



The Comparison of Total Fumonisin and Total Aflatoxin Levels in Biscuit and Cookie Samples in Babol City, Northern Iran

*Issa GHOLAMOUR AZIZI¹, *Samaneh ROUHI²*

1. Dept. of Mycology Sciences, Babol Branch, Islamic Azad University, Babol, Iran
2. Dept. of Microbiology Science, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran

*Corresponding Author: Tel: +98 9353929575 Email: roohi.samaneh@yahoo.com

(Received 25 Sep 2012; accepted 15 Mar 2013)

Abstract

Background: Fumonisin and aflatoxins are mycotoxins that are produced by *Fusarium* and *Aspergillus* genus respectively. Due to the toxicity of aflatoxin and fumonisin and its effects on human and animals' health, the purpose of this study was analysis of total fumonisin and total aflatoxin contamination in biscuit and cookie samples in Babol City, Northern Iran.

Methods: Thirty biscuit (n=15) and cookie (n=15) samples were randomly collected at supermarkets in Babol City in winter 2011. Competitive ELISA was conducted for total fumonisin and total aflatoxin separately.

Results: Out of 30 biscuit and cookie samples, 28 (93.4%) samples were contaminated with <2ppm of total fumonisin and 2 (6.6%) showed infection with 2-4ppm of this toxin. The highest contamination rate of total fumonisin was 2.3 ppm in biscuit samples. In addition, none of the samples was infected by > 4ppm of total fumonisin. From 30 samples, 26 (86.7%) were contaminated with <4ppb of total aflatoxin and 4 (13.3%) were positive in total aflatoxin with ≥4ppb and highest contamination rate was found 7.9 ppb in biscuit samples. Contamination rate of samples by total aflatoxin was higher than total fumonisin.

Conclusion: Since biscuits and cookies are extensively used among all ages of humans, consumption of contaminated food causes different diseases in human. Therefore, determination, management, and prevention of mycotoxins according to the climatic conditions should be considered.

Keywords: Total fumonisin, Total aflatoxin, Biscuit, Cookie, Iran

Introduction

Fumonisin is a group of mycotoxins that are produced by *Fusarium verticillioides*, *F. proliferatum*, *F. nygamai* and *Alternaria alternate* f. sp. *Lycopersici* (1). Fumonisin causes serious diseases such as equine leukoencephalomalacia (ELEM), porcine pulmonary edema (PPE) and hydrothorax in pigs, liver cancer in rats and esophageal cancer in humans and neural tube defects in infants. This toxin disrupt sphingolipid metabolism (2, 3). International Agency for Research on Cancer (IARC) has listed fumonisin as a category 2B carcinogen (4).

The recommended maximum level of fumonisin in human foods is 2–4 ppm according to Food and Drug Administration (FDA) and Iranian maximum tolerated level is 1 mg/kg (=ppm) for total fumonisin (2, 3). This toxin is produced in corn, wheat, sorghum, asparagus, rice, beer and beans (3).

Aflatoxins are most toxic mycotoxins and can be found in peanuts, nuts, copra, soya, corn, rice, cotton, grain, wheat, grains, fruits and red pepper (1, 4, 5). The main aflatoxins include B₁, B₂, G₁ and G₂ that are produced by *Aspergillus flavus*, *A.*

parasiticus and *A. nomius*. AFM₁ and AFM₂ are oxidative metabolites of AFB₁ and AFB₂ and are produced by liver microsomal enzymes of some species of mammals such as dairy cattle that consume contamination feedstuffs with aflatoxins (6, 7). Aflatoxins are mutagenic, teratogenic, carcinogenic and immunosuppressive. This toxin listed in group I carcinogen by IACR (8).

The recommended maximum level of aflatoxins in human foods is 4 ppb according to European Community and Codex Alimentarius (9). Iranian maximum tolerated level is 15 ng/g (=ppb) for total aflatoxin (8).

Of 51 maize samples that was collected from four provinces in Iran (Fars, Kermanshah, Khuzestan, Mazandaran), AFB₁ was detected in 80% of the maize samples obtained from Mazandaran Provinces (5.67 ± 4.75 µg/kg) (10). In other study of 70 samples of flour and wheat that were tested, 17 samples (24.28%) had too much pollution with aflatoxin and ochratoxin (11). ELISA method in human food samples showed that 2% of samples were contaminated with fumonisin and 3% were contaminated with aflatoxin (12). Of 43 cereal samples in India, 21(48%) samples were contaminated with total fumonisin (13).

