JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2009, 57, 3955–3960 3955
DOI:10.1021/jf803786h

Fumonisin Determination in Tunisian Foods and Feeds. ELISA and HPLC Methods Comparison

Ridha Ghali,* Hayet Ghorbel, and Abderrazak Hedilli

Laboratory of Biology and Toxicology, Research Unit, Toxicology and Environment, Centre of Urgent Medical Aid of Tunis, 99UR07-04 Tunis, Tunisia

A survey for fumonisins B1 and B2 (FB1 and FB2, respectively) was performed on 180 samples of high-consumption food commodities and 15 feed samples randomly collected from various regions of Tunisia. The determination of fumonisin level was performed by an in-house validated high-performance liquid chromatography and an enzyme-linked immunosorbent assay (ELISA) methods. Detection limit by ELISA for fumonisins sum was 25 μg/kg, and those by HPLC were 50 μg/kg for FB1 and 70 μg/kg for FB2. Recoveries of fumonisins spiked at 130 μg/kg ranged from 68.5 to 75.6% by ELISA, whereas those by HPLC for FB1 at 400 μg/kg and for FB2 at 300 μg/kg varied from 75.2 to 90.5%. Naturally occurring fumonisins were found in 10.5% of food samples with levels ranging from 70 to 2130 μg/kg. All contaminated samples contained FB1, and 31.5% of them contained FB2. In addition, the most contaminated commodities were corn foods and sorghum, whereas no fumonisin contamination was found in any nut or rice samples. For analyzed feed samples, fumonisins were detected in 86.6% of them with concentrations ranging from 50 to 2800 μg/kg. In addition, the performance of analytical methods was investigated in a comparison between ELISA and HPLC results for samples analyzed by both methods.

KEYWORDS: ELISA; feed; foods; fumonisin; HPLC; immunoaffinity; Tunisia

INTRODUCTION

Fumonisins are naturally occurring mold toxins produced mainly by *Fusarium verticillioides*, *Fusarium proliferatum*, and *Gibberella fujikuroi* (*1*). They consist of a group of seven structurally related analogues (*2*), but only fumonisins B1 (FB1), B2 (FB2), and B3 (FB3) have been reported to occur worldwide as natural contaminants. Fumonisins are potentially hazardous to humans and animals; they cause various diseases: liver and kidney toxicity, carcinogenicity, immunosuppression, and neurotoxicity (*23*). The exposure to fumonisin has been related to leukoencephalomalacia in equines, pulmonary edema in pigs, and hepatocellular carcinoma in rats (*4*, *5*). Most of the toxicities have a close relationship to the disruption of sphingolipids metabolism (*6*). Fumonisins are also suspected as a possible cause of esophageal cancer for humans in some areas of Africa and China (*7*), and FB1 was classified by the international agency for research on cancer in the 2B carcinogens group as possibly carcinogenic to humans (*8*). Fumonisin occurrence in human foods is widespread; contamination of maize (*Zea mays*) and maize-based products have been well-documented (*9*), but other cereals such as barley, wheat, rice, and sorghum have also been shown to contain fumonisin (*10*). These commodities are widely consumed in Tunisia and are a possible source of human exposure to mycotoxins, but there is no available information about the natural occurrence of fumonisin in Tunisian foodstuffs. Limits for fumonisin in foods are defined. The European Commission enforced the limits of FB1 and FB2 sum in maize and maize-based products only, with the following levels: 2000 μg/kg for unprocessed maize; 1000 μg/kg for maize flour, grits, germ, and refined oil; 400 μg/kg for direct human consumption maize-based foods; and 200 μg/kg for processed maize-based foods (*11*). There are currently no legal fumonisin limits for other commodities such as spices, nuts, and dried fruits. For animal feed, the total fumonisin maximal tolerable levels recommended by the U.S. FDA in maize and maize-based feeds are 5 mg/kg for equids and rabbits, 60 mg/kg for ruminants (cattle, sheep, goats), and 100 mg/kg for poultry (chickens, turkeys, ducklings) (*12*). Several analytical methods were used for fumonisin determination in food, including thin layer chromatography (*13*), enzyme-linked immunosorbent assay (*14*), and high-performance liquid chromatography linked to fluorescence detection used for confirmatory analysis (*15*, *16*). The objective of this study is to describe a sensitive HPLC method for fumonisin analysis in some food matrices by comparing it with an ELISA method and to evaluate the occurrence of fumonisin in some Tunisian foods and feeds.

