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Research Article

Free and bound fumonisins in gluten-free food products

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In this work a multiresidual LC-ESI-MS/MS method for the simultaneous detection of free and bound fumonisins is described, which allowed for a very low LOD and a very good recovery for all the analytes. The method was applied to the determination of free and bound fumonisins in several gluten-free products from the Italian market. Free fumonisins were found to occur in 90% of the samples: the overall median value was below the EU legal limit for foods for human consumption (800 μg/kg). Nonetheless, fumonisins occurred in several samples at concentrations above the legal limit, reaching also very strong contamination levels (maximum concentration level: 3310 μg/kg). Anyway, considering the limited diet of people suffering of the celiac disease or allergic to other wheat proteins, the incidence of fumonisin contamination may be envisaged as problematic. Furthermore, bound fumonisins were found to be present in all the analysed samples at similar or even higher amounts than the free forms. In many cases the sum of free and bound fumonisins exceeded the EU legal limit for total fumonisins also for those samples characterized by a low contamination of free fumonisins, thus opening a new important task to be addressed for the risk assessment in this field.

Keywords: Bound fumonisins / Free fumonisins / Gluten free products / Masked mycotoxins / MS

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1 Introduction

Fumonisins are a group of structurally related *Fusarium* mycotoxins produced mainly by *F. verticilloides* and *F. proliferatum*. Among several homologues, fumonisins B1, B2 and B3 (FB1, FB2 and FB3, respectively) are the major mycotoxins produced in corn. These compounds are characterized by a 20 carbon aminopolyhydroxyalkyl chain diesterified with propane-1,2,3-tricarboxylic acid (tricarballylic acid (TCA)) (Fig. 1) [1, 2].

Fumonisins may cause several diseases in animals, as well as hepatocarcinogenic, hepatotoxic, nephrotoxic and cytotoxic effects in mammals. Moreover, there is evidence of a high incidence of human esophageal cancer associated with FB1 exposure [3]. For these reasons, in the last months European Union has enforced the new legislation for fumonisins in food: according to this regulation the limits for total fumonisins in unprocessed maize (4000 μg/kg), maize for direct human consumption (1000 μg/kg), maize-based breakfast cereals and snacks (800 μg/kg) and in baby-food (200 μg/kg) have been established (EC No 1126/2007).

The problem of fumonisin contamination is further complicated by the fact that hydrolysed forms and, more recently, bound forms were detected in food products [4–6]. Indeed, although fumonisins are relatively heat stable and persist through most of the conditions used in food manufacturing, the TCA side chains may be removed by alkaline treatment, yielding the hydrolysed analogues (hydrolysed fumonisin Bs (HFBs)) [2]. The toxicity of hydrolysis products is so far still ambiguous: several studies reported the lower acute toxicity of HFB1 when compared to FB1, whereas other researches underlined the higher absorption of the less polar hydrolysed derivative, which may be better absorbed by the intestinal mucosa [7, 8]. Moreover, experimental evidence showed that, despite the low absorption and bioavailability of FB1 after oral administration, toxic effects were recorded also after ingestion of low contaminated feed: this led to formulate the hypothesis that FB1-derivatives may be present and preferentially

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Abbreviations: FB1, fumonisin B1; **FB2,** fumonisin B2; **FB3,** fumonisin B3; **HFB,** hydrolysed fumonisin B; **MRM,** multiple reaction monitoring