Since Mazandaran region provide a very good conditions for the growth of fungi and occurrence of mycotoxins such as fumonisin and aflatoxin have a important role in human health and since biscuit and cookie are extensively used among all ages of humans, purpose of this study was the analysis of total aflatoxin and total fumonisin in biscuit and cookie samples in Babol City (Mazandaran Province, northern Iran).

Materials and Methods

Samples

In this study, 30 biscuit (n=15) and cookie (n=15) samples were randomly collected from supermarkets in Babol City in winter 2011 and total aflatoxin (B₁, B₂, G₁ and G₂) and total fumonisin (B₁, B₂ and B₃) contamination were analyzed in this samples separately (Due high price of kits and lack of supporting of institution and easy access to

Kits and economic problems, we had confined ourselves to 30 samples).

Procedure of extraction

Samples were grounded to powder and then 20 g of each sample was mixed with 100 cc of 70% methanol in blender and then was shaken in Erlenmeyer flask continuously for 3 min. After settled, the extract was filtered with Whatman No.1 (3).

Procedure of ELISA

Contamination in the samples was measured in Mycology Laboratory of Islamic Azad University, Babol branch in Iran using competitive ELISA method and Agraquant total aflatoxin and total fumonisin assay kits (taken from Romer Singapore Company). Therefore, 200 µl of enzyme-conjugated solutions were added to uncoated- antibody microplate wells and then 100 µl of each standard solutions and samples extract were added to it. Of these 100 µl solutions were transferred to coated-antibody microplate wells and were incubated at room temperature in 20-25°C for 10 min.

Toxins in samples and control standards competed with enzyme conjugate for binding to solid phase antibody. After washing step, 100µl of enzyme substrate was added to wells and incubated at room temperature for 5 min.

After this step, blue color was observed in wells. Then 100 µl of stop solution was added to wells and blue color changed into yellow. Dye concentration is inversely related to concentration of toxin in the samples and standards.

ELISA reader in the wavelength of 450-630 nm analyzed toxins concentration and wells absorbance. Toxin concentration in the samples was compared with standard concentrations and absorption by using of a standard curve. Then Information was analyzed by analysis of variance (ANOVA) by using of SPSS software package ($P<0.05$).

Results

Out of 15 biscuit samples, 14 (93.4%) were contaminated with total fumonisin with <2ppm.

In addition, 1 (6.6%) sample showed 2-4 ppm of contamination. None of the samples was contaminated with > 4ppm. The highest contamination rate of total fumonisin was 2.3 ppm in biscuit samples (Mean \pm Se: 0.67 ± 0.16). Also 14 (93.4%) of 15 cookie samples were contaminated with total fumonisin <2 ppm and only 1 (6.6%) sample was infected with 2-4 ppm of this toxin and such as biscuit samples, none of the cookie samples were contaminated with >4 ppm of this toxin and highest contamination rate in samples was 2.1 ppm for cookie samples (Mean \pm Se: 0.71

± 0.17). Out of 30 biscuit and cookie samples 28 (93.4%) were contaminated with <2 ppm of total fumonisin and 2 (6.6%) showed infection with 2-4 ppm of this toxin. The highest contamination rate among the all biscuit samples was 2.3 ppm that was higher than Iran Standard Institute (Mean \pm Se: 0.69 ± 0.12). Despite the contamination of samples, there was no contamination higher than the standard limit of EU. (Acceptable limits of total fumonisin are 2-4 ppm and 1 mg/kg according to WHO, Institute of Standards, and Industrial Research of Iran (ISIRI) respectively) (Table 1).

Table 1: Distribution of total fumonisin contamination in samples

Samples	n	< 2ppm		2-4ppm		> 4ppm		Mean \pm Se	SD	Max	Min
		n	%	n	%	n	%				
Biscuit	15	14	93.4	1	6.6	0	0	0.67 ± 0.16	0.63	2.3	0
Cookie	15	14	93.4	1	6.6	0	0	0.71 ± 0.17	0.67	2.1	0
Sum	30	28	93.4	2	6.6	0	0	0.69 ± 0.12	0.64	2.3	0

N: Number sample, SD: Standard deviation

Also of 15 biscuit samples 13 (86.7%) were contaminated with <4 ppb of total aflatoxin and 2 (13.3%) were contaminated with ≥ 4 ppb that were more than acceptable limit in EU. The highest level of aflatoxin contamination in biscuits was 7.9 ppb (Mean \pm Se: 1.65 ± 0.57). In 15 cookie samples 13 (86.7%) were contaminated with <4 ppb of total aflatoxin and 2 (13.3%) were positive for

contamination (≥ 4 ppb) that were more than acceptable limit in EU and highest rate of infection was 4.1 ppb with this toxin in cookies (Mean \pm Se: 1.45 ± 0.33). From 30 samples 26 (86.7%) were contaminated with <4 ppb of total aflatoxin and 4 (13.3%) were positive for contamination with this toxin.

