Anal Bioanal Chem (2006) 384: 1214–1220
DOI 10.1007/s00216-005-0295-z

ORIGINAL PAPER

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Determination of fumonisins B1 and B2 in Portuguese maize and maize-based samples by HPLC with fluorescence detection

Received: 18 November 2005 / Revised: 19 December 2005 / Accepted: 21 December 2005 / Published online: 8 February 2006

Abstract Fumonisins B_1 (FB_1) and fumonisin B_2 (FB_2) are the main members of a family of mycotoxins produced by *Fusarium verticillioides*, *Fusarium proliferatum*, and other fungi species of the section *Liseola*. The present work shows the results of comparative studies using two different procedures for the analysis of fumonisins in maize and maize-based samples. The studied analytical methods involve extraction with methanol/water, dilution with PBS, and clean-up through immunoaffinity columns. Two reagents (*o*-phthaldialdehyde and naphthalene-2,3-dicarboxaldehyde) were studied for formation of fluorescent derivatives. The separation and identification were carried out by high-performance liquid chromatography with fluorescence detection. The optimized method for analysis of fumonisins in maize involved extraction with methanol/water (80:20), clean-up with an immunoaffinity column, and derivatization with naphthalene-2,3-dicarboxaldehyde (NDA). The limit of detection was 20 μg kg^{-1} for FB_1 and 15 μg kg^{-1} for FB_2. Recoveries of FB_1 and FB_2 ranged from 79% to 99.6% for maize fortified at 150 μg kg^{-1} and 200 μg kg^{-1}, respectively, with within-day RSDs of 3.0 and 2.7%. The proposed method was applied to 31 samples, and the presence of fumonisins was found in 14 samples at concentrations ranging from 113 to 2,026 μg kg^{-1}. The estimated daily intake of fumonisins was 0.14 μg kg^{-1} body weight per day.

Keywords Fumonisins B_1 and B_2 · Maize · Maize derivatives · HPLC

Introduction

Fumonisins (FBs) are a group of toxins comprising fumonisins B_1 (FB_1), B_2 (FB_2), and B_3 (FB_3). FBs are structurally analogous to sphingosine [1] and are mainly yielded by *Fusarium verticillioides*, *Fusarium proliferatum*, and other fungi species of the section *Liseola* [2]. FB_1 is the most abundant and the most toxic fumonisin, followed by FB_2 [3]. FBs has been linked with an increased risk of oesophageal cancer in humans in the Transkei region of South Africa and China [1] and are possibly connected with neural tube defects (NTD) in South Texas, USA [4]. FB_1 is classified by International Agency for Research on Cancer (IARC) as possibly carcinogenic in humans (Group 2B) [5].

The wide geographical distribution of the fumonisin-producing maize pathogen *F. verticillioides* and its endophytic nature in maize have resulted in fumonisins occurring naturally in maize or products containing maize worldwide [6]. High levels of fumonisins in maize have been found in many countries, including Morocco [7], Brasil [8], Nigeria [9], and South Africa [10]. In Europe sporadic studies have been recently published [11].

Maize (*Zea mays* L.) is one of the most important crops in Portugal, representing a maize area and production of 126,000 ha and 665,000 Mt, respectively [12].

In Portugal, a single investigation of FB_1 and FB_2 in nine naturally contaminated maize hybrids, originating from the 1992 crop from the Agricultural School of Coimbra, was reported in international references, and revealed a high frequency of contamination, 100% [13].

Since the discovery of these mycotoxins and their characterization in 1988, there has been significant progress in the analytical methods used for their detection [14].

Recently, Lino et al. [15] reviewed the analytical methodology available for the determination of fumonisins in foods. These methods generally involve liquid extraction, with mixtures of polar solvents, such as methanol/water [16, 17], acetonitrile/water [18], methanol/acetonitrile/water [19], and acetonitrile/NaH_2PO_4 [20]. A clean-up procedure is frequently applied that usually employs solid-phase extraction (SPE) columns such as with reversed-phase columns [18, 20], strong anion exchange columns (SAXs) [8, 21], and immunoaffinity columns (IACs) [17, 22] that present higher specificity. Different derivatization

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reagents have been reported, namely, *o*- phthaldialdehyde (OPA)—the most used [8, 9, 21], naphthalene-2,3-dicarboxaldehyde (NDA) [19, 20, 23], and 4-fluoro-7-nitrobenzofurazan (NBD-F) [24]. Detection and quantification have preferentially been performed using HPLC with fluorescence or mass spectrometry detection [8, 9, 21, 22, 25–28] although other chromatographic, electrophoretic, and immunological methods have been reported [15].

