**Report Number:C03061**

# INVESTIGATION OF THE LOSS OF PARENT FUMONISIN MYCOTOXINS DURING FOOD PROCESSING

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# 1 SUMMARY

***SPONSOR***

The work in this report was funded by the UK Food Standards Agency (Project Number C03061).

***BACKGROUND***

The fumonisins (FB1, FB2 and $FB_3$) frequently occur in maize that is intended for food production in the UK. Recent research (carried out during the course of a joint FSA/DEFRA funded project entitled 'The Fate of Fusarium Mycotoxin During Food Processing') investigated the fate of several groups of Fusarium mycotoxin (trichothecenes, zearalenone and fumonisins) that can occur in cereals used to make food products in the UK. Fumonisins have shown significant losses in certain processes, i.e. are undetectable using conventional analytical techniques.

***OBJECTIVE***

The aim of this research project is to investigate this apparent loss of parent fumonisin mycotoxins in both model systems and during food processing, to identify the fate of the fumonisin molecules and determine the significance in terms of the UK consumers' exposure to this class of mycotoxin. This is important information when assessing the exposure of UK consumers to this class of mycotoxin.

***APPROACH***

The approach in this project was to use model systems to investigate the loss of parent fumonisin (FB1, FB2 and FB3) during the physical and chemical steps that these compounds undergo during food processes that result in the "loss" of parent fumonisins. The knowledge of the complexity of fumonisin determination in processed food products was utilised to investigate the fate of the "lost" parent fumonisins. Using a combination of GC/MS and LC/MS/MS the chemical nature of the breakdown/masked products was investigated.

***MAIN RESULTS***

A full literature review was on-going throughout the project showing an increased amount of published information on the occurrence of bound toxins. Bound fumonisin occurrence is not restricted to thermally treated products. Bound fumonisins were detected in raw maize, intermediate and final products showing their occurrence at levels ranging from 15 to 78% higher than those found for parent fumonisins.

*Cornflakes*

Corn grits contained parent, hydrolysed and bound fumonisins, the overall fumonisins levels being significantly higher than the conventionally analysed parent level. Corn flakes contained only very low level of parent, hydrolysed and bound fumonisins (loss of between 87-95%). The cooked grits that had been supplied in some runs also showed much reduced total fumonisin equivalents (in line with levels in cornflakes) indicating that this step gives rise to the reduction.

*Extruded Snacks*

For extruded snacks based on maize grits the results for the analysis of extruded snacks showed a greater variability than cornflakes. Grits used for the process had a low proportion of bound toxins (0-20% of total fumonisin equivalents). Levels of total fumonisin equivalents drop at the extrusion step of the process. The post drying intermediate and the final product have a much higher proportion of bound fumonisins (between 27-79% of total fumonisin equivalents).

For extruded snacks based on maize flour and pellet processing:
Maize flour (in most cases) had a higher total fumonisin content than the maize grits used in other food products, in maize flour the proportion of bound fumonisin was 10-13% of the total fumonisin equivalents. The pellet had a much reduced total fumonisin equivalent. Both the pellet and the products have much higher proportion of bound fumonisin (42-75% of total fumonisin equivalents for the pellet and 44-79% for the snack product). Conventional analysis of fumonisins in the snack product would under determine the total fumonisins present.

*Tortilla Chips*

FB concentrations in the chips were reduced significantly from 33- 76% in the chips, compared to that in the maize flour. Hydrolysed fumonisins and parent fumonisins were detected in the raw materials and final products but were not fully accounted for in thermally treated processes. The change of total fumonisin equivalents during total production is much less that in the two extrusion processes.

*Simple Model Systems*

Maize - hydrolysed and bound fumonisins were determined in the ground maize sample, the total fumonisin equivalents being 36% higher than the conventionally analysed parent fumonisins.
Spiked Wheat Flour - the wheat flour contained no fumonisins.
After extraction all added fumonisin was recovered (within limits of analytical uncertainty) though a very low level of hydrolysed material was detected. Determination of bound fumonisins showed a range of 7-15% of the added fumonisins had become bound, primarily to the protein fractions of the wheat.
Simple Sugars - significant reductions were obtained when salt and sucrose were added into ground maize (9-75% reduction) whereas salt alone caused a reduction of 19-40%, glucose and fructose did not significantly affect the FB1 (4-26% reduction). FB1 bound to the sucrose. The data showed that using naturally contaminated ground maize treated with various salt/sucrose combinations a significant drop in the levels of parent fumonisins was noted in some cases, however when the total fumonisin equivalent were analysed the majority of the fumonisins present were accounted for. In some combinations the degree of hydrolysis and binding was much increased.
Heat - the heating experiments showed that:

- Parent fumonisins decreased after both 10mins (36% decrease) and 4hours (66%)
- No increase in hydrolysed fumonisins
- Total bound fumonisin increased after 4 hours at 120°C.
- Overall decrease in total fumonisin equivalents of between 23-31%.

Corn Pasta - the production of a simple corn pasta resulted in a decrease in parent fumonisins, but when total fumonisins equivalents were determined all the naturally occurring fumonisins were accounted for, the levels of both hydrolysed and bound having increased.

## ***CONCLUSION***

A full literature review was on-going throughout the project showing an increased amount of published information on the occurrence of bound toxins.

Bound and hydrolysed fumonisins were investigated in both simple model systems and food processes. The presence of the breakdown/masking of parent fumonisins in retail food products was investigated. Bound fumonisin occurrence is not restricted to thermally treated products. Bound fumonisins were detected in raw maize, intermediate and final products showing their occurrence at levels ranging from 15 to 78% higher than those found for parent fumonisins. Hence the sum of free and bound toxins could exceed the EU legal limits for total fumonisins.

In some thermally heated processes there is an apparent loss of fumonisins not accounted for by hydrolysis, protein binding or other binding.

Limited and sometimes ambiguous toxicological information is available but there are still gaps in the literature. An external activity by ILSI Europe evaluating masked mycotoxins may lead to a workshop in 2011/12 focussing on the toxicological aspects and we are a member of this expert group.

Reports on the ability of *Aspergillus niger* species to produce fumonisins have continued to appear. *Aspergillus niger* is one of the most commonly reported fungi recovered from foods, responsible for the post harvest decay of fresh fruits, it is commonly extracted from nuts, cereals, pulses and oilseeds.

Recent findings have shown that some *Aspergillus niger* isolates are able to produce fumonisins in high quantities on agar media with a low water activity. Several agricultural products fit this criterion, including dried vine fruits, dates and figs. No UK food products have been included in these surveys.

# 2 ABBREVIATIONS

| | |
|---|---|
| ACN | Acetonitrile |
| AR | Analytical Reagent |
| COSHH | Control of Substances Hazardous to Health |
| LC/MS | Liquid Chromatography with Mass Spectrometry |
| LC/MS/MS | Liquid Chromatography/ Mass Spectrometry/Mass Spectrometry (tandem) |
| FB1 | Fumonisin B1 |
| FB2 | Fumonisin B2 |
| $FB_3$ | Fumonisin $B_3$ |
| H2O | Water |
| HPLC | High performance liquid chromatography |
| MeOH | Methanol |
| SAX | Strong anion exchange |
| OPA | ortho-phthaldialdehyde |
| id | Internal diameter |
| nd | Not Detected |
| ppb or µg/kg | parts per billion or microgram/kilogram |
| ppm or µg/g | parts per million or microgram/gram |
| PAS | Premier Analytical Services |
| TDI | Tolerable Daily Intake |
| NMR | Nuclear magnetic resonance |

# 3 INTRODUCTION

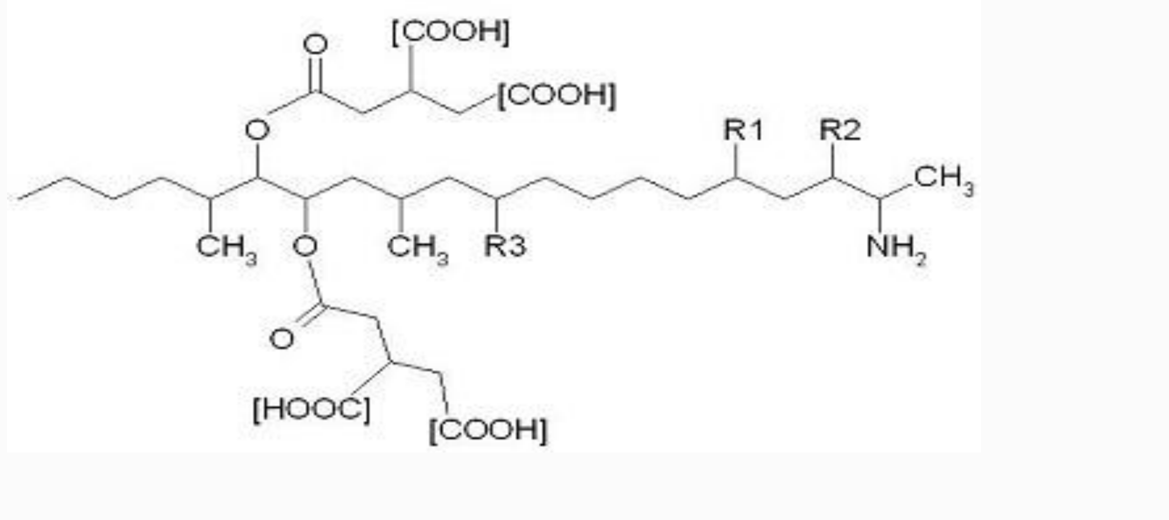
## 3.1 Background

Fusarium mycotoxins frequently occur in cereal grains, including maize, that are intended for food production in the UK. One such class of mycotoxins are the fumonisins such as $B_1$, $B_2$, $B_3$ and $B_4$ that are produced by various fungi of the genus *Fusarium,* primarily by *Fusarium verticillioides* (formerly named *F.moniliforme*) and the related *F. proliferatum*, although other fungal species including *F. napiforme*, *F. dlamini* and *F. nygamai* are also able to produce fumonisins (US-NTP, 1999; WHO-IPCS, 2000). Fumonisins were only identified during the mid-1980's, although their effects on horses had been recognised for at least 150 years before. Though over 15 different fumonisins have been identified, the most significant (and those accounting for most of the fumonisins naturally occurring in maize) are FB1, FB2 and FB3.[1, 2]

## 3.2 Structure

The structure of fumonisins is based on a long hydroxylated hydrocarbon chain containing methyl and amino groups. Two hydroxyl groups are esterified to two propane-1, 2, 3-tricarboxylic acids. Fumonisin B1 differs from fumonisin B2 in that it has an extra hydroxyl group at the 10 position as shown in Figure 1.

**Figure 1: Fumonisin Structure**




Fumonisin B1: R1= OH; R2= OH; R3= OH
Fumonisin B2: R1= H; R2= OH; R3= OH
Fumonisin B3: R1= OH; R2= OH; R3= H

Fumonisin B1 (FB1) has the empirical formula $C_{34}H_{59}NO_{15}$ (relative molecular mass: 721). The pure substance is a white hydroscopic powder that is soluble in water, acetonitrile-water or methanol. Fumonisins are soluble in polar solvents because of the 4 free carboxyl groups, the hydroxyl groups and the amino group. Their insolubility in many organic solvents such as chloroform and hexane commonly used in mycotoxin analysis partly explains the difficulty in their original identification. Fumonisins B1 and B2 are stable in methanol if stored at –18 ºC but steadily degrade at 25 ºC and above. However, they are reported to be stable over a 6-month period at 25 ºC in acetonitrile-water (1:1).

## 3.3 Analytical

It is important that the methods used for the surveillance of mycotoxins give an accurate measure of the amount of that mycotoxin in the raw material or foods tested, so that the exposure of the consumer to that toxin can be accurately determined and effectively managed. Since the discovery of fumonisin mycotoxins much research has been conducted to enable the robust determination of levels of these compounds in cereal raw materials, foods and feeds. Analytical techniques investigated have included TLC, ELISA, GC/MS, capillary electrophoresis and HPLC with fluorescence detection. The majority of studies have been performed using HPLC analysis of a fluorescent derivative. Lack of a suitable chromophore in the molecule means that it must be derivatised with reagents such as p-anisaldehyde, fluorescamine or o-phthaldialdehyde to allow detection by HPLC. There are currently two official methods of the AOAC for the determination of fumonisins by HPLC, one using SAX clean up and one immunoaffinity clean up. More recently LC/MS techniques have become increasingly used because of their high sensitivity and selectivity and is the method of analysis chosen for this project.

## 3.4 Occurrence

*F.verticillioides* and *F.proliferatum* are common fungi associated with maize causing 'Fusarium kernel rot' an important plant disease in hot climates. 'Fusarium kernel rot' may also be induced by *F. graminearum* and a strong relationship exists between insect damage, temperature stress, and fungal invasion, especially in cultivars grown outside their area of adaptation. As *F. verticillioides* and *F. proliferatum* grow over a wide range of temperatures but only at relatively high water activities ($a_w > 0.9$), fumonisins are formed in maize prior to harvest or during the early stage of storage. Except under extreme conditions, the concentrations of fumonisins do not increase during storage.

When the fumonisins were first identified, it was considered that their occurrence was confined to maize. Subsequently, their presence is being noted in a range of products, which include rice, sorghum and navy beans, but so far in much lower concentrations than are common in maize.

Surveillance has shown that fumonisins may be present in a number of finished foods, such as polenta, maize-based breakfast cereals and beer and snack products. They have not been detected in milk, meat or eggs. Recent findings on the occurrence of fumonisin producing fungi and fumonisins in other foods commodities are discussed later in this report.

## 3.5 Toxicity

Fumonisin B1 is known to cause a range of species-specific toxic responses such as leucoencephalomalacia (ELEM) in horses, pulmonary oedema in swine as well as hepatocarcinogenic, hepatotoxic, nephrotoxic and cytotoxic effects in rodents.

Fumonisins are considered to be toxic principally because of their effects on sphingolipid synthesis. Alteration in sphingolipid base ratios occurs almost immediately after exposure because fumonisin inhibits ceramide sythetase. The range of effects that fumonisins cause in mammals appears to be species-related. ELEM was firstly linked to the presence of *Fusarium moniliforme* in feed and more recently to the presence of fumonisins. In equines, affected animals commonly lose appetite, become lethargic and develop neurotoxic effects after a period of ingesting contaminated feed. Autopsy shows oedema in the brain and liquefaction of areas within the cerebral hemispheres. The liver is also generally affected and, in severe cases, gross liver lesions may be seen with fibrosis of the centrilobular areas.

In humans epidemiology studies in countries where maize is a staple component of the diet, indicate a possible correlation with oesophageal cancer. In addition there is a possibility that fumonisins are connected to infant neural tube defects in parts of the USA.

Fumonisins were considered by the Joint FAO/WHO Committee on Food Additives in 2001 and this body allocated a group provisional maximum tolerable intake (PMTDI) for fumonisins B1, B2 and B3 of 2 µg/kg of body weight per day on the basis of the no observed effect level (NOEL) and a safety factor of 100.

The EC Scientific Committee on Food expressed an opinion about FB1 in October 2000, this was updated in 2003. The recommendation was that in order to assess whether there is a potential human health problem in the EU more occurrence data was required and that this formed part of the SCOOP activity on Fusarium toxins.

