Determination of *N*-(Carboxymethyl)fumonisin B_1 in Corn Products by Liquid Chromatography/Electrospray Ionization—Mass Spectrometry†

W. Seefelder, M. Hartl, and H.-U. Humpf*

Lehrstuhl für Lebensmittelchemie, Universität Würzburg, Am Hubland, 97074 Würzburg, Germany

It is well-known that fumonisin B_1 (FB_1) in corn meal decreases during baking, frying, and cooking, but it is still not exactly clear how heating affects the formation of *N*-(carboxymethyl)fumonisin B_1 (NCM-FB_1), the reaction product of FB_1 and reducing sugars. In model experiments corn grits were spiked with FB_1 (2 mg/kg) and D-glucose (50 g/kg) or sucrose (50 g/kg) and manufactured into extrusion products at various temperatures (160−180 °C) and moisture levels (16−20%). A liquid chromatography/electrospray ionization−mass spectrometry method using isotopically labeled fumonisin FB_1-d_6 as an internal standard was developed for the determination of NCM-FB_1. For sample cleanup solid-phase C18 cartridges were used. The detection limit achieved with this method was 10 ng/g (signal-noise ratio = 3:1) using the protonated molecule $[M + H]^+$ signal of NCM-FB_1 (*m*/*z* 780) in the selected ion monitoring mode. Low concentrations of NCM-FB_1 (29−97 ng/g) were detected in all samples spiked with D-glucose and FB_1, whereas those spiked with FB_1 and sucrose showed only NCM-FB_1 in samples produced at 180 °C (NCM-FB_1 = 27 ng/g). Various corn-containing food samples from the German market were analyzed for the presence of NCM-FB_1, FB_1, and hydrolyzed fumonisin B_1 (HFB_1). All samples were contaminated with FB_1 (22−194 ng/g) and HFB_1 (5−247 ng/g). Six of nine samples contained NCM-FB_1 in low concentrations ranging from 10 to 76 ng/g. From these data and the low toxicity of NCM-FB_1 it can be concluded that the significance of NCM-FB_1 in food seems to be a minor one.

Keywords: *Fumonisins; N-(carboxymethyl)fumonisin B_1; liquid chromatography/electrospray ionization−mass spectrometry; reducing sugars; corn products; mycotoxin*

INTRODUCTION

Fumonisins, fungal toxins produced by *Fusarium moniliforme* (= *F. verticillioides*), are one of the most common contaminants in corn-based foods and feeds worldwide (*1*). Toxicological studies of the most prevalent of the fumonisins, fumonisin B_1 (FB_1), proved clearly its causality for some diseases in animals after the consumption of *F. moniliforme*-contaminated corn such as equine leukoencephalomalacia (ELEM) in horses (*2*), pulmonary edema in swine (*3*), and hepatotoxic, nephrotoxic, and carcinogenic effects in rats (*4*, *5*). In addition, a positive correlation in a number of epidemiological studies between dietary fumonisins and human esophageal cancer rates in Africa and China has been reported (*6*, *7*). Most recently a National Toxicology Program (NTP) long-term feeding study provided clear evidence for the carcinogenic activities of FB_1 in female mice and male rats (*8*). The mode of action of FB_1 is believed to be the inhibition of ceramide synthase, a key enzyme in sphingolipid metabolism, which is responsible for the acylation of sphinganine and sphingosine. This disruption of the biosynthethic pathway of sphingolipid biosynthesis leads to increased levels of sphingolipid precursors and decreased levels of complex sphingolipids (*9*, *10*). This elevation in sphinganine, a highly bioactive compound, initiates a cascade of cellular alterations that may contribute to the toxicity and carcinogenicity of this mycotoxin (*11*, *12*).