*Address correspondence to this author at the Laboratory of Biology and Toxicology, Centre of Urgent Medical Aid of Tunis, Montfleury, 1008 Tunis, Tunisia (telephone/fax 216 71 241554; e-mail ghaliridha_ba@yahoo.fr).

MATERIALS AND METHODS

Reagents and Instruments. Lyophilized fumonisin B1 and B2 mixtures were supplied by R-Biopharm, Rhone Ltd. (Glasgow,

 Published on Web 3/19/2009

U.K.). Stock solutions at 100 μg/mL FB1 and 30 μg/mL FB2 were prepared in acetonitrile/water (50:50) solution and kept in the dark at −5 °C. Working standard solutions for calibration and spiking experiments were prepared immediately before use by diluting the stock solution with an acetonitrile/water (50:50) solution mixture. Orthophthalic dialdehyde (OPA, 1 mg/mL methanol solution), 2-mercaptoethanol, sodium phosphate, orthophosphoric acid, and boric acid were provided by Sigma Chemicals (St. Louis, MO). HPLC grade methanol, hexane, and acetonitrile were from Merck (Darmstadt, Germany). Double-distilled and demineralized water was obtained through a Milli-Q quality water system (Millipore, Bedford, MA). The RIDASCREEN fumonisin ELISA test kits and the Fuminoprep immunoaffinity columns (IAC) were from R-Biopharm, Rhone Ltd. Immunoaffinity columns were used with a vacuum manifold (Supelco, Sigma-Aldrich, St. Louis, MO). Sodium dihydrogen phosphate buffer (0.1 M and pH 3.3) was prepared by dissolving 3.45 g of NaH_2PO_4 in 250 mL of distilled water. The pH was then adjusted at 3.3 by adding 1 M orthophosphoric acid. Borate buffer (0.1 M, pH 10) was prepared by dissolving 3.1 g of boric acid in 475 mL of distilled water; the pH was adjusted to 10, and the final volume was completed by water to 500 mL.

The HPLC apparatus consisted of a Waters HPLC system (Milford, MA) including a gradient pump (Waters 600) connected to an automatic sampler (Waters 717) and a Waters model 477 fluorescence detector. This system was piloted by a Millennium 4.0 data system. The analytical column Symmetry Spherisorb ODS2 (250 × 4 mm, 5 μm; Waters), kept at 25 °C, was used for fumonisin determination. A microplate washer and ELISA 96-well plate reader were used for fumonisin ELISA analysis. A centrifuge (Heraeus CHRIST, LABOFUGEL, Germany) and a coffee mill SCM (Sibata, Tokyo, Japan) were used during the extraction steps.

Samples. A total of 180 representative human food samples and 15 feed samples were collected during the years 2005 and 2006 from local markets and traditional home reserves in Tunisia. The selected food commodity groups were cereals, including wheat, barley, maize, sorghum, rice, and their derived products; spices (cumin, red pepper, and black pepper); and nuts, such as peanuts and pistachios. Feed samples are maize-based products for ruminants and poultry. Samples were stored in plastic bags at 4 °C until grinding and analysis.

Methods. *ELISA Fumonisin Analysis*. RIDASCREEN_-Fumonisin test kits were used for the analysis. Mycotoxin extraction and tests were performed according to the manufacturer's instructions (*17*). About 5 g of the ground sample was extracted by 25 mL of methanol 70%. The extract was filtered through a Whatman filter, and 1 mL from the filtered sample was then diluted by 13 mL of distilled water. About 50 μL of diluted filtrate per well was used for the ELISA test. The optical density was measured at 450 nm using an ELISA 96-well plate reader, and all standard and sample solutions were analyzed in duplicate wells. The evaluation of ELISA data and the mycotoxin concentrations for samples were performed using the software program Rida-softwin (R-Biopharm, Rhone Ltd.). Recoveries were determined by spiking negative samples of analyzed food at 130 μg/kg for the fumonisins sum (100 μg/kg FB1 and 30 μg/kg FB2). Results were not corrected for recoveries.