Table 2: Distribution of total aflatoxin contamination in samples

Samples	n	< 4ppb		≥ 4 ppb		Mean \pm Se	SD	Max	Min
		n	%	n	%				
Biscuit	15	13	86.7	2	13.3	1.65 ± 0.57	2.20	7.9	0
Cookie	15	13	86.7	2	13.3	1.45 ± 0.33	1.26	4.1	0
Sum	30	26	86.7	4	13.3	1.55 ± 0.32	1.77	7.9	0

N: Number sample, SD: Standard deviation

The highest contamination rate was found 7.9 ppb in biscuit samples (Mean \pm Se: 1.55 ± 0.32). However, there was no contamination higher than the standard limit of Iran. (Acceptable limit of total aflatoxin is 4ppb and 15 ng/g according to Codex Alimentarius and ISIRI, respectively) (Table 2).

Contamination rate of samples by total aflatoxin was higher than the total fumonisin. In addition, there was a significant relationship between biscuit samples and contamination ($P < 0.05$). Contamination in biscuit samples was higher than the contamination in cookie samples.

Discussion

Fungi have been found to contaminate a wide variety of important agricultural products worldwide. Fungal metabolites have toxic, mutagenic and teratogenic effects and have a considerable role in human health (8).

Fumonisin are mycotoxins mainly produced by fungi of the genus *Fusarium* and have been shown to be hepatocarcinogenic and nephrocarcinogenic in animals. Aflatoxins are a group of mycotoxins produced by strains of *Aspergillus* and are most potent hepatocarcinogen in mammals. These toxins contaminated agricultural and food products especially cereals and cereal products (8, 14).

A Study in Malaysia by ELISA method in grains samples showed that from 80 samples 100% were contaminated with FB1(15). In our study out of 15 biscuit samples 93.4% were contaminated with total fumonisin <2 ppm and 6.6% showed 2-4 ppm of contamination. 93.4% cookie samples were contaminated with total fumonisin <2 ppm and only 6.6% sample was infected with 2-4 ppm of this toxin.

In Iran (Mazandaran province) in 42 samples of wheat flour, 7.1% were contaminated with total fumonisin (0-4.5 ppm) (3). In other study from 38 corn samples, FB1 was between 1.19-12.95 mg/kg (14). In 11 corn grain samples, all of the samples were contaminated with FB1 (1270-3980 ng/g), FB2 (190-1175 ng/g) and FB3 (155-960 ng/g) (16). Positive contamination levels by fumonisin were higher than our study. Different reasons such as environmental factors including different geographical areas, temperature, humidity and rainfall prior to and during preharvest and harvest periods influence on levels of fumonisin might be involved (17).

A research conducted by using of liquid chromatography-mass spectrometry (LC-MS) method on bread made of wheat flour and corn at Portuguese bakeries showed that of 80 samples 24 (30%) were contaminated with FB1 (0-448 µg/g), 25 (30.1%) with FB2 (0-270 µg/g) and other types of fumonisin (0-550 µg/g) (4).

In our study, contamination in biscuit samples was higher than contamination in cookie samples.

Fumonisin was reduced after cooking extrusion at 190 °C for 60 min approximately 50–70% (18, 19). In addition, hygienic condition in the bakeries and confectioneries and workers health in these environments can affect on the levels of contamination in different foods (3). Also from 15 biscuit samples, 86.7% were contaminated with <4 ppb of total aflatoxin and 13.3% were contaminated with ≥4 ppb, which were more than acceptable limit. The highest level of aflatoxin in biscuits was 7.9 ppb. In 15 cookie samples, 86.7% were contaminated with <4ppb of total aflatoxin and 13.3% were positive for contamination (≥4ppb). Of 30 samples, 26 samples were contaminated with <4ppb of total aflatoxin and 4 were positive for contamination with ≥ 4ppb of total aflatoxin and highest contamination was founded 7.9ppb in biscuit samples and was higher than the EU standard.

