African Journal of Microbiology Research Vol. 6(8), pp. 1815-1818, 29 February, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.1392
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Fumonisin contamination based on flour quality used in bakeries and confectioneries in Qaemshahr (city of the Northern Iran)

S. Roohi[1], I. Gholampour Azizi[2] and M. Hashemi[1]*

[1]Department of Microbiology, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran.
[2]Department of Mycology, Babol Branch, Islamic Azad University Babol, Iran.

Accepted 27 December, 2011

Fumonisins are the mycotoxin that is produced by *Fusarium* species which are found in cereals and cereal-based foods products. This toxin causes esophageal cancer in humans, leucoencephalomalasia in horses and pulmonary edema in swine, other hosts include cattle, chicken and other pets. In this study, 42 samples of wheat flour at bakeries and confectioneries were collected in Qaemshahr during spring in 2011. Data were analyzed by competitive enzyme-linked immunosorbent assay (ELISA) for total fumonisin. Out of 21 wheat bakery samples, 2 (9.5%) (Mean: 0.30) were contaminated with total fumonisin. Among 21 samples of wheat flour at confectioneries, 1 (4.8%) (Mean: 0.29) was contaminated with total fumonisin. There was a significant relationship between total fumonisin contamination level and the type of wheat flour, and the months applying statistical test.

Key words: *Fusarium*, fumonisin, wheat flour, bakery, confectionery.

INTRODUCTION

Fumonisins are a group of non-fluorescent, water-soluble and polar mycotoxins (Richard, 2007) and at least 15 related fumonisin compounds have been identified (Kumar et al., 2008). The most important species producing this toxin include, *Fusarium moniliforme*, *Fusarium prolifratume, Fusarium nygamai* and *Alternaria alternaria* (Richard, 2007; Plattner et al., 1991; Amadi and Adeniyi, 2009). Fumonisins are classified into four groups (A, B, C and P), but B group (B_1, B_2 and B_3) have only been identified as important and natural products (Krnjaja et al., 2009). These toxins inhibit the biosynthesis of sphingosine and sphingolipids compounds, because the alcohol part of polyhydric in fumonisins is similar to amino alcohol complex in sphingosine (Martins et al., 2008; Bankole and Adebanjo, 2003). In 1998 in South Africa, fumonisin was separated from *F. moniliforme* after prevalence of leucoencephalomalasia in horses (Segvicklaric et al., 2001). Consumption of food contaminated with 37 to 122 ppm of fumonisin causes leucoencephalomalasia in horses, and disease diagnosed by the presence of necrotic lesions in the white matter of the interior cerebral hemispheres (Giannitti et al., 2011). Cattle are generally less susceptible than other species. Feeding of 150 ppm of this toxin to cattle causes liver damage (Vincelli and Parker, 2002). Esophageal cancer in Transkei of South Africa (Tseng and Liu, 2001), Northern Italy and Linxian region of China (Wang et al., 2008) has been reported. Neural tube defect in South Texas is related to consumption of the corn contaminated by fumonisin (Lino et al., 2007). According to Food and Drug Administration (FDA), the maximum amount of grains with fumonisin contamination is 2 to 4 ppm (Menniti et al., 2010). Maize is the major production that is contaminated with this toxin (Sreenivasa et al., 2006; Fandohan et al., 2003). But in some cases, it is also found in wheat, banana (Al-Hazmi, 2009), sorghum, asparagus, rice, beer and beans (Creppy, 2002).

Roger (2011) found that 78.5% sorghum opaque beer samples were positive for FB_1. Salem and Ahmad (2010) detected mycotoxin in food from Jordan by enzyme-

*Corresponding author. E-mail: fungi_azad@yahoo.com. Tel: (+98)1113290352.

Table 1. Distribution of positive number in wheat flour of bakeries in months.

Month	N	Positive	Percentage	Mean	SD	Max.	Min.
April	7	1	14.3	0.54	1.44	4.3	0
May	7	1	14.3	0.55	1.44	4.5	0
June	7	0	0	0.54	1.44	3.7	0
Total	21	2	9.50	0.30	1.44	4.5	0

N: Number sample, SD: Standard deviation.

linked immunosorbent assay (ELISA). Aflatoxins, deoxynivalenol, ochratoxin A and fumonisins were detected with a contamination of 3, 4, 25 and 2%, respectively.