Sample Extraction and Cleanup for HPLC Analysis. About 10 g of ground sample was added to 1 g of sodium chloride and extracted with 40 mL of an acetonitrile/methanol/water (25:25:50 v/v/v) mixture by vigorous shaking during 30 min in a 300 mL sealed flask. The extract was filtered through filter paper, and the filtrate was collected and centrifuged for 15 min at 4000*g*. Eight milliliters of the supernatant was diluted with 32 mL of PBS, mixed well, and filtered through a microfiber filter paper. After immunoaffinity Fumoniprep column conditioning with PBS, 10 mL of diluted extract was passed through this column at a flow rate of 3 mL/min. The column was washed with 10 mL of PBS, and air was forced through the column to push out all of the washing solution. Methanol (1.5 mL) was passed through the column to elute bound fumonisin, and the eluate was collected in a sample vial. Water (1.5 mL) was passed through the column and collected in the same sample vial to give a total volume of 3 mL.

Fumonisin Derivatization. To prepare fumonisin B1 and B2 derivatives, about 200 μL of sample extract or standard solution was mixed with 200 μL of derivatization solution. The mixture was mixed and kept for 1 min to react before the injection of 100 μL of the derivatized eluate into the HPLC system. Derivatization solution was prepared as follows: 5 mL of 1 mg/mL OPA reagent was mixed with 10 μL of 2-mercaptoethanol and 2 mL of borate buffer (pH 10). The pH was verified and adjusted to 10 if needed. This solution was kept in the dark at 4 °C.

Fumonisin HPLC Determination. Fumonisin determination was carried out under isocratic conditions, with a mobile phase constituted of methanol/0.1 M sodium dihydrogen phosphate (77:23) at a flow rate of 1 mL/min. A C_{18} analytical HPLC column showed most efficient for fumonisin separation. The detection of FB1 and FB2 was performed under fluorescence at 335 and 440 nm, respectively, for excitation and emission wavelengths. Fumonisin quantification was performed on the basis of the peak area corresponding to each toxin compared with the standard. The FB1 and FB2 identities were confirmed in all positive samples. IAC elute was spiked with a fumonisin standard solution (500 μg/kg for FB1 and 150 μg/kg for FB2) and analyzed by HPLC. Spiked and nonspiked chromatograms were then compared.

Method Validation. The analytical HPLC method validation was carried out on the basis of the harmonized guidelines single-laboratory validation of analysis methods (*18*). This method was initially validated by the analysis of the replicate of standard solutions and spiked samples ($n = 4$) for a variety of wheat, maize, rice, and sorghum. The method linearity was verified by linear regression analysis in the ranges from 125 to 4000 μg/kg for FB1 and from 75 to 1200 μg/kg for FB2. Detection and quantification limits (signal-to-noise ratio of 3) were estimated by analyzing decreased concentrations of standard solutions (LOQ was calculated as 2 LOD). Blank samples analyzed previously by both ELISA and HPLC methods and showing no fumonisin contamination were used for extraction recoveries and precision calculation. They were spiked with fumonisin standard solutions at 1000 and 400 μg/kg for FB1 and at 300 and 120 for FB2 and analyzed in triplicate over three consecutive days. Spiked samples were allowed to equilibrate for 1 h prior to extraction. Extraction recoveries were calculated by comparison of peak areas obtained after the extraction with those from working standard solutions at the intended final concentrations.

Statistical Analysis. Normal distribution of toxin contents, means, standard errors, and validation data were analyzed by an SPSS software program (SPSS Institute, Inc., 2000, version 10.0). The calibration curve used for quantification was calculated by the least-squares method. Multisample ANOVA test was used for the determination of statistical significance of averages differences. A P value of < 0.05 was accepted as significant.

RESULTS AND DISCUSSION

ELISA Method Performance. Recovery rates of fumonisin analyzed by ELISA method for artificially contaminated wheat, maize, rice, and sorghum samples at 130 μg/kg for the fumonisins sum (100 μg/kg FB1 and 30 μg/kg FB2) are summarized in **Table 1**. According to the manufacturer's description, the fumonisins (FB1 + FB2) recovery rate in corn meal samples spiked with 50 and 500 μg/kg is about 60% and the detection limit is 25 μg/kg (*17*). This limit is found to be lower than that of the ELISA method described by Abouzied et al. (*19*), which had a detection limit of 100

μg/kg, but it is higher than the FB1 detection limit (5 μg/kg) for the direct competitive ELISA method reported by Park et al. (*14*).