The data was published in April 2003, 13 countries provided data (including the UK) on 16 different Fusarium mycotoxins including FB1, FB2 and FB3. The overview is shown in Table 1

**Table 1: SCOOP Fumonisin Data Summary**

| Mycotoxin | Number of samples | Positive samples (%) | Main items contained |
|---|---|---|---|
| Fumonisin B1 | 3863 | 46% | Corn (66%), corn flour (79%), corn-based products (31%), corn flakes (46%) |
| Fumonisin B2 | 1010 | 42% | Corn (51%) |
| Fumonisin B3 | 239 | 35% | (Not reported) |

As part of the SCOOP exercise consumption data supplied was used to calculate the average daily intakes calculated as percentage of TDI-values as shown in Table 2.

**Table 2: Range of Average Daily Intake Calculated as a % of TDI**

| Mycotoxin | TDI (µg/kg bw/day | Population | Adults | Infants |
|---|---|---|---|---|
| FB1 + FB2 | 2 | 0.8-13.2% | 0.1-14.1% | 22.3% |

The report concluded that based on the data supplied the average daily intakes across Europe are well below the group TDI; higher intakes were noted for young children.

## 3.6 Regulatory

In the European Union, Commission Regulation 1881/2006 as amended by 1126/2007 has established regulatory limit for fumonisins in foodstuffs based on a sum of FB1 and FB2 as show in Table 3.

**Table 3: European Regulatory Limits for Fumonisins**

| **MAXIMUM LIMITS FOR CERTAIN CONTAMINANTS IN FOODSTUFFS** | | |
|---|---|---|
| **CONTAMINANT** | **FOODSTUFF** | **MAXIMUM LEVELS (µg/kg)** |
| FUMONISINS (Sum of FB1 and FB2) | | |
| Unprocessed maize (with the exception of maize for wet milling) | | 4000 |
| Milling fraction of maize with particle size >500µ | | 1400 |
| Milling fraction of maize with particle size ≤500µ such as maize flour and maize meal | | 2000 |
| Maize intended for direct human consumption<br>Maize based food with the exception of those below: | | 1000 |
| Maize based breakfast cereals and cereal snacks | | 800 |
| Maize based foods for infants and young children | | 200 |

## 3.7 Hidden Fumonisins

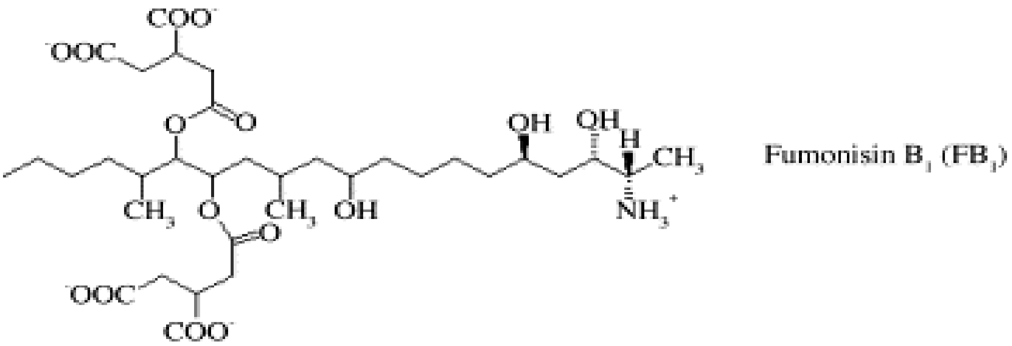
In recent years several strands of investigation have given rise to the hypotheses that cereal based food may contain so called hidden fumonisins. Earliest findings concerned tortilla production. One of the major processes undergone by maize in certain parts of the world is tortilla manufacture involving a nixtamalization (alkaline treatment) step. It was recognised that this process caused a lowering of fumonisin levels. In other studies the consumption of feed with only low fumonisin levels still led to toxin effects, leading to the suggestion of hidden/ bound toxins[9, 15].

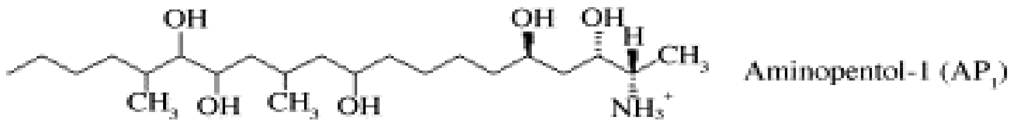
### 3.7.1 Hydrolysed Fumonisin

Hydrolysed fumonisin B1 was first discovered when corn was nixtamalised as part of the tortilla flour production process. Fumonisins contain a long chain aminopentol backbone (AP1) with two ester linked tricarballyic acids. HFB1 (also called AP1) originates from FB1 (Figure 2) by hydrolysis.

At 100°C, calcium hydroxide (0.01M) caused the loss of two tricarballylic acid moieties of FB1 (C14 and C15) and formation of hydrolysed FB1 (HFB1). HFB2 is the hydrolysis product of FB2. The amino pentol compound has been found to occur in masa (tortilla flour) tortilla chips and canned sweet corn. The toxicity of the hydrolysed fumonisin has been found to be higher than that of the unhydrolysed parent in mammalian cell culture and leaf bioassay.

**Figure 2 : Chemical structures of FB1 and HFB1 ($AP_1$).**




### 3.7.2 Bound Fumonisins

A further consideration is the possibility that the toxins (or their breakdown products) might be masked within the food matrix, for example bound to proteins. The bound (or hidden) toxins might be non-extracted by conventional treatments but released in the gastrointestinal tract [11].

Corn based foods that are processed with heat (e.g. corn flakes, corn chips) can contain up to 46% protein bound material, which is not extracted with conventional solvent mixtures.

To overcome this analytical issue, samples are first analysed for parent fumonisins (and hydrolysed fumonisin) and then the remaining material is re-extract for the assessment of bound toxins. Using this approach analysts have reported the presence of both protein bound and other bound toxins. To differentiate, the second stage extraction utilises a detergent (SDS) followed by alkaline hydrolysis to determine protein bound material, and alkaline hydrolysis only to release all the bound material. Both approaches then include an OASIS clean up of the resulting HFBs and conventional end point determination[10]. Using this technique Park *et al* [4] found 14 out of 15 cornflake samples contained $FB_1$ (range 13-237µg/kg). In addition all samples contained bound fumonisins (range 22-176µg/kg) reported as $FB_1$ equivalents. On average there was 1.3 times more bound than unbound fumonisin.

## 3.8 Project Aims

The overall aim of the project was to study the loss of parent fumonisin mycotoxins (Figure 1) during the food processes that have been identified as causing an apparent loss of fumonisins. The proposal was to simulate the chemical and physical processes encountered in the selected processes and studying the effect on fumonisins (FB1 and FB2).

The work programme comprised the following elements:

- Agreement of detailed work plan with the FSA, scope of processes to be studied
- Investigation of the presence of the breakdown/masking of parent fumonisins in food products from the agreed processes
- Setting up of a model system for food manufacture
- Application of the model system for assessing fumonisin losses to cereals
- Assessment of the likely toxicological impact of breakdown products observed

The following report gives the details of the study undertaken and the results obtained, throughout the findings are discussed in light of other workers recently published work in the same area.

# 4 GENERAL METHODOLOGY

## 4.1 Safety

All procedures described in this report were conducted at Premier Analytical Services (PAS) within the Bioanalytical Chemistry trace analysis laboratory. All laboratory workers have been trained for work with mycotoxins. The work is carried out according to detailed PAS Internal Section Safety Procedures and Control of Substances Hazardous to Health (COSHH) risk assessments are prepared prior to undertaking specific jobs. All potential mycotoxin contaminated material was treated with 10-50% sodium hypochlorite solution prior to disposal.

## 4.2 Materials and Chemicals

All reagents were analytical (AR) grade. All solvents were of HPLC grade purchased from Romil Chemical Company. Immunoaffinity columns and phosphate buffered saline (pH 7.4) tablets were purchased from R-Biopharm Rhone Ltd (Glasgow, UK).

## 4.3 Mycotoxin Standards

Solid fumonisin B1, B2 and B3 standards for this project were purchased from MRC (Medical Research Council, South Africa)[20].

### 4.3.1 Preparation of Hydrolysed Fumonisins Standards

Hydrolysed fumonisin standards are not available commercially. Published methods are available for the preparation of standards from parent fumonisins [4].

The standards were prepared from parent fumonisin standards. Aliquots (1ml of 150µg/ml) standards were mixed with 1ml IM potassium hydroxide solution. The mixture was heated for 1hour at 70°C. After cooling the standard was assessed by HPLC with fluorescence detection and by LC/MS. The working hydrolysed fumonisins standards were prepared by diluting the stock with acetonitrile/water (1/1).

All standards were kept at -20°C when not in use.

## 4.4 Preparation of samples

All samples in this survey were ground and thoroughly mixed to ensure homogeneity prior to analysis. After homogenisation the sample was stored in a freezer at –16°C. Samples were allowed to defrost to ambient temperature prior to analysis and any remaining sample returned to –16°C immediately after analysis.

## 4.5 Analytical Methodology

Fumonisins were determined by LC-MS/MS [5] using UKAS accredited method BA-TM-31.

Hydrolysed fumonisins can be analysed alongside the conventional analysis i.e. they can be extracted with the same extraction solvents (particularly acetonitrile/water (1/1)), cleaned up on the solid phase extraction columns and determined by HPLC after OPA derivatisation. In earlier work it was shown that it was possible to analyse both parent and hydrolysed fumonisins in the same sample at the limits of determination required, using LC/MS/MS. An advantage of the use of this instrument for this particular application is that due to the enhanced sensitivity it is possible to analyse samples extracts directly, i.e. no clean up step is required.

### 4.5.1 Extraction of Samples

For parent and hydrolysed fumonisins a 25g sample was extracted with 100ml acetonitrile:water (50:50) by shaking for 120 minutes. The extract was then filtered and fumonisins/hydrolysed fumonisins determined directly by LC-MS/MS using electrospray ionization (ESI) and tandem mass spectrometry (MS/MS), combined with liquid chromatography (LC).

### 4.5.2 HPLC-MS/MS

HPLC-MS/MS analyses were performed on a Waters Acquity Ultra Performance system coupled to a Quattro Premier XE Mass Spectrometer. The equipment was operated in electrospray positive ionisation mode.

The analytical column was a Waters Acquity UPLC BEH C18 1.7µm, 2.1 x 100mm. The column oven was set at +40 ºC. The mobile phase consisted of a mixture acetonitrile/water both containing 0.1% formic acid. The elution was performed by raising the acetonitrile content from 10% to 90% in 12 min, the acetonitrile was kept constant for 2 min, then reduced to initial value in 3 min. The flow rate was 400 µL/min, while the injection volume was 10 µL. The column eluent was directly transferred into the mass spectrometer operated in electrospray positive ionisation mode.

**Table 4: LC-MS/MS Conditions**

| | |
|---|---|
| Final sample solvent | Acetonitrile/water (1/1) |
| Mobile phase | 0.1% formic acid in water (A) /0.1% formic acid in acetonitrile (B) gradient. |
| Column type | Waters Acquity UPLC BEH C18 1.7µm, 2.1 x 100mm |
| Column temperature | 40°C |

| Time (mins) | % A | % B |
|---|---|---|
| 0 | 90 | 10 |
| 3 | 90 | 10 |
| 10 | 30 | 70 |
| 10.1 | 10 | 90 |
| 12 | 10 | 90 |
| 12.1 | 90 | 10 |
| 15 | 90 | 10 |

The eluent from the UPLC column was directed into the electrospray source of a Quattro Premier XE tandem quadrupole mass spectrometer operated in positive ionisation, multiple reaction monitoring (MRM) mode. Table 6 shows the MRM transitions monitored for each compound. The monitoring of two transitions (if possible) allows the presence of a mycotoxin contaminant to be confirmed.

The limit of quantification for each fumonisin is 10µg/kg.

For this investigation the limit of detection was pushed as low as possible in order to detect trace levels of parent fumonisins and hydrolysed fumonisins in a range of matrices. The limit of detection for both parent and hydrolysed fumonisins were as follows:

**Table 5: Limit of Determination (LOD)**

| Toxin | Parent Fumonisins (µg/kg) | Hydrolysed Fumonisins (µg/kg) |
|---|---|---|
| B1 | 10 | 10 |
| B2 | 10 | 10 |
| B3 | 10 | 10 |

**Acceptable recovery 70-110%**

**Table 6: Monitored Ions used in LC-MS/MS**

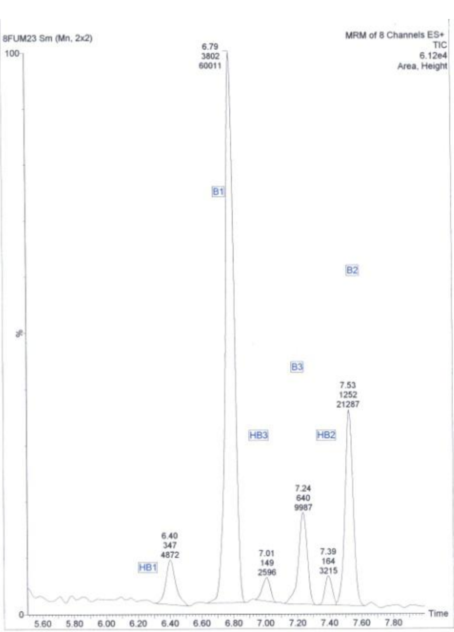
| | Parent Ion (m/z) | Product Ion (m/z) | Cone Voltage (V) | Collision Voltage (V) |
|---|---|---|---|---|
| **Fumonisin B1** | 722 | 334 | 50 | 40 |
| | 722 | 352 | 50 | 40 |
| **Fumonisin B2** | 706 | 336 | 50 | 40 |
| | 706 | 318 | 50 | 40 |
| **Fumonisin B3** | 706 | 372 | 50 | 40 |
| | 706 | 354 | 50 | 40 |
| **Hydrolysed Fumonisin B1** | 406 | 352 | 30 | 21 |
| | 406 | 57 | 30 | 40 |
| **Hydrolysed Fumonisin B2** | 390 | 256 | 35 | 22 |
| **Hydrolysed Fumonisin B3** | 390 | 95 | 35 | 29 |

#### *4.5.2.1 Analysis of hydrolysed fumonisin*

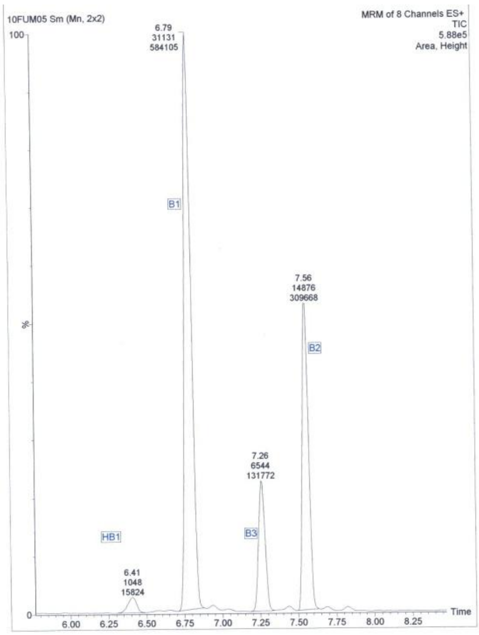
In previous work the LC/MS/MS analysis of extracts, without the need for clean-up showed that hydrolysed fumonisins are naturally occurring in maize, cornflakes and the tortilla samples. This method was suitable to extract both parent fumonisins and hydolysed fumonisins for the concurrent determination of both hydrolysed fumonisins and parent fumonisins at the limits of determination required (see section 4).

