0.10)	0.02 (0.79)	0.08 (0.41)
Humidity	summer	0.07 (0.49)	0.24 (0.01)	0.21 (0.03)	0.20 (0.04)	0.21 (0.03)
	winter	0.13 (0.18)	0.13 (0.20)	0.33 (0.00)	0.01 (0.85)	0.24 (0.01)
Storage duration of wheat	summer	0.12 (0.21)	0.05 (0.62)	0.04 (0.63)	0.08 (0.42)	0.06 (0.50)
	winter	0.26 (0.00)	0.13 (0.21)	0.15 (0.14)	0.15 (0.13)	0.15 (0.12)
Storage duration of flour	summer	0.12 (0.21)	0.05 (0.62)	0.04 (0.63)	0.08 (0.42)	0.06 (0.50)
	winter	0.26 (0.00)	0.13 (0.21)	0.15 (0.14)	0.15 (0.13)	0.15 (0.12)

*Each cell represents Pearson correlation coefficient (P-value)

Discussion

The results showed that the incidences of aflatoxin in wheat flour in winter and summer were respectively about 99% and 70%. Aflatoxin B1 contamination levels in the winter has seen higher than the summer (77% vs. 33%). The level of aflatoxin contamination in milk in 14 states in Iran in winter was significantly higher than in summer (12). The mean levels for total aflatoxins in samples in winter and summer were 0.99 ng /g and 0.82 ng/g, respectively. The levels of aflatoxins were in ISIR's recommended range for aflatoxin levels (11). In a study wheat flour samples were analyzed by using ELISA, the mean level of aflatoxin B1 was 0.93 ng/g and 6.5% of samples were higher than the maximum limits set by the worldwide regulations for aflatoxin B1 (13). In another study, 2.54% of samples were contaminated with aflatoxin, and afalatoxin B1, G1 detected in 2.54 and 3.39% of samples, respectively. Total aflatoxin and aflatoxin B1 levels in samples ranged between (1.3 – 7.1 ng/g) and (1.36 -1.78 ng/g), respectively (9).

Zinedine et al. reported the mean level of total aflatoxin and aflatoxin B1 in wheat flour as 0.07 ng/gr and 0.07 ng/g, respectively, and did not found aflatoxin B2, G1, G2 in any sample (14). In Malaysia, 21.7% of samples were contaminated with aflatoxin and aflatoxin G2, G1, B2, B1 detected in 1.2 %, 4.8 %, 4.8 %, 13.3 % of samples, respectively (15). In another study, 41 samples of wheat were analyzed and 59% of samples was contaminated with aflatoxin and the incidence of aflatoxin G2, G1, B2, B1 were 42%, 12%, 37% and 12%, respectively (16). In the study of Halt et al. the mean level of aflatoxin B1 was 16.3 ng/g (8).

Despite the level of aflatoxins in flour samples was not higher than the limits set by Iran Standard Institute but regarding worldwide regulations and the results of many other studies, there were impermissible high aflatoxin level in a considerable number of our samples (10, 14, 17).

Geographical location and climatic characteristics and favorable conditions of temperature and humidity, close to the Caspian Sea, agricultural practices, soil characteristics, type of wheat, sampling method and wheat and flour storage condition can affect the contamination level of flour.

Baliukoniene et al. showed that storage conditions have very significant impact on levels of aflatoxin (18). In the summer temperature and humidity are higher and very favorable to mycotoxin production but as can be seen in Table 2 aflatoxin levels

in winter was higher than summer. Storage duration of wheat is an important factor in level of aflatoxin contamination in the winter. Because of the wheat harvest was in the first half of year and kept in stocks for months, this storage duration can be a considerable result on high aflatoxin contamination in the winter of the year. Our results also showed that the relationship between humidity and the rate of total aflatoxin was significant.

Basilico showed that fungal growth and toxin production were interaction between fungi, host and environment that can influence the types and quantity of toxin produced by the fungus (19). It is known that the crop varies, weather pattern, temperature, humidity, water activity, level of oxygen, poor storage condition, inadequate drying, activity of insects or rodents and other problems affect *Aspergillus* growth and aflatoxin production in storage products stored (20, 21).

Conclusion

We found that the level of aflatoxins in flour samples was not higher than the limits set by Iran Standard Institute. But, regarding worldwide regulations and the results of many other studies, a considerable number of our samples were contaminated with impermissible high levels of aflatoxins, especially aflatoxin B1. In our study humidity, was the most important factor associated with aflatoxin contamination of flour.

Due to the important role of aflatoxin contamination, especially negative effect of aflatoxin B1 on public health, efforts should be conduct to prevent the contamination, since prevention is the most economical and practical approach. Controlling environmental factors such as storage duration, temperature and humidity can help us with achieving this goal.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

Acknowledgements

This research was supported by Golestan Research center of Gastroenterology and Hepatology, Golestan University of Medical Sciences (GOUMS) (project number: 2015). It was also conducted as thesis for obtaining MD degree in college of medicine, GOUMS. The authors declare that there is no conflict of interest.