HPLC Method Performances. The proposed HPLC method enabled the fumonisin quantification in analyzed commodities with higher selectivity and sensitivity. The use of the mixture of methanol/0.1 M sodium dihydrogen phosphate (77:23) as mobile phase associated with a C_{18} HPLC stationary phase allowed the most satisfactory fumonisin separation with a maximum peak resolution and respective retention times of 5.28 and 11.20 min. The best fluorescence signals in terms of signal-to-noise ratio and sensitivity were obtained with 335 nm excitation and 440 nm emission wavelength combinations. The reproducibility of FB1 and FB2 retention times was tried with a standard solution analyzed six times. The relative standard deviations are 1.36 and 2.83% for FB1 and FB2 retention times, respectively. Linear regression analysis was performed with a correlation coefficient "*r*" of 0.998 for FB1 and of 0.995 for FB2. The lower limits of detection by HPLC are 50 μg/kg for FB1 and 75 μg/kg for FB2. In other investigations, the fumonisin detection limits ranged from 30 to 50 for FB1 (*15*, *14*), and they are 500 μg/kg for B1 and B2 fumonisins sum (*20*). FB1 and FB2 quantification limits are, respectively, 100 and 150 μg/kg. The fumonisin recovery rates for wheat, maize, rice, and sorghum are quite efficient, ranging from 75.2 ± 2.5 to 90.5 ± 2.4% (**Table 2**). The average value found for recoveries is in accordance with the European Committee acceptable range (*21*). Fumonisin recoveries calculated in our study are slightly lower than those obtained by Mateo et al. (*16*), who described recoveries ranging from 97.0 ± 2.9 to 105.0 ± 5.9% for cereal samples. The highest recovery averages are obtained for rice samples. The FB1 recovery rates are more important than those for FB2, which varied from 83.5 ± 3.5 to 90.5 ± 2.4% for the 400 μg/kg spiking level. For maize, FB1 rates are, respectively, 83.5 ± 1.9 and 87.7 ± 3.8%, results in accordance with those described in a previous study that reported 81.4 ± 1.5% as average recovery for maize samples spiked at 500 μg/kg (*15*). Contrarily to the earlier publications, only 10 g of food sample was used for fumonisin analysis. The significant reduction of sample quantity allowed both the cleanup process and the use of toxic solvents to be reduced, which permitted cleaner chromatograms to be obtained and overcame any potential saturation of IAC. Relative inter- and intraday variabilities (respectively, RSDr and RSDR) were calculated to express the method precision. The proposed HPLC method proved to be reproducible with a relative intraday standard deviation (RSDr) ranging from 2.4 to 4.4% for nine replicates. For interday variability, the average standard deviation (RSDR) is 5.37%, expressing a good reproducibility for three days.

For the 95 test samples analyzed with both ELISA and HPLC methods, contamination frequencies obtained by using the ELISA method are higher than those obtained by using the HPLC method but with lower fumonisin levels (**Table 3**). Of 37 samples positive for fumonisin by ELISA, 25 were positive by HPLC, whereas 12 samples that showed a weak contamination by the ELISA method (< 25 μg/kg) were shown to be negative or below the HPLC detection limits. The results in naturally contaminated food and feed samples indicated that both methodologies gave comparable levels. There is concordance between the 25 sample measurements identified as positive by the two methods (**Figure 1**). A *t* test and a linear regression analysis showed no significant difference between ELISA and HPLC measurements (= 0.978, $P < 0.005$). Although less specific, the ELISA is shown to be faster, more economical, and a useful first step in identifying positive samples before confirmation by HPLC. The HPLC assay is less sensitive, but it allowed the separation of fumonisin and gave more accurate quantitative results for each.