**Figure 3: LC/MS/MS Determination of Parent and Hydrolysed Fumonisins**

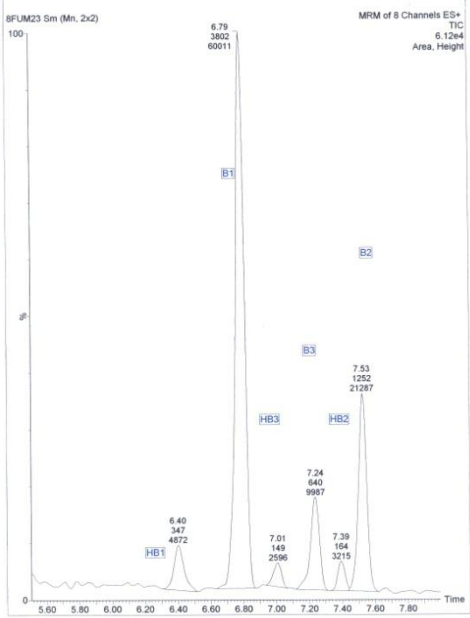
**a) Mixed Standard**



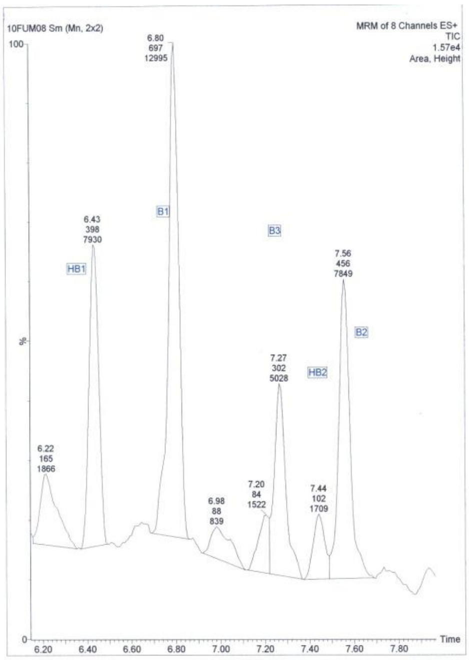

**b) Tortilla Test Material**




**c) Maize**




**d) Cornflakes**




Key: B1, B2, B3 = fumonisin B1, B2, B3 and HB1, HB2, HB3 = hydrolysed B1, B2, B3

#### *4.5.2.2 Analysis of Bound Fumonisins*

***Protein Binding-Chemical Disruption***

To explore the occurrence of protein bound fumonisins in the processed food test materials the method of Park *et al* [4] had previously been assessed and was used in this study. The samples were extracted for parent and hydrolysed fumonisins by extracting 25g sample with 100ml acetonitrile:water (50:50) and shaking for 120 minutes. The extract was then filtered and the solid residue re-extracted a further three times to remove any residual parent or hydrolysed fumonisins Protein bound fumonisins were extracted with 1% SDS solution by taking 10g of the remaining solid residue and extracting with 25ml of 1% SDS (sodium dodecylsulphate). In order to carry out the analysis it was necessary to separate the protein-fumonisin complex by complexing 5ml of the SDS extract with 2ml of 1% methylene blue and then hydrolysing with 5ml of 2N KOH to yield the hydrolysed fumonisins (HFB1, HFB2 and HFB3).

Table 8 shows the repeatability data.

***Total Bound***

A more rigorous approach was taken to the extraction of total fumonisins as fully hydrolysed material, using a combination of alkaline treatment (KOH) and a heating step. The samples were extracted for parent and hydrolysed fumonisins by extracting 25g sample with 100ml acetonitrile:water (50:50) and shaking for 120 minutes. The extract was then filtered and the solid residue re-extracted a further three times to remove any residual parent or hydrolysed fumonisins. Total bound fumonisins were extracted by taking 10g of the remaining solid residue with 25ml 2N KOH and heated at 60°C for 1 hour. Clean up was performed on an OASIS polymeric solid-phase extraction column and the bound fumonisins were determined by LC-MS/MS as HFB1, HFB2 and HFB3.

Table 8 shows the repeatability data.

**Table 8: Summary of Bound Fumonisin Investigation**

| **Test Materials** | **Conventional Extracted Fumonisins** | **Protein Bound Fumonisins Equivalents** | **Total Bound Fumonisins Equivalents** |
|---|---|---|---|
| | | **(μg/kg)** | |
| **Tortilla** | | | |
| Replicate 1 | 345 | 86 | 121 |
| Replicate 2 | 362 | 102 | 133 |
| Replicate 3 | 344 | 86 | 119 |
| Replicate 4 | 360 | 98 | 115 |
| Replicate 5 | 356 | 108 | 126 |
| Replicate 6 | 342 | 101 | 135 |
| **Mean** | **351** | **97** | **125** |
| **Cornflakes (maize grits)** | | | |

| **Test Materials** | **Conventional Extracted Fumonisins** | **Protein Bound Fumonisins Equivalents** | **Total Bound Fumonisins Equivalents** |
|---|---|---|---|
| | **(µg/kg)** | | |
| Replicate 1 | <10 | 12 | 18 |
| Replicate 2 | <10 | 10 | 22 |
| Replicate 3 | <10 | 11 | 17 |
| Replicate 4 | <10 | 14 | 16 |
| Replicate 5 | <10 | 10 | 12 |
| Replicate 6 | <10 | 12 | 21 |
| **Mean** | **<10** | **12** | **18** |

# 5 RESULTS

## 5.1 Literature Review

A full literature review of published information on hidden or bound mycotoxins was carried out in 2009 (see Appendix 1). This was used to select the processes for further study.

Key information from this review is summarised below:

Fumonisins were reported to be largely thermostable.
Reduction during processing (as opposed to redistribution as seen during physical processing of other mycotoxins) was reported to occur:

- Cornflakes
- Extruded products
- During nixtamalsation

Reports on the reaction and binding of fumonisins mycotoxins showed that:
The presence of sugars had significant effects on parent fumonisins, binding occurs and the bound moieties are not detected by conventional analytical techniques, these reports are discussed in more detail in Section 5.3.2 of this report.

### 5.1.1 Summary of Published Information

During the course of this investigation more information has been published about both the analysis and occurrence of hidden fumonisins in maize and maize products, reviews of this information are included in this report alongside data generated in this study.

In addition regular literature reviews on fumonisins have been performed; additional information of potential interest to the Food Standards Agency is included in this report.

### 5.1.2 Choice of Processes for Further Study

Based on findings in FSA sponsored work and published literature it was agreed with the FSA that the processes for study were to be:

- Cornflake production
- Extruded maize snacks
- Tortilla chips

## 5.2 Commercial Food Product Analysis

The aim was to investigate the breakdown/masking of parent fumonisins in food products from the processes identified in the literature review.

Each commercial process that had been identified as giving rise to loss of parent fumonisins (i.e. cornflake production, maize snack production) was examined using the analytical methods developed in an earlier FSA funded project and detailed above.

A range of maize based food products were purchased from local retail outlets by PAS. All samples were ground and thoroughly mixed to ensure homogeneity prior to analysis and stored in a freezer at –16°C. Samples were allowed to defrost to ambient temperature prior to analysis and returned to –16°C immediately after analysis.

### 5.2.1 Parent and Hydrolysed Fumonisin Investigation

The samples were analysed for parent fumonisins and hydrolysed fumonisins and the results are shown in Tables 9a to 9c.

**Table 9a: Summary of Parent and Hydrolysed Fumonisin Investigation: Cornflakes**

| Laboratory Code | Sample Description | Parent Fumonisins (μg/kg) | | | Hydrolysed Fumonisins (μg/kg) | | |
|---|---|---|---|---|---|---|---|
| | | FB1 | FB2 | FB3 | HFB1 | HFB2 | HFB3 |
| 09B-02204 | Cornflakes | 95 | 20 | <10 | <10 | <10 | <10 |
| 09B-02205 | Organic Cornflakes | 17 | <10 | <10 | <10 | <10 | <10 |
| 09B-02206 | Cornflakes | 31 | <10 | <10 | <10 | <10 | <10 |
| 09B-02207 | Cornflakes | 41 | <10 | <10 | <10 | <10 | <10 |
| 09B-02208 | Cornflakes | 30 | <10 | <10 | <10 | <10 | <10 |
| 09B-02209 | Cornflakes | 31 | <10 | <10 | <10 | <10 | <10 |
| 09B-02210 | Frosted Flakes | 15 | <10 | <10 | 11 | <10 | <10 |
| 09B-00699 | Cornflakes | 15 | <10 | <10 | <10 | <10 | <10 |
| 09B-02203 | Organic Cornflakes (EXTRUDED) | 131 | 22 | 13 | <10 | <10 | <10 |

**Table 9b: Summary of Parent and Hydrolysed Fumonisin Investigation: Extruded Maize Snacks**

| Laboratory Code | Sample Description | Parent Fumonisins (µg/kg) | | | Hydrolysed Fumonisins (µg/kg) | | |
|---|---|---|---|---|---|---|---|
| | | FB1 | FB2 | FB3 | HFB1 | HFB2 | HFB3 |
| 09B-02211 | Maize Snacks Mixed | <10 | <10 | <10 | <10 | <10 | <10 |
| 09B-02212 | Cheesy Maize snacks | 72 | 23 | 10 | <10 | <10 | <10 |
| 09B-02213 | Maize Snacks | <10 | <10 | <10 | <10 | <10 | <10 |

**Table 9c: Summary of Parent and Hydrolysed Fumonisin Investigation: Tortilla Chips**

| Laboratory Code | Sample Description | Parent Fumonisins (µg/kg) | | | Hydrolysed Fumonisins (µg/kg) | | |
|---|---|---|---|---|---|---|---|
| | | FB1 | FB2 | FB3 | HFB1 | HFB2 | HFB3 |
| 09B-02214 | Tortilla Chips | 105 | 39 | 11 | 70 | 12 | <10 |
| 09B-02215 | Lightly Salted Tortilla Chips | 156 | 35 | 17 | 74 | 18 | <10 |
| 09B-01499 | Chilli Flavoured Tortilla Chips | 11 | <10 | <10 | <10 | <10 | <10 |

#### *5.2.1.1 Summary*

Parent fumonisins were detected in the maize based food products at levels ranging from 11 to 208µg/kg and hydrolysed fumonisins were detected in the cornflakes and tortilla chips at levels ranging from 11 to 92µg/kg.

### 5.2.2 Bound Toxins

The same range of maize based food products were also analysed for bound toxins, as described in Section 4, parent and hydrolysed fumonisins are extracted from the sample prior to treatments to recover the protein and or other bound toxins. The results of this investigation are shown in Table 10a-10c.

**Table 10a: Summary of Bound Fumonisin Investigation Traditional Cornflakes**

| Laboratory Code | Sample Description | Parent Fumonisins Extracted Conventionally | Protein Bound Fumonisins Equivalents | Total Bound Fumonisins Equivalents |
|---|---|---|---|---|
| | | (µg/kg) | | |
| 09B-02204 | Cornflakes | 123 | 25 | 37 |
| 09B-02205 | Organic Cornflakes | 22 | <10 | <10 |
| 09B-02206 | Cornflakes | 39 | <10 | 12 |
| 09B-02207 | Cornflakes | 52 | 13 | 20 |
| 09B-02208 | Cornflakes | 38 | 10 | 13 |
| 09B-02209 | Cornflakes | 40 | <10 | 12 |
| 09B-02210 | Frosted Flakes | 19 | <10 | <10 |
| 09B-00699 | Cornflakes | 19 | <10 | <10 |
| 09B-02203 | Organic Cornflakes (EXTRUDED) | 166 | 10 | 15 |

**Table 10b: Summary of Bound Fumonisin Investigation Maize Snacks**

| Laboratory Code | Sample Description | Parent Fumonisins Extracted Conventionally | Protein Bound Fumonisins Equivalents | Total Bound Fumonisins Equivalents |
|---|---|---|---|---|
| | | (µg/kg) | | |
| 09B-02211 | Maize Snacks Mixed | <10 | <10 | <10 |
| 09B-02212 | Cheesy Maize snacks | 105 | <10 | <10 |
| 09B-02213 | Maize Snacks | <10 | <10 | <10 |

**Table 10c: Summary of Bound Fumonisin Investigation Tortilla chips**

| Laboratory Code | Sample Description | Parent Fumonisins Extracted Conventionally | Protein Bound Fumonisins Equivalents | Total Bound Fumonisins Equivalents |
|---|---|---|---|---|
| | | (µg/kg) | | |
| 09B-02214 | Tortilla Chips | 155 | 35 | 54 |
| 09B-02215 | Lightly Salted Tortilla Chips | 208 | 20 | 42 |
| 09B-01499 | Chilli Flavoured Tortilla Chips | 13 | <10 | <10 |

#### *5.2.2.1 Summary*

Total bound fumonisins were detected in the maize based food products at levels ranging from 12-54µg/kg fumonisin equivalents. The results for each sample type are discussed in more detail below, covering parent, hydrolysed and bound fumonisins.

### **5.2.3** Discussion

The results from both series of analyses can be combined to show total recoverable fumonisins in each food product. In Tables 11-13 below the results are consolidated and total fumonisin equivalents shown.

**Table 11: Comparative Levels of Hidden Fumonisins in Cornflakes**

| Laboratory Code | Sample Description | Conventional Extraction | | Bound | | Total Fumonisin Equivalents |
|---|---|---|---|---|---|---|
| | | Parent Fumonisins | Hydrolysed Fumonisins | Protein Bound Fumonisins Equivalents | Total Bound Fumonisins Equivalents | |
| | | (µg/kg) | | | | |
| 09B-02204 | Cornflakes | 123 | <10 | 25 | 37 | 169 |
| 09B-02205 | Organic Cornflakes | 22 | <10 | <10 | <10 | 30 |
| 09B-02206 | Cornflakes | 39 | <10 | <10 | 12 | 59 |
| 09B-02207 | Cornflakes | 52 | <10 | 13 | 20 | 81 |
| 09B-02208 | Cornflakes | 38 | 11 | 10 | 13 | 62 |
| 09B-02209 | Cornflakes | 40 | <10 | <10 | 12 | 61 |
| 09B-02210 | Frosted Flakes | 19 | 11 | <10 | <10 | 35 |
| 09B-00699 | Cornflakes | 19 | nd | <10 | <10 | 23 |

| Laboratory Code | Sample Description | Conventional Extraction | | Bound | | Total Fumonisin Equivalents |
|---|---|---|---|---|---|---|
| | | Parent Fumonisins | Hydrolysed Fumonisins | Protein Bound Fumonisins Equivalents | Total Bound Fumonisins Equivalents | |
| | | (µg/kg) | | | | |
| 09B-02203 | Organic Cornflakes (EXTRUDED) | 166 | 11 | 10 | 15 | 192 |

**Key points**

- Only one sample of cornflakes is known to be extruded (09B-02203), this contained the highest amount of fumonisins 192µg/kg fumonisin equivalents.
- Hydrolysed fumonisins were detected in all but one cornflake sample, the range being 0-36% of conventionally extractable fumonisins.
- Bound fumonisins were found in all samples, protein bound material, accounted for most of the bound toxins. The total bound fumonisin as a % of total fumonisin equivalents ranged from 0-25%.
- The total fumonisin equivalent for all samples has been calculated, the additional fumonisin recovered with the analysis of both hydrolysed and bound toxins ranged from 13% (extruded cornflakes) to 31% (frosted flakes).