The objective in the present study was to optimize a sensitive and accurate method for determination of FBs in maize and derivatives by HPLC with pre-column derivatization and fluorescence detection, and to provide data on the occurrence of FB_1 and FB_2 in 31 maize and maize product samples consumed in central Portugal. The method was useful for assessing the potential contribution of dietary exposure to maize products in Portuguese consumers.

Materials and methods

Apparatus and chromatographic conditions

The high-performance liquid chromatography (HPLC) apparatus used consisted of a Gilson 307 pump (Gilson Medical Electronics, Villiers-le-Bel, France), one 50-μL Rheodyne 7125 injector (Cotati, CA, USA), a 5-μm C_{18} Nucleosil 120 guard column KS (30 mm × 4-mm i.d.), and a 5-μm C_{18}Nucleosil 120 column (250 mm × 4.6-mm i.d.). A Perkin Elmer LS45 spectrofluorimeter (Perkin Elmer, Beaconsfield, UK) operating at an excitation wavelength of 420 nm and an emission wavelength of 500 nm was used.

The results were recorded on a 3990 integrator (Hewllet-Packard, Philadelphia, PA). The mobile phase (acetonitrile/water/acetic acid 61:38:1 v/v/v) was maintained at a flow rate of 1 mL min^{-1}.

Isocratic analysis under the conditions described above allowed the elution of FB_1 and FB_2 with a retention time of about 7.36 min and 13.34 min, respectively, when NDA derivatization was used.

A Meditronic S-599 centrifuge (Selecta, Barcelona, Spain), Retsh vortex mixer (Haan, Germany), and a Sonorex RK 100 ultrasonic bath (Berlin, Germany) were also used.

Chemicals

HPLC-grade acetonitrile (ACN) and methanol were purchased from Carlo Erba (Milan, Italy). Acetic acid, hydrochloride acid, sodium hydroxide, potassium chloride, potassium dihydrogen phosphate, anhydrous disodium hydrogen phosphate, potassium cyanide, sodium tetraborate, and sodium chloride were obtained from Merck (Darmstadt, Germany). Water was prepared from a Milli Q system (Millipore, Bedford, MA, USA). FB_1 and FB_2 standards, naphthalene-2,3-dicarboxaldehyde (NDA), and 2-mercaptoethanol (MCE) were obtained from Sigma Chemicals Co (St. Louis, USA). *o*-Phthaldialdehyde (OPA) was obtained from Carlo Erba (Milan, Italy).

FumoniTest immunoaffinity columns were from Vicam (Watertown, USA).

Phosphate buffer solution (PBS) was prepared from 0.2 g potassium chloride, 0.2 g potassium dihydrogen phosphate, 1.2 g anhydrous disodium hydrogen phosphate, and 8.0 g sodium chloride to 990 mL distilled water, adjusted to pH 7.0 with 25% HCl, and the solution was made up to 1 L.

The OPA derivatization reagent was prepared by mixing 40 mg OPA, 1 mL of methanol, 5 mL of a 0.1 M aqueous sodium tetraborate, and 50 μL of 2-mercaptoethanol. The naphthalene-2,3-dicarboxaldehyde (NDA) solution was prepared at 0.5 mg mL^{-1} in acetonitrile. The potassium cyanide was prepared at 0.13 mg mL^{-1}; 0.05M sodium borate solution was adjusted to pH 9.5 with 1 N NaOH.

The mobile phase consisted of acetonitrile/water/acetic acid (61:38:1 v/v/v).