References

1. El-Serag HB (2012). Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology*, 142(6):1264-1273.
2. Stoloff L (1977). Aflatoxins-an overview. In : Mycotoxins in human and Animal Health.(eds j.v. Rodricks CW, Hosseltine and MA Mehlman), Pathotox Publishers, Park Forest South, Illinois, pp. 7-28.
3. Smela ME, Hamm ML, Henderson PT, Harris CM, Harris TM, Essigmann JM (2002). The aflatoxin B1 formamidopyrimidine adduct plays a major role in causing the types of mutations observed in human hepatocellular carcinoma. *Proceedings of the National Academy of Sciences*, 99: 6655-60.
4. Rawal S, Kim JE, Coulombe R Jr (2010). Aflatoxin B1 in poultry: toxicology, metabolism and prevention. *Res Vet Sci*, 89(3):325-31.
5. Caldas ED, Silva SC, Oliveira JN (2002). Aflatoxins and ochratoxin A in food and the risks to human health. *Revista de Saude Publica*, 36: 319-23.
6. International Agency for Research on Cancer (IARC) (1993). Evaluation of Carcinogenic Risks of Chemical to Humans. Some naturally-occuring substances: Food items and constituence. Heterocyclic Aromatic Amines and Mycotoxins. IARC monographs, Lyon, France, pp. 359-62.
7. Ayalew A, Fehrmann H, Lepschy J, Beck R, Abate D (2006). Natural occurrence of

mycotoxins in staple cereals from Ethiopia. *Mycopathologia*, 162: 57-63.
8. Halt M (1994). Aspergillus flavus and aflatoxin B1 in flour production. *Eur J Epidemiol*, 10: 555-8.
9. Hedayati MT, Mohammadpour RA (2005). The Aspergillus flavus and aflatoxin contamination in wheat samples of Mazandaran province, Iran. *Behbood*, 9: 52-61.
10. FAO (2004). Worldwide Regulations for Mycotoxins in Food and Feed in 2003. FAO Food and Nutrition Paper, vol. 81. Food and Agriculture Organization of the United Nations, Rome, Italy.
11. ISIRI (2002). Food and Feed- Mycotoxins: Maximum tolerated level. 1st ed. Institute of standards and industrial research of Iran (ISIRI), Tehran, Iran, pp. 3-20.
12. Tajkarimi M, Shojaee Aliabadi F, Salah Nejad M, Pursoltani H, Motallebi AA, Mahdavi H. Seasonal study of aflatoxin M1 contamination in milk in five regions in Iran. *Int J Food Microbiol*, 2007 30;116(3):346-9.
13. Azizi G, Gholampour Azizi I, Khoushnevis SH, Hashemi SJ (2007). Aflatoxin M1 level in pasteurized and sterilized milk of Babol city, Iran. *Tehran University Medical Journal*, 65: 20-4.
14. Zinedine A, Juan C, Soriano JM, Molto JC, Idrissi L, Manes J (2007). Limited survey for the occurrence of aflatoxins in cereals and poultry feeds from Rabat, Morocco. *Int J Food Microbiol*, 115: 124-27.
15. Abdullah N, Nawawi A, Othman I (1998). Survey of fungal counts and natural occurrence of aflatoxins in Malaysian starch-based foods. *Mycopathologia*, 143: 53-8.
16. Giray B, Girgin G, Engin AB, Aydin S, Sahin G (2007). Aflatoxin levels in wheat samples consumed in some regions of Turkey. *Food Control*, 18: 23-9.
17. Aydin A, Gunsen U, Demirel S (2008). Total Aflatoxin, Aflatoxin B1 and Ochratoxin A Levels in Turkish Wheat Flour. *Journal of Food and Drug Analysis*, 16: 48-53.
18. Baliukoniene V, Bakutis B, Stankevicius H (2003). Mycological and mycotoxicological evaluation of grain. Annals of Agricultural and Environmental Medicine, 10: 223-7.
19. Basilico JC (1995). Mycotoxinas in food. PhD thesis. National University of Litoral, Santa Fe, Argentina.
20. OBrain GR, Georgianna DR, Wilkinson JR, Yu J, Abbas HK, Bhatnagar D, Cleveland TE, Nierman W, Payne GA (2007). The effect of elevated temperature on gene transcription and aflatoxin biosynthesis. *Mycologia*, 99: 232-39.
21. Cotty P, Jaime-Garcia R (2007). Influences of climate on aflatoxin producing fungi and aflatoxin contamination. *Int J Food Microbiol*, 119: 109-15.