Researchers in Croatia by ELISA showed that one of six wheat samples was infected by total aflatoxin (0-2.6 µg/kg) and total fumonisin was not detected in samples (20). In our study none of the samples was infected by > 4ppm of total fumonisin but total aflatoxin was higher than total fumonisin.

in Iran from 35 pre-harvest maize samples, incidence of AFB1, AFB2 and total aflatoxins in maize samples were 66%, 54% and 63% with mean of 9.5 ± 16.3 , 1.7 ± 2.6 and 10.4 ± 18.4 ng/g, respectively (21). Physical properties of samples and presence of different species spores and toxin-producer species can affect on the type and percent of fungi and toxins in our study samples in compared to other studies (1, 22, 23).

In different cereal processed products such as wheat-based crisp bread, 73% to 114% of samples were positive for total aflatoxin (24). Total aflatoxin contamination in our samples was 86.7% and is comparable with this study. In Pakistan in 70 samples of processed foods such as biscuit (10 samples), AFB1 was observed in 3 samples of biscuits (0.31 ± 0.01 µg/kg) (25).

In 200 wheat flour samples in Iran, total aflatoxin levels of samples were 0.82 and 1.99 ng/g in summer and winter, respectively. AFB1 levels were detected in 3.1%, 7.4% over permissible limits by

worldwide regulations in samples collected in summer and winter, respectively (8). Mycotoxin contamination may occur during the process and under practical conditions. Different months for analyzes make different amounts of toxin in the samples (3, 15, 24). For example, aflatoxin contamination rate in cold months is higher than hot months or *Fusarium* species are not capable to survive in the low oxygen and low pH in environment (25-27).

Food commodities are often contaminated with more than one mycotoxin, because mold species produced different mycotoxins at the same time (24). Some mycotoxins can be produced by more than one mold species or some fungal species are capable to produce several types of mycotoxins (28). Different analytic methods that are used by researchers to analyze mycotoxins are another reason for discrepancy between the results of different researches. In result, amount and type of toxins in diets may be different (29). In end, by using of different methods in agriculture and protection of farm products in harvest or after harvest, storage and packaging food, applying fungicides and hygienic condition can reduce amount of fungi growth and mycotoxin in the environment (3, 30).

Conclusion

None of the samples was contaminated by >4ppm of total fumonisin and 13.3% samples were positive for total aflatoxin with \geq 4ppb. Fumonisin and aflatoxin contamination in biscuit and cookie is harmful for humans' health, so toxicology, prevention and control of these toxins should be considered.

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

Faculty of Veterinary and Microbiology Sciences and Research Branch, Islamic Azad University, Babol, Iran supported the study. The authors declare that there is no conflict of interest.

References

1. Richard John L (2007). Some major mycotoxins and their mycotoxicoses—An overview. *Int J Food Microbiol*, 119(1-2): 3-10.
2. 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 (1):25–28.
3. Roohi S, Gholampour Azizi I, Hashemi M (2012). Fumonisin contamination based on flour quality used in bakeries and confectioneries in Qaemshahr (city of the Northern Iran). *Af J Microbiol Res*, 6 (8): 1815-1818.
4. Lino CM, Silva LJG, Pena A, Fernández M, Manes J (2007). Occurrence of fumonisins B1 and B2 in broa, typical Portuguese maize bread. *Int J Food Microbiol*, 118(1):79-82.
5. Rahimi E (2010). A study on contamination of Aflatoxin M₁ in milk and infant milk products in Iran. *American-Eurasian J Toxicol Sci*, 2 (2): 109-111.
6. Sherif SO, Salama EE, Abdel-Wahhab MA (2009). Mycotoxins and Child Health: The Need for Health Risk Assessment. *Int J Hyg Environ Health*, 212 (4):347-368.
7. Devi Upadhaya S, Park MA, Ha JK (2010). Mycotoxins and Their Biotransformation in the Rumen: A Review. *Asian-Aust J Anim Sci*, 23 (9): 1250 – 1260.
8. Taheri N, Semnani S, Roshandel G, Namjoo M, Keshavarzian H, Chogan AG, et al (2012). Aflatoxin Contamination in Wheat Flour Samples from Golestan Province, Northeast of Iran. *Iranian J Publ Health*, 41 (9): 42-47.
9. Commission of the European Communities (2001). Setting maximum levels contaminants in foodstuffs. *Commission Regulation (EC)*, No: 466/2001, 8th March.
10. Ghiasian SA, Shephard GS, Yazdanpanah H (2011). Natural Occurrence of Aflatoxins from Maize in Iran. *Mycopathologia*, 172 (2):153-160.

11. Mahmoudi M, Aryaee P, Ghanbari M, Ansari H, Nourafcan H (2012). The Determination of Aflatoxin and Ochratoxin of Flour and Wheat in Northern Iran. *International Conference on Environment, Agriculture and Food Sciences (ICEAFS'2012)*, August 11-12, 2012 Phuket (Thailand).
12. Salem NM, Ahmad R (2010). Mycotoxins in food from Jordan: Preliminary survey. *Food Control*, 21 (8): 1099-1103.
13. 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 (1): 71-78.
14. Yazdanpanah H, Eskandari Gheidari P, Zarghi A, Mirkarimi SK (2005). Survey of fumonisin B1 contamination of corn in Northern Iran during 2000. *Iran J Pharmaceut Res*, 4 (4): 221-225.
15. Reddy KRN, Salleh B (2011). Co-Occurrence of Mould and Mycotoxin in Corn Grains Used for Animal Feeds in Malaysia. *J Anim Vet Adv*, 10 (5): 668-673.
16. Yazdanpanah H (2006). Mycotoxin Contamination of Foodstuffs and Feedstuffs in Iran. *Iran J Pharmaceut Res*, 5 (1): 9-16.
17. Gong HZ, Ji R, Li YX, Zhang HY, Li B, Zhao Y, et al (2009). Occurrence of Fumonisin B1 in Corn from the Main Corn-Producing Areas of China. *Mycopathologia*, 167 (1): 31-36.
18. Wan Norhasima WM, Abdulmir AS, Abubakr F, Son R, Norhafniza A (2009). The Health and Toxic Adverse Effects of *Fusarium* Fungal Mycotoxin, Fumonisin, on Human Population. *Am J Infect Dis*, 5 (4): 273-281.
19. Meister U (2001). Investigations on the change of fumonisin content of maize during hydrothermal treatment of maize. Analysis by means of HPLC methods and ELISA. *Eur Food Res Technol*, 213 (3):187-193.
20. Segvic Klaric M, Cvetnic Z, Pepeljnjak S, Kosalec I (2009). Co-Occurrence of Aflatoxins, Ochratoxin A, Fumonisin, and Zearalenone in Cereals and Feed, Determination by Competitive Direct Enzyme-Linked Immunosorbent Assay and Thin-Layer Chromatography. *Arch Hig Rada Toksikol*, 60 (4): 427-434.
21. Hadiani MR, Yazdanpanah H, Amirahmadi M, Soleimani H, Shoeibi Sh, Khosrokhavar R (2009). Evaluation of Aflatoxin Contamination in Maize from Mazandaran Province in Iran. *J Med Plants*, 8 (5): 109-114.
22. Krnjaja V, Levic J, Stankovic S (2009). Ubiquity of Toxicogenic Fungi and Mycotoxins in Animal Feeds in Republic of Serbia. *J Biotechnol Anim Husbandry*, 25 (5-6): 477-497.
23. Pereyra CM, Alonso VA, Rosa CAR, Chiacchiera SM, Dalcerioand AM, Cavaglieri LR (2008). Gliotoxin Natural Incidence and Toxicogenicity of *Aspergillus Fumigatus* Isolated From Corn Silage and Ready Dairy Cattle Feed. *World Mycotoxin J*, 1 (4): 457-462.
24. Lattanzio VM, Gatta SD, Suman M, Visconti A (2011). Development and in-house validation of a robust and sensitive solid-phase extraction liquid chromatography/tandem mass spectrometry method for the quantitative determination of aflatoxins B1, B2, G1, G2, ochratoxin A, deoxynivalenol, zearalenone, T-2 and HT-2 toxins in cereal-based foods. *Rapid Commun Mass Sp*, 25 (13):1869-80.
25. Mushtaq M, Sultana B, Anwar F, Zargham Khan M, Ashrafuzzaman M (2012). Occurrence of Aflatoxins in Selected Processed Foods from Pakistan. *Int J Mol Sci*, 13 (7): 8324-8337.
26. Mansfield MA, Kuldau GA, (2007). Microbiological and Molecular Determination of Mycobiota in Fresh and Ensiled Maize Silage. *Mycologia*, 99 (2): 269-278.
27. Ayar A, Sert D, Hilmi Con A (2007). A Study on the Occurrence of Aflatoxin in Raw Milk Due to Feeds. *J Food Safety*, 27 (2): 199-207.
28. Mostrom DVM, Jacobsen BJ (2010). Ruminant Mycotoxicosis. *Vet Clin North Am Food Anim Pract*, 27 (2): 144-315.
29. Sassahara M, Pontes Netto D, Yanaka EK (2005). Aflatoxin Occurrence in Foodstuff Supplied to Dairy Cattle and Aflatoxin M₁ in Raw Milk in the North of Parana State. *Food Chem Toxicol*, 43 (6): 981-984.
30. 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 (7): 546-552.