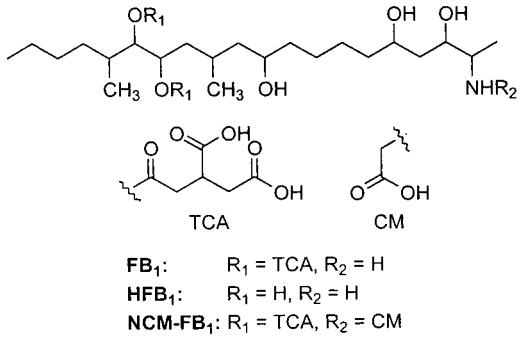

Figure 1. Structures of fumonisin B_1 (FB_1) hydrolyzed fumonisin B_1 (HFB_1), and *N*-(carboxymethyl)fumonisin B_1 (NCM-FB_1).

Although FB_1 is relatively heat stable and persists through most of the conditions used in food manufacturing, it may undergo reactions in food systems that alter its chemical structure and toxicity. For instance, removing the two tricarballylic acid side chains from the 20-carbon backbone of FB_1 during alkaline treatment results in the formation of hydrolyzed fumonisin B_1 (HFB_1), which can be found in nixtamalized corn meal (masa), tortilla chips, and canned yellow corn (*13*, *14*) and reveals greater cytotoxicity than FB_1, although it is less toxic in vivo (*15*). Another conversion product of FB_1 that may be formed during food processing is *N*-(carboxymethyl)fumonisin B_1 (NCM-FB_1) (Figure 1). It is known as the principal reaction product following the heating of FB_1 with reducing sugars (*16*). The

* Corresponding author (telephone +49 931 888 5483; fax +49 0931 888 5484; e-mail humpf@pzlc.uni-wuerzburg.de).

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formation of a stable Schiff base has been suggested to be a common Maillard reaction product between FB_1 as the aliphatic primary amine and a reducing sugar (*17*), and the structure was finally identified by Howard et al. (*16*). The toxicity of NCM-FB_1 is not known, nor has it been proved that it diminishes or alters the FB_1 toxicity. It was shown for the *N*-acyl derivatives of FB_1 and FB_2 that blocking the amino group prevents toxicity in primary rat hepatocyte cultures as well as in vivo (*15*). Purified *N*-acyl FB_1 is also not an inhibitor of ceramide synthase (*18*). Besides, there have been some toxicological studies with heating products between FB_1 and fructose, but the chemical structures of these reaction products have not been described. Whereas FB_1 promoted hepatocarcinogenesis in diethylnitrosamine (DEN)-initiated rats, an equimolar amount of unidentified reaction products of FB_1 and fructose caused no development of altered hepatic foci (*19*), although FB_1−fructose products are absorbed to a greater extent than FB_1 in rats (*20*, *21*). Using the brine shrimp assay for the toxicity assessment of fumonisins, NCM-FB_1 was 100-fold less effective than FB_1 (*22*).

Another remarkable fact is that FB_1 in corn meal decreases during baking, frying, and cooking (*23*−*26*), but it is still not exactly clear how heating affects fumonisins during food processing; in addition, the relevance of NCM-FB_1 as a possible reaction product of FB_1 in heat-treated food remains undefined. For this reason corn grits were spiked with FB_1 (2 mg/kg), D-glucose (50 g/kg), or sucrose (50 g/kg) and extruded under typical industrial conditions at various temperatures (160−180 °C) and moisture levels (16−20%). For quantitative determination of NCM-FB_1, FB_1, and HFB_1, isotopically labeled FB_1-d_6 was used as an internal standard. The samples were extracted with acetonitrile/methanol/water (25:25:50) and then purified using C18 solid-phase extraction cartridges. Liquid chromatography/electrospray ionization−mass spectrometry (LC/ESI-MS) in combination with selected ion monitoring (SIM) was used for the simultaneous quantification of NCM-FB_1, FB_1, and HFB_1.

MATERIALS AND METHODS

Analytical Standards. FB_1 was purchased from Alexis Biochemicals (Grünberg Germany). $NCFB_1$ was produced from FB_1 according to the method of Howard et al. (*16*) and further purified using C18 solid-phase extraction cartridges and an acetonitrile/formic acid gradient. d_6-Labeled fumonisin B_1 (FB_1-d_6) was isolated from *F. moniliforme* culture material as described previously (*27*). Stock solutions were prepared by dissolving 1 mg of the reference compound in 1 mL of acetonitrile/water (1:1) and further dilutions. Fumonisins are potential carcinogens and should be handled with care.