Fumonisin Detection in Food and Feed Samples. During the experiment, the presence of fumonisin was checked in 195 food and feed samples. On the basis of the HPLC results (**Table 4**), fumonisins are found in 10.5% of food samples with levels ranging from 70 to 2130.0 μg/kg. FB2 was detected in 31.5% of contaminated samples containing FB1. In accordance with worldwide studies, maize-based food is the most FB1 contaminated (52.9%). For FB1, rates range from 75.0 to 1700.0 μg/kg, with an average concentration of 309.7 ± 374 μg/kg. FB2 concentrations varied from 214 to 430 μg/kg, with an average level of 226.1 ± 113 μg/kg. This result is in accordance with the work of Zinedine et al. (*15*) work, which reported that 50% of corn samples intended for human consumption in Morocco were contaminated with FB1. However, we should note that fumonisin levels detected in our study are definitely lower than those reported in other countries. Indeed, in Morocco, the average of FB1 rates in corn samples is 1930 μg/kg, the highest value being 5960 μg/kg (*15*). In a Spanish study, among cereals, the highest levels of fumonisins were detected in maize. FB1 and FB2 were, respectively, detected in 79.5 and 14.6% of samples with average levels of 3300 μg/kg for FB1 and 1700 μg/kg for FB2 (*22*). In Brazil, the FB1 and FB2 rates in corn meal samples ranged from 1100 to 15300 μg/kg for FB1 and from 200 to 3900 μg/kg for FB2 (*23*). Among the studied samples, two maize-based food samples included fumonisin contents higher than the European maximum

Table 1. ELISA Recovery Rates of Fumonisins (B1 and B2) for Wheat, Maize, Rice, and Sorghum

commodities spiked at 130 μg/kg FB1 and FB2	recovery ± RSD (%), $n = 3$[a]
maize	68.5 ± 3.9
rice	72.4 ± 4.8
sorghum	75.6 ± 3.7
wheat	69.4 ± 4.2

[a] RSD, relative standard deviation; *n*, number of samples.

Table 2. Average Recovery for Fumonisins B1 and B2 in Wheat, Maize, Rice, and Sorghum Analyzed by HPLC

	av recovery ± RSDr (%), $n = 3$[a]			
commodity	FB1 at 1000 μg/kg	FB1 at 400 μg/kg	FB2 at 300 μg/kg	FB2 at 120 μg/kg
maize	83.5 ± 1.9	87.7 ± 3.8	79.5 ± 3.6	82.7 ± 2.8
rice	85.7 ± 2.8	90.5 ± 2.4	82.2 ± 3.2	83.4 ± 2.3
sorghum	75.3 ± 2.9	83.5 ± 3.5	75.2 ± 2.5	76.1 ± 4.1
wheat	88.4 ± 4.8	89.5 ± 4.4	83.5 ± 5.1	84.5 ± 4.6

[a] RSDr, intraday relative standard deviation; *n*, number of samples.

Table 3. Results for Samples Analyzed by both ELISA and HPLC Methods for Fumonisins Sum

	ELISA		HPLC	
commodity	positive/analyzed samples	mean level ± SD[a] (μg/kg)	positive/analyzed samples	mean level ± SD (μg/kg)
all commodities	37/95	440.6 ± 537	25/95	510.2 ± 658
food samples	24/80 (12 < LD)[b]	407.2 ± 547	12/80	458.8 ± 584
feed samples	2/15	471.3 ± 547	2/15	557.6 ± 740
wheat	6/25 (5 < LD)[b]	300	1/25	380
barley	1/13	89.0	1/13	132.0
maize	11/18 (2 < LD)[b]	487.4 ± 616	9/18	540.4 ± 658
spices	3/9 (2 < LD)[b]	111	1/9	130
rice	0/8		0/8	
sorghum	3/7 (3 < LD)[b]		0/7	

[a] SD, standard deviation. [b] Positive samples inferior to ELISA quantification limit and excluded in mean level determination. LD, limit of detection.