The stock solutions, prepared in the FB_1 and FB_2 vials purchased from Sigma Chemicals, were made in 1 mL acetonitrile/water (50:50) at 1,000 μg mL^{-1}. Intermediate solutions were prepared at 50 μg mL^{-1}, diluting 250 μL of stock solution with 5 mL acetonitrile/water (50:50). For fortification assays, one work solution was prepared with acetonitrile/water (50:50) at 5 μg mL^{-1} for both fumonisins. For determination of the calibration curve, two work solutions were prepared at 50 μg mL^{-1} and 5 μg mL^{-1}. The others were prepared by diluting one of these solutions at the following concentrations: 5.0, 2.5, 1.0, 0.5, and 0.25 μg mL^{-1}. All solutions were kept in amber flasks to protect from light.

Sampling

A total of 31 samples were purchased in commercially available sizes from March to July 2005 at shops, health food stores, and supermarkets located in the city of Coimbra. The following commodities were collected: yellow maize (n=9), white maize (n=2), maize flour (n=3), maize semolina (n=3), maize starch (n=3), sweet maize (n=11). Samples, except for maize flour, maize semolina, and maize starch, were finely milled in the switching apparatus.

Recoveries

The recoveries of fumonisins from maize and maize starch were determined by spiking the ground sample of each food (three replications) with known amounts of fumonisins at final concentrations between 100 μg kg^{-1} and 250 μg kg^{-1}.

Extraction and clean-up procedures

Method A

Ground samples (50 g) were mixed with 5 g NaCl and placed into a blender jar. One hundred mL CH_3OH/H_2O

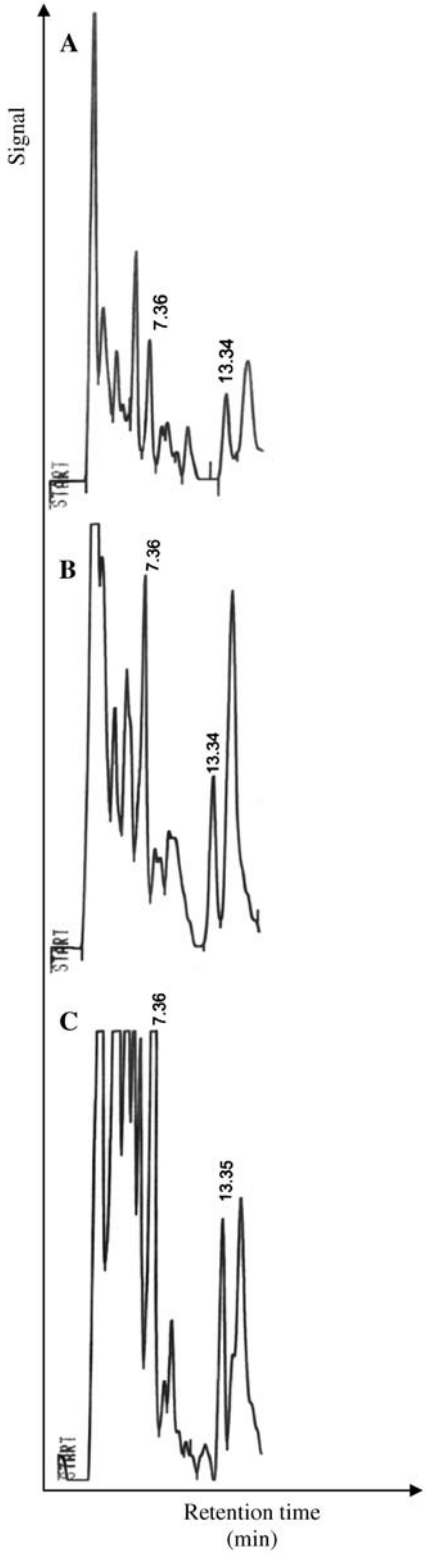

Fig. 1 Liquid chromatography spectrofluorimetric chromatogram of fumonisin B_1 and B_2 standard (**a**) (retention time 7.36 and 13.34 min for FB_1 and FB_2, respectively), one fortified sample at 250 μg kg^{-1} for FB_1 and 200 μg kg^{-1} for FB_2 (**b**), and one contaminated sample (**c**), obtained using the optimized method

(80:20 v/v) was added. The mixture was blended at high speed for 5 min, filtered through a Whatman N°1 filter paper, and collected in a clean vessel. Ten mL of filtered extract was transferred to another vessel and eluted with 40 mL PBS. The extract was filtered through microfiber filter (Whatman 934-AH, 110 mm). Ten mL was added to a FumoniTest IAC attached onto a vacuum manifold. The column was washed with 10 mL PBS, and fumonisins were eluted with 1.5 mL methanol. After evaporation under a gentle nitrogen stream at 60°C, the residue was redissolved in 50 μL methanol/water (50:50 v/v).