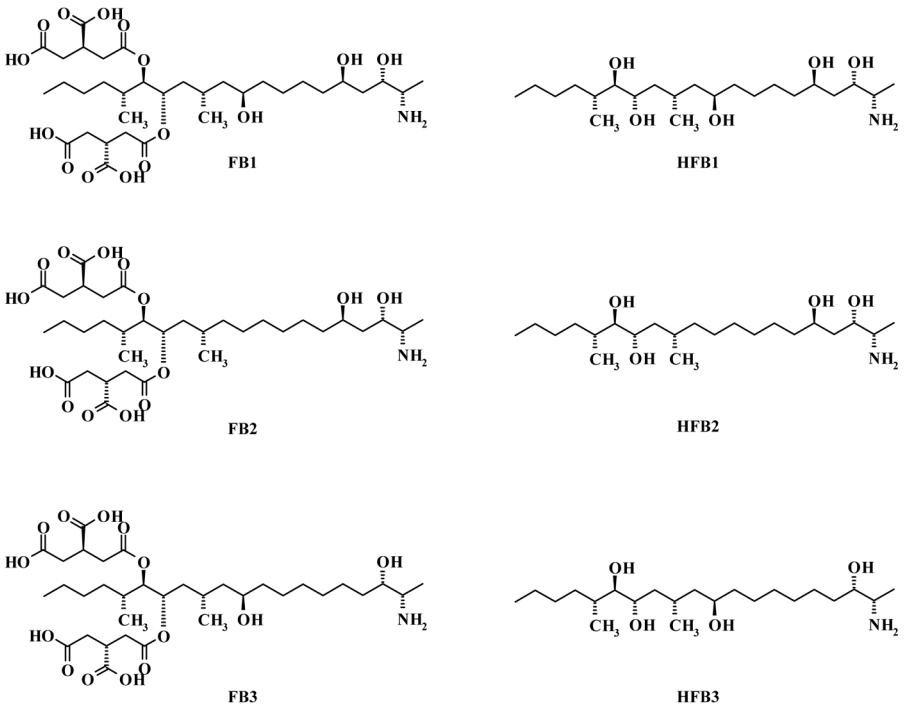

Figure 1. Chemical structures of FB1, FB2 and FB3 as well as their hydrolysed derivatives HFB1, HFB2 and HFB3.

absorbed, then reconverted to the active forms in the body [9]. In an experiment using radiolabelled FB1 added to corn meal dough, Shier *et al*. [10] found that only 37% of the radioactivity was detected by the conventional analysis after roasting, while an additional 46% was extracted by a solution of sodium dodecyl sulphate (SDS), a detergent used to dissolve proteins. Moreover, the author partially characterized the covalent binding of radiolabelled FB1 to corn proteins and starch. Seefelder *et al*. [11] demonstrated that upon thermal treatment FB1 reacts with methyl-β-D-glucopyranoside and protected amino acids, as model compounds for starch and proteins, respectively. Adducts are formed *via* the tricarballylic acid moieties. Moreover, recently large amounts of *N*-fatty acylated derivatives of fumonisins have been detected in tortilla chips by means of radioactive studies [12].

Recently, several authors [5, 6] demonstrated the presence of bound fumonisins in corn-based foods by using HPLC-FLD and HPLC-MS. Protein-bound fumonisins are extractable with SDS, which is removed by liquid–liquid partition and HFB1 released by hydrolysis is cleaned-up by OASIS HLB extraction columns. Total-bound fumonisin is also purified by using the same clean-up column after alkali-hydrolysis of the food sample. Compared with FB1 detected by traditional analysis, about 1.3 and 0.9 times more FB1 in total bound and protein forms, respectively, are detected in alkali-processed corn-based food.

Although the determination of fumonisins by RP-HPLC using fluorescence detection and precolumn derivatization with orthophthalaldehyde (OPA) and 2-mercaptoethanol has been adopted by AOAC International as an official method for corn analysis [13], in the last years MS-based methods became the technique of election for fumonisin analysis, allowing for multiresidual determinations without immunoaffinity clean-up or derivatization steps [14, 15].

Since fumonisins contamination occurs mainly in maize, a higher exposure to these contaminants may be suffered by people affected by gluten allergy (*e.g.*, celiac disease), being their diet particularly based on gluten-free cereals such as maize. Although several surveys have been reported for corn-based foods [16, 17], only one study was published about fumonisins occurrence in gluten-free products, showing a very diffuse contaminations [18]. In this study, 88% of the analysed samples were found to be positive with FB1 contamination levels up to 1.8 mg/kg. Unfortunately, the recently enforced limits do not take into account special corn-based foods for gluten-free diets and, moreover, the limits are established only for FB1 and FB2, without considering the occurrence of their bound derivatives in food.

Very recently, we optimized the alkaline hydrolysis conditions for the indirect quantification of bound fumonisins and developed a method for the simultaneous determination of FB1, FB2, FB3 and its hydrolysed derivatives HFB1, HFB2 and HFB3 [19]. The procedure was applied for a preliminary survey on several corn-based products, showing that bound-fumonisins occurrence is not restricted to thermally treated products, but may be also found in raw products.

In the present study, we applied our multiresidual method to gluten-free corn-based foods purchased from the market, in order to determine the overall fumonisin contamination. Moreover, the occurrence of free and bound fumonisins have been evaluated in several corn-based products.

2 Materials and methods

2.1 Chemicals

FB1, FB2 and FB3 standard solutions (50 μg/mL) were purchased from Biopure (Tulln, Austria). All solvents used (LC grade) were obtained from Carlo Erba (Milan, Italy); bidistilled water was produced in our laboratory utilizing an Alpha-Q system (Millipore, Marlborough, MA, USA).