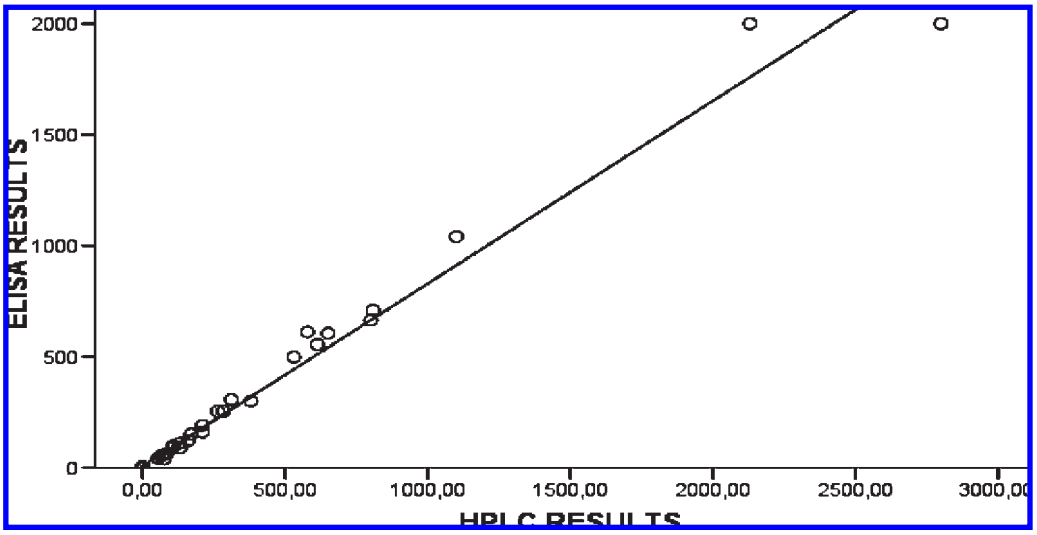

Figure 1. Correlation of measurements of fumonisins by HPLC and ELISA for 25 samples identified as positive by both methods. (There is an overall agreement between the two methods with $r^2 = 0.978$ at a significance level of $P < 0.005$.)

Table 4. Contamination Frequency and Average Level of HPLC Analyzed Commodities

	fumonisins sum (B1 + B2)			FB1	FB2
commodity (no. of samples)	no. of positive samples	contamination values range (μg/kg)	mean level ± SD[a] (μg/kg)	mean level ± SD (μg/kg)	mean level ± SD (μg/kg)
all commodities (195)	32	55−2800	452.8 ± 597	362.1 ± 466	201.0 ± 140
food samples (180)	19	70−2130.0	381.1 ± 485	309.7 ± 374	226.1 ± 113
feed samples (15)	13	55−2800	557.6 ± 740	438.6 ± 584	182.1 ± 163
wheat[b] (46)	2	70−380	225.0 ± 219	225.0 ± 219	0
barley[b] (24)	1	132	132	132	
maize[b] (17)	9	84−2130	540.4 ± 658	442.6 ± 508	293.3 ± 118
dried fruits (20)	0	0			
spices (13)	2	70−130	100.0 ± 42	100.0 ± 42	0
rice (11)	0	0			
sorghum (49)	5	80−629	319.2 ± 227	223.8 ± 133	159.0 ± 69

[a] SD, standard deviation. [b] Positive samples inferior to ELISA quantification limit and excluded in mean level determination.

limit (400 μg/kg), and only one sample of unprocessed maize exceeded the European tolerable limit of fumonisin (2000 μg/kg) (*11*). For analyzed feed samples, the contamination frequencies and levels are more important. Fumonisins are detected in 86.6% of the investigated samples with a concentration ranging from 55 to 2800 μg/kg. FB1 is present in all contaminated samples and FB2 in 53.3% of these samples with respective average levels of 438.6 ± 584 and 182.1 ± 163 μg/kg. A similar widespread contamination of animal feed with fumonisins was reported in other countries (*24*). In most instances, the predominant fumonisin was FB1. In South Africa, FB1 was detected with higher levels varying from 4000 to 11000 μg/kg, whereas in India, FB1 was detected in all analyzed samples at lower levels ranging from 20 to 260 μg/kg (*25*). No feed sample exceeded the fumonisin maximal tolerable levels recommended by the FDA in maize-based feeds for ruminants (6000 μg/kg) or poultry (100000 μg/kg) (*12*). However, some of the detected values in our study are of toxicological significance because a level as low as 1000 μg/