Method B

Ground samples (25 g) were extracted with 40 mL CH_3OH/H_2O (80:20 v/v), centrifuged for 15 min at 2,500 *g*. The remaining solid was extracted twice with 30 mL each. The three extracts were combined and filtrated (Whatman N°1 paper). For clean-up, 10 mL of filtrate diluted with 40 mL phosphate-buffered saline was filtrated through glass microfiber. Twenty mL was added to a FumoniTest IAC attached onto a vacuum manifold. The column was washed with 10 mL PBS, and fumonisins were eluted with 2 × 1.5 mL methanol. After evaporation under a gentle nitrogen stream at 60°C, the residue was reconstituted with 50 μL methanol/water (50:50 v/v).

Derivatization

Five hundred microliter 0.05 M sodium borate buffer (pH 9.5 adjusted with 1 N NaOH), 500 μL sodium cyanide reagent, and 150 μL NDA reagent (0.5 mg mL^{-1} ACN) were added to the reconstituted residue. The mixture was heated for 15 min at 60°C in a heating bath and cooled to room temperature.

Results and discussion

The calibration curves were obtained using the linear least-squares regression procedure for the peak area plotted versus the concentration. The linearity for FB_1 and FB_2, in the working standard solutions at four determinations of

Table 1 Comparison of different chromatographic conditions with OPA at λ_{exc}=335 nm and λ_{em}=440 nm

Volumes of OPA (μL)	Quantity of standard (ng)	Mobile phase	Flow rate (mL min^{-1})
100	1,000	A=ACN/H_2O/CH_3 COOH (49.5:49.5:1.0)	1.0
100	50	B=ACN/H_2O/CH_3 COOH (59.0:40.0:1.0)	1.0

Table 2 Comparison of different chromatographic conditions with NDA at λ_{exc}=420 nm and λ_{em}=500 nm

Mobile phase	Flow rate (mL min^{-1})
A = 65% ACN/CH_3COOH (99:1) + 35% H_2O/ CH_3COOH (99:1)	1.0
B = ACN/H_2O/CH_3COOH (63:36:1)	1.0
C = ACN/H_2O/CH_3COOH (60:39:1)	1.0
D = ACN/H_2O/CH_3COOH (59:40:1)	0.9
E = ACN/H_2O/CH_3COOH (62:40:1)	1.0
F = ACN/H_2O/CH_3COOH (61:38:1)	1.0

five concentration levels, between 0.25 and 5.0 μg mL^{-1}, which corresponds to 0.52 ng and 10.4 ng injected, was good as shown by the fact that the correlation coefficients (r^2) were 0.984 and 0.994 for FB_1 and FB_2, respectively.

Figure 1 shows the HPLC spectrofluorimeter chromatograms of the FBs standard, one sample and one sample fortified using method B. FB_1 and FB_2 standards were initially analyzed using pre-column derivatization with *o*-phthaldialdehyde (OPA) and reversed-phase HPLC separation with an isocratic elution followed by fluorescence detection. Different chromatographic conditions were used (Table 1). When mobile phase A was used, according to Bittencourt et al. [8], long retention times were obtained: 12.57 for FB_1 and 33.10 for FB_2. The proportion of acetonitrile was increased and the retention times decreased to 5.49 and 10.09 for FB_1 and FB_2, respectively.

Several fluorescent derivatives have been reported. Although OPA-MCE is currently the most commonly used derivatization reagent it has the disadvantage of exhibiting time-dependent degradation, leading to the rapid decay of fluorescence after derivatization [29–31]. This disadvantage was overcome by the use of naphthalene-2,3-dicarboxaldehyde (NDA) that has been reported in several studies [19–21, 29, 32].

