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Fumonisin level in corn-based food and feed from Linxian County, a high-risk area for esophageal cancer in China

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Abstract

The level of mycotoxin fumonisins in corn-based food and feed collected from Linxian County, a high-risk area for esophageal cancer in China, has been analyzed using high-performance liquid chromatographic coupled with evaporative laser scattering detector (HPLC-ELSD). A total of 104 corn kernel samples were obtained from local households, granaries, wholesale markets (central markets), and retail markets (stores and supermarkets). Fumonisin B_1 (FB_1) was detected in the samples from households, granaries, central markets, and stores, with a positive rate of 61.5%, 50%, 33.3%, and 17%, respectively. No fumonisin was detected in samples from the supermarket. The highest FB_1 levels (0.30–3.20 μg/g; mean, 1.42 μg/g) were found in samples from the granary, followed by household (0.25–1.80 μg/g; mean, 0.73 μg/g), central market (0.25–1.10 μg/g; mean, 0.51 μg/g), and store (0.22–0.34 μg/g; mean, 0.28 μg/g). Among the 80 corn kernel samples collected from local households, 18 of 24 (75.0%) moldy samples contained high levels of FB_1 (0.28–3.30 μg/g; mean, 1.58 μg/g), and 20 of 56 (35.7%) apparently healthy samples contained low levels of FB_1 (0.21–0.82 μg/g; mean, 0.46 μg/g). As the central market plays an important role in trade of corn-based food and feed in China, a total of 115 corn-based food and feed samples were collected from the local central market. The highest FB_1 levels (0.30–3.13 μg/g; mean, 1.50 μg/g) were found in feed, followed by unprocessed food (0.31–0.63 μg/g; mean, 0.47 μg/g) and processed food (0.21–0.28 μg/g; mean, 0.25 μg/g). The positive incidence of FB_1 in feed, unprocessed, and processed food were 53.6%, 33.3% and 17.9%, respectively. In conclusion, the results showed that corn-based food and feed from Linxian County contained low level of FB_1 (<2 μg/g) in general, but efforts should be made to control the fumonisin contamination in corn kernels stored in granaries and households.

Keywords: Fumonisins, HPLC-ELSD, Corn products

1. Introduction

Fumonisins are a class of mycotoxins produced mainly by *Fusarium moniliforme* (*synonym F. verticillioides*), a primary fungal contaminant of corn and corn-derived products throughout the world. So far, twenty eight fumonisin analogs have been characterized (Rheeder, Marasas, & Vismer, 2002). They are classified into four main groups, the A, B, C, and P-series fumonisins. The B-series fumonisins are the most abundant analogs produced by *F. moniliforme* strains, with fumonisin B_1 (FB_1) being the most abundant and toxic constituent. FB_1 accounts for approximately 70% of total fumonisins found in nature ([Marasas, 2001] and [Nelson et al., 1993]).

Elevated fumonisin level in corn has become an area of concern since ingestion of fumonisin-contaminated corn has been associated with livestock loss and human health risks. Fumonisins were reported to cause leukoencephalomalacia (ELEM) in horses (Marasas, 2001), pulmonary oedema syndrome (PES) in pigs (Harrison, Colvin, Greene, Newman, & Cole, 1990), and hepatocarcinoma in rats (Gelderblom, Abel,

& Smuts, 2001). In addition to the diseases in livestock, consumption of corn-based food contaminated with fumonisins is known to epidemiologically relate to human esophageal cancer (HEC) in people from the Transkei region of South Africa and the Linxian region of China ([Chu and Li, 1994], [Rheeder et al., 1992] and [Sydenham et al., 1990]). It was also reported that gestational exposure to fumonisins may result in birth defects, such as neural tube defects (Hendricks, 1999). Several modes of action have been postulated to explain fumonisin-induced toxicity, but the primary hypothesis involves disruption of de novo sphingolipid biosynthesis (Merrill, Sullards, Voss, & Riley, 2001). Fumonisins are specific inhibitors of sphinganine *N*-acyltransferase (ceramide synthase), a key enzyme in the biosynthesis of sphingolipids.