2.2 Hydrolysed FB1, FB2 and FB3 preparation

A standard solution of the three main fumonisins (50 μg/mL of each, 5 mL) was prepared in ACN/water 1:1 and evaporated to dryness. The residue was redissolved in 2 M KOH (5 mL) in an ambered vial, then allowed to react overnight at room temperature. After hydrolysis, the mixture was extracted three times by liquid–liquid partition using ACN (5 mL each aliquot). The organic phase were pooled, evaporated under N_2 stream and redissolved in 1 mL methanol. The reaction yield was checked by LC-MS, by monitoring the conversion of FB1 to HFB1 and the absence of side products, and it was found to be higher than 99%. Calibration curves were prepared by proper dilution of the standard solution, assuming the total conversion of the native compounds to the hydrolysed forms. The concentration of the hydrolysed form was calculated starting from the concentration of the native standard by applying a proper conversion factor.

2.3 Sample collection

Gluten-free products were obtained from the market. All the products were purchased from retail shops specialized in diet food and were labelled as 'gluten-free approved'. Each commercial package was entirely ground and carefully mixed, then a subsample (5 g) underwent to the extraction procedure.

2.4 Sample preparation for the analysis of free fumonisins

Aliquots (5 g) of finely ground corn-based products were homogenized in a high speed blender (Ultraturrax T25, IKA, Stauffen, Germany) with 50 mL of water/methanol (30:70 v/v) for 10 min at 6000 rpm, then stirred for 60 min. The solid residue was then extracted again in the same way. The extracts were then pooled and filtered through Whatman no. 4 filter papers. The filtrate (4 mL) was evaporated to dryness by rotavapour and the residue was dissolved in 2 mL of methanol before LC-MS/MS analysis.

2.5 Sample preparation for the analysis of total fumonisins

Each aliquot (5 g) of finely ground corn-based products underwent to alkaline hydrolysis at room temperature: the sample was added with 50 mL 2 M KOH, homogeneized by Ultraturrax for 10 min and then stirred for 60 min. Afterwards, the aqueous phase was extracted twice with 50 mL ACN. After centrifugation, the organic phases were pooled and evaporated by rotavapour. The residue was dissolved in 2 mL methanol and analysed by LC-MS/MS.

2.6 LC-MS/MS analysis

The LC-MS/MS system consisted of a 2695 Alliance (Waters, Milford, MA, USA) equipped with a Quattro™ triple quadrupole mass spectrometer with an electrospray source (Micromass, Waters, Manchester, UK). Chromatographic conditions were the following: column, C_{18} XTerra Waters narrow bore (250 mm × 2.1 mm, 5 μm) equipped with a C_{18} precolumn cartridge; flow rate, 0.2 mL/min; column temperature, 30°C; injection volume, 5 μL. Gradient elution was performed using water (eluent A) and methanol (eluent B), both acidified with 0.2% formic acid: 0–2 min, isocratic step 30% B, switched to the waste in order to wash out the salts and to focus the analytes on the C_{18} precolumn cartridge; 2–5 min to 45% B; 5–25 min to 90% B; 25–35 min isocratic step 90% B, 35–36 min to 30% B; finally, a re-equilibration step at 30% B (initial conditions) for 20 min was performed (total analysis time, 56 min). The LC-MS/MS method parameters have been already described in details by Dall'Asta *et al*. [19]. Briefly, MS parameters were the following: ESI+ (positive ion mode); capillary voltage, 3.2 kV; extractor voltage, 3 V; source block temperature, 120°C; desolvation temperature, 160°C; desolvation and cone gas (nitrogen) 650 and 70 L/h, respectively. Detection was performed using a multiple reaction monitoring (MRM) mode, by monitoring three transitions for each analyte, as reported in Table 1. The main transition was used for quantification, while two more transitions were chosen as qualifiers.

Recovery experiments and detection limits have been already reported by Dall'Asta *et al*. [19]. Linearity and matrix-matched calibration experiments were based on the analysis of spiked corn samples not containing fumonisins either as free or bound. The spiking experiments were performed at six concentration levels in the range 50–5000 μg/kg (three determinations at each level were performed). For the free forms, the spiked fumonisins were determined as such. For the bound forms, the spiked samples were previously submitted to hydrolysis procedure and then the hydrolysed forms were determined.

Table 1. MRM conditions for LC-ESI-MS/MS analysis of fumonisins

Compound	Cone (V)	Precursor ion [M + H]+	Main transition	CE (eV)	First qualifier	CE (eV)	Second qualifier	CE(eV)
FB1	50	722.4	352.4	35	334.4	35	704.4	35
FB2	50	706.4	336.5	35	354.0	35	688.6	35
FB3	50	706.4	336.5	35	354.0	35	688.6	35
HFB1	35	406.5	334.5	20	370.5	15	388.5	15
HFB2	35	390.5	318.5	20	354.5	15	372.5	15
HFB2	35	390.5	318.5	20	354.5	15	372.5	15

CE: collisional energy.