Fusarium and *Aspergillus* were isolated at high levels in maize grains produced in Karnataka (India) for the post harvest (Sreenivasa et al*.,* 2011).

Liu et al. (2005) in corn products of the total 76 samples, 11 samples (14.5%) were detected with FB_1. In Bulgaria, fumonisin was measured in 91 maize samples that were 94.7% positive (Manova and Mladnova, 2009). Since wheat is the most consumed grain in the world, and Iran, and according to the climatic conditions in province in our study and storage conditions for fungal growth, contamination of wheat flour by health-threatening agents, such as types of mycotoxins, should be considered, seriously. The purpose of this study is the analysis of fumonisin contamination in wheat flour used at bakeries and confectioneries in Qaemshahr, Northern Iran.

MATERIALS AND METHODS

Samples

In this study, 21 wheat flour samples at bakeries and 21 wheat flour samples at confectioneries (7 samples per month: April, May and June) were randomly collected in spring 2011 in Qaemshahr (city of the Northern Iran).

Procedure of extraction

20g of each sample was mixed with 100 cc of 70% methanol and was shaken in Erlenmeyer flask continuously for 3 min, then filtered through Whatman No. 1 filter paper (Sahar et al., 2009).

Procedure of ELISA

The mycotoxin in the samples were determined using the competitive enzyme-linked immunosorbent assay by AgraQuant total fumonisin (B1, B2 and B3) assay kit obtained from Romer Singapore, Company. At first step, 200 µl of enzyme conjugated was added to uncoated wells with antibody. In the second step, 100 µl of the standard solutions and samples were added to wells, respectively, then 100 µl of the solutions were transferred to antibody-coated wells and were incubated at the room temperature (37°C) for 15 min. Fumonisin in samples competed with enzyme conjugate to connect to antibody in solid phase. After addition substrate, blue color was observed in wells. After addition of 100 µl of stop solution to each well, blue color in wells was changed into yellow color. Data were analyzed by the ELISA reader at 450 and 630 nm and were compared with standard. Data were analyzed by analysis of variance (ANOVA) utilizing Statistical Package for Social Sciences (SPSS) software package. There was a significant relationship between total fumonisin contamination level and type of wheat flour and the months applying statistical test (Salem and Ahmad, 2010; Sahar et al., 2009; Roger, 2011).

RESULTS

In this study, 18 samples out of 21 samples of wheat flour at bakeries were contaminated by different amounts of fumonisin. This toxin amount in 6 samples in each month was in the value of the FDA's limit (The recommended maximum level of fumonisins in human foods is 2 to 4 ppm according to FDA). 2 samples with 4.3 ppm toxin concentration in April and 4.5 ppm in May were contaminated above the limit set by FDA. In June there was no positive sample (Tables 1 and 3). 17 samples of 21 wheat flour samples at confectioneries were contaminated with fumonisin. In April and May, 6 samples, respectively and 5 samples in June, were contaminated with different amounts of fumonisin. In one sample with 4.1 ppm concentration in April, fumonisin contamination was above the limit of FDA. In May and June there was no positive sample for fumonisin (Tables 2 and 3). The results show that 26 samples were contaminated with less than 2 ppm of fumonisin (negative contamination) and 13 samples were contaminated with 2 to 4 ppm of this toxin and 3 samples were contaminated with more than 4 ppm (positive contamination) (Table 4).

DISCUSSION

Fumonisins are carcinogenic mycotoxins which have hepatotoxic and nephrotoxic effects in humans and animals (Sharma et al., 2004; Voss et al., 2007). This toxin is produced by *Fusarium* species in food storage. Decreasing fungal growth and mycotoxin formation in wheat is essential because it is consumed by humans and animals (Gholampour and Azarmi, 2009). Results in this study showed that, 3 samples out of 42 samples were contaminated by total fumonisin. 2 samples in bakeries in April and May, respectively and 1 sample in

Table 2. Distribution of positive number in wheat flour of confectionaries in months.

Month	N	Positive	Percentage	Mean	SD	Max.	Min.
April	7	1	14.3	0.55	1.46	4.1	0
May	7	0	0	0.50	1.33	3.5	0
June	7	0	0	0.51	1.36	3.4	0
Total	21	1	4.8	0.29	1.33	4.1	0

N: Number sample, SD: Standard deviation.