The major high-risk areas for HEC in China are located in Henan and Hebei Provinces, with Linxian County in the Henan Province being the highest-risk area. Several surveys have been conducted concerning the natural occurrence of fumonisin in corn samples collected from households in Linxian County. Chu and Li (1994) detected high level of FB_1 (18–155 μg/g; mean, 74 μg/g) in all moldy samples from households in Linxian County, and relatively lower level of FB_1 (20–60 μg/g; mean, 35.3 μg/g) in all normal samples from the same households. However, lower incidence and level of FB_1 were also reported in other surveys conducted in Linxian County by (Yoshizawa et al., 1994) and (Wang and Zhu, 2002). The content of FB_1 varied greatly among different surveys conducted in Linxian County, which may be due to the different methods used for fumonisin analysis. As reported recently (Shephard, Van der Westhuizen, Gatyeni, Katerere, & Marasas, 2005), fumonisins detected in food using indirect analytical methods could sometimes produce false positive results, and it is important to have more definitive techniques to validate the results, because Linxian County is a main corn-producing and HEC-high-risk area in China.

Moreover, corns produced by farmers in Linxian County are not only stored and consumed by themselves in household, but also stored in granary or traded in local wholesale and retail markets. The trade of corn-based foods and feeds has been increasing in the local markets. However, limited information is available on fumonisin contamination in corn-based foods and feeds traded in Linxian County. It is important to investigate fumonisin contamination in various sources. The objective of this work was to use HPLC-ELSD to directly identify and measure fumonisins in corn and corn-based food and feed from different sources in Linxian County.

2. Materials and methods

2.1. Chemicals

High-performance liquid chromatography (HPLC)-grade acetonitrile was from EM Science (Darmstadt, Germany) or from Fisher Scientific (Pittsburgh, PA). Standard FB_1, FB_2, FB_3, and FB_4 were gifts from Ronald D. Plattner (US Department of Agriculture [USDA], Peoria, Ill.).

2.2. Sample collection

Corn kernel samples were collected from local farmers (household), granaries, central markets, stores, and supermarkets, while unprocessed corn kernels, processed corn foods, and corn-based feed were taken from local central market in Linxian County, Henan Province, in the period from July 2005 to May 2006. The processed corn foods included corn grit, corn flour, corn meal, corn tortilla chips and vacuum-packaging cooked corn. The samples were sent to the laboratory as soon as they were collected, and tested upon arrival or stored at −20 °C to arrest any fumonisin formation up to the time of analysis.

2.3. Determination of fumonisin

Analysis of fumonisins was performed according to our former method (Bojja, Cerny, Proctor, & Du, 2004), with minor modifications.

2.3.1. Extraction and clean up

A 10 g aliquot of the sample was placed in a flask containing 25 ml acetonitrile/water (1:1, v/v). Samples were placed in an orbital shaker overnight, filtered and a 10-ml aliquot was transferred to a 50 ml centrifugal tube and added 40 ml deionized water. Clean up of the extract was performed in 300 mg Amberlite XAD-4, previously conditioned with 2 ml of methanol and 20 ml of deionized water. After the initial washing with 200 ml deionized water, the extract was eluted with 3 ml 100% methanol. The eluent was dried under vacuum with freezing at −65 °C and stored at −20 °C, up to the moment of quantification.

2.3.2. Chromatographic quantification of fumonisins by HPLC-ELSD analysis

Samples were dissolved in 200 μl water and filtered through a 0.2 μm syringe-filter. A 20 μl aliquot was injected directly into the HPLC-ELSD system and used for quantification of the fumonisins. The HPLC system was a ProStar, Model 210 (Varian Walnut Creek, CA) with a column of Alltima C18LL, 5 μm, 250 × 4.6 mm inner diameter (Alltech, Deerfield, Ill.). The HPLC-ELSD method conditions were performed according to our previous studies with some modifications (Bojja et al., 2004). The mobile phases were (A) water-TFA (100:0.025, v/v) and (B) acetonitrile-TFA 100:0.025, (v/v), with a gradient of 0–20% B in A in the first 5 min, 20–40% B from 5 to 10 min, 40–80% B from 10 to 15 min, 80% B from 15 to 20 min, and 80–0% B from 20 to 25 min. The flow rate was 1.0 ml/min. The conditions set for ELSD (ELSD 2000, Alltech, USA) were 45 °C of drift tube temperature, 2.0-l/min nitrogen gas flow, and gain value of 1 in the impactor-on mode.