3 Results and discussion

3.1 Method performance

The LC-MS/MS analysis was performed using a C_{18} column and a water–MeOH mobile phase acidified with 0.2% HCOOH, using a gradient elution. A chromatogram of the six analytes in a naturally contaminated corn sample is depicted in Fig. 2.

The method gave a good linearity over a wide range for all the analytes in the matrix. The matrix-matched calibration was performed using corn flour for FBs and HFBs in the range 50–5000 μg/kg.

In order to estimate the eventual matrix effect, the peak areas of each matrix-matched standard calibration curves obtained for corn flour samples were compared to normal standard calibration curves using two-tailed paired *t*-test at 95% confidence limit, as reported in Table 2.

Concerning the matrix-matched calibrations for the determination of free forms, the $|t|$-values of these curves which were all lower than a critical value ($p = 0.05$) of 2.032 ($n = 36$) imply that the data of these two sets are not significantly different. On the contrary, the data set obtained for the determination of bound forms showed $|t|$-values higher than the critical one ($p = 0.05$) of 2.032 ($n = 36$). This matrix effect is probably due to ion suppression/enhancement phenomena induced by salts deriving from the alkaline hydrolysis procedure. Thus, for bound fumonisins determination, a calibration check was run for each sample batch and the results were properly corrected. Although suppression of the signal was recorded only after a great number of injection ($n > 100$), the source was routinely cleaned after 50 injections.

LOQ were evaluated in maize flour as 5 μg/kg for FB1 and FB2, 12 μg/kg for FB3 and 70 μg/kg for HFB1, HFB2 and HFB3. All recoveries in maize flour were in the range of 92–98%, considering two contamination levels (500 μg/kg, 2000 μg/kg).

The repeatability of the method was tested by analysing the spiked corn flour samples at two contamination levels (500 and 2000 μg/kg) and both intra-assay (six extractions at each level on the same day) and intermediate precision (six extractions at each level on two days) were calculated.

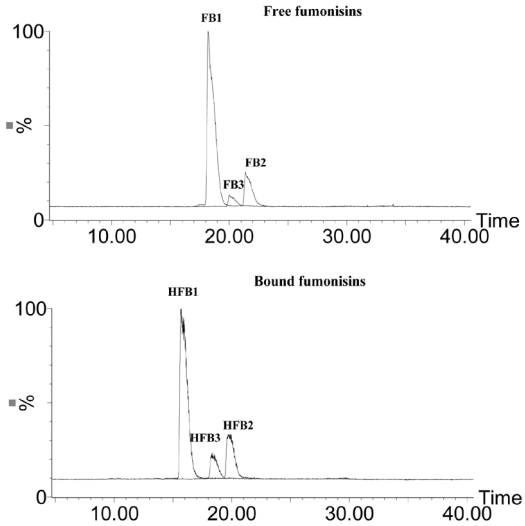

Figure 2. MRM chromatogram of a corn sample naturally contaminated with free (a) and bound (b) fumonisins (determined as HFBs after alkaline hydrolysis).

The results were satisfactory at all the concentration levels, the RSDs being lower than the critical value obtained by the Horwitz equation [20, 21] for the considered concentration levels, as reported in Table 3.

3.2 Free fumonisins in gluten-free corn-based food

The LC-MS/MS method was applied to the analysis of several gluten-free commercial products from the Italian market. Samples were collected from supermarket, organic food and chemist shops.

A screening for the occurrence of free fumonisins was performed by checking the simultaneous contamination of FB1, FB2 and FB3 as well as their hydrolysed forms HFB1, HFB2 and HFB3, which may arise from food processing.