Table 3. Distribution of positive number in wheat flour of bakeries and confectionaries in samples.

Sample	N	Positive	Percentage	Mean	SD	Max.	Min.
Bakery	21	2	9.5	0.30	1.37	4.5	0
Confectionery	21	1	4.8	0.29	1.33	4.1	0
Total	42	3	7.1	0.21	1.33	4.5	0

N: Number sample, SD: Standard deviation.

Table 4. Distribution of fumonisin in samples.

Sample	>2 ppm		2 - 4 ppm		> 4 ppm	
	N	Percentage	N	Percentage	N	Percentage
Bakery	13	64.9	6	28.5	2	9.5
Confectionary	13	64.9	7	33.3	1	4.8
Total	26	64.9	13	30.9	3	7.1

N: Number sample, SD: Standard deviation.

confectionary in April contained above the limit recommended by FDA. Also, statistical test showed more contamination in bakeries flour samples than confectionaries flour samples. Yazdanpanah (2006) in a study in September in Mazandaran province, Northern Iran, reported that, in 11 corn grain samples collected, all of the samples were contaminated with FB_1 (1270 to 3980 ng/g), FB_2 (190 to 1175 ng/g) and FB_3 (155 to 960 ng/g). Also, the corn grains which were collected in Isfahan province in Iran, all samples (8) were contaminated with FB_1 (10 to 590 ng/g) and FB_2 (50 to 75 ng/g) and 2 (25%) were contaminated with FB_3 (50 to 75 ng/g). In a study of Ghiasian et al. (2009) on samples of corn that was imported from various countries to Iran repositories reported that 100% of 33 samples, were contaminated with FB_1 (87 to 200 µg/g) and only 2 (6.6%) were contaminated with FB_2 (22 to 53 µg/g) and no FB_3 was found. In our study, only 3 (7.1%) samples among 42 collected samples showed positive contamination with fumonisin.

Studies done in Iran showed that cotamination levels were higher than in our study. Samples conducted during different months for fumonisin analyzes, ecological conditions, climate and environmental factors (temperature, humidity and pH) and other factors, such as hygienic condition in the bakeries and confectioneries, and workers health in this environments, can be effective on the levels of contamination in the environment considered in our study as compared to the other studies (Saini et al., 2011). Sanchis et al. (1994) in Spain showed that in 50 samples of cereal-based foods, such as wheat flour, 8 (12%) samples were contaminated with FB_1 (0 to 200 ng/g). FB_2 was not detected in the samples. Chandra et al. (2010) in India showed that among 43 maize samples, 21 (48%) were contaminated by total fumonisin. Study on the snack samples in Saudi Arabia by Al-Hazmi (2009) showed that approximately 12 (24%) samples of 45 samples were positive to FB_1 (30 to 1330 ppb). De Girolamo et al. (2010) in children cereal-based food samples in Italy reported that 18 (97%) of 19 samples, were contaminated with FB_1 (80 to 800 µg/g) and 14 (78%) of samples showed FB_2 (20 to 200 µg/g) contamination. Lino et al. (2007) conducted a research by using liquid chromatography-mass spectrometry (LC-MS) method on bread made of wheat flour and corn at Portuguese bakeries. In this study, of 80 samples, 24 (30%) were contaminated with FB_1 (0 to 448 µg/g), 25 (30/1%) with FB_2 (0 to 270 µg/g) and other types of fumonisin (0 to 550 µg/g). According to our study, the amount of fumonisin was less at bakeries. In this study fermentation and temperature between 100 to 125°C were less effective in reducing fumonisins and only temperature above 150°C was effective for reducing fumonisins. Since our samples were not cooked flour, it is not the reason for the difference. Segvicklric et al. (2009) in Croatia, using competitive direct-enzyme-linked immunosorbent assay (CD-ELISA) and thin layer chromatography (TLC) method on cereals, and feed collected such as wheat samples reported by CD-ELISA 10 (27%) (200 to 20700 µg/kg) and by TLC 4 (10.8%) (2600 to 25000 µg/kg) of 37 samples were contaminated with total fumonisin. Reddy and Salleh (2009) in Malaysia by ELISA showed that all the 80 samples of corn were contaminated with FB_1 (261 to 2420 µg/kg).