**Table 12: Comparative Levels of Hydrolysed Fumonisins in Maize Snacks - Extruded**

| Laboratory Code | Sample Description | Conventional Extraction | | Bound | | Total Fumonisin Equivalents |
|---|---|---|---|---|---|---|
| | | Parent Fumonisins | Hydrolysed Fumonisins | Protein Bound Fumonisins Equivalents | Total Bound Fumonisins Equivalents | |
| | | (µg/kg) | | | | |
| 09B-02211 | Maize Snacks Mixed | <10 | <10 | <10 | <10 | <10 |
| 09B-02212 | Cheesy Maize snacks | 105 | <10 | <10 | <10 | 113 |
| 09B-02213 | Maize Snacks | <10 | <10 | <10 | <10 | <10 |

**Key points**

- Hydrolysed fumonisins were not detected in any of the extruded snacks.

**Table 13: Comparative Levels of Hydrolysed Fumonisins in Maize Snacks-Tortilla**

| Laboratory Code | Sample Description | Conventional Extraction | | Bound | | Total Fumonisin Equivalents |
|---|---|---|---|---|---|---|
| | | Parent Fumonisins | Hydrolysed Fumonisins | Protein Bound Fumonisins Equivalents | Total Bound Fumonisins Equivalents | |
| | | (µg/kg) | | | | |
| 09B-02214 | Tortilla Chips | 155 | 89 | 35 | 54 | 298 |
| 09B-02215 | Lightly Salted Tortilla Chips | 208 | 98 | 20 | 42 | 348 |
| 09B-01499 | Chilli Flavoured Tortilla Chips | 13 | <10 | <10 | <10 | 13 |

**Key points**

- Hydrolysed fumonisins were found in 2 of the 3 tortilla samples, the range being 32-36% of conventionally extractable fumonisins. The third sample had a very low level of parent fumonisins.

- Bound fumonisins were found in the two samples, protein bound material accounted for some of the bound toxins. The total bound fumonisin as a % of total fumonisin equivalents ranged from 12-18%.

- The total fumonisin equivalent for all samples has been calculated, the additional fumonisin recovered with the analysis of both hydrolysed and bound toxins ranged from 40-48%.

### 5.2.4 Conclusions

Bound fumonisins were determined as the hydrolysed fumonisins. The results showed in cornflakes a 7-25% increase in fumonisins detected as the bound form, 7% in extruded snacks and an increase of 12-18% for tortilla chips.

## 5.3 Model Systems

Following the completion of the literature review and the determination of bound/hidden fumonisins in retail food samples, key model systems for additional study were agreed. The gathered information on the commercial processes demonstrated that ingredients such as sugars, starch, salt and protein are components that should be studied as part of the model system investigations.

Aim: To set up a simple model system containing selected food ingredients.

The use of model systems was proposed so as to have a phased approach to the identification of possible breakdown or bound products of the parent fumonisins. As discussed above techniques are available to determine some of the known breakdown products of parent fumonisins and some of the bound compounds; however the purpose of the model systems is to facilitate the search for potentially unknown breakdown products. A simple model system was used to simulate the chemical and physical conditions encountered during food processing but in the absence of the food ingredients. As a first stage simple solution of sugars (mono and disaccharides), starch and protein were prepared and any binding assessed at the second stage the model systems mimicked the time and temperature/pressure profiles of food processes. Any losses seen in this system would be simpler to identify due to the absence of co-extractive cereal compounds.

The same conditions were utilised in the presence of food ingredients, so showing a) that the process conditions are reasonable, i.e. producing recognisable food products and b) allowing the targeted search for any breakdown products discovered.

In addition binding of parent fumonisins in these conditions would be studied using the techniques described above.

This approach had been utilised as part of the FSA/DEFRA funded sister project The Fate of Fusarium Mycotoxins During Commercial Food Processing conducted at the University of Bristol but has not been applied to fumonisins as the University have not got the analytical capability for this class of mycotoxins.

During the FSA/DEFRA project several maize processing systems had been studied on a commercial scale by collaborators and loss of parent fumonisins noted (breakfast cereal manufacture, snack production) and it is these processes (temperature profiles, pressure treatments, and cooking techniques) that were mimicked in the pilot scale food processing facilities available.

In order to investigate the interactions between analyte and matrices the occurrence of fumonisin derivatives was investigated. Proteins mask the presence of hidden fumonisins, which cannot be directly determined by LC-MS/MS but enzymatic digestion and alkaline hydrolysis releases the entrapped FBs by cleaving the ester bonds between the tricarballylic acid groups and the polyhydroxyamino backbone to release hydrolysed fumonisins (HFB1). In addition hidden fumonisins were also detected in the extract obtained with the extraction solvent used for fumonisin determination.

*Extraction of extractable fumonisins*
25g sample was extracted with 100ml acetonitrile:water (1:1) by shaking for 120mins. The extract was filtered and analysed by LC-MS/MS

*Extraction of total fumonisins*
25g sample was shaken with 100mls (2m KOH) for 120mins, 100ml acetonitrile added and 2ml was evaporated to dryness and redissolved in acetontrile:water (1:1), filtered and analysed by LC-MS/MS. Fumonisins after sample hydrolysis were measured as sum of HFB1, HFB2 and HFB3.

Hidden fumonisins were calculated as the difference between extractable FBs and total FBs value. The extract was also treated under alkaline conditions to check whether hidden forms were co-extracted together with commonly detectable forms. Extractable fumonisins, total fumonisins found in each extract after hydrolysis and total fumonisins found in each sample after hydrolysis were calculated.

Fumonisin detection falls into three categories.

1. Extractable fumonisins – the sum of FB1, FB2, FB3 detected in each sample from common extraction conditions.
2. Total fumonisins – the sum of FB1, FB2, FB3 in each sample after hydrolysis obtained by measuring HFB1, HFB2, HFB3.
3. Hidden fumonisins – the difference between total fumonisins and extractable fumonisins.

The occurrence of parent, hydrolysed and bound fumonisins in food products was determined.

### 5.3.1 Simple Model

**Standards**

Stability in aqueous solutions, different temperature, addition of food materials and pH changes) that might be the cause of the loss of parent fumonisins.

The stability of fumonisins standards was investigated and the effect of time and temperature on fumonisin B1 (FB1) stability in aqueous solutions at pH 4, 7, and 10 was determined. FB1 was least stable at pH 4 followed by pH 10 and 7, respectively. At >150 °C, >85% of FB1 was lost after processing for 60 min, regardless of pH.

**Characterisation of Naturally Contaminated Maize**

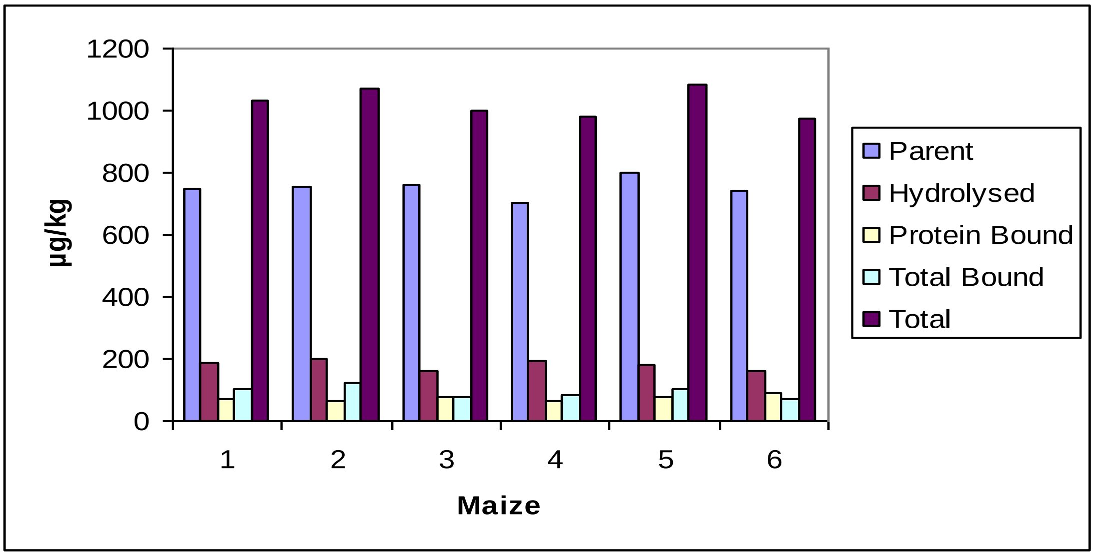
Naturally contaminated ground maize (0.5mm) with a total fumonisin (B1+B2+B3) concentration of 750µg/kg was initially analysed to determine the fumonisin levels prior to any processing.

**Table 14: Total Fumonisins in Naturally Contaminated Ground Maize**

| Sample Description | Conventional Extraction | | Bound | | Total Fumonisin Equivalents |
|---|---|---|---|---|---|
| | Parent Fumonisins | Hydrolysed Fumonisins | Protein Bound Fumonisins Equivalents | Total Bound Fumonisins Equivalents | |
| | (µg/kg) | | | | |
| Maize replicate 1 | 746 | 185 | 74 | 104 | 1035 |
| Maize replicate 2 | 752 | 201 | 65 | 120 | 1073 |
| Maize replicate 3 | 760 | 163 | 80 | 75 | 998 |
| Maize replicate 4 | 704 | 195 | 66 | 82 | 981 |

| Sample Description | Conventional Extraction | | Bound | | Total Fumonisin Equivalents |
|---|---|---|---|---|---|
| | Parent Fumonisins | Hydrolysed Fumonisins | Protein Bound Fumonisins Equivalents | Total Bound Fumonisins Equivalents | |
| | (µg/kg) | | | | |
| Maize replicate 5 | 800 | 180 | 75 | 102 | 1082 |
| Maize replicate 6 | 744 | 159 | 90 | 72 | 975 |
| Mean | 751 | 181 | 75 | 93 | 1024 |

**Figure 4: Total Fumonisins in Naturally Contaminated Ground Maize**




Hydrolysed and bound fumonisin were determined in the ground maize sample, the total fumonisin equivalents being 36% higher than the conventionally analysed parent fumonisins. This finding is in line with the results of other workers who have determined bound fumonisins in non-thermally treated maize[39]. Dall'Asta *et al* hypothesised that plant metabolism might be responsible for the transformation of the fumonisin formed by the contaminating fungi into bound conjugates due to the effect of chemical compartmentalisation exerted by the plant towards xenobiotics produced by the contaminating fungi. The findings of this study, i.e. the presence of bound fumonisins in raw maize, are in line with this hypothesis. These levels are still below the EC legislation limits for the parent fumonisins (B1+B2).

**Spiked Wheat Flour**

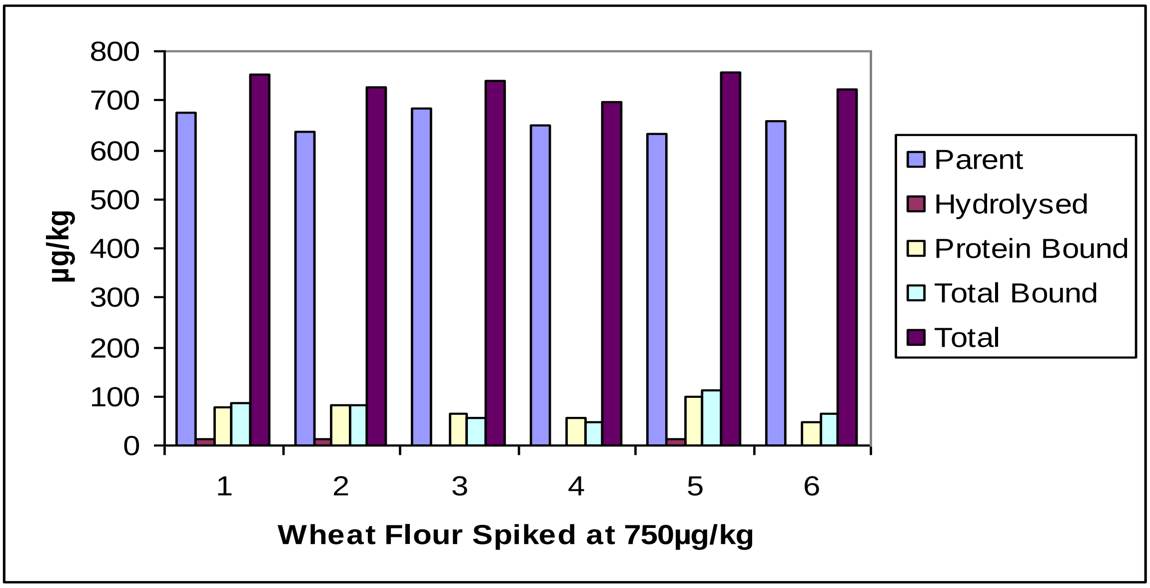
Wheat flour was selected for use as an inert food component, as no fumonisins occur in this material. This was confirmed by analysis.

Wheat flour was spiked with fumonisins standard at a total fumonisin level (B1 plus B2) of 750µg/kg (a level comparable to that found in the naturally contaminated maize) and left overnight as room temperature (in the dark). The parent, hydrolysed and bound forms were then analysed.

**Table 15: Fumonisins in Wheat Flour spiked with Fumonisins**

| Sample Description | Conventional Extraction | | Bound | | Total Fumonisin Equivalents |
|---|---|---|---|---|---|
| | Parent Fumonisins | Hydrolysed Fumonisins | Protein Bound Fumonisins Equivalents | Total Bound Fumonisins Equivalents | |
| | (µg/kg) | | | | |
| Wheat flour | <10 | <10 | <10 | <10 | <10 |
| Wheat Flour spike 1 | 675 | 11 | 78 | 85 | 751 |
| Wheat Flour spike 2 | 635 | 12 | 81 | 82 | 729 |
| Wheat Flour spike 3 | 685 | <10 | 65 | 55 | 740 |
| Wheat Flour spike 4 | 649 | <10 | 55 | 48 | 697 |
| Wheat Flour spike 5 | 632 | 15 | 98 | 112 | 759 |
| Wheat Flour spike 6 | 658 | <10 | 48 | 66 | 724 |
| **Mean Spike** | **656** | **<10** | **71** | **75** | **733** |

**Figure 5: Fumonisins in Wheat Flour spiked with Fumonisins**




The wheat flour contained no fumonisins.

After extraction all added fumonisin was recovered (within limits of analytical uncertainty) though a very low level of hydrolysed material was detected. Determination of bound fumonisins showed a range of 7-15% of the added fumonisins had become bound, primarily to the protein fractions of the wheat.