In quantitative analyses, a volume of 20 μl standard FB_2 FB_3 and FB_4 samples (1 μg/μl) was injected for HPLC-ELSD analysis, and standard FB_1 (10 μg/μl) was diluted in four different concentrations of 0.15, 0.3, 0.6, 1.2 μg/μl with the same method above. The peak area from the responding peak was integrated using on-system tools provided by Varian. At least 2 injections were made for each concentration, and the peak areas were then plotted against the absolute amounts of FB_1 used to obtain a standard curve. Under the established conditions, standard FB_1 gave a peak at a retention time of 16.26 min. Retention times for standard FB_3, FB_2 and FB_4 were 16.9, 17.2, and 17.9 min, respectively.

The method gave a linear response in the range of 3–24 μg FB_1 with $R^2 = 0.9828$. In five independent spiking experiments, where a known amount of standard FB_1 was added to the food sample, the recovery rate was also determined. The rates varied from 88.6% to 112.7%, with an average of 102.6% and a standard deviation of 8.9% ($n = 5$). The limit of determination was 3 ng/μl for FBs.

3. Results and discussion

Linxian County is one of the main maize-producing areas in Henan Province. Corn produced in Linxian County is either stored by local farmers and in granaries, or traded in local markets. The newly harvested corn cobs without husk, are usually dried under the sun on the ground floor in order to reduce the moisture content; then the sufficiently dried cobs are shelled, and the corn kernels are either stored or traded. Local farmers usually store the corn kernels in gunnysacks or put them in cement tanks inside the house without any ventilated equipment, for household storage. As for granary storage, no ventilated equipment is used either. The corn kernels are stored in cement tanks after wrapped with plastic film, and the top of cement tanks are then covered with straw mat to avoid insect and rodent attack. The newly harvested corn kernels with good visual quality and low moisture content, are usually traded in the markets. In all the corn samples analyzed, FB_1 was the only fumonisin detected, and no detectable levels of FB_2, FB_3 and FB_4 were found. In previous studies, the co-occurrence of FB_1 and FB_2 in corn (Yoshizawa et al., 1994), corn-based food (Bittencourt, Oliveira, Dilkin, & Corrêa, 2005), and feed (Sanchis et al., 1995), with FB_1 being the predominant fumonisin have been reported. In our test, only FB_1 was detectable in corn samples, which agrees with some other surveys in Linxian County ([Chu and Li, 1994], [Wang et al., 2000] and [Wang and Zhu, 2002]).

We collected a total of 104 corn kernel samples from local households, granaries, central markets, stores and supermarkets for fumonisin analysis. The highest incidence of FB_1 contamination (61.5%) was found in samples from households, followed by from granaries (50%), central markets (33.3%), and stores (17.0%). No FB_1 was detected in samples from supermarkets. The highest FB_1 level (0.30–3.20 μg/g; mean, 1.42 μg/g) was found in samples from granaries, followed by households (0.25–1.80 μg/g; mean, 0.73 μg/g), central markets (0.25–1.10 μg/g; mean, 0.51 μg/g), and stores (0.22–0.34 μg/g; mean, 0.28 μg/g) (Table 1).

No moldy corn kernel samples were found in the central and retail markets during our sampling, and all the samples collected were apparently healthy corn without any visible mold contamination, which may explain the low incidence and levels of FB_1 in corn samples traded in the markets in the present survey. As most of the moldy samples were from households, we conducted another survey, randomly collecting 80 samples from household and dividing them into two groups, moldy and normal healthy, according to their surface character. FB_1 analysis of the samples showed that high levels of FB_1 (from 0.28 to 3.30 μg/g; mean, 1.58 μg/g) were found in 18 of 24 (75%) moldy samples, while low levels (from 0.21 to 0.82 μg/g; mean, 0.46 μg/g) were found in 20 of 56 (35.7%) apparently healthy samples (Table 2), which suggested that even corn samples with normal surface character might contain fumonisin.