kg was associated with disease syndromes in livestock (*24*). Dried fruits (nuts) and rice showed to be not contaminated with fumonisin. In other parts of the world, rice is not considered to be a contamination matrix for fumonisin (*20*). Compared to contamination frequencies described for Spanish wheat and barley (*22*), lower fumonisin levels were found in barley, spices, and wheat with respective contamination frequencies of 4.16, 1.53, and 4.34%. In Korea, FB1 was found in 6% of investigated barley food samples with an average level of 16 μg/kg (*14*). For sorghum, fumonisins were detected with a relatively higher frequency (10.2%) and mean level (319.2 $\pm$ 227 μg/kg). FB1 occurrence in sorghum ranging from 110 to 150 μg/kg was reported in Brazil with a contamination frequency of 74.2% (*26*). This cereal is highly consumed in Tunisia as a breakfast ingredient and baby food and showed high aflatoxins and ochratoxin A contaminations (*27*). Statistical analysis made by ANOVA test showed significant differences between fumonisin mean contents in analyzed food groups ($P < 0.05$). This result confirms the influence of the fungus nutriment source on the produced mycotoxin type and level already described (*28*).

Due to the possible health implications of fumonisin contamination, valid and reliable analytical methods are essential. In this work, an HPLC method for fumonisin analysis was validated and compared to an existing ELISA method. Both methods are used for fumonisin B1 and B2 assessment in some Tunisian foods and feeds. This survey reported that some products are contaminated by fumonisin with concentrations above the legal limit. FB1 and FB2 are detected in investigated food and feed samples with respective contamination frequencies of 10.5 and 86.6%. In addition, FB1 is the predominant fumonisin present with an important level in some commodities, especially in corn-based food and in feed samples.

ABBREVIATIONS USED

ANOVA, analysis of variance; ELISA, enzyme-linked immunosorbent assay; FB1, fumonisin B1; FB2, fumonisin B2; FB3, fumonisin B3; FDA, U.S. Food and Drug Administration; IAC, immunoaffinity columns; LOD, limit of detection; LOQ, limit of quantification; ODS, octadecylsilane; OPA, orthophthalic dialdehyde; PBS, phosphate-buffered saline; R^2, correlation coefficient; RSDR, interday relative standard deviation; RSDr, intraday relative standard deviation.

LITERATURE CITED

(1) Leslie, J. F. Introductory biology of *Fusarium moniliforme*. *Adv. Exp. Med. Biol*. **1996**, *392*, 153–164.

(2) Branham, B. E.; Plattner, R. D. Alanine is a precursor in the biosynthesis of fumonisin B1 by *Fusarium moniliforme*. *Mycopathologia* **1993**, *124*, 99–104.

(3) Gelderblom, W. C.; Cawood, M. E.; Snyman, S. D.; Marasas, W. F. Fumonisin B1 dosimetry in relation to cancer initiation in rat liver. *Carcinogenesis* **1994**, *15*, 209–214.

(4) Colvin, B. M.; Harrison, L. R. Fumonisins induced pulmonary oedema and hydrothorax in swine. *Mycopathologia* **1992**, *117*, 79–82.

(5) Ross, P. F.; Ledet, A. E.; Owens, D. L.; Rice, L. G.; Nelson, H. A.; Osweiler, G. D.; Wilson, T. M. Experimental equine leukoencephalomalacia, toxic hepatosis, and encephalopathy caused by corn naturally contaminated with fumonisins. *J. Vet. Diagn. Invest*. **1993**, *5*, 69–74.

(6) Voss, K. A.; Plattner, R. D.; Bacon, C. W.; Norred, W. P. Comparative studies of hepatotoxicity and fumonisin B1 and B2 content of water and chloroform/methanol extracts of *Fusarium moniliforme* strain MRC 826 culture material. *Mycopathologia* **1990**, *112*, 81–92.

(7) Pitt, J. I. Toxigenic fungi and mycotoxins. *Br. Med. Bull*. **2000**, *56*, 184–192.

(8) International Agency for Research on Cancer. *Ochratoxin A and Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins*; Monographs on the Evaluation of Carcinogenic Risk to Humans 56;IARC: Lyon, France, 1993; pp 489−452.

(9) Jimenez, M.; Huerta T.; Mateo, R. Secondary metabolites produced by *Fusarium*. *Appl. Environ. Microbiol*. 1997, *63*, 364−372.

(10) Mateo, J. J.; Jiménez, M. Trichothecenes and fumonisins produced in autoclaved tiger nuts by strains of *Fusarium sporotrichioides* and *Fusarium moniliforme*. *Food Microbiol*. **2000**, *17*, 167–176.

(11) European Commission. Commission regulation setting maximum levels for certain contaminants in foodstuffs. *Off. J. Eur. Commission* 2006, *L364*, 5−24.