### 5.3.2 Effect of Simple Sugars

Seefelder *et al*.[39] studied the binding of fumonisin to food matrix components e.g. saccharides and proteins during thermal processing. Three types of models were considered: a mono and disaccharide model; a starch model; and a protein model. α-D-glucose and sucrose, α-D-glucopyranoside, N-α-acetyl-L-lysine methyl ester and BOC-L-cysteine methyl ester were the reagents, respectively. The reactions were carried out using fumonisin B1 and its hydrolyzed derivative at 150°C. The hydrolyzed products obtained were analysed by LC-MS/MS with electrospray ionisation. The chemical structures were confirmed by nuclear magnetic resonance NMR. The experiments indicated that tricarboxylic acid side chains are responsible for binding fumonisins to polysaccharides and proteins.

**Simple Binding Experiments**

Several workers had already performed model experiments on the binding of FB1 to glucose and sucrose. Binding had been shown to occur with the formation of various Maillard type reaction products [40, 16].

Castells *et al* [27] had studied the reduction of fumonisin B1 in corn flour with salt, malt and sugar in their formulation .Two levels of both sodium chloride (0.4% and 2%) were added to the unextruded corn flour, and six levels of sucrose (3-10%) were used. The addition of sucrose at the lowest salt content (0.4%) as well as salt, either at 0.4% or at 2%, led to a significant decrease of FB1 levels in extruded samples. Decontamination rates depended on the concentrations of added ingredients and ranged from 2% to 92%. The greatest reductions in FB1 content were achieved with extrusion cooking with a high salt content, whilst the lowest reductions were the result of processing corn flour with low contents of both salt and sucrose. Salt at 2% was the most effective ingredient in reducing FB1 content of the final extruded food. The authors only reported on the parent fumonisins levels. An experiment following a similar approach was conducted.

The stability of FBs was investigated using simple food matrix components to investigate potential binding of parent fumonisins. Naturally contaminated ground maize contaminated with FB1, FB2 and FB3 was used to demonstrate the effect of simple sugars.

Naturally contaminated ground maize (0.5mm) with a total fumonisin (B1+B2+B3) concentration of 750µg/kg was used. The following was added to 1kg of ground maize:

- Salt was added at two levels (0.4% and 2%)
- Sucrose (2%, 10%)
- Glucose (2%, 10%)
- Fructose (2%, 10%)

600ml of water was added to each respective mixture and thoroughly mixed until an homogenous dough was obtained. The dough was placed in the fridge at 4°C for 48hours.

The doughs were first analysed for parent fumonisin, the results are presented in Table 16a and Figure 6a and 6b.

**Table 16a: Effect of the addition of salt, glucose and sucrose on Parent Fumonisin Levels in Naturally Contaminated Maize Flour**

| Addition | FB1 (µg/kg | FB2 (µg/kg) | $FB_3$ (µg/kg) | Total/% reduction µg/kg |
|---|---|---|---|---|
| Maize Flour | 541 | 133 | 77 | 751 |
| Maize Dough | 490 | 120 | 65 | 675 (11%) |
| 0.4% salt | 450 | 101 | 58 | 609 (19%) |
| 2% salt | 322 | 75 | 55 | 452 (40%) |
| 2% sucrose | 514 | 126 | 70 | 710 (6%) |
| 10% sucrose | 486 | 100 | 59 | 645 (14%) |
| 2% glucose | 531 | 130 | 74 | 735 (3%) |
| 10% glucose | 525 | 118 | 76 | 719 (4%) |
| 2% fructose | 521 | 121 | 81 | 723 (4%) |
| 10% fructose | 532 | 109 | 52 | 693 (8%) |
| 0.4% salt + 2% sucrose | 503 | 116 | 68 | 687 (9%) |
| 0.4 % salt + 10% sucrose | 378 | 90 | 50 | 518 (31%) |
| 2% salt + 2% sucrose | 159 | 40 | 20 | 219 (70%) |
| 2% salt + 10% sucrose | 134 | 35 | 22 | 189 (75%) |
| 0.4% salt + 10% glucose | 401 | 98 | 58 | 557 (26%) |
| 0.4% salt + 10% fructose | 476 | 102 | 68 | 646 (14%) |

(Note: all results corrected for moisture content).

A small decrease in parent fumonisin levels was seen in the dough and in doughs to which sugars were added (3-14%).

More significant reductions were obtained when salt was added into ground maize (19-40% reduction) and dough produced.

The addition of salt and higher levels of sugars resulted in a great reduction in the level of parent fumonisin determined. The largest decrease was seen when the salt was added at 2% and sucrose at 10%.

**Figure 6a: Effect of the addition of salt, glucose and sucrose on Parent Fumonisin Levels in Naturally Contaminated Maize Flour**

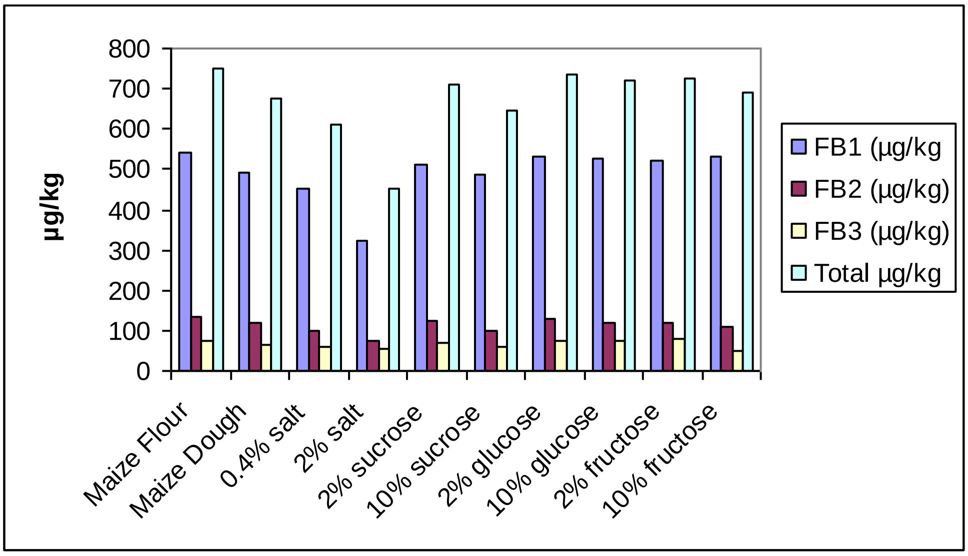
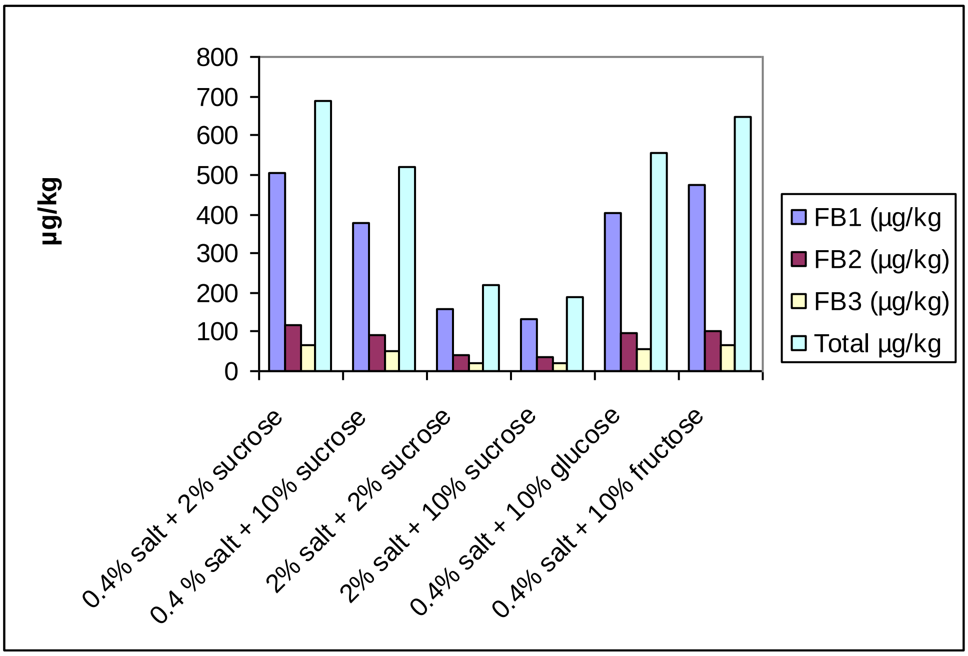


**Figure 6b: Effect of the addition of salt, glucose and sucrose on Parent Fumonisin Levels in Naturally Contaminated Maize Flour**




The doughs were then analysed for "hidden" fumonisins (in line with procedures described in Section 4). Hydrolysed, protein and other bound fumonisin were determined. The results of this analysis are presented in Table 16b and Figure 7a and 7b.

**Table 16b: Effect of the addition of salt, glucose and sucrose on Total Fumonisin Levels in Naturally Contaminated Maize Flour**

| Sample Description | Conventional Extraction | | Bound | | Total Fumonisin Equivalents |
|---|---|---|---|---|---|
| | Parent Fumonisins | Hydrolysed Fumonisins | Protein Bound Fumonisins Equivalents | Total Bound Fumonisins Equivalents | |
| | (µg/kg) | | | | |
| **Maize Flour** | 751 | 181 | 75 | 93 | 1025 |
| **Maize Dough** | 675 | 175 | 82 | 125 | 975 |
| **0.4% salt** | 609 | 155 | 120 | 190 | 954 |
| **2% salt** | 452 | 256 | 146 | 202 | 910 |
| **2% sucrose** | 710 | 185 | 60 | 82 | 977 |
| **10% sucrose** | 645 | 132 | 71 | 136 | 913 |
| **2% glucose** | 735 | 165 | 65 | 101 | 1001 |

| Sample Description | Conventional Extraction | | Bound | | Total Fumonisin Equivalents |
|---|---|---|---|---|---|
| | Parent Fumonisins | Hydrolysed Fumonisins | Protein Bound Fumonisins Equivalents | Total Bound Fumonisins Equivalents | |
| | (µg/kg) | | | | |
| **10% glucose** | 719 | 199 | 75 | 98 | 1016 |
| **2% fructose** | 723 | 164 | 81 | 105 | 992 |
| **10% fructose** | 693 | 185 | 54 | 125 | 1003 |
| **0.4% salt + 2% sucrose** | 687 | 221 | 99 | 102 | 1010 |
| **0.4 % salt + 10% sucrose** | 518 | 236 | 120 | 164 | 918 |
| **2% salt + 2% sucrose** | 219 | 321 | 350 | 426 | 966 |
| **2% salt + 10% sucrose** | 189 | 345 | 330 | 454 | 988 |
| **0.4% salt + 10% glucose** | 557 | 192 | 175 | 215 | 964 |
| **0.4% salt + 10% fructose** | 646 | 198 | 91 | 110 | 954 |

**Figure 7a: Effect of the addition of salt, glucose and sucrose on Total Fumonisin Levels in Naturally Contaminated Maize Flour**

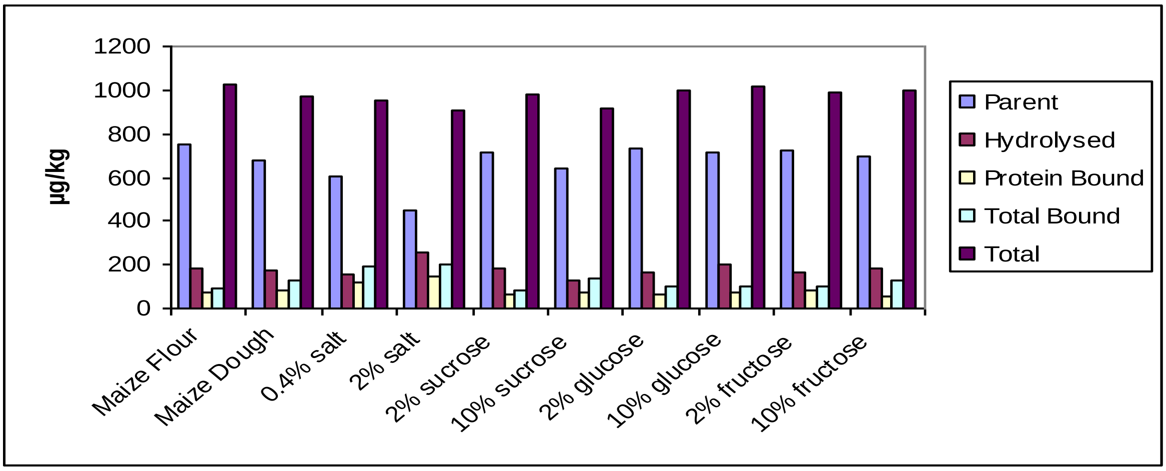
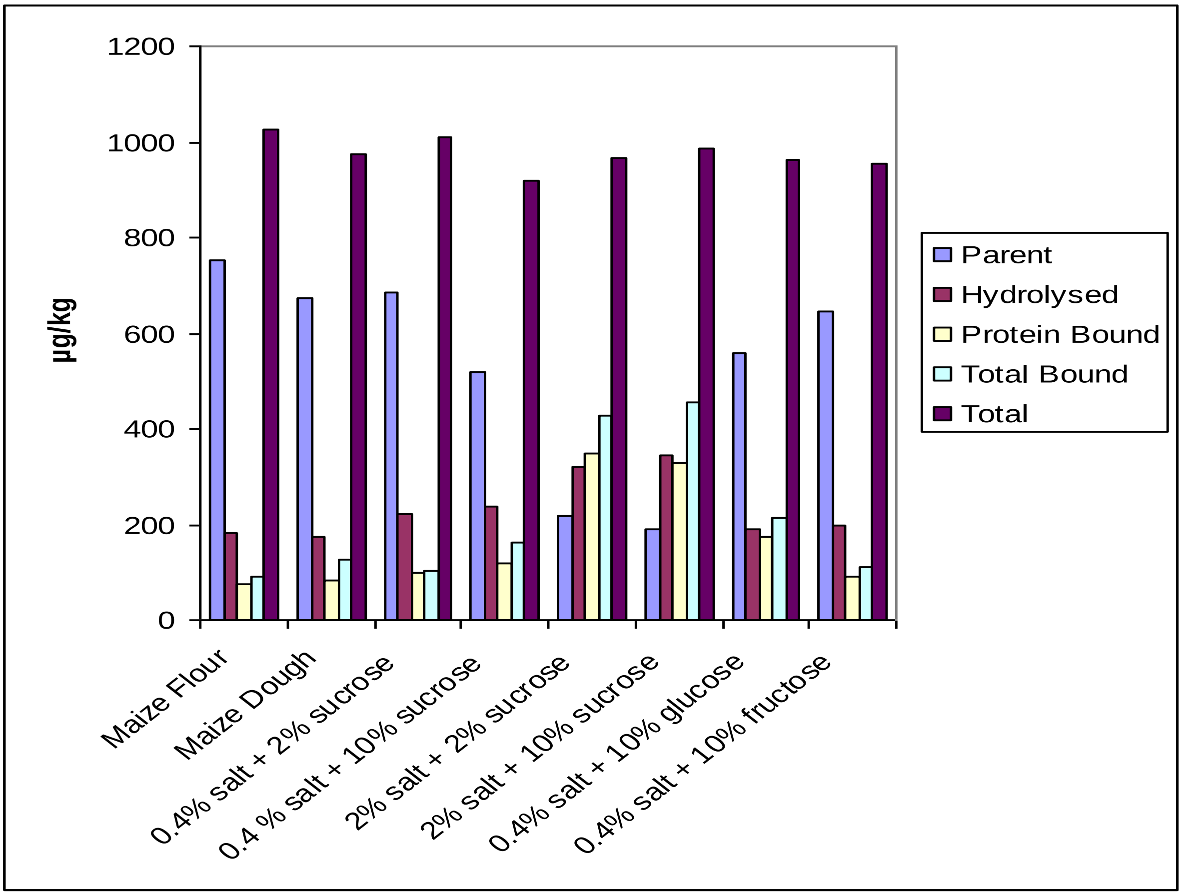


**Figure 7b: Effect of the addition of salt, glucose and sucrose on Total Fumonisin Levels in Naturally Contaminated Maize Flour**




The maize flour used for this investigation contained hydrolysed and bound fumonisins; the total fumonisin equivalent was 36% higher than the parent fumonisins.