The analyses showed a diffuse contamination, since in 82% of the samples were detected FBs and/or HFBs: in particular, 33 out of 40 samples were found to be contaminated above the LOD (4 μg/kg for FB1, 8 μg/kg for FB2 and FB3 and 20 μg/kg for HFBs). Although 7 out of 40 sample were

Table 2. Matrix effect evaluation (corn flour): comparison between standard and matrix calibration curves for FB1, FB2, FB3, HFB1, HFB2 and HFB3

	Analyte	Standard calibration curves			Matrix calibration curves			*t*-value
		Slope	*Y* intercept	r^2	Slope	*Y* intercept	r^2	
Free fumonisins	FB1	14.1	−37.9	0.9978	14.3	23.9	0.9988	0.131 ($p = 0.896$)
	FB2	7.6	−10.3	0.9985	6.7	26.7	0.9931	0.260 ($p = 0.796$)
	FB3	4.9	88.1	0.9975	5.6	26.4	0.9932	0.365 ($p = 0.717$)
	HFB1	4.0	−18.3	0.9998	3.4	−17.9	0.9997	0.225 ($p = 0.823$)
	HFB2	3.8	57.5	0.9999	3.3	52.2	0.9998	0.249 ($p = 0.804$)
	HFB3	4.0	−16.3	0.9992	4.2	−18.0	0.9993	0.015 ($p = 0.988$)
Bound fumonisins	HFB1	5.2	−50.6	0.9975	10.4	−406.0	0.9963	2.500 ($p = 0.017$)
	HFB2	4.6	−86.1	0.9887	7.3	8.7	0.9919	2.190 ($p = 0.035$)
	HFB3	4.7	−103.6	0.9912	8.0	147.0	0.9943	2.350 ($p = 0.025$)

Linear regression parameters of free and bound fumonisins for standard and matrix calibration curves: range from 50–5000 μg/kg (six points, triplicate analyses). |*t*|-values of two-tailed paired *t*-test at 95% confidence limit: $|t_{crit}| = 2.032$ (34 degree of freedom).

Table 3. The method precision expressed as RSD of spiked corn samples.

	RSD% at 2000 μg/kg					RSD% at 500 μg/kg				
	Day 1[a)]	Day 2[a)]	Day 3[a)]	Overall[b)]	Acceptable value[c)]	Day 1[a)]	Day 2[a)]	Day 3[a)]	Overall[b)]	Acceptable value[c)]
FB1	7.2	5.1	6.4	6.2	14.1	7.7	9.8	12.8	10.1	18.9
FB2	7.8	9.3	7.2	8.1		4.2	5.1	10.1	6.5	
FB3	6.5	6.1	4.4	5.7		5.8	6.6	13.2	8.5	
HFB1	4.5	4.4	7.9	5.6		12.2	10.5	10.9	11.2	
HFB2	4	4.7	10.5	6.4		11.4	7.6	12.5	10.5	
HFB3	5.1	4.5	12.4	7.3		13.2	11.1	16.5	13.6	

a) Intra-assay precision of data analysed within the same day ($n = 6$).
b) Intermediate precision of data analysed on different day ($n = 3$).
c) RSD values obtained from Horwitz equation ($RSDr = 0.67 \times 2(1 - 0.5 \log C)$).

contaminated above the EU legal limit for human consumption (800 μg/kg), the overall median value was below the EU limit. Nonetheless, fumonisins occurred in several samples at concentrations above the legal limit, reaching also very strong contamination levels (maximum concentration level: 3310 μg/kg).

Gluten-free pasta and breads ($n = 17$) showed a very low contamination, with a maximum concentration of 554 μg/kg. Also extruded products ($n = 7$) showed a low contamination, although in one case a large FB contamination was detected (2250 μg/kg). On the contrary, corn flours ($n = 7$) seem to be highly contaminated with a median value of 1020 μg/kg, which is above the legal limit. As far as gluten-free snacks ($n = 9$), the collected data showed a diffuse contamination with a median value of 354 μg/kg. Although this value is below the legal limit, the amount of corn flour in these products is small, thus suggesting the use of strongly contaminated flours as ingredients. The collected data are reported in Table 4 and in Fig. 3.

Moreover, in almost all the analysed products, also the hydrolysed derivatives HFB1, HFB2 and HFB3 were found, although at a lower concentration than native fumonisins. The comparison between native fumonisins and hydrolysed derivatives contents is reported in Fig. 3.

It is worth noting that, although the EU legal limit for total fumonisins enforced for corn-based products for human consumption is 800 μg/kg, the products considered in the present study are dedicated to people suffering from foodborne illness such as celiac disease or allergies: a diet specifically based on such products potentially exposes them to higher risk than normal population. For this reason, further studies concerning the risk assessment for these particular consumers should be performed and, when necessary, specific regulation should be enforced.