Derivatization reaction with NDA was performed according to the methods of Chu and Li [32] and Bennett and Richard [29], with some modifications. The best conditions were obtained using 150 μL of derivatization reagent and replacing the redissolution of the residue by 50 μL methanol/water (50:50). Different mobile phases were also experimented (see Table 2). The best results were obtained with ACN/H_2O/CH_3COOH (61:38:1 v/v/v).

NDA with KCN formed a highly fluorescent derivative which was relatively stable over 24 h [30].

Table 3 Stability of NDA fluorescent derivatives (ng and %)

Fumonisins	1 day		1 day		8 days	
FB_1 ng (%)	1.04 (100)	1.15 (110)	2.08 (100)	1.65 (79.4)	2.08 (100)	1.41 (67.7)
FB_2 ng (%)	1.04 (100)	0.66 (62.9)	2.08 (100)	1.56 (75.0)	2.08 (100)	0.67 (32.1)

Studies on the stability of NDA fluorescent derivatives were performed with the same standard, after 24 h and 8 days (Table 3). This study demonstrated that after 1 day, the stability of NDA-FB_1 and NDA-FB_2 decreased slightly. However, a minor increase in the percentage of NDA-FB_1 was observed, as reported by Bennett and Richard [29]. After 8 days, the loss of FB_2 was more significant than that of FB_1. NDA-FBs derivatives have higher stability than OPA derivatives, as reported by Sydenham et al. [33] and Williams et al. [31]. NDA also tends to give higher values at lower toxin levels present in maize samples [32].

The methanol/water (80:20 v/v), previously applied by Cortez-Rocha et al. [17] used as an extraction solvent, was successfully used to extract fumonisins from maize and maize-based products.

The accuracy was determined by calculating the mean recovery values used for each fortification level (Table 4). The recovery values for FB_1, using the method B, were 79.0% and 98.5% for fortification levels at 150 and 250 μg kg^{-1}, respectively. For FB_2, recovery values were 98.4% and 99.6% for fortification levels at 100 and 200 μg kg^{-1}, respectively. The precision was calculated from intraday repeatability (n=3) and interday repeatability (3 days). For FB_1, the intraday repeatability obtained oscillated between 3.0 and 3.9% for fortification levels at 150 and 250 μg kg^{-1}, respectively. For FB_2, intraday repeatability was 2.7 and 10.4% at 200 and 100 μg kg^{-1}, respectively. The 3-day repeatability (n=9) obtained for FB_1, oscillated between 10.0 and 14.0% for fortification levels at 150 and 250 μg kg^{-1}, respectively. For FB_2 the corresponding values were 15.1 and 15.8% at 200 and 100 μg kg^{-1}, respectively.

These values were lower, about 50%, using method A either with normal filtration [19, 22] or vacuum filtration. The filtration process therefore required modification, since the slurry produced after extraction clogged the Whatman N°1 filter paper, with or without vacuum, leading to losses. Due to the characteristics of the sample, an efficient process for separating the matrix residue from the solvent extract was essential. Centrifugation was crucial to improve this step [34]. Moreover, the time expended when method B was applied was much lower. The centrifugation step allowed good separation between sample residue and extraction solution when 2,500 *g* was applied in contrast to what happened with 500 *g* and 1,000 *g*. Attempts were made to optimize the analytical

Table 4 Accuracy and intra-assay validation results (n=3) and inter-assay (n=9) obtained with the optimized method

Fumonisins	Fortification level (μg kg^{-1})	Recovery mean (%)	RSD within-day (%)	RSD between-day (%)
FB_1	150	79.0	3.0	10.0
	250	98.5	3.9	14.0
FB_2	100	98.4	10.4	15.8
	200	99.6	2.7	15.1

methodology. Extraction of 25 g of a contaminated sample, spiked with 100 μg kg^{-1} of FB_1 and FB_2, centrifuging twice using 50 mL of extraction solvent, resulted in recoveries of 61.5% for FB_1 and 95.6% for FB_2. As the result obtained for FB_1 was low, centrifugation was tried three times, and FBs were eluted from IAC twice with 1.5 mL methanol.