The data showed that using naturally contaminated ground maize treated with various salt/sucrose combinations and determination of parent fumonisins a significant drop in levels was noted in some cases, however when the total fumonisin equivalent were analysed the majority of the fumonisins present were accounted for. The profile of hydrolysed fumonisin/bound fumonisin was similar in most combinations, however in the 2% salt/10% sucrose dough the level of bound fumonisin was greater than hydrolysed (and parent) fumonisins.

These results are similar to those reported by Castells et al [40], these workers reported significant reductions in FB1 levels in presence of salt/sucrose. The results presented here additionally include the determination of bound/hydrolysed fumonisin (which was not included in Castells work) and show that almost all the decrease can be attributed to hydrolysis and binding.

### 5.3.3 Effect of heat

Literature reports indicated that fumonisins (in both culture extracts and dry corn) demonstrated high thermal stability [15, 16]. However studies on the thermal stability of fumonisins in food matrices had shown large reductions in parent fumonisin levels.

The experiment described above was developed to investigate if the addition of salt and sucrose (at levels typically used in food systems) and applying heat had an enhanced impact of fumonisin levels.

A. 10min at 110-120°C

The binding of fumonisin to food matrix components e.g. saccharides and proteins during thermal processing was investigated.

Maize flour naturally contaminated with fumonisins at 750µg/kg with a moisture content of 14% was used. Maize flour (930g) was mixed with a mixture of salt (20g) and sucrose (50g) and water and cooked in a pressure cooker at a temperature of 110-120°C for 10min.

**Table 17: Effect of Heat 10mins at 110-120°C**

| Sample Description | Conventional Extraction | | Bound | | Total Fumonisin Equivalents |
|---|---|---|---|---|---|
| | Parent Fumonisins | Hydrolysed Fumonisins | Protein Bound Fumonisins Equivalents | Total Bound Fumonisins Equivalents | |
| | (µg/kg) | | | | |
| **Maize Flour** | 751 | 181 | 75 | 93 | 1025 |
| replicate 1 | 510 | 124 | 102 | 186 | 820 |
| replicate 2 | 450 | 99 | 86 | 199 | 748 |
| replicate 3 | 380 | 75 | 77 | 150 | 605 |
| replicate 4 | 420 | 85 | 99 | 125 | 630 |
| replicate 5 | 511 | 102 | 66 | 131 | 744 |
| replicate 6 | 590 | 79 | 78 | 122 | 691 |
| Mean | 477 | 94 | 85 | 152 | 706 |

**Figure 8a: Effect of Heat 10mins at 110-120°C**

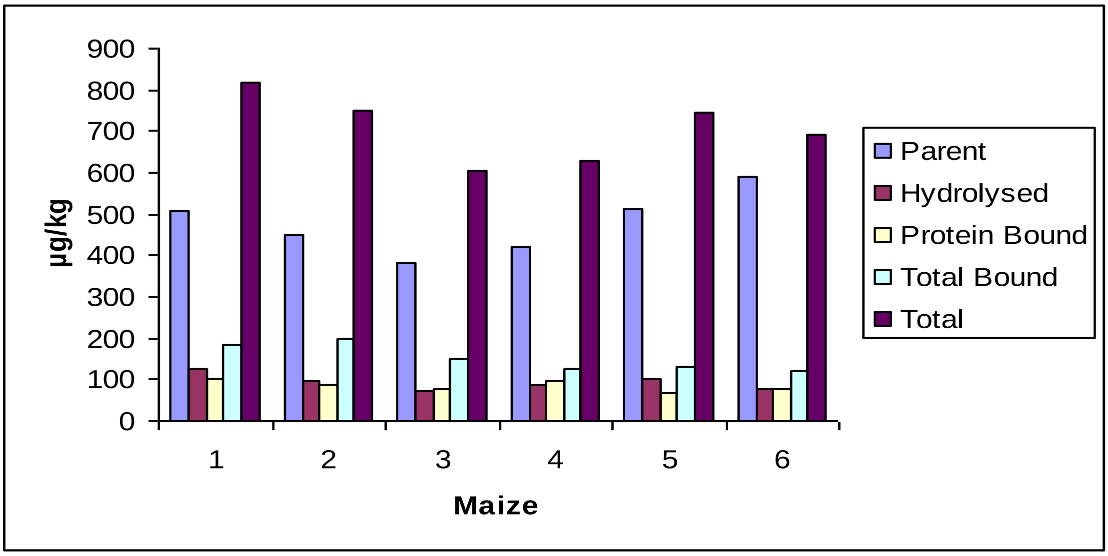
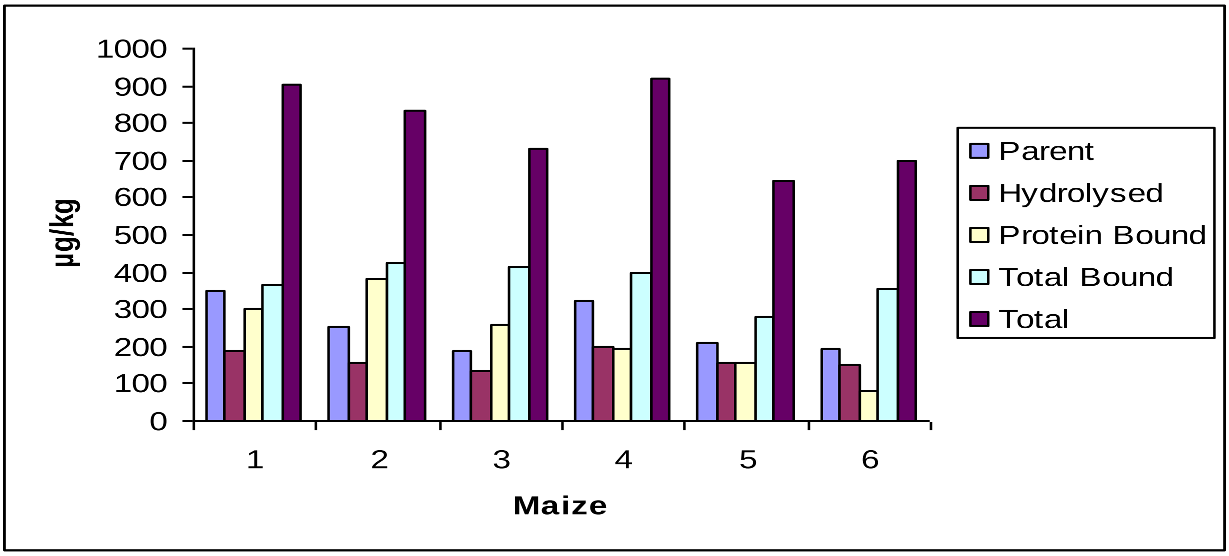



**Table 17b: Effect of Heat 4 hour at 120°C**

| Sample Description | Conventional Extraction | | Bound | | Total Fumonisin Equivalents |
|---|---|---|---|---|---|
| | Parent Fumonisins | Hydrolysed Fumonisins | Protein Bound Fumonisins Equivalents | Total Bound Fumonisins Equivalents | |
| | (µg/kg) | | | | |
| **Maize Flour** | 751 | 181 | 75 | 93 | 1025 |
| replicate 1 | 352 | 186 | 299 | 366 | 904 |
| replicate 2 | 252 | 154 | 382 | 425 | 831 |
| replicate 3 | 186 | 132 | 256 | 412 | 730 |
| replicate 4 | 321 | 199 | 195 | 399 | 919 |
| replicate 5 | 211 | 154 | 154 | 282 | 647 |
| replicate 6 | 196 | 148 | 78 | 354 | 698 |
| Mean | 253 | 162 | 227 | 373 | 788 |

**Figure 9: Effect of Heat 4 hour at 120°C**




## Conclusions

The heating experiments described above showed that:

- Parent fumonisins decrease after both 10mins (36% decrease) and 4hours (66%)
- No increase in hydrolysed fumonisins
- Total bound fumonisin increased after 4 hours at 120°C.
  Overall decrease in total fumonisin equivalents of between 23-31%, this decrease is greater than seen after the addition of salt and sugar and no heat treatments as demonstrated in the earlier experiments.

### 5.3.4 Food Products

#### *5.3.4.1 Corn pasta*

Corn pasta was selected as a starting point as this is the simplest food system involving use of maize flour, slurry, mixing and drying. No pH changes and minimal heat treatment. Full characterisation (free fumonisins, bound/hidden fumonisins data) of cereal raw materials to be subjected to laboratory scale processing was initially investigated.

Maize flour naturally contaminated with fumonisin at 750µg/kg with a moisture content of 14.2% was used. Maize pasta was prepared using a pasta maker on a laboratory scale. The pasta was made from a simple dough (maize flour, water, eggs, oil and salt) kneaded and left to stand for 48 hours.

*Preparation of pasta*
1kg of flour, 40g of salt, 25g of olive oil and 10eggs were placed in a Blixer 4 food processor and mixed until the flour looked like breadcrumbs. The mixture was then kneaded into a dough by hand. The dough was wrapped in clingfilm and put it in the fridge for 48hours. The dough was analysed without any further heat treatment.

**Table 18a: Parent Fumonisins in Pasta Dough**

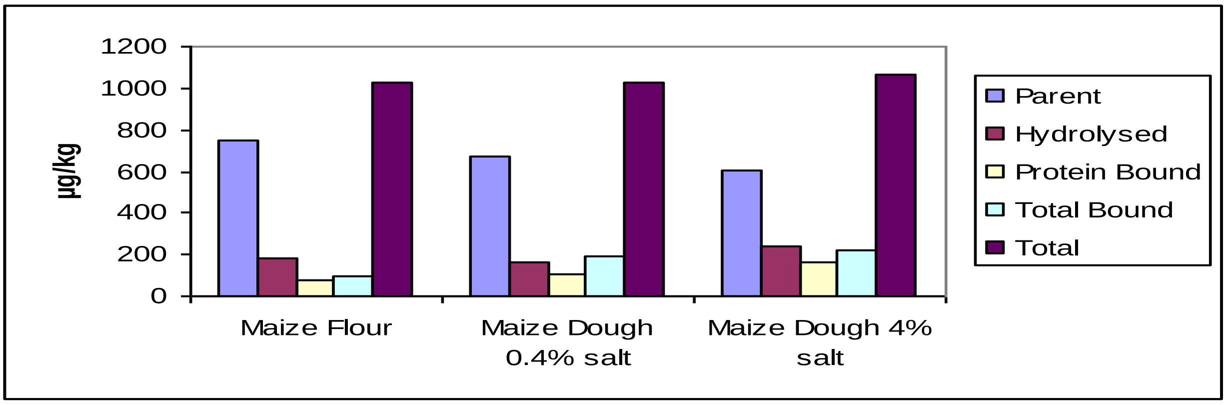
| Addition | FB1 (µg/kg) | FB2 (µg/kg) | $FB_3$ (µg/kg) | Total/% reduction µg/kg |
|---|---|---|---|---|
| **Maize Flour** | 541 | 133 | 77 | 751 |
| **Maize Dough 0.4% salt** | 490 | 120 | 65 | 675 (11%) |
| **Maize Dough 4% salt** | 405 | 101 | 58 | 609 (25%) |

The mean reduction in FB1 levels when processing with 4% salt was 25% of the initial content.

**Table 18b: Total Fumonisins in Pasta Dough**

| Sample Description | Conventional Extraction | | Bound | | Total Fumonisin Equivalents |
|---|---|---|---|---|---|
| | Parent Fumonisins | Hydrolysed Fumonisins | Protein Bound Fumonisins Equivalents | Total Bound Fumonisins Equivalents | |
| | (µg/kg) | | | | |
| **Maize Flour** | 751 | 181 | 75 | 93 | 1025 |
| **Maize Dough 0.4% salt** | 675 | 162 | 102 | 194 | 1031 |
| **Maize Dough 4% salt** | 609 | 236 | 166 | 222 | 1067 |

**Figure 10: Total Fumonisins in Pasta Dough**



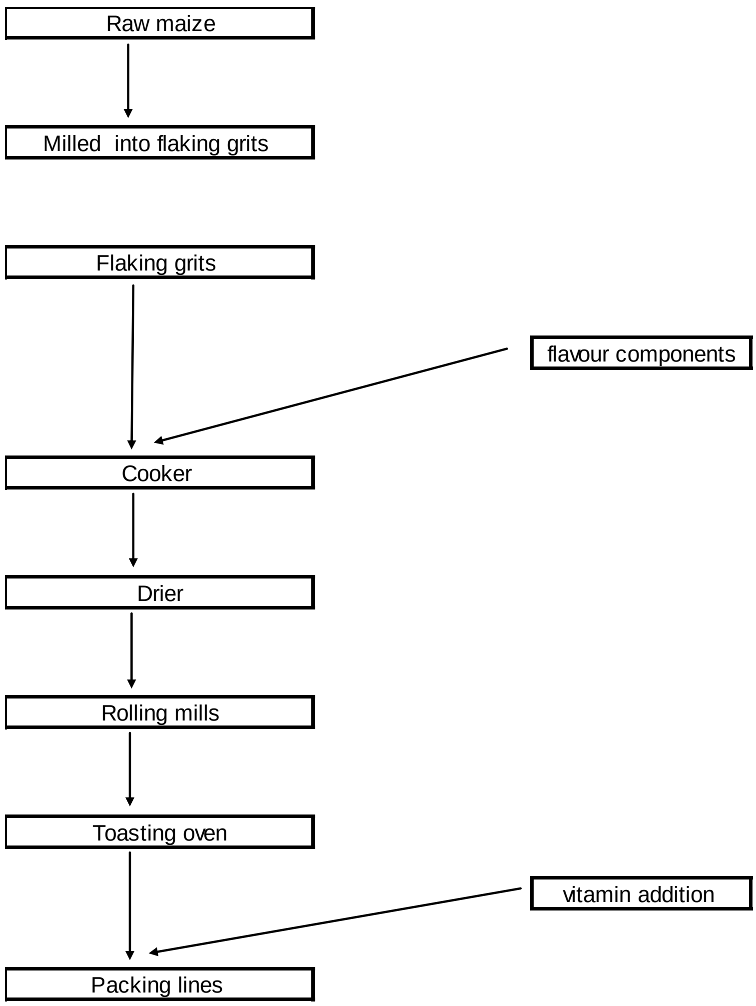

The production of a simple corn pasta resulted in a decrease in parent fumonisins, but when total fumonisins equivalents were determined all the naturally occurring fumonisins were accounted for, the levels of both hydrolysed and bound having increased.