Reagents. Water, acetonitrile, and methanol, all of HPLC grade, and trifluoroacetic acid (TFA) were from Merck (Darmstadt, Germany). All other chemicals (of analytical purity) were obtained from Fluka (Deisenhofen, Germany) or Sigma-Aldrich (Steinheim, Germany). C18 solid-phase extraction cartridges (500 mg, 3 mL) were from ICT (Bad Homburg, Germany). Food samples were purchased from local markets.

Extrusion Cooking. A laboratory-scale single-screw extruder 20 D (Brabender, Duisburg, Germany) was used, as were a compression ratio of 1:4 and a 4-mm-diameter cylindrical die. Corn grits with an initial moisture content of 11.8% were spiked with FB_1 (2 mg/kg), D-glucose (50 g/kg), or sucrose (50 g/kg). Moisture levels of 16, 18, and 20% were obtained by adding different amounts of distilled water and mixing in a Hobart mixer NCM-20 (Hobart GmbH, Offenburg, Germany). Prior to the addition of distilled water to the samples, FB_1 was added to the water to provide the final FB_1 concentration (2 mg/kg based on the initial moisture content of 11.8%) in the samples. The samples were extruded at various temperatures (160, 170, and 180 °C) and screw speeds (all samples at 200 rpm, samples 9 and 10 at 220 rpm, and samples 11 and 12 at 180 rpm) (see Tables 1 and 2). The feed section of the extruder was kept at 80 °C. Extrusion products were purchased from and prepared at the Institut für Lebensmittel- und Umweltforschung e.V. (Bergholz-Rehbrücke, Germany).

Table 1. Process Conditions and Concentrations[a] of Formed NCM-FB_1[b] and Remaining FB_1 in Extrusion Products from Corn Grits Spiked with FB_1 (2 mg/kg) and Sucrose (50 g/kg)

sample	moisture (%)	temp (°C)	FB_1 (ng/g)	NCM-FB_1 (ng/g)
1[c]	18	170[c]	26.0 ± 12.6	nd[d]
3	18	160	729.7 ± 9.6	nd
5	16	170	522.3 ± 10.1	nd
7	20	170	588.5 ± 6.5	nd
9	18	170	563.9 ± 5.8	nd
11	18	170	654.7 ± 12.25	nd
13	18	180	501.8 ± 35.6	27.1 ± 4.8

[a] Based on dry weight. [b] HFB_1 was not detectable in any of the samples. [c] Blank (sample was not spiked with FB_1). [d] Not detected.

Table 2. Process Conditions and Concentrations[a] of Formed NCM-FB_1[b] and Remaining FB_1 in Extrusion Products from Corn Grits Spiked with FB_1 (2 mg/kg) and D-Glucose (50 g/kg)

sample	moisture (%)	temp (°C)	FB_1 (ng/g)	NCM-FB_1 (ng/g)
2[c]	18	170[c]	11.0 ± 3.3	nd[d]
4	18	160	351.7 ± 9.1	45.9 ± 1.4
6	16	170	146.0 ± 4.5	44.7 ± 4.7
8	20	170	251.4 ± 13.6	88.6 ± 10.9
10	18	170	251.1 ± 15.6	53.4 ± 5.5
12	18	170	232.4 ± 9.6	29.6 ± 1.12
14	18	180	158.8 ± 16.3	96.8 ± 14.5

[a] Based on dry weight. [b] HFB_1 was not detectable in all samples. [c] Blank (sample was not spiked with FB_1). [d] Not detected.