The limit of detection obtained using the NDA derivatization procedure, at a signal-to-noise ratio of 3:1, was 20 μg kg^{-1} for FB_1 and 15 μg kg^{-1} for FB_2.

Application to real samples

The method B was successfully applied to 31 samples of maize and maize-based products, and the presence of FBs was verified in 14 samples (45%). The analyzed samples revealed high contamination levels, between nd and 1,569 μg kg^{-1} for FB_1 and between nd and 457 μg kg^{-1} for FB_2. In all groups of studied commodities, the mean concentration of FB_2 is lower than FB_1 (Table 5), which is usual in other studies [11, 35].

In our study, 67% of yellow maize and 100% of with maize samples (Table 5), which corresponds to 72.7% for all maize samples, were contaminated with FBs. These results are according to the only known study in Portugal for FB_1 and FB_2 in nine naturally contaminated maize hybrids, originating from the 1992 crop from the Agricultural School of Coimbra [13], which presented one incidence of 100%. The mean concentrations in the present study for the totality of maize samples were 329 μg kg^{-1} for FB_1 and 131 μg kg^{-1} for FB_2, which are much lower when compared to the studies in Portuguese maize samples in 1992: 1,031 μg kg^{-1} for FB_1 and 1,077 μg kg^{-1} for FB_2 [13].

White maize samples, used for the preparation of *cachupa*, a traditional dish from Cabo Verde, presented higher levels of both fumonisins than yellow samples, 638 μg kg^{-1} versus 421 μg kg^{-1}.

The average contamination levels found in maize samples, 329 μg kg^{-1} for FB_1 and 131 μg kg^{-1} for FB_2, are lower than those reported in Spain (4,800 μg kg^{-1} for FB_1 and 1,900 μg kg^{-1} for FB_2), UK (3,046 μg kg^{-1} for FB_1 and 1,268 μg kg^{-1} for FB_2), and Morocco (1,930 μg kg^{-1} for FB_1) [36, 35, 7]. However the percentage of positive samples in our study (72.7%) is similar to that reported in Spain (87.3%). In Nigeria similar levels of contamination have been reported (495 μg kg^{-1} for FB_1 and 114 μg kg^{-1} for FB_2) [9].

Maize flour samples presented the highest mean concentration of FB_1 contamination, 822 μg kg^{-1}. FB_2 presented a mean value of 173 μg kg^{-1}. Higher values have been reported in France (1,113 μg kg^{-1} for FB_1) [25], Italy (FB_1 ranged from 10 to 2,870 μg kg^{-1} and FB_2 from 10 to 420 μg kg^{-1}) [26], and Brazil (2,100 μg kg^{-1} for FB_1 and 700 μg kg^{-1} for FB_2) [8], whereas in Argentina the mean contamination of FB_1 and FB_2 were lower: 358 μg kg^{-1} and 122 μg kg^{-1}, respectively [37].

Results obtained for FB_1 (118 μg kg^{-1}) and FB_2 (nd) in maize semolina are in agreement with those reported by

Table 5 Prevalence and levels of fumonisins in maize and maize products from Portugal

Sample type	No of analyzed samples	No (%) of positive samples	Detection rate FB_1:FB_2	Range of FB_1 (μg kg^{-1})	Mean (μg kg^{-1})	Median (μg kg^{-1})	Range of FB_2 (μg kg^{-1})	Mean (μg kg^{-1})	Median (μg kg^{-1})	Range of FB_1+FB_2 (μg kg^{-1})	Mean (μg kg^{-1})	Median (μg kg^{-1})
Yellow maize	9	6 (67)	6:5	nd–871	322	167	nd–272	99	112	nd–1,061	421	279
White maize	2	2 (100)	1:2	nd–725	363	363	113–437	275	275	113–1,162	638	375
Total maize samples	11	8 (72.7)	7:7	nd–871	329	167	nd–437	131	113	nd–1,162	460	279
Maize flour	3	2 (67)	2:2	nd–1,569	822	898	nd–457	173	62	nd–2,026	995	960
Maize semolina	3	2 (67)	2:0	nd–183	118	171	nd	–	–	nd–183	118	171
Maize starch	3	0 (0)	0:0	nd	–	–	nd	–	–	nd	–	–
Sweet maize	11	2 (18)	2:0	nd–523	64	0	nd	–	0	nd–523	64	0

Piñeiro et al. [38] in Uruguay (105 μg kg^{-1} for FB_1 and nd for FB_2), Pittet et al. [39] in Switzerland (260 μg kg^{-1} for FB_1 and 100 μg kg^{-1} for FB_2), and Broggi et al. [37] in Argentina (135 μg kg^{-1} for FB_1 and 39.1 μg kg^{-1} for FB_2).