### *5.3.4.2 Cornflake Production - traditional.*

As discussed in the literature review section above a key food process that is reported to lead to a drop in levels of parent fumonisins is the traditional production of cornflakes. This process has more complex ingredients and heating and drying steps. Since we were unable to make traditional cornflakes the work was carried out on the LINK samples we had stored frozen.

Maize flaking grits were supplied on contract from a miller in the LINK project and confined to specific silos from which batches were with drawn for processing. A simplified flow chart for the manufacture of cornflakes is shown in Figure 11.

**Figure 11: Cornflake Process**




The process of each batch was tracked through the process by timing. Flaking grits of 4-6mm in size together with other ingredients, which included approximately 1% of a smaller size maize grit added for flavouring purposes, were placed in a cooker of about 1,000kg capacity. This was heated under steam pressure above 100°C for about 1 hour to soften the grits. After emptying the cooker, the wet product is dried to 10-14% moisture in hot air after which the flakes are rolled and elongated. The flakes are then toasted in a very hot rotating oven for about 30seconds. The flakes are then sprayed with vitamins to give the finished product. Details of the process are subject to commercial confidentiality. Samples of the raw grits and cornflakes were collected from each run. Additionally samples of the cooked maize and dried cooked maize grits were taken from a few consignments.

A 1kg sample of maize grits entering each of 10 cookers was collected and combined to give a 10kg composite. Manufacture was carried out on a continuous process and sampling was performed at regular intervals. At each sampling stage, 1 kg quantity was collected from each cooker, drier or toasting oven, combining the ten increments to give the composite sample.

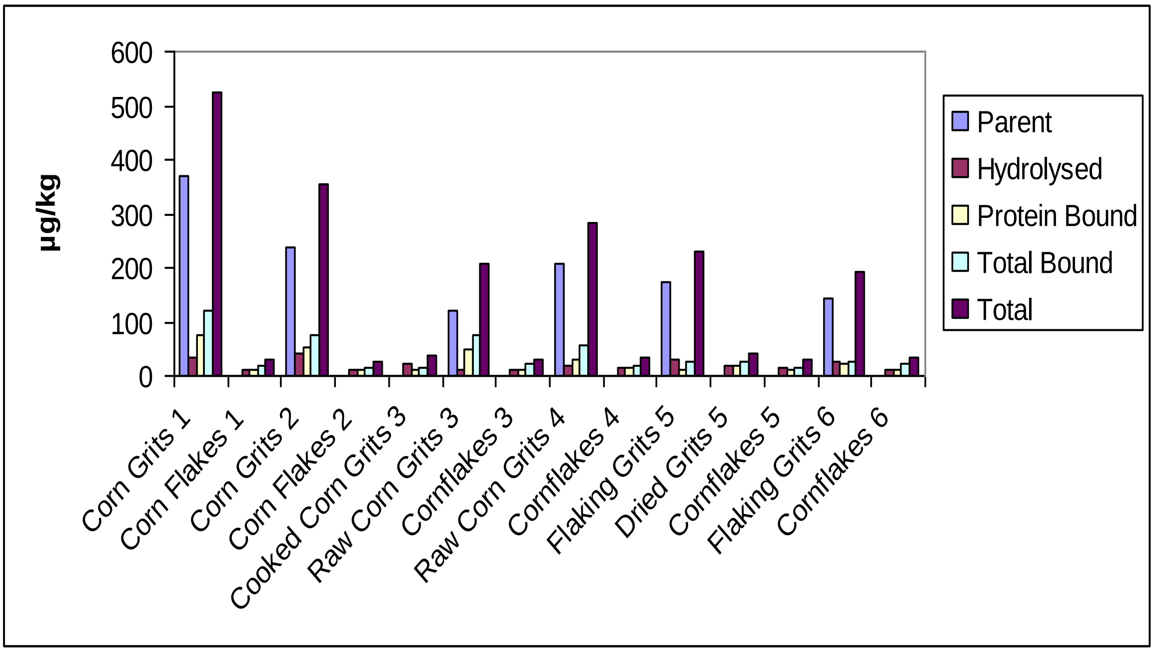
Previous work had shown that the mean reduction from intake maize to maize grits for FB1 and FB2 was 94% and a further reduction from grits to cornflakes was greater than 93% [6].

The stability of FBs during processing of corn flakes was investigated by analysis of the naturally contaminated raw material (maize), intermediate product and final product for parent and total fumonisin equivalents

**Table 19: Total Fumonisins in Cornflakes (Six Production Batches Studied)**

| Sample Description | Conventional Extraction | | Bound | | Total Fumonisin Equivalents |
|---|---|---|---|---|---|
| | Parent Fumonisins | Hydrolysed Fumonisins | Protein Bound Fumonisins Equivalents | Total Bound Fumonisins Equivalents | |
| | (µg/kg) | | | | |
| 1. Corn Grits | 369 | 35 | 75 | 120 | 524 |
| 1. Corn Flakes | <10 | 12 | 13 | 19 | 31 |
| 2. Corn Grits | 236 | 42 | 52 | 75 | 353 |
| 2. Corn Flakes | <10 | 11 | 11 | 14 | 25 |
| 3. Cooked Corn Grits | <10 | 22 | 10 | 15 | 37 |
| 3. Raw Corn Grits | 122 | 10 | 50 | 75 | 207 |
| 3.Cornflakes | <10 | 11 | 12 | 21 | 32 |
| 4. Raw Corn Grits | 207 | 20 | 32 | 55 | 282 |
| 4. Cornflakes | <10 | 15 | 14 | 19 | 34 |
| 5. Flaking Grits | 175 | 30 | 13 | 26 | 231 |
| 5. Dried Grits | <10 | 18 | 20 | 25 | 43 |
| 5. Cornflakes | <10 | 16 | 10 | 14 | 30 |
| 6. Flaking Grits | 142 | 25 | 23 | 25 | 192 |
| 6. Cornflakes | <10 | 10 | 12 | 24 | 34 |

**Figure 12: Total Fumonisins in Cornflakes**




As reported in the LINK scheme report and associated publication the milling process results in a redistribution of the fumonisin mycotoxins into different milling fractions, the mean reduction from intake maize to maize grits for FB1 and FB2 was 94%.

This study has taken the maize grits and cornflakes and analysed the parent fumonisins and total fumonisin equivalents.

The results showed that:

- Corn grits contained parent, hydrolysed and bound fumonisins, the overall fumonisins levels being significantly higher than the conventionally analysed parent level
- Corn flakes contained only very low level of parent, hydrolysed and bound fumonisins (loss of between 87-95%)
- The cooked grits that had been supplied in some runs also showed much reduced total fumonisin equivalents (in line with levels in cornflakes) indicating that this step gives rise to the reduction.

Other workers have studied cornflake fumonisins levels. The interpretation of the published data is complicated by that fact that not all cornflakes are produced by the same process; some are extruded from maize flour.

De Girolamo *et al*[41] studied the effect of processing on fumonisins in cornflakes but only analysed the parent molecules. Dall'Asta *et al*[38] have performed studies on cornflakes on retail sale in Italy, hence no process intermediates were available. The products were analysed for bound and free fumonisins, mean levels of parent and total equivalent fumonisin were higher than in the UK samples and the amount of bound derivative was found to be very close or even higher than the free/parent toxin.

There is no published information on the nature of the as yet unidentified breakdown products. Results presented here suggest that the cooking step may play a role. The heating experiments described above do give rise to a reduction in total fumonisin equivalents but not as large a drop as seen in cornflakes.

#### *5.3.4.3 Maize snacks*

Most cereals contain a large amount of starch. In its natural form, the starch is insoluble, tasteless and unsuited for human consumption. To make it digestible and acceptable, it must be cooked. Cooking or gelatinisation of starch in the traditional cereal process is controlled by time, temperature and availability or presence of water.

For most corn-based breakfast cereals and extruded snacks, dry-milled corn meal is used. Cornmeal, corn grits and corn flour are all different forms of dry-milled dent corn, and in general vary only in particle size distribution. Selection of the granulation depends upon the type of snack, breakfast cereal and type of extruder. For example, for fine texture and cell structure, or softer bite, a fine granulation of corn meal is desired. Mostly, de-germed corn is used in breakfast cereals and extruded snacks because it expands better than whole corn.

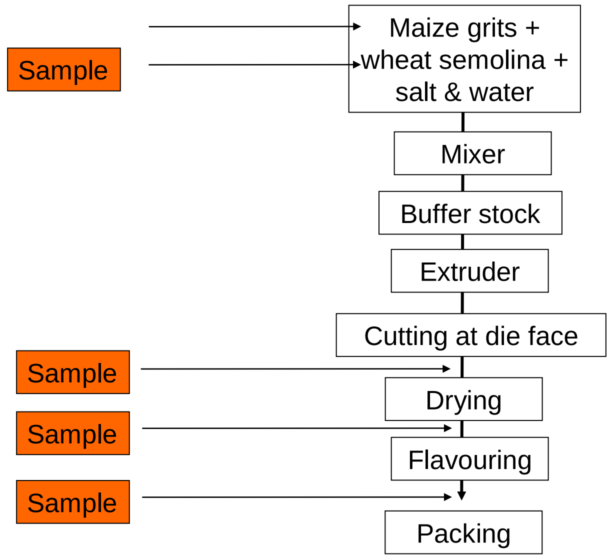
Extrusion is simply the operation of shaping a dough-like material by forcing it through a restriction or die. Extruders can be used to cook, form, mix, texturise and shape food products. During extrusion, the cooking temperature could be as high as 100-170°C but only for 20-40 seconds.

Due to difficulty of preparing extruded snacks in house, an alternative source of raw materials, intermediates and final product has been accessed, as utilised in Fusarium Processing LINK scheme.[8] These samples enabled the mass balance of fumonisins across the process to be determined.

#### *5.3.4.4 Expanded snack via direct extrusion processing*

This was manufactured from a mixture of maize grits and wheat semolina together with other minor ingredients. After mixing and holding at ambient temperature for approximately one hour, the mixture was cooked in a single screw extrusion process involving heat between 90°C and 130°C for less than a minute. The extrudate was dried at about 170°C for 80 seconds.

## Figure 13: Maize Snack Process

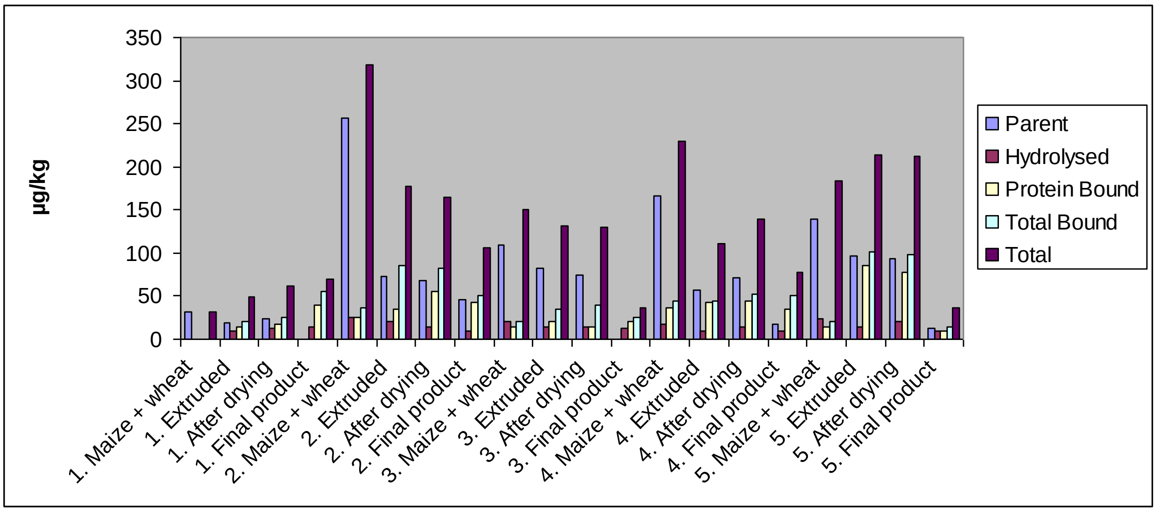



## Table 19: Total Fumonisins in Extruded Snacks

| Sample Description | Conventional Extraction | | Bound | | Total Fumonisin Equivalents |
|---|---|---|---|---|---|
| | Parent Fumonisins | Hydrolysed Fumonisins | Protein Bound Fumonisins Equivalents | Total Bound Fumonisins Equivalents | |
| | (µg/kg) | | | | |
| 1. Maize + wheat | 31 | <10 | <10 | <10 | 31 |
| 1. Extruded | 19 | 10 | 15 | 20 | 49 |
| 1. After drying | 24 | 12 | 18 | 25 | 61 |
| 1. Final product | <10 | 15 | 40 | 55 | 70 |
| 2. Maize + wheat | 257 | 25 | 25 | 36 | 318 |
| 2. Extruded | 73 | 20 | 35 | 85 | 178 |
| 2. After drying | 68 | 15 | 55 | 82 | 165 |
| 2. Final product | 46 | 10 | 42 | 50 | 106 |
| 3. Maize + wheat | 110 | 21 | 15 | 20 | 151 |
| 3. Extruded | 82 | 15 | 20 | 35 | 132 |
| 3. After drying | 75 | 15 | 15 | 40 | 130 |

| Sample Description | Conventional Extraction | | Bound | | Total Fumonisin Equivalents |
|---|---|---|---|---|---|
| | Parent Fumonisins | Hydrolysed Fumonisins | Protein Bound Fumonisins Equivalents | Total Bound Fumonisins Equivalents | |
| | (µg/kg) | | | | |
| 3. Final product | <10 | 12 | 20 | 25 | 37 |
| 4. Maize + wheat | 166 | 18 | 36 | 45 | 229 |
| 4. Extruded | 57 | 10 | 42 | 44 | 111 |
| 4. After drying | 72 | 15 | 45 | 52 | 139 |
| 4. Final product | 17 | 10 | 35 | 50 | 77 |
| 5. Maize + wheat | 140 | 24 | 15 | 20 | 184 |
| 5. Extruded | 97 | 15 | 85 | 102 | 214 |
| 5. After drying | 94 | 20 | 78 | 98 | 212 |
| 5. Final product | 12 | 10 | 10 | 15 | 37 |

**Figure 14: Total Fumonisins in Extruded Snacks**




The results for the analysis of extruded snacks showed:

- Greater variability than cornflakes
- Grits used for the process had a low proportion of bound toxins (0-20% of total fumonisin equivalents)
- Levels of total fumonisin equivalents drop at the extrusion step of the process
- The post drying intermediate and the final product have a much higher proportion of bound fumonisins (between 27-79% of total fumonisin equivalents)

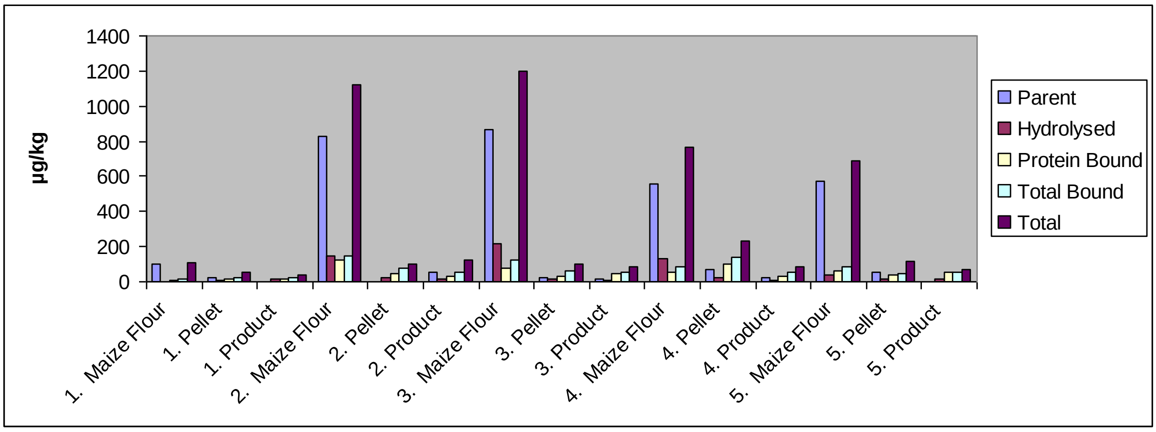
#### *5.3.4.5 Expanded snack via pellet processing*

This was manufactured from a mixture of maize flour and potato starch in an approximately 3:1 proportion together with other minor ingredients. After mixing for 12 minutes at ambient temperature, the mixture was cooked in an extrusion process involving heating for 7 minutes at up to 130°C and then formed into a rope and cut thinly into pellets after cooling. The pellets were dried between 40°C and 70°C for 2 hours. To form the product, the dried pellets were fried briefly in hot oil at 160-180°C and drained.