Apparatus. Chromatographic separation was performed by an Applied Biosystems 140b HPLC pump (Bai, Bensheim, Germany). For sample injection a SunChrom Triathlon autosampler (SunChrom, Friedrichsdorf, Germany) was used. LC/ESI-MS analyses were conducted on a TSQ 7000 tandem mass spectrometer system equipped with an ESI interface (Finnigan MAT, Bremen, Germany). Data acquisition and mass spectrometric evaluation were carried out on a personal DECstation 5000/33 (Digital Equipment, Unterföhring, Germany) with ICIS 8.1 software (Finnigan MAT).

Analytical Procedures. *Sample Preparation.* Commercially available food samples (corn flakes, tortilla chips, and nacho chips) and extrusion products were finely ground in a laboratory blender, and to 2.5 or 5 g subsamples was added a known amount of FB_1-d_6 (10−100 ng/g), serving as an internal standard for quantification. The samples were then extracted by blending for 3 min with 10 mL of methanol/acetonitrile/water (25:25:50) in an Ultra-Turrax TM disperser followed by centrifugation at 4000 rpm for 10 min. The supernatant was adjusted to pH 3.5 with 1 N hydrochloric acid (HCl), and 4 mL was applied to a C18 cartridge preconditioned with 2 mL of methanol and 1 mL of water. After each column had been washed with 2 mL of methanol/water (1:3) and 1 mL of methanol/water (1:1), it was eluted with 1 mL of methanol containing 5% acetic acid (v/v). The eluate was evaporated under a gentle stream of nitrogen and redissolved in 100 μL of acetonitrile/water (30:70). For alkali treatment of the extrusion products, the samples were extracted three times with 10 mL of methanol/0.1 N HCl (3:1) by blending in an Ultra-Turrax TM disperser followed by centrifugation to remove free FB_1. The obtained residue was then homogenized with 8 mL of 2 N KOH using a disperser and incubated for 30

Table 3. Concentrations of FB_1, HFB_1, and NCM-FB_1 in Various Processed Corn Products from the German Market

sample	FB_1 (ng/g)	HFB_1 (ng/g)	NCM-FB_1 (ng/g)
tortilla chips 1	21.9 ± 3.5	5.4 ± 0.8	nd[a]
tortilla chips 2	124.1 ± 4.8	165.6 ± 20.6	16.6 ± 4.5
tortilla chips 3	117.6 ± 5.5	246.5 ± 13.6	21.3 ± 7.8
corn flakes 1	67.6 ± 6.3	56.1 ± 3.0	76.0 ± 0.8
corn flakes 2	76.4 ± 4.4	102.0 ± 4.1	nd
corn flakes 3	45.5 ± 6.6	39.8 ± 5.8	nd
corn flakes 4	25.3 ± 2.9	79.6 ± 32.5	nd
nacho chips 1	147.9 ± 6.8	15.5 ± 0.4	26.5 ± 4.8
nacho chips 2	194.0 ± 9.1	115.2 ± 3.7	18.3 ± 7.0
nacho chips 2	42.2 ± 2.6	60.3 ± 24.3	9.6 ± 1.0

[a] Not detected.

min at 50 °C. The supernatant was adjusted to pH 3.5 with 2 N HCl, and 3 mL was applied to a C18 cartridge as described above.