Fumonisins were not detected in maize starch. Piñeiro et al. [39] detected neither FB_1 nor FB_2 in the analyzed maize starch samples from Uruguay. This fact can be explained by the wet-milling process that leads to the migration of fumonisins into aqueous solutions during steeping [6].

Sweet maize samples presented the lowest contamination, 64 μg kg^{-1} in 18% of the analyzed samples for FB_1, with no detection for FB_2. Low contamination levels were also reported by Truckess et al. [40]: 70 μg kg^{-1} for canned maize and 98 μg kg^{-1} for frozen maize; Pittet et al. [39] reported 70 μg kg^{-1} for FB_1 and nd for FB_2 in Switzerland; and Piñeiro et al. [38] reported 65 μg kg^{-1} for FB_1 and nd for FB_2, in Uruguay.

The comparison between countries in Europe is somehow difficult regarding the few recent reports of contamination by fumonisins in these kinds of goods.

One maize flour sample exceeded the recommended limit of 2,000 μg kg^{-1} proposed by the European Mycotoxin Awareness Network [41], and two maize samples exceeded the maximum allowable concentration of FB_1+FB_2 established by Switzerland, 1,000 μg kg^{-1} [42].

For maize samples and maize products collected in a central zone of Portugal, the average sample contamination of FB_1 + FB_2 was 303 μg kg^{-1}. Assuming that the estimation of average daily intake of maize in the Portuguese population is 27.9 g per person per day [43] and that an adult body weights 60 kg, the estimated daily intake (EDI) of fumonisins in this study was 0.14 μg kg^{-1} body weight per day. These values are lower than the 2 μg kg^{-1} established by Commission Regulation (EC) No 856/2005[44].

According to data from Portuguese Food Balance, maize consumption in 2003 reached 10.19 kg per person per year, which means 27.90 g per person per day, second place in the EU rank of total consumption of cereals [43]. Accepting this, the daily intake would reach, on average, 0.14 μg kg^{-1} body weight per day or 0.98 μg kg^{-1} body weight per week, a close value (70%) to the estimated total intake of FB_1 in the European diet, 1.4 μg kg^{-1} body weight per week [45]. The provisional maximum tolerable daily intake (PMTDI) for FB_1+FB_2 is 2 μg kg^{-1} body weight per day. This data was not overlapped for the most contaminated sample, representing 47.1% of the PMTDI reported by EMAN [41], but represents 118% when TDI of 0.8 μg kg^{-1} body weight per day, proposed by Gelderblom et al. [46] is chosen as reference.

Conclusions

Extraction with methanol/water, centrifugation, and dilution with PBS allows the supernatant to be applied onto an IAC column, making it possible to achieve low limits of detection. Stability of NDA fluorescent derivatives overwhelmed the time-dependent degradation presented by OPA. This optimized analytical methodology provides good results in terms of accuracy, repeatability, intermediate precision and sensitivity, and has been shown to be reliable for determination of FB_1 and FB_2 in maize and maize products presenting limits of detection of 20 μg kg^{-1} and 15 μg kg^{-1} for FB_1 and FB_2, respectively.

The application of the procedure to 31 samples from the central Portugal has demonstrated that 45% of the samples were contaminated, FB_1 contamination levels were higher than FB_2, and some maize samples and maize flour exceed the recommended limits.

None of the analyzed samples exceeded the provisional maximum tolerable daily intake. The estimated daily intake demonstrated that FBs do not represent a real concern for consumers.

Acknowledgements This work was carried out with the financial support, which is gratefully acknowledged, from the FCT and FEDER/ POCTI. The authors are also gratefully recognized to FCT for a PhD fellowship granted to Liliana Silva.

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