(12) U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition. Fumonisin Levels in Human Foods and Animal Feeds. Guidance for Industry, Final guidance; Rockville, MD, Nov 2001; available at http://www.cfsan.fda.gov/dms/ fumongu2.html.

(13) George, E. R.; Charles, E. C.; Harry, C. M. A rapid, sensitive thin layer chromatography procedure for the detection of fumonisin B1 and B2. *J. Vet. Diagn. Invest*. **1992**, *4*, 326–329.

(14) Park, J. W.; Kim, E. K.; Shon, D. H.; Kim, Y. B. Natural co-occurrence of aflatoxin B1, fumonisin B1 and ochratoxin A in barley and corn foods from Korea. *Food. Addit Contam*. **2002**, *19*, 1073–1080.

(15) Zinedine, A.; Brera, C.; Elakhdari, S.; Catano, C.; Debegnach, F.; Angelini, S.; De Santis, B.; Faid BenlemLih Minardi, M.; Miraglia, V. Natural occurrence of mycotoxins in cereals and spices commercialized in Morocco. *Food Control* **2006**, *17*, 868–874.

(16) Mateo, J. J.; Mateo, R.; Hinojoa, M. J.; Llorensa, A.; Jimenez, M. Liquid chromatographic determination of toxigenic secondary metabolites produced by *Fusarium* strains. *J. Chromatogr., A* **2002**, *955*, 245–256.

(17) R-Biopharm Rhone Ltd. Method Fumoniprep IFU (P31.V4). Doc 1.07.03, 2003.

(18) Thompson, M.; Ellison, S. L. R.; Wood, R. Harmonized guidelines for single-laboratory validation of methods of analysis. *Pure Appl. Chem*. **2002**, *74*, 835–855.

(19) Abouzied, M.; Askegard, S.; Bird, C.; Miller, B. M. Development of a rapid quantitative ELISA for determination of the mycotoxin fumonisin in food and feed. *J. Clin. Ligand Assay* **1995**, *18*, 145–149.

(20) Abbas, H.; Cartwright, R.; Shier, W.; Abouzied, M.; Rice, L.; Ross, P.; Sciumbato, G.; Meredith, F. Natural occurrence of fumonisins in rice with *Fusarium* sheath rot disease. *Plant Dis*. **1998**, *82*, 22–25.

(21) European Committee for Standardization, CEN/TC 275/WG5 N195, 1999.

(22) Castella, G.; Bragulat, M. R.; Cabanes, F. Surveillance of fumonisins in maize-based feeds and cereals from spain. *J. Agric. Food Chem*. **1999**, *47*, 4707–4710.

(23) Bittencourt, A. B. F.; Oliveira, C. A. F.; Dilkin, P.; Corrêa, B. Mycotoxin occurrence in corn meal and flour traded in São Paulo, Brazil. *Food Control* **2005**, *16*, 117–120.

(24) Shephard, G.; Thiel, P. G.; Stockenstrom, S.; Sydenham, E. W. Worldwide survey of fumonisin contamination of corn and corn-based products. *J. AOAC Int*. **1996**, *79*, 671–687.

(25) Placinta, C. M.; D'Mello, J. P. F.; Macdonald, A. M. C. A review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. *Anim. Feed Sci. Technol*. **1999**, *78*, 21–37.

(26) Da Silva, J. B.; Pozzi, C. R.; Mallozzi, M. A. B.; Ortega, E. M.; Corrêa, B. Mycoflora and occurrence of aflatoxin B_1 and fumonisin B_1 during storage of Brazilian sorghum. *J. Agric. Food Chem*. **2000**, *48*, 4352–4356.

(27) Ghali, R.; Hmaissia-khlifa, K.; Ghorbel, H.; Maaroufi, K.; Hedilli, A. Incidence of aflatoxins, ochratoxin A and zearalenone in Tunisian foods. *Food Control* **2008**, *19*, 921–924.

(28) Meri, K.; Marika, J.; Aldo, R. The effect of substrate on mycotoxin production of selected *Penicillium* strains. *Int. J. Food Microbiol*. **2005**, *99*, 207–214.

Received for Review December 7, 2008. Accepted March 05, 2009. Revised manuscript received March 5, 2009.