Our results indicated that fumonisin levels of all samples were significantly lower compared to the maximum level of FDA listed (2–4 ppm for human food) (FDA, 2002). Specifically, the samples from supermarket were free of FB_1 contamination, although FB_1 contamination was slightly more serious in corn kernel samples from local households and granaries than those from local markets. Several previous surveys have been conducted concerning the natural occurrence of

Table 1. Fumonisin contamination of corn from different sources

Source	Samples		Level of FB_1 in positive samples	
	Number	Positive (incidence)	Mean (μg/g)	Range (μg/g)
Household	26	16 (61.5%)	0.73 ± 0.18[1]	0.25–1.80
Granary	24	12 (50.0%)	1.42 ± 1.51	0.30–3.20
Central market	36	12 (33.3%)	0.51 ± 0.28	0.25–1.10
Store	12	2 (17.0%)	0.28 ± 0.08	0.22–0.34
Supermarket	6	0	–[2]	–

[1] Mean ± standard derivation.
[2] Below detection limit.

Table 2. Fumonisin contamination of corn samples from household

Surface character	Samples		Level of FB_1 in positive samples	
	Number	Positive (incidence)	Mean (μg/g)	Range (μg/g)
Moldy samples	24	18 (75.0%)	1.58 ± 1.21	0.28–3.30
Normal samples	56	20 (35.7%)	0.46 ± 0.20	0.21–0.82

fumonisin in corn samples collected from households in Linxian County. Chu and Li (1994) detected high level of FB_1 in concentrations ranging from 18 to 155 μg/g (mean 74 μg/g) in all moldy samples from households in Linxian County, and relatively lower level of FB_1 in concentrations ranging from 20 to 60 μg/g (mean 35.3 μg/g) was detected in all normal samples from the same households. However, lower incidence and level of FB_1 were also reported in another survey conducted in Linxian County by Yoshizawa and his colleagues, who described FB_1 contamination frequencies of 48% with average FB_1 level of 0.872 μg/g (Yoshizawa et al., 1994). A recent report by Wang and Zhu (2002) showed that FB_1 at the concentrations ranging from 1.07 to 2.56 μg/g was detected in 50% of moldy corn samples collected from households of Jingtou village in Linxian County, while low level (0.21–0.737 μg/g) of FB_1 was detected in 10.5% of 19 normal samples from the same households. Both the incidence and level of FB_1 in either moldy or healthy corn samples from household in Linxian County in the current survey were much lower than those reported by Chu and Li (1994), but similar to those reported by (Wang and Zhu, 2002) and (Wang and Zhu, 2002).

The variation of FB_1 content among different surveys in corn from households in Linxian County may be related to the climate conditions during and after harvest. For example, harvest occurring during a rainy period favours for fungal growth and fumonisin production (Jackson & Jalonski, 2004). Our results indicate, that although some helpful means have been adopted to control the fumonisin contamination of corn from household in Lianxian County in recent years, there are still many areas requiring improvements. For example, moldy corn is still harvested, stored, and consumed by the rural population in Linxian County, which represents a health risk for the people.

A direct detection method was used to determine the level of fumonisin contaminations in corn from granaries and markets in Linxian County. The results suggested that both the incidence and level of FB_1 in corn samples from granaries were higher than those of markets, with FB_1 level as high as 3.20 μg/g being determined. Although fumosinsin formation is believed to occur predominately in corn before harvest, it was also reported that the toxins could be formed during post-harvest storage, especially when corn was inadequately stored at high temperature and high relative moisture (Jackson & Jalonski, 2004). The relatively heavy FB_1 contamination in corn from granary might be related to the storage environment and method. It is easy to create partial high moisture in corn stored in such a non-ventilated storage system as granary, which is suitable for fungal (*Fusarium*) growth and mycotoxin production. A simple and effective method to reduce fumonisin contamination is to keep corn kernels sufficiently dried (moisture content less than 13%) before storage to ensure unfavourable conditions for fungal growth. To do this, the corn cob without husk can either be dried on the floor during sunny days, or put them on an indoor platform during rainy period before storing them in households or granaries.