Mass Spectrometric Analysis of NCM-FB_1, FB_1, and HFB_1. For LC/ESI-MS chromatrographic separations were carried out on a Waters Symmetry C18 column (150 × 2.1 mm i.d., 5 μm; Waters, Milford, MA) using a binary gradient. Solvent A was 0.05% TFA in water (v/v), and solvent B was 0.05% TFA in acetonitrile (v/v). HPLC was programmed as follows: 0 min, 70% A; 15 min, 55% A; 17 min, 1% A. The column was washed for 3 min with 100% of solvent B after each injection and equilibrated for 5 min at the starting conditions. The flow rate was set to 200 μL/min, and the injection volume was 10 μL. For pneumatically assisted electrospray ionization, the spray capillary voltage was set to 3.5 kV and the temperature of the heated capillary acting simultaneously as repeller electrode (20 V) was 240 °C. Nitrogen served both as sheath (70 psi; 1 psi = 6894.76 Pa) and as auxiliary gas (10 units). The mass spectrometer was operated in the SIM mode, detecting the protonated molecular ions $[M + H]^+$ of NCM-FB_1 (*m/z* 780), FB_1 (*m/z* 722), HFB_1 (*m/z* 406), and FB_1-d_6 (*m/z* 728) (0−5.5 min, *m/z* 406, 722, and 728; 5.5−17 min, *m/z* 722, 728, and 780; total scan duration of 1.0 s, respectively). Positive results of NCM-FB_1 in the SIM mode were confirmed in the selected reaction monitoring mode (SRM) detecting the protonated molecular ion $[M + H]^+$ of NCM-FB_1 (*m/z* 780) in quadrupole 1 and the typical product ion at *m/z* 410 in quadrupole 3 (collision gas argon at 2.1 mTorr, collision energy = 35 eV, scan duration = 1.0 s). Quantitative evaluations were based on the peak area ratios of NCM-FB_1, FB_1, or HFB_1 in comparison to that of FB_1-d_6, which served as an internal standard. Product ion spectra of NCM-FB_1 were recorded using argon as collision gas at a pressure of 2.1 mTorr and a collision energy of 35 eV.

System Calibration, Limit of Detection (LOD), and Recovery. The analytical system was calibrated with a standard curve for NCM-FB_1, which was prepared as follows: 10 μL from standard solution mixtures of NCM-FB_1 (10−60 ng/g) and FB_1-d_6 in various weight ratios (1:5 up to 1:1) were analyzed in the SIM mode (each concentration was injected at least three times). The resulting peak area ratios of the ions *m/z* 780 (NCM-FB_1) to *m/z* 728 (FB_1-d_6) were plotted against the weight ratios. The LOD was determined with standard solutions as well as in matrix by spiking a blank extrusion product sample, known to contain no NCM-FB_1, with NCM-FB_1 and FB_1-d_6. The samples were prepared as described above, and the LOD was specified to be 10 ng/g with a signal/noise ratio of 3:1. Recoveries were determined by adding 100 ng/g NCM-FB_1 to a blank extrusion product sample following the analytical procedure as described above (recovery = 50−60%). All analyses were performed in duplicate and injected at least two times. The results in Tables 1−3 represent the average of four analyses (derived from duplicate sample cleanup, each injected two times) ± standard deviation (SD). The calibration for FB_1 and HFB_1 was performed as described previously (*13*, *28*).

RESULTS AND DISCUSSION

For the analytical determination of fumonisin mycotoxins the majority of the already existing methods uses the technique of precolumn derivatization of the amino group with *o*-phthalaldehyde. However, this methods require the C2 primary amino group and will therefore not detect NCM-FB_1 (Figure 1). For this reason we decided to develop an analytical method based on LC/ESI-MS for the determination of NCM-FB_1 in corn-containing food. Howard et al. (*16*) indicated the formation of NCM-FB_1 by heating FB_1 and reducing sugars in a buffer medium and detected NCM-FB_1 in raw corn. Thus, it was interesting to know how heating of FB_1 under different conditions, as they are relevant for the manufacturing of corn products, would affect the formation of NCM-FB_1. In model experiments corn grits were spiked with FB_1 (2 mg/kg) and D-glucose (50 g/kg) or sucrose (50 g/kg). Blank samples were prepared only with D-glucose (50 g/kg) or sucrose (50 g/kg). All samples were manufactured into extrusion products under typical industrial conditions. The incubation of D-glucose, a reducing sugar, with FB_1 results in the formation of NCM-FB_1, whereas the incubation of FB_1 with sucrose, a nonreducing sugar, shows no reaction product (*16*). Both sugars can be found in many foods, for example, corn flakes and popcorn, and were chosen for this study to observe any difference in the formation of NCM-FB_1 during the extrusion process. Besides time and the presence of a reducing sugar, temperature is an important component that influences the formation of NCM-FB_1. For this reason corn grits were treated in our studies at different temperatures during the manufacturing process (Tables 1 and 2). Also, it was important to know if the moisture content influences the formation of NCM-FB_1. For the quantification of NCM-FB_1 formation during the heating process a sensitive and reliable method is needed. Our own results (*13*, *27*, *28*) as well as other studies (*29*) have proven the combination of LC/ESI-MS to be a very useful tool for the analysis of fumonisins. They are effectively ionized by the electrospray process, resulting mainly in protonated molecules $[M + H]^+$. Figure 2A shows a typical electrospray mass spectrum of NCM-FB_1 with the $[M + H]^+$ signal at *m/z* 780 and the sodium adduct $[M + Na]^+$ at *m/z* 802, demonstrating a similar behavior compared to other fumonisins. From these data NCM-FB_1 was quantified by electrospray ionization mass spectrometry using isotopically labeled fumonisin (FB_1-d_6). The high accuracy of the quantitative analysis using FB_1-d_6 has recently been proven for the simultaneous determination of FB_1 and HFB_1 (*13*). The product ion spectrum of NCM-FB_1, obtained by collision-induced dissociation (CID) of the protonated molecule using argon as collision gas, revealed signals at *m/z* 428, 410, and 392 generated by the loss of water and the TCA side chains (Figure 2B). The signal at *m/z* 410 is most specific and was employed for the selected reaction monitoring (SRM) mode (*27*). This technique guarantees high selectivity because coeluting and interfering matrix compounds are excluded from detection. Due to the relatively high molecular mass of fumonisins, the SIM is almost as sensitive as the SRM mode and not perturbed by background noise. Furthermore, the SIM mode allows analysis of fumonisins on benchtop single-quadrupole LC-MS instruments, which are available in many laboratories. For this reason method development and

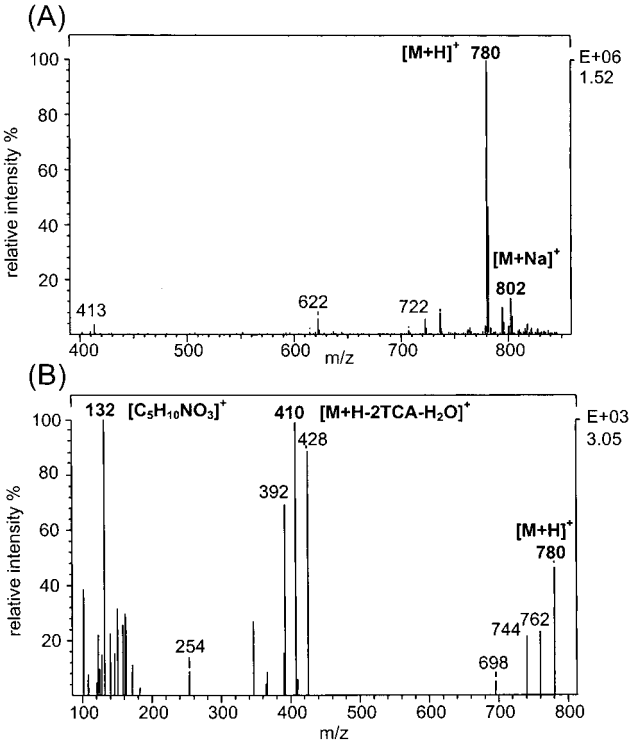

Figure 2. (A) Electrospray mass spectrum of NCM-FB_1 and (B) product ion spectrum of NCM-FB_1, obtained by CID (35 eV, 2.1 mTorr Ar) of the precursor ion *m/z* 780 $[M + H]^+$.

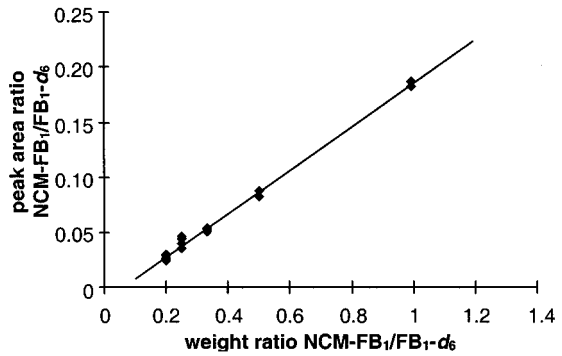

Figure 3. Calibration curve for NCM-FB_1, showing the relationship between the weight ratios of NCM-FB_1 to FB_1-d_6 and the resulting peak area ratios.

validation were performed in our studies in the SIM mode and positive results confirmed using the SRM technique.

It could be indicated in our studies that quantification by LC/ESI-MS with FB_1-d_6 as an internal standard is also a sensitive method for a reliable determination of NCM-FB_1. First, a calibration curve was made with pure mixtures of standard solutions of NCM-FB_1 (10−60 ng/g) and FB_1-d_6 in different weight ratios (1:5, 1:4, etc., up to 1:1), each injected at least three times. The peak area ratios were plotted against the corresponding mass ratio, and the resulting diagram showed a linear curve with a correlation coefficient of $r = 0.997$ and an average response factor of 5.38 (Figure 3). The reason for the relatively high response factor is that the amino group of NCM-FB_1 is blocked and thus ionization in the electrospray process is still possible but to a lower extent compared to FB_1 or FB_1-d_6. For sample cleanup we used a method that had been proven to be most effective for the simultaneous determination of FB_1 and HFB_1 (*13*) working with a mixture of acetonitrile/methanol/water

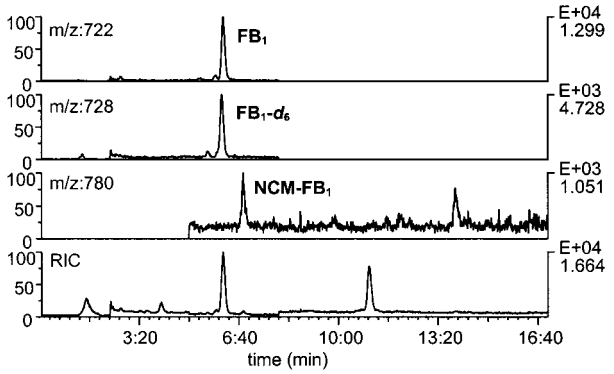

Figure 4. LC/ESI-MS analysis of an extrusion product sample containing 45.9 ng/g NCM-FB_1. Monitored *m/z* ratios were 780 (NCM-FB_1), 722 (FB_1), and 728 (FB_1-d_6). RIC, reconstructed ion chromatogram.

(25:25:50) for sample extraction and a C18 cartridge for subsequent cleanup. Although the use of strong anion exchanger (SAX) columns would be more selective for NCM-FB_1, we decided to use C18 material in order to analyze simultaneously HFB_1. Analytes were separated on a reversed phase column with an acetonitrile/water gradient (see Materials and Methods). Using our standard gradient for fumonisins (*13*) NCM-FB_1 could not be separated from other fumonisins, so a suitable gradient had to be developed. Figure 4 shows the LC/ESI-MS chromatogram of an extrusion product (sample 4) demonstrating the baseline separation of NCM-FB_1 (6.81 min) and FB_1/FB_1-d_6 (6.13 min). The LOD and recovery for NCM-FB_1 were determined by spiking blank extrusion products [containing only D-glucose (50 g/kg) and no FB_1] with NCM-FB_1. Using standard solutions, amounts of 400 pg of NCM-FB_1 could be detected, whereas in the presence of a food matrix 10 ng/g NCM-FB_1 was required for detection with a signal/noise ratio of 3:1. Recoveries of NCM-FB_1 ranged from 50 to 60% and are in the same range as found for HFB_1 in corn-containing food (*13*). The use of SAX columns for cleanup would improve the recovery, but as mentioned above the determination of HFB_1 is not possible. Besides NCM-FB_1, all extrusion products were simultaneously analyzed for HFB_1 and FB_1, as described previously (*13*, *28*). The results are listed in Tables 1 and 2.

The results for the samples spiked with sucrose (Table 1) or D-glucose (Table 2) have two facts in common: First, samples that were treated at the lowest temperature show the highest concentrations of remaining FB_1. Second, high temperatures lead to relatively high amounts of NCM-FB_1. In samples treated with sucrose, a nonreducing sugar, NCM-FB_1 could be detected only in extrusion products that were heated at 180 °C (NCM-FB_1 = 27.1 ng/g), whereas NCM-FB_1 was detected in amounts between 29.6 and 96.8 ng/g in all samples treated with D-glucose, as expected. Although only 14 samples were analyzed, it seems that different moisture levels influence the fate of FB_1 and the formation of NCM-FB_1, as can be seen from all samples produced at 170 °C. It is noticeable that among all samples treated with temperatures of 170 °C those with the lowest moisture content (16%) show the highest loss of FB_1. Among the D-glucose samples produced at 170 °C, sample 8 (moisture content of 20%) shows the highest concentration of NCM-FB_1 (88.6 ng/g). Using different screw speeds (180, 200, and 220 rpm) had not much influence on the results. Furthermore, it could be

demonstrated that the fate of FB_1 in heat-treated corn products cannot be explained only with the formation of NCM-FB_1 because the concentrations are too low. HFB_1 was not detectable in any of the samples. The recovery of the total added FB_1 (expressed as the sum of the percentage of remaining FB_1 and determined NCM-FB_1, which was calculated as FB_1) ranged from approximately 23 to 32% in sucrose-spiked samples, in which nearly no NCM-FB_1 was detected, and from 7 to 15% in D-glucose-spiked extrusion products. This means that recovery of added FB_1 in extrusion products prepared with sucrose was nearly 2 times as high as in samples prepared with D-glucose. Thus, further studies have to be done to determine if the reaction of FB_1 with reducing sugars leads to products different from NCM-FB_1 and if these products could explain the discrepancy between the percentage of remaining FB_1 in extrusion products containing sucrose or D-glucose. Unclear as well is still the binding of FB_1 to proteins, polysaccharides, or other food ingredients. As the results in Tables 1 and 2 show, more than half of the spiked FB_1 is removed during the heating process. Our own experiments prove that alkali treatment of the extrusion products releases HFB_1 (up to 15% of the total added FB_1) from the samples, which could be an indication of bound forms of FB_1. These results are in agreement with data recently published by Resch and Shier (*30*). They showed that alkali treatment of cornstarch, which was spiked with FB_1 and heated, released HFB_1 and other compounds. First attempts to clear the binding of FB_1 to proteins and starch have also been made (*30*).

To compare our results from the spiked extrusion products with corn-containing food, we analyzed various samples from the German market for NCM-FB_1, FB_1, and HFB_1. The results are listed in Table 3. All samples contained FB_1 and HFB_1 ranging from 5.4 to 246.5 ng/g. Concerning NCM-FB_1 the results are in agreement with those from the model experiments, and it was found in six samples in very low concentrations (9.6−76 ng/g). From these data and the low toxicity of NCM-FB_1 it can be concluded that the significance of NCM-FB_1 in food seems to be a minor one.

CONCLUSION

Model experiments using corn grits spiked with FB_1 and D-glucose or sucrose were performed to determine the significance of NCM-FB_1 formation relating to the fate of FB_1 during the extrusion process. An LC/ESI-MS method using isotopically labeled fumonisin FB_1-d_6 as an internal standard was developed for the quantification of NCM-FB_1, FB_1, and HFB_1. NCM-FB_1 was detected in all extrusion products spiked with D-glucose and FB_1, whereas those spiked with FB_1 and sucrose showed only low concentrations of NCM-FB_1 in samples produced at temperatures of 180 °C. Various food samples were analyzed for the presence of NCM-FB_1. The results clearly show the low significance of NCM-FB_1 as a reaction product of FB_1 and reducing sugars in heat-treated food. The fate of FB_1 in heat-treated corn products cannot be explained with the formation of NCM-FB_1. Further investigations are necessary to elucidate the fate of FB_1 and its reactions during heat treatment. This is the first report of a screening for NCM-FB_1 in corn-containing products from the German market.

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