Using different methods in agriculture and protection of farm product, in harvest or after harvest and storage, all must be in accordance with the principles of agriculture (Fandohan et al., 2005). Applying fungicides can reduce the amount of fungi growth and mycotoxin in the environment (Kollu et al., 2009). However, results in this study can be effective in mycotoxins analysis and management practices in storing grains for detection and reducing mycotoxins.

REFERENCES

Amadi JE, Adeniyi DO (2009). Mycotoxin production by fungi isolated from stored grains. Afr. J. Biotechnol,. 8: 1219-1221.

Al-Hazmi NA (2009). Occurrence of Fumonisin B_1 in Imported and Local Corn Based-snacks Collected from Jeddah, Saudi Arabia. Global. J. Biotech. Biochem., 4: 193-200.

Bankole SA, Adebanjo A (2003). Mycotoxins in food in West Africa: current situation and possibilities of controlling it. Afr. J. Biotechnol., 2: 254-263.

Chandra Nayaka S, Udaya Shankar AC, Niranjana SR, Wulff EG, Mortensen CN, Prakash HS (2010). Detection and quantification of fumonisins from *Fusarium verticillioides* in maize grown in southern India. World. J. Microb. Biot., 26: 71–78.

Creppy EE (2002). Update of survey, regulation and toxic effects of mycotoxins in Europe. Toxicol. Lett., 127: 19–28.

De Girolamo A, Pereboom-de Fauw D, Sizoo E, Van Egmond HP, Gambacorta L, Bouten K, Stroka J, Visconti A, Solfrizzo M (2010). Determination of fumonisins B_1 and B_2 in maize-based baby food products by HPLC with fluorimetric detection after immunoaffinity column clean-up. World Mycotox., J. 3: 135-146.

Fandohan P, Hell K, Marasas WFO, Wingfield MJ (2003). Infection of maize by *Fusarium* species and contamination with fumonisin in Africa. Afr. J. Biotechnol., 2: 570-579.

Fandohan P, Gnonlonfin B, Hell K, Marasas WFO, Wingfield MJ (2005). Impact of indigenous storage systems and insect infestation on the contamination of maize with fumonisins. Afr. J. Biotechnol., 5: 546-552.

Giannitti F, Diab SS, Pacin AM, Barrandeguy M, Larrere C, Ortega J, Uzal FA (2011). Equine leukoencephalomalacia (ELEM) due to fumonisins B_1 and B_2 in Argentina. Pesq. Vet. Bras., 31: 407-412.

Ghiasian SA, Aghamirian MR, Maghsood AH, Shephard GS, Van der Westhuizen L (2009). Occurrence of fumonisins in maize imported into Iran during 2001–2002. Mycotox. Res., 25: 25–28.

Kumar V, Basu MS, Rajendran TP (2008). Mycotoxin research and mycoflora in some commercially important agricultural commodities. Crop. Prot. 27: 891–905.

Gholampour Azizi I, Azarmi M (2009). Determination of Zearalenone and Ochratoxin in Foodstuffs. World Appl. Sci. 7: 1388-1391.

Krnjaja V , Levic J, Stankovic S (2009). Ubiquity of toxigenic fungi and mycotoxins in animal feeds in republic of Serbia. Biotechnol. Anim. Husband., 25: 477-491.

Kollu NR, Baireddy VB, Girisham Sivadevuni G, Solipuram MR (2009). Influence of volatile compounds and food preservatives in the management of fumonisins (B_1) production by *Fusarium moniliforme*. Afr. J. Biotechnol., 8: 4207-4210.

Lino CM, Silva LJG, Pena A, Fernandez M, Manes J (2007). Occurrence of fumonisins B1 and B2 in broa, typical Portuguese maize bread. Int. J. Food. Microbiol., 118: 79–82.

Liu FM, Chen PC, Fu YM, Shih DYC (2005). Determination of Fumonisin B_1 and B_2 in Corn Products. J. Food. Drug. Anal., 13: 273-278.

Martins HM, Almeida I, Marques MF, Guerra MM (2008). Fumonisins and deoxynivalenol in corn-based food products in Portugal. Food. Chem. Toxicol., 46: 2585–2587.

Menniti AM, Gregori R, Neri F (2010). Activity of natural compounds on *Fusarium verticillioides* and fumonisin production in stored maize kernels. Int. J. Food. Microbiol., 136: 304–309.