**Table 20: Total Fumonisins in Extruded Snacks**

| Sample Description | Conventional Extraction | | Bound | | Total Fumonisin Equivalents |
|---|---|---|---|---|---|
| | Parent Fumonisins | Hydrolysed Fumonisins | Protein Bound Fumonisins Equivalents | Total Bound Fumonisins Equivalents | |
| | (µg/kg) | | | | |
| 1. Maize Flour | 97 | <10 | 10 | 15 | 112 |
| 1. Pellet | 23 | 10 | 15 | 25 | 58 |
| 1. Product | <10 | 12 | 15 | 25 | 37 |
| 2. Maize Flour | 825 | 150 | 120 | 150 | 1125 |
| 2. Pellet | <10 | 25 | 50 | 75 | 100 |
| 2. Product | 55 | 15 | 30 | 55 | 125 |
| 3. Maize Flour | 864 | 216 | 80 | 120 | 1200 |
| 3. Pellet | 23 | 12 | 32 | 62 | 97 |
| 3. Product | 19 | 10 | 45 | 55 | 84 |
| 4. Maize Flour | 554 | 132 | 55 | 82 | 768 |
| 4. Pellet | 70 | 25 | 100 | 140 | 235 |
| 4. Product | 22 | 10 | 30 | 55 | 87 |
| 5. Maize Flour | 571 | 35 | 60 | 85 | 691 |
| 5. Pellet | 53 | 15 | 35 | 49 | 117 |
| 5. Product | <10 | 15 | 52 | 55 | 70 |

**Figure 14: Total Fumonisins in Extruded Snacks**




For extruded snacks based on maize flour and pellet processing:

- Maize flour (in most cases) had a higher total fumonisin content than the maize grits used in other food products, in maize flour the proportion of bound fumonisin was 10-13% of the total fumonisin equivalents.
- The pellet had a much reduced total fumonisin equivalent
- Both the pellet and the products have much higher proportion of bound fumonisin (42-75% of total fumonisin equivalents for the pellet and 44-79% for the snack product).
- Conventional analysis of fumonisins in the snack product would under determine the total fumonisins present.

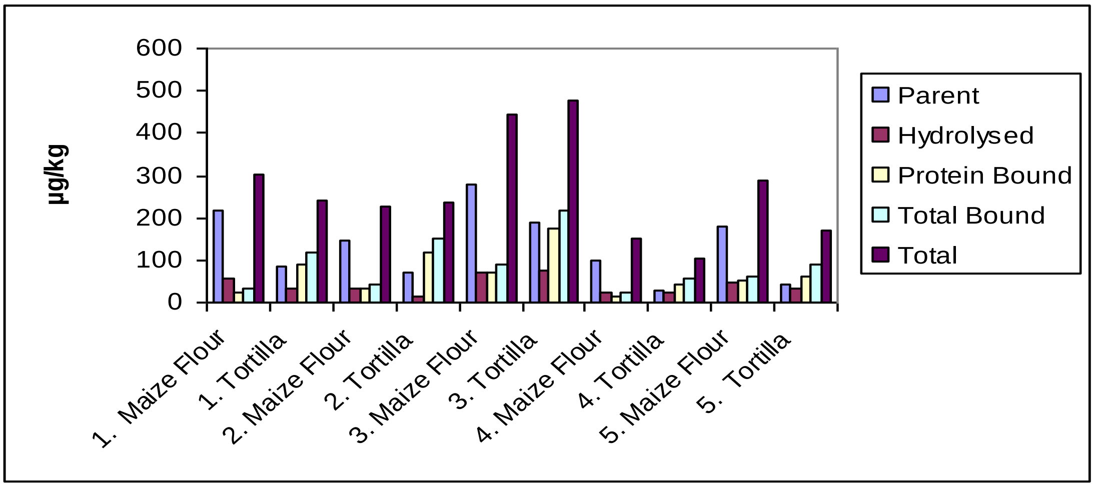
#### *5.3.4.6 Tortilla Chip*

Tortilla chips were prepared from a mixture of maize flours mixed with water at ambient temperature, the mix formed into a thin sheet and tortilla pieces cut from the sheet before drying for 20 seconds at 260°C. These were then fried in oil at 170-175°C.

**Table 21: Total Fumonisins in Tortilla Chips**

| Sample Description | Conventional Extraction | | Bound | | Total Fumonisin Equivalents |
|---|---|---|---|---|---|
| | Parent Fumonisins | Hydrolysed Fumonisins | Protein Bound Fumonisins Equivalents | Total Bound Fumonisins Equivalents | |
| | (µg/kg) | | | | |
| 1. Maize Flour | 218 | 55 | 22 | 31 | 304 |
| 1. Tortilla Unflavoured Finished Products | 85 | 34 | 88 | 120 | 239 |
| 2. Maize Flour | 148 | 35 | 35 | 44 | 227 |
| 2. Tortilla Unflavoured Finished Products | 73 | 15 | 120 | 150 | 238 |
| 3. Maize Flour | 281 | 70 | 72 | 92 | 443 |
| 3. Tortilla Unflavoured Finished Products | 189 | 75 | 175 | 215 | 479 |
| 4. Maize Flour | 100 | 25 | 15 | 25 | 150 |
| 4. Tortilla Unflavoured Finished Products | 27 | 22 | 42 | 55 | 104 |
| 5. Maize Flour | 181 | 45 | 52 | 62 | 288 |
| 5. Tortilla Unflavoured Finished Products | 43 | 35 | 60 | 92 | 170 |

**Figure 15: Total Fumonisins in Tortilla Chips**




The fate of fumonisins during the manufacture of fried tortilla chips was studied.

- FB concentrations in the chips were reduced significantly from 33- 76% in the chips, compared to that in the maize flour.

- Hydrolysed fumonisins and parent fumonisins were detected in the raw materials and final products but were not fully accounted for in thermally treated processes.
- The change of total fumonisin equivalents during total production is much less that in the two extrusion processes.

## 5.4 Toxicological impact of breakdown products observed

The toxicity of parent fumonisin mycotoxins has been and continues to be studied in great depth and has been summarised in Section 3.5. Much less is known about the toxicity and bioavailability of the hidden fumonisins.

During the course of this investigation hydrolyzed fumonisins and bound fumonisins have been identified in raw maize and maize based foodstuffs. The toxicity of these compounds is discussed below.

### 5.4.1 Hydrolysed fumonisins

As discussed earlier hydrolysed fumonisins consist of the aminopentol backbone of the parent molecule i.e. loss of the tricarboxylic (tricarballyic) acid groups. Initially this hydrolysis was thought to be a potential detoxification route. However in some mammalian cell cultures and leaf bioassays HFB and HFB2 were more toxic than FB1 and FB2[10].

In an oral administration study in rats neither HFB1 (nor the TCA side chains) were found to be hepatoxic or act as cancer initiators. It was suggested that this might be due to lack of absorption from the gut that was possibly mediated by the TCA group[11]. This finding has been confirmed more recently. *Fusarium verticillioides* culture material (CM) was nixtamalized as is (NCM) or after mixing with ground corn (NCMC). Additional portions were sham nixtamalized without

(SCM) or with corn (SCMC). Nixtamalization and sham nixtamalization reduced $FB_1$; CM, NCM, and SCM diets contained 9.08, 2.08, and 1.19 ppm, respectively. $FB_1$ was further reduced in the NCMC (0.49 ppm) but not the SCMC (1.01 ppm) diets compared to their NCM and SCM counterparts. Equivalent weights of the cooked products, uncooked CM, corn (UC) or nixtamalized UC (NUC) were fed to rats for up to three weeks. Kidney lesions in the NCM-fed group were less severe than in the CM-fed, positive control group and no lesions were found in the NCMC and other groups. Group kidney sphinganine (biomarker of fumonisin exposure) concentrations decreased in the order: CM (absolute concentration (nmol/g) = 600-800) > NCM (400-600) > SCM and SCMC (30-90) > NCMC, UC and NUC (<8). Together, these results suggest that mycotoxin-corn matrix interactions during nixtamalization reduce the bioavailability and toxicity of $FB_1$.[12]

In summary, the toxicity of the hydrolysed fumonisins is still ambiguous, they have been reported to show lower acute toxicity when compared to the parent molecule, but other research has shown the higher absorption of the less polar hydrolysed derivative which is hypothesised may be better absorbed by the intestinal mucosa[9].

### 5.4.2 Bound fumonisins

As discussed in Section 5 of this report fumonisins can bind to proteins and other matrix components and are analytically detectable after disruption of these associations.

Soon after the first determination of bound fumonisins their potential as a food safety concern was raised, as it was considered possible that such hidden compounds might be expected to be released in the gastrointestinal tract[17]. Some reports from animal studies suggested that dietary fumonisin-glucose adducts were less toxic to swine than free fumonisin.

Acute and sub acute intraperitoneal doses of fumonisin B1 (FB1) were administered to test the efficacy of the $FB_1$-glucose reaction products in detoxifying $FB_1$ in swine. Analysis of serum aspartate aminotransferase, γ-glutamyltransferase, and total bilirubin showed protection against $FB_1$ toxicity by the $FB_1$-glucose reaction products. The levels of sphinganine and sphinganine/sphingosine ratios in serum and liver as well as pathologic findings provided definitive evidence of protection against the $FB_1$ toxic effects by this detoxification procedure[18,].

The effects of fumonisin B-glucose reaction products in swine diets were examined. Pigs were fed diets containing 528 μmol of total fumonisin B/kg (FB), 528 μmol of total FB-glucose adducts/kg (FB-G, 122 μmol of unreacted FB/kg), or 0 μmol of total FB/kg for 15 days to test the efficacy of the FB-G reaction products in detoxifying FB. Weight gain in FB pigs was lower than in FB-G or controls, which was correlated with feed intake reduction in FB pigs. Serum aspartate aminotransferase, γ-glutamyltransferase, and total bilirubin in FB pigs were higher than in FB-G or control pigs. Serum sphinganine/shingosine ratios in FB pigs were higher than in FB-G or control pigs. Microscopic examination of tissues from FB pigs showed generalized liver necrosis and apoptosis with marked cellular pleomorphism and disorganized hepatic cords. The liver and kidneys in the FB-G

group appeared to be normal. Tissues of controls were free of lesions. Results suggest that dietary FB-G products are less toxic to swine and may provide a detoxification approach in instances of widespread FB grain contamination[19].
Cytotoxicity and lipid peroxidation were studied in monkey kidney cells (Vero cells). After 24 h exposure, $FB_1$ revealed an $IC_{50}$ (median inhibitory concentration) of 55 ± 7 μm with neutral red uptake, but no $IC_{50}$ was obtained after N-(carboxymethyl)fumonisin $B_1$ exposure at the studied concentrations. Lipid peroxidation was assessed and findings showed that the transformation products exhibit lower cytotoxicity than fumonisin $B_1$ and lipid peroxidation may be involved in the cytotoxicity induced by both toxins[21].

Corn grits spiked with 30 μg/g fumonisin $B_1$ and two batches of grits fermented with Fusarium verticillioides (batch 1 contained 33 μg/g, and batch 2 contained 48 μg/g fumonisin $B_1$), which were extruded by a single-screw extruder with and without glucose (10%, dry weight basis) supplementation were fed to rats. Control groups were fed uncontaminated grits. Extrusion with glucose more effectively reduced fumonisin $B_1$ concentrations of the grits (75 to 85%) than did extrusion alone (10 to 28%). With one exception, the fumonisin $B_1$-spiked and fermented extrusion products caused moderately severe kidney lesions and reduced kidney weights, effects typically found in fumonisin-exposed rats. Lesions in rats fed the least contaminated grits (batch 1) after extrusion with 10% glucose were, however, significantly less severe and not accompanied by kidney weight changes. Therefore, extrusion with glucose supplementation is potentially useful for safely reducing the toxicity of fumonisins in corn-based products and studies to determine the optimal conditions for its use are warranted[22].

The extent to which these bound forms are bioavailable has recently been studied[23]. The aim was to determine the bioaccessibility of total bound $FB_1$ (TB $FB_1$) (percentage of TB $FB_1$, released from corn flakes to the chyme) after in vitro digestion. Two samples of corn flakes washed with solvents were incubated with gastrointestinal tract solutions simulating saliva plus stomach and duodenal juices. After hydrolysis of the chyme with KOH, TB $FB_1$ was determined as hydrolyzed $FB_1$ ($HFB_1$). The bioaccessibility of TB $FB_1$ in chyme from corn flakes was 37-64%, indicating that these derivatives should be considered in evaluation of exposure to fumonisin.

The study of the toxicity of the newly identified bound fumonisin compounds is at an early stage and more research is required to be able to full robust conclusion on the foods safety implications of the results.

## 5.5 Occurrence of Fumonisins in Other Commodities

As part of the literature review in 2009 the following key points were made regarding fumonisin occurrence.

- Maize and maize products are the major source of fumonisins, produced by Fusarium species.

- Fumonisin $B_2$ production by *Aspergillus niger* reported, with fumonisin reported to occur naturally in coffee beans
- Figs reported to contain $FB_1$ and $FB_2$.
- Fumonisin carryover into cow's milk reported.
- Single report of occurrence of $FB_1$ in wheat (one sample from Japan).

Since this review was completed the literature concerning fumonisin occurrence in non maize raw materials has continued to expand.

### 5.5.1 *Aspergillus niger*, dried fruits, grapes and wine

Reports on the ability of *Aspergillus niger* species to produce fumonisins have continued to appear. *Aspergillus niger* is one of the most commonly reported fungi recovered from foods, responsible for the post harvest decay of fresh fruits, it is commonly extracