

Occurrence of Fumonisin B₁ in Imported and Local Corn Based-snacks Collected from Jeddah, Saudi Arabia

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Abstracts: Corn based-snacks which are a type of food that very popular among children and the contamination of this type of food may be hazardous to those susceptible young age consumers. Corn is the main source of contamination in this food. Fumonisin is among the hazardous mycotoxin that may exist in corn. Therefore, the aim of this study was to assess the level of Fumonisin B₁ and fungal contamination in 15 different snack types (45 samples) of imported and locally produced snacks in order to determine the source of contamination whether it is from the field or during processing. HPLC was used to determine the level of Fumonisin B₁ and the fungal count was also determined using malt extract agar medium. The results showed that no fungal contamination was detected in all samples indicating the good manufacturing practices were followed in the production of these types of snacks. However, 24% of the total collected samples were positive for Fumonisin B₁. All the positive samples were imported snacks and the positive samples constitute 40% of the imported samples. Among the positive samples 50% were exceeding the permissible limits of both the FDA and EU. The country source of contaminated samples was from Malaysia and USA. However, other samples from Malaysia and USA were free of Fumonisin B₁. Positive samples were found to be in certain types and all the samples in these types were positive with a close concentration of Fumonisin B₁. This means that contamination is a type dependant.

Key word: Fumonisin B₁ • Corn based-snacks • HPLC detection • Fungal contamination

INTRODUCTION

Fumonisin is a group of naturally occurring metabolites produced by several *Fusarium* species [1-3], isolated and characterized for the first time in 1988 [4-5]. *Fusarium moniliforme* and *F. proliferatum* strains have been isolated frequently from corn, corn-based food and feedstuffs [1,6,7] as well as from other grains, such as barley and wheat [8-11] and fruits, such as banana [12]. They are diesters of propane-1,2,3-tricarboxylic acid (tricarballic acid, TCA) and various 2-amino-12,16-dimethylpoly-hydroxyeicosanes in which the hydroxyl groups on C₁₄ and C₁₅ are esterified with a terminal carboxyl moiety of the TCA. In addition, various partially [13] and fully hydrolyzed forms are known, the latter being found in maize subjected to alkaline hydrolysis, as in the making of Mexican tortillas [14-15]. The natural occurrence of Fumonisin in corn or corn-based food and feeds has been studied in detail [16-20]. While these mycotoxins are found in other commodities [21-24].

Animal and human health problems related to these mycotoxins are almost exclusively associated with the consumption of contaminated maize or products made from maize [2,25]. The human health effects of Fumonisin are uncertain. However Fumonisin is suspected risk factors for esophageal [25] and liver [26] cancers, neural tube defects [27-28] and cardiovascular problems [29] in populations consuming relatively large amounts of food made with contaminated maize. While causality between Fumonisin and human disease is unproven, this is not the case for animals. Consumption of moldy maize has long been a recognized cause of equine leukoencephalomalacia (ELEM) and over the years, experiments have demonstrated that *F. verticillioides* -contaminated feeds and Fumonisin B₁ (FB₁) can induce ELEM [30]. Similarly, *F. verticillioides* -contaminated feeds and FB₁ have been shown to be cardio toxic and cause pulmonary edema in pigs, a syndrome termed porcine pulmonary edema or PPE [31-32]. Cattle and poultry are considerably less sensitive to Fumonisin

than horses, pigs, rabbits or laboratory rodents [2]. Their natural occurrence in home-grown corn was statistically associated with the high rate of human esophageal cancer in Africa [6,33-35] in northern Italy [36], in Iran [37], the Southeast of the United States [6,38] and with the promotion of primary liver cancer in certain endemic areas of the People's Republic of China [39-41].

Fumonisin B₁ (FB₁) has been classified as a potential carcinogen for humans (Group 2B) by the International Agency for Research on Cancer [42]. Furthermore, these compounds have been implicated in a food borne disease outbreak characterized by abdominal pain, borborygmi and diarrhea in a few villages in India due to the consumption of contaminated corn and sorghum [43]. A review of legislation of Fumonisin in foods has been recently published by Soriano and Dragacci [44]. The USFDA Center for Food Safety and Nutrition has issued guidance levels for Fumonisin in maize and maize-byproducts used in animal feeds [45]. The levels vary by species, reflecting their relative sensitivities to Fumonisin. The European Union (Commission of European Communities) has also recommended guidance levels for Fumonisin in animal feed materials and formulated feeds. Like the FDA guidance, the European Union recommendations vary according to species. An overview of the Fumonisin, their bioavailability, toxicology and other considerations contributing to establishment of the guidance levels follows [46].

Regarding this potential risk, the scientific committee for food (SCF) from the European Commission has established a tolerable daily intake of 2 µg.kg⁻¹ body weight per day for the total FB₁, FB₂ and FB₃, alone or in combination. To reduce the intake of Fumonisin, the European Commission has set action limits of 4000 µg Fumonisin/kg for unprocessed corn and 200 µg Fumonisin/kg for processed corn-based foods and baby foods for infants and young children [47].

The problems and risks associated with Fumonisin contamination have resulted in the development of precise, reliable and sensitive methods for its determination in corn and corn-based foods [48]. In this way, since its discovery and characterization in 1988, the analytical methods applied in their detection have been improved successfully [49].

The objective of this study was to assess the level of Fumonisin in a survey of 15 different types of imported and locally produced snacks in Saudi Arabia in order to detect if there is a contamination source by Fumonisin in this type of food mainly consumed by children. Also fungal contamination was explored to detect if there is improper manufacturing practices during the process of this snacks.

MATERIALS AND METHODS

Materials: Fifteen different types of snacks (45 samples) at various retail outlets were collected at Jeddah, Saudi Arabia during 2009. The samples stored below 4°C before analyzing.

Mycological Analyses: Fungal counts of the snacks sample were performed by weighting out 1 g of the finely ground sample into the first tube of each of two dilution series of 1:101 to 1:106 in sterile distilled water. Aliquots (1 ml) of each dilution were dispensed into individual sterile 9-mm-diameter Petri dishes, mixed with molten sterilized malt extract agar (15 ml), allowed to cool and incubated at 25°C in the dark for 7 days. The developing fungal colonies were microscopically identified and the number of colonies expressed as colony forming units per gram of the ground sample (cfu.g⁻¹) [50-54].

Extraction of Fumonisin B₁: 100 ml methanol: H₂O (3:1) was added to 50 g grinded sample and blended at 5 min. Then centrifuged for 10 min at 500 xg and filtered. Filtrate was adjusted to 5.8 pH. 10 ml of filtered extract was applied to cartridge of silica-based strong anion exchange (SAX) and Fumonisin was eluted with 10 ml acetic acid-methanol (1+99), then evaporated under nitrogen steam at 60°C. The residue was redissolved in 200 µl methanol, then 225 µl of orthophthalaldehyde was added to 25 µl of the final extract and 10 µl was injected into LC within 1 min [55].

HPLC Conditions: HPLC Agilent 1100 system equipped with quaternary pump model G1311A and autosampler model G1329A. C18 column (150X4.6 mm), 5 µm was used for Fumonisin B₁ separation and determination. Mobile phase consists of methanol and 0.1 M NaH₂PO₄ (77+23). pH was adjusted to 3.3 with H₃PO₄ and the flow rate was adjusted to 1 ml.min⁻¹. Fumonisin was detected using fluorescence detector set at 335 and 440 nm as excitation and emission wavelengths after reaction with o-phthalaldehyde (OPA) [55].

RESULTS AND DISCUSSION

The importance of this study is because it deals with some type of corn based-snacks which is a type of food that very popular among children and the safety of this type of food is of great concern among the scientist and the public. Corn is the main source of contamination in this type of food. Fumonisin are among the hazardous

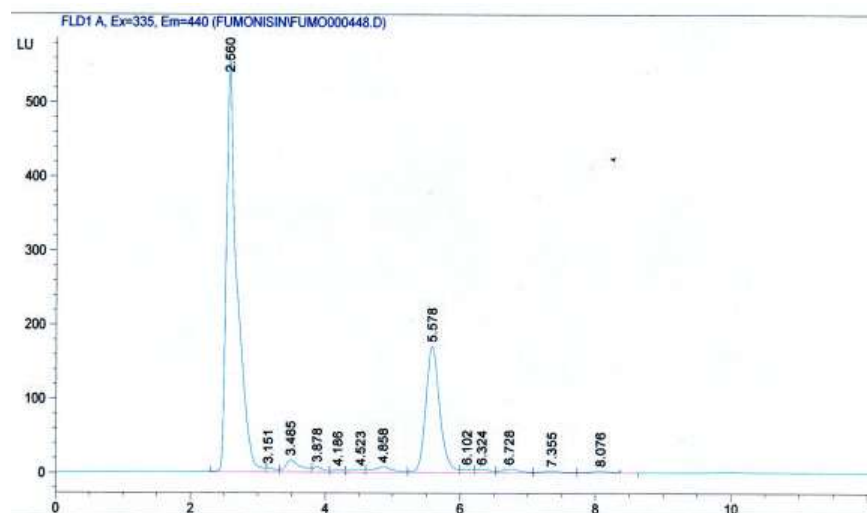


Fig. 1: HPLC chromatogram of fumonisin B₁ standard with a retention time of 5.57 and a 2.58 peak of the residual OPA that was used for a fluorescence reaction. C18 column (150 x 4.6 mm), 5 μ m was used for separation and methanol+0.1 M NaH₂PO₄ (77+23) as a mobile phase. pH was adjusted to 3.3 with H₃PO₄ and the flow rate was set to 1 ml.min⁻¹. Fumonisin B₁ was detected using fluorescence detector set at 335 and 440 nm as excitation and emission wavelengths after reaction with OPA

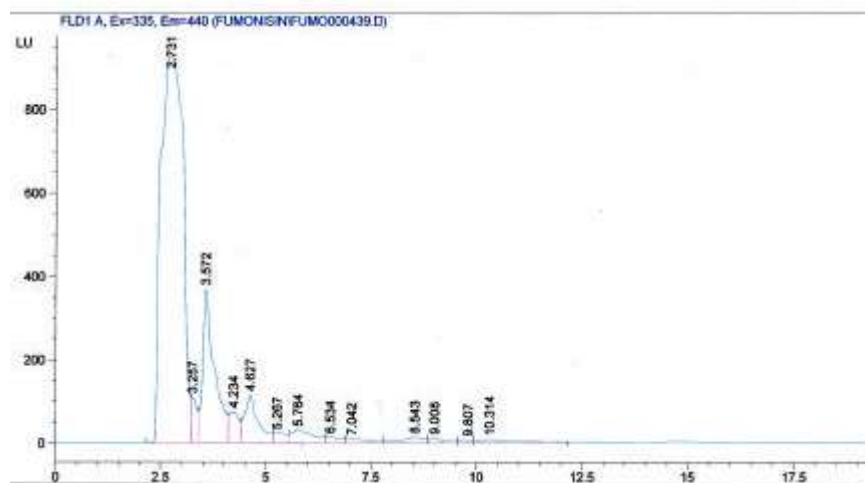


Fig. 2: HPLC chromatogram of the lowest concentration of fumonisin B₁ detected (30 ppb) in the snack sample with a retention time of 5.8 and a 2.7 peak of the residual OPA

mycotoxin that may exist in corn. Those young age consumers are more susceptible to food contamination with Fumonisin than other consumers.

Toxicological effects of Fumonisin B₁ include leukoencephalomalacia in horses, renal lesions in rats and a possible association with human esophageal cancer. Fumonisin B₁ is classified in Group 2B by IARC[56-57].

This is why this study was conducted to assess the level of Fumonisin B₁ and fungal contamination in 45 snack samples of imported and locally produced 15 different types. As a result, it can be determined if there is

a contamination source by Fumonisin B₁ in this type of food or if there is improper manufacturing practices during the process of this snacks in case of the detection of fungal contamination.

In order to detect minor level of Fumonisin B₁ a sensitive HPLC method was used in the analysis of the collected samples for Fumonisin B₁. Fig. (1) shows the HPLC chromatogram of Fumonisin B₁ standard indicating that standard peak had a retention time of 5.57 minutes after the huge peak of the OPA at 2.58 minutes. Fig. (2) showed the lowest concentration of Fumonisin B₁

detected in the tested samples where the Fumonisin B₁ Peak shows at 5.7 min. and OPA peak 2.7 with 0.1 shift in the retention time.

A major concern in controlling risk associated with mycotoxin contaminated foodstuffs is the reliability of assessing exposure levels [58]. Sensitive methods to detect Fumonisin in foods involve extraction into organic solvents and enrichment using C-18 and/or ion exchange column [59]. These are derivatives with a fluorescent label, usually o-phthalaldehyde (OPA), prior to quantization by HPLC. The liquid chromatography method utilising OPA for the determination of FB₁, FB₂ and FB₃ in corn has been adopted by AOAC International [60].

Fig. 1 - HPLC chromatogram of fumonisin B₁ standard with a retention time of 5.57 and a 2.58 peak of the residual OPA that was used for a fluorescence reaction. C18 column (150 x 4.6 mm), 5µm was used for separation and methanol+0.1 M NaH₂PO₄ (77+23) as a mobile phase. pH was adjusted to 3.3 with H₃PO₄ and the flow rate was set to 1 ml.min⁻¹. Fumonisin B₁ was detected using fluorescence detector set at 335 and 440 nm as excitation and emission wavelengths after reaction with OPA.

Fig. 2- HPLC chromatogram of the lowest concentration of fumonisin B₁ detected (30 ppb) in the snack sample with a retention time of 5.8 and a 2.7 peak of the residual OPA.

Although a number of immunochemical methods for the analysis of Fumonisin in food have been developed, a lack of sensitivity in many of the immunoassays, combined with poor correlations with physico-chemical methods HPLC, GC-MS, generally restrict immunological methods to qualitative screening, or to immunoaffinity approaches to enrich, prior to quantitation [61]. Despite the existence of highly sophisticated analytical instrumentation that can be used for measuring Fumonisin in foods, accurate exposure assessment is

problematic, with study design and representative sampling being of importance in epidemiological studies [62,63]. The results indicating that 24% of the total collected samples were positive for Fumonisin B₁ (Table 1). All the positive samples were imported samples. This means that the 12 positive samples constitute 40% of the imported samples. The main source of contamination by Fumonisin B₁ in these snacks is corn and being the 40% of the imported samples were contaminated should draw the attention of the source of this snacks and the source of the corn used in this industry. Other reports showed that corn samples were contaminated with Fumonisin B₁ as well as other mycotoxins [64].

Tracing the source of the contaminated snacks (Table 2) showed that 50% of the samples imported from Asia were positive. Part of the samples that considered being among the Asian samples was imported from United Arab Emirates where the real source of the contaminated corn is not known. However, those samples from the United Arab Emirates showed lower level of Fumonisin B₁ (30-70 ppb) than that from Malaysia (1800-1850 ppb). Also 50% of positive samples were detected in the samples from USA with a Fumonisin B₁ level ranged from 1250 to 1330 ppb. No Fumonisin B₁ were detected in the samples imported from Europe. The result showing that the local manufactured snacks had no detectable level of Fumonisin B₁ clearly indicated that the source of used corn in this industry was of high grade as well as those imported from Europe. Similar level of Fumonisin B₁ to those detected in this study were found in corn samples of other studies. Zinedine *et al.* [64], reported in their study that the collected 20 corn samples had a mean level of Fumonisin B₁ reached 1930 µg/kg. To explore whether the contamination of Fumonisin B₁ may not be due to the source of the exporting country but rather related to certain types of snacks that has been using low grade of corn contaminated with different concentration of

Table 1: Range of Fumonisin B₁ level in local and imported snacks collected from in supermarket of Jeddah in Saudi Arabia

Type	Local	Imported
No of samples	5×3 =15	10×3 = 30
No of samples type	5	10
No of +ve samples	0	12
% +ve samples	0	40%
Range of detected Fumonisin B ₁	0	ND-1850 ppb
Mean ± SE	-	320.7 ± 116.8

Table 2: Source of tested samples with their level of Fumonisin B₁

Samples source	No. of samples	No. of +ve samples	Range of Fumonisin (ppb)	Mean ± SE
Australia	3	0	0	-
Europe	3	0	0	-
Asia	18	9	ND -1850	640 ± 295.9
USA	6	3	ND -1330	1287 ± 23.3

Positive samples: 3 USA (1250-1330 ppb), 3 Malaysia (1800-1850 ppb), 6 United Arab and Emirates (30-70ppb)

Table 3: Level Fumonisin B₁ in local and imported snacks in supermarket of Jeddah in Saudi Arabia

Sample Type	Type source	Range of Fumonisin B ₁ level	Mean ± SE
1	Saudi Arabia	ND	-
2	Saudi Arabia	ND	-
3	Saudi Arabia	ND	-
4	Saudi Arabia	ND	-
5	Saudi Arabia	ND	-
6	Australia	ND	-
7	Europe	ND	-
8	United Arab Emirates	30-50 ppb	38 ± 6.0
9	United Arab Emirates	ND	-
10	Malaysia	1800-1850 ppb	1823 ± 14.5
11	Malaysia	ND	-
12	United Arab Emirates	40-70 ppb	58 ± 9.3
13	United Arab Emirates	ND	-
14	USA	1250-1330 ppb	1287 ± 23.3
15	USA	ND	-

Number of samples for each type = 3

Fumonisin B₁. So comparing the different types of snacks from the same source, Table (3) showed that not all the types coming from United Arab Emirates were positive meaning that contamination is type related rather than country source related. Also, the fact that all the samples in one type were positive with a close concentration values confirm that contamination is a type related. The real hazard to found a contamination by Fumonisin is the possibility of the co-occurrence of other mycotoxins in the same samples whether from *Fusarium* toxins (Zeralenone, T2, DON...etc) or *Aspergillus* toxins (Aflatoxins, Ochratoxins,...etc). Abbas *et al.* [65], reported that a severe infestation by aflatoxin-producing fungi diminished food quality of southern United States corn in 1998. Corn hybrids (65) naturally infected with *Fusarium* spp. and *Aspergillus* spp. were evaluated from 1998 to 2001 for resistance to mycotoxin contamination. Kernel corn samples were assayed at harvest for Aflatoxins and Fumonisin. In 1998, samples from all hybrids exceeded the maximum levels permitted by United States Food and Drug Administration guidelines with mean levels: 21–699 ppb for Aflatoxins and mean levels: 23–79 ppm for Fumonisin. Samples from hybrids planted in the same and other locations in Arkansas in 1999 and 2001 were shown to contain aflatoxin levels ranging from not detected to 255.3 ppb and Fumonisin levels from 0.3 to 83.6 ppm.

The Fumonisin levels in 2001 were very high in all hybrids, ranging from 8 to 83.6 ppm while aflatoxin levels were low ranging from 5 in most hybrids to 131 ppb. The presence of aflatoxin B₁ and B₂ in samples was confirmed by thin layer chromatography and liquid chromatography/mass spectrometry and Fumonisin B₁, B₂, B₃, B₄ and C₄ by liquid chromatography/mass spectrometry. During the period studied, a positive correlation was observed between aflatoxin and

Fumonisin levels, indicating that natural infection with *Fusarium* spp. did not appear to protect against aflatoxin production [65].

All samples proved to be free from fungal spores and no fungal count was detected on the malt extract agar media. This result indicated that all samples were processed under good manufactured practices and the detected Fumonisin B₁ in the tested samples were due to the use of contaminated corn.

CONCLUSIONS

The results indicated in this study should draw the attention of the probable contamination of young age consumed food with one or more of mycotoxins with levels that may exceed the permissible levels set by international agencies. This fact should alarm the official authorities to set a national standards and a monitoring program that will be of great help to detect such type of hazards in food.

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