3.3 Bound fumonisins in gluten-free corn-based food

After a screening of free fumonisins in gluten-free foods, we tried to evaluate whether bound fumonisins also occurred in processed corn-based products. In particular, 21 low-contaminated gluten-free products, already ana-

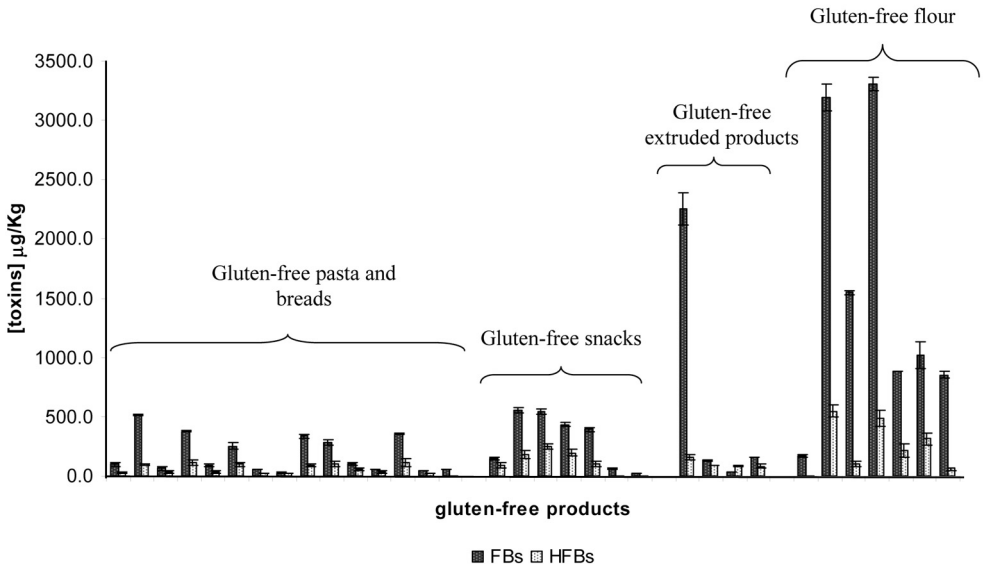

Figure 3. Total fumonisins (FB1, FB2 and FB3) and total HFBs (HFB1, HFB2 and HFB3) contamination in gluten-free corn-based products.

Table 4. Occurrence of free fumonisins in gluten-free foods: median values and maximum concentrations

		Fumonisins (FB1 + FB2 + FB3)		HFBs (HFB1 + HFB2 + HFB3)	
Gluten-free products	Positive	Median (µg/kg)	Max concentration (µg/kg)	Median (µg/kg)	Max concentration (µg/kg)
Snacks (n = 9)	(7/9)	354	554	89	245
Pasta and breads (n = 17)	(15/17)	66	513	22	127
Extruded products (n = 7)	(4/7)	39	2250	31	458
Flours (n = 7)	(7/7)	1020	3310	259	621

lysed for free FBs and HFBs, were analysed for bound fumonisins: pasta and breads (n = 11), snacks (n = 4) and corn flakes (n = 6). An aliquot of each product was hydrolysed for the determination of total fumonisins, according to the method described in Section 2. All fumonisins, in these conditions, were transformed in hydrolysed fumonisins. Thus, the amount of bound fumonisins was indirectly calculated by subtracting the amount of free FB1 and HFB1 found before the hydrolysis from the total fumonisins content.

The results are reported in Figs. 4a and b.

The data showed the occurrence of bound fumonisins in all the analysed samples at higher levels than the free forms (see Table 5). In gluten-free pasta and breads (n = 11) with a very low contamination for free fumonisins, the bound fumonisins level was higher with a maximum concentration of 1530 µg/kg. The occurrence of bound fumonisins was found to be significative for all the analysed samples, with a median value of 148 µg/kg. As far as gluten-free snacks (n = 4), the data indicate a strong contamination also for bound fumonisins, being both the maximum and the median concentrations (4740 and 1430 µg/kg, respectively) above the legal limit.

Table 5. Occurrence of bound fumonisins in gluten-free foods: median values and maximum concentrations

	Bound fumonisins	
Gluten-free products	Median (µg/kg)	Max concentration (µg/kg)
Snacks (n = 4)	1430	4740
Pasta, bread, flour (n = 11)	148	1530
Corn flakes (n = 6)	89	245

It is noticeable that for both these glutenfree product categories (snacks and bread and pasta) the median values for bound fumonisins (148 and 1430 µg/kg) were higher than the level obtained for free fumonisins: these data clearly indicate that the occurrence of bound or masked mycotoxins should be considered during risk assessment trials.