Corn kernels are processed to varieties of corn-based foods and feed besides being directly consumed as food. We conducted another survey of fumonisins contamination in corn-based food and feed samples from central markets of Lianxian County. The results showed that FB_1 was detected in the ranges from 0.21 to 0.28 μg/g (mean, 0.25 ± 0.04 μg/g) in 7 of 39 (17.9%) processed corn food samples. No fumonisin was detected in all corn flour, corn meal and corn tortilla chip samples, only one of six vaccum-packaging cooked corn cob samples detected positive FB_1 at the level of 0.28 μg/g. However, FB_1 was detected at the concentration ranging from 0.21 to 0.27 μg/g in 6 of 12 (50%) corn grit samples (Table 3). Our results showed that less or no contamination of fumonisin was found in the processed corn products, which is coincident with previous reports ([Weidenbörner, 2001] and [Humpf and Voss, 2004]). Several previous studies were conducted to investigate the effect of processing method on fumonisn content. It was reported that the use of alkaline solution, water and temperature, used in the prepared corn-based foods might contribute to variations in the occurrence of fumonisin (Soriano & Dragacci, 2004). Cleaning corn to remove damaged or moldy kernels reduces fumonisins in foods while milling increases their concentration in some and reduces their concentration in other products (Jackson & Jalonski, 2004). Humpf and Voss (2004) found that baking, frying, and extrusion cooking of corn at high temperatures (≥190 °C) also reduced fumonisin concentrations in foods, with the amount of reduction achieved depending on cooking time, temperature, recipe, and other factors. It was also suggested that the fumonisin level in corn-based products was reduced during processing because fumonisins become 'hidden' and not recoverable under currently used extraction/purification procedures (Kim, Scott, & Lau, 2003). There are only a few reports on fumonisin contamination in corn-based products in China, while relatively more surveys are conducted in the occurrence of fumosins in corn-based products in some other countries (Soriano

Table 3. Fumonisin contamination of corn-based foods and feeds from central market

Use purpose	Samples		Level of FB_1 in positive samples	
	Number	Positive (incidence)	Mean (μg/g)	Range (μg/g)
Processed food	39	7 (17.9%)	0.25 ± 0.04	0.21–0.28
Corn grit	12	6 (50%)	0.23 ± 0.03	0.21–0.27
Corn flour	6	0	–	–
Corn meal	9	0	–	–
Corn tortilla chips	6	0	–	–
Vacuum-packaging cooked corn cob	6	1	0.28	0.28
Unprocessed food	48	16 (33.3%)	0.47 ± 0.12	0.31–0.63
Feed	28	15 (53.6%)	1.50 ± 1.24	0.30–3.13

and Dragacci, 2004; and Weidenbörner, 2001). Moreover, the consumption of corn is different from other countries in China as some special processing methods are used. It will be interesting to further investigate the effect and mechanism of different processing methods on fumonisin level of corn-based foods.

High levels of FB_1 (1.50 ± 1.24 μg/g) were found in 15 of 28 (53.6%) feed samples with the maximal level of 3.13 μg/g, which suggests that the feed produced in Linxian County need some more suitable practice for fumonisin control. In Linxian County, the corn kernels with good visual quality and low moisture content are directly traded in the market or used to made corn-based foods, while those with relatively poor quality are stored and consumed by the farmers or used to produce feed. Our results indicated that the fumonisin contamination is more serious in corn feed than in corn-based foods obtained from central markets. The Food and Drug Administration of USA has announced guidance levels for total fumonisin levels in corn products, 2–4 ppm for human foods and 5–100 ppm for animal feeds depending on the species and the proportion of the contaminated material in the total diets (FDA, 2002). Therefore, the feed traded in central markets of Linxian County is still safe according to this guidance standard.

4. Conclusion

FB_1 was the only fumonisin detected by HPLC-ELSD in all corn-based food and feed samples collected in Linxian County, a high-risk area for esophageal cancer in China. No fumonisin was detected in corn samples from local supermarket, and processed corn foods (corn flour, corn meal, and corn tortilla chips) from local central markets. Low average levels of FB_1 (<2 μg/g) were found in all corn samples collected locally, and all corn-based food and feed samples from local central markets, although relatively high levels of FB_1 were detected in corn samples from local granaries, and moldy corn samples from local households with maximal FB_1 level of 3.20 and 3.30 μg/g, respectively. The results indicate that new practices should be adopted to control the fumonisin contamination of corn kernels stored in granaries and households.

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