Manova R, Mladnova R (2009). Incidence of zearalenone and fumonisins in Bulgarian cereal production. Food Control. 20: 362-365.

Sharma N, He QP, Sharma R (2004). Augmented fumonisin B1 toxicity in co-cultures: evidence forcrosstalk between macrophages and non-parenchymatous liver epithelial cells involving proinflammatory cytokines. Toxicology, 203: 239-251.

Plattner RD, Frank Ross P, Reagor J, Stedelin J, Rice LG (1991). Analysis of corn and cultured corn for fumonisin B1 by HPLC and GC/MS by four laboratories. J. Vet. Diagn. Invest., 3: 357-358.

Richard JL (2007). Some major mycotoxins and their mycotoxicoses—An overview. Int. J. Food Microbiol., 119: 3-10.

Roger DD (2011). Deoxynivanol (DON) and fumonisins B_1 (FB_1) in artisanal sorghum opaque beer brewed in north Cameroon. Afr. J. Microbiol. Res., 5: 1565-1567.

Reddy KRN, Salleh B (2009). Co-Occurrence of moulds and mycotoxins in corn grains use for animal feed Malaysia. J. Anim. Vet. Adv., 10: 668-673.

Segvic M, Pepeljnjak S (2001). Fumonisins and their effects on animal health- A brief review. Vet. Arhiv., 71: 299-323.

Sreenivasa MY, Sharmila Dass R, Charith Raj AP, Janardhana GR (2006). Molecular Detection of Fumonisin Producing *Fusarium* Species of Freshly Harvested Maize Kernels Using Polymerase Chain Reaction (PCR). Taiwania, 51: 251-257.

Sreenivasa MY, Dass RS, Raj APC, Janrdhana GR (2011). Mycological Evaluation of Maize Grains produced in Karantaka (India) for the post harvest Fungal Contamination. Word Appl. Sci. J., 13: 688-692.

Sahar N, Ahmed M, Parveen Z, Ilyas A, Bhutto A (2009). Screening of Mycotoxins in Wheat, Fruits and Vegetables Grown in Sindh, Pakistan. Pak. J. Bot., 41: 337-341.

Salem NM, Ahmad R (2010). Mycotoxins in food from Jordan: Preliminary survey. Food Control, 21: 1099- 1103.

Saini K, Kalyani S, Surekha M, Reddy SM (2011).Temperature as a factor in the elaboration of mycotoxins by two fungi in groundnut fodder. Int. J. Biotechnol. Mol. Bio. Res., 2: 90-92.

Sanchis V, Abadias M, Oncins L, Sala N, Vinas I, Cane R (1994). Occurrence of Fumonisins B1 and B2 in Corn-Based Products from the Spanish Market. Appl. Environ. Microb., 60: 2147-2148.

Segvicklaric M, Cvetnic Z, Pepeljnjak S, Kosalec I (2009). Co-occurrence of Aflatoxins, Ochratoxin A, Fumonisins, and Zeralenone in Cereals and Feed, Determined By Comppetitive Direct Enzyme-Linked Immunosorbent Assay and Thin-Layer Chrmomatography. Arh. Hig. Rada. Toksikol, 60: 427-434.

Tseng T, Liu C (2001). Occurrence of fumonisin B in maize imported into Taiwan. Int. J. Food. Microbiol., 65: 23–26.

Vincelli P, Parker G (2002). Fumonisin, Vomitoxin, and Other mycotoxins in Corn Produced by fusarium fungi. University of Kentucky College of Agriculture, Lexington and Kentucky State University, Frankfort. available at www.ca.uky.edu. Issued, pp. 1-8.

Voss KA, Smith GW, Haschek WM (2007). Fumonisins: Toxicokinetics, mechanism of action and toxicity. Anim. Feed. Sci. Tech., 137: 299–325.

Wang J, Zhou Y, Liu W, Zhu X, Du L, Wang Q (2008). Fumonisin level in corn-based food and feed from Linxian County, a high-risk area for esophageal cancer in china. Food. Chem., 106: 241–246.

Yazdanpanah H (2006). Mycotoxin Contamination of Foodstuffs and Feedstuffs in Iran. Iran J. Pharmaceut. Res., 1: 9-16.