Gluten-free corn flakes showed a diffused contamination at a median level of 89 µg/kg for bound fumonisins: in this

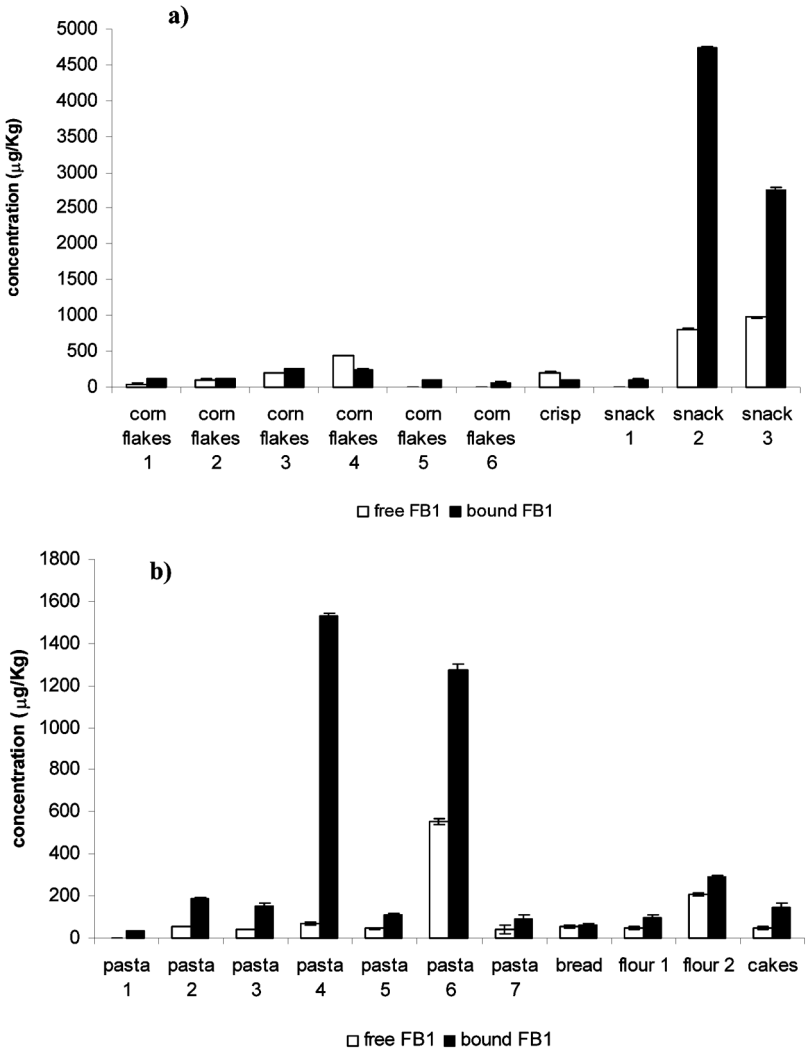

Figure 4. Free and bound fumonisins in gluten-free corn-based products: corn flakes and snacks (4a), pasta, bread and flour (4b).

case, it should be noticed that the bound fumonisins concentration reported as median level is closer to that obtained for free forms and the maximum level was significantly lower (bound forms: 245 μg/kg; free forms: 2250 μg/kg).

It is also to be remarked that after hydrolysis the total fumonisins contamination is significantly higher than the EU legal limit for 4 out of 21 samples.

4 Concluding remarks

In conclusion, the direct LC-MS/MS method without sample purification allows for the determination of fumonisins FB1, FB2 and FB3 as well as their hydrolysed forms HFB1, HFB2 and HFB3 in corn and in corn-based products. The proposed method is simple, accurate, reliable, less time-consuming and more sensitive then the common HPLC-FLD method, giving an LOD for fumonisins in corn flour products of 4 μg/kg, which is in the same range of those obtained by using other LC-MS/MS methods, although our procedure does not require a sample clean-up.

Concerning free fumonisins, the collected data clearly showed the common occurrence of fumonisins in maize-based products, with contamination level comparable to the EU legal limit of 800 μg/kg. Anyway, considering the limited diet of people suffering of the celiac disease or allergic to other wheat proteins, the incidence of fumonisin contamination may be envisaged as problematic for this category of consumers, demanding for a more detailed study of this problem.

Moreover, the developed procedure for bound fumonisins allowed to determine bound forms in the samples, showing their occurrence in all the analysed samples at levels higher than those found for the free forms. Furthermore several samples, which were found to be acceptable for the EU limits, were found contaminated above the limit when also the bound forms are considered. These data are actually of high concern: toxicological studies and larger

surveys should be performed in order to better define the eventual incidence of these bound forms and to collect more reliable data for risk assessment.

The authors have declared no conflict of interest.

5 References

[1] Sewram, V., Mshicileli, N., Shephard, G., Vismer, H. F., *et al*., Production of fumonisin B and C analogues by several fusarium species. *J. Agric. Food Chem.* 2005, *53*, 4861 – 4866.

[2] Murphy, P. A., Hendrich, S., Hopmans, E. C., Hauck, C. C., *et al*., Effect of processing on fumonisin content of corn. *Adv. Exp. Med. Biol.* 1996, *392*, 323 – 334.

[3] Creppy, E. E., Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicol. Lett.* 2002, *127*, 19 – 28.

[4] Scott, P. M., Lawrence, G. A., Determination of hydrolyzed fumonisin B1 in alkali-processed corn foods. *Food Addit. Contam.* 1996, *13*, 823 – 832.

[5] Kim, E.-K., Scott, P. M., Lau, B. P.-Y., Hidden fumonisin in corn flakes. *Food Addit. Contam.* 2003, *20*, 161 – 169.

[6] Park, J. W., Scott, P. M., Lau, B. P.-Y., Lewis, D. A., Analysis of heat-processed corn foods for fumonisins and bound fumonisins. *Food Addit. Contam.* 2004, *21*, 1168 – 1178.

[7] Caloni, F., Spotti, M., Pompa, G., Zucco, F., *et al*., Evaluation of fumonisin B1 and its metabolites absorption and toxicity on intestinal cells line Caco-2. *Toxicon* 2002, *40*, 1181 – 1188.

[8] Humpf, H. U., Schmelz, E. M., Meredith, F. I., Vesper, H., *et al*., Acylation of naturally occurring and synthetic 1-deoxysphinganines by ceramide synthase. *J. Biol. Chem.* 1998, *273*, 19060 – 19064.

[9] Shier, W. T., The fumonisin paradox: A review of research on oral bioavailability of fumonisin B1, a mycotoxin produced by fusarium moniliforme. *J. Toxicol. Toxin Rev.* 2000, *19*, 161 – 187.

[10] Shier, W. T., Abbas, H. K., Badria, F. A., Structure-activity relationships of the corn fungal toxin fumonisin B1: Implications for food safety. *J. Nat. Toxins* 1997, *6*, 225 – 242.

[11] Seefelder, W., Knecht, A., Humpf, H.-U., Bound fumonisin B1: Analysis of fumonisin-B1 glyco and amino acid conjugates by liquid chromatography-electrospray ionization-tandem mass spectrometry. *J. Agric. Food Chem.* 2003, *51*, 5567 – 5573.

[12] Shier, W. T., Abbas, H. K., Abou-Karam, M., Badria, F. A., Resch, P. A., Fumonisins: Abiogenic conversions of an environmental tumor promoter and common food contaminant. *J. Toxicol. Toxin Rev.* 2003, *22*, 591 – 616.

[13] Sydenham, E. W., Shephard, G. S., Thiel, P. G., Liquid chromatographic determination of fumonisins B1, B2, and B3 in foods and feeds. *J. AOAC Int.* 1992, *75*, 313 – 318.

[14] Sforza, S., Dall'Asta, C., Marchelli, R., Recent advances in mycotoxin determination in food and feed by hyphenated chromatographic techniques/mass spectrometry. *Mass Spectrom. Rev.* 2006, *25*, 54 – 76.

[15] Zoellner, P., Mayer-Helm, B., Trace mycotoxin analysis in complex biological and food matrices by liquid chromatography-atmospheric pressure ionization mass spectrometry. *J. Chromatogr. A* 2006, *1136*, 123 – 169.

[16] Caldas, E. D., Silva, A. C. S., Mycotoxins in corn-based food products consumed in Brazil: An exposure assessment for fumonisins. *J. Agric. Food Chem.* 2007, *55*, 7974 – 7980.

[17] Silva, L. J. G., Lino, C. M., Pena, A., Molto, J. C., Occurrence of fumonisins B1 and B2 in Portuguese maize and maize-based foods intended for human consumption. *Food Addit. Contam.* 2007, *24*, 381 – 390.

[18] Ostry, V., Ruprich, J., Determination of the mycotoxin fumonisins in gluten-free diet (corn-based commodities) in the Czech Republic. *Cent. Eur. J. Public Health* 1998, *6*, 57 – 60.

[19] Dall'Asta, C., Galaverna, G., Aureli, G., Dossena, A., Marchelli, R., A LC/MS/MS method for the simultaneous quantification of free and masked fumonisins in corn and corn-based products. *World Mycotoxin Journal* 2008, *1*, 1 – 10.

[20] EC Regulation No 401/2006, *Off. J. Eur. Union* 2006, L70/12.

[21] Commission Decision 2002/657/CE, *Off. J. Eur. Union* 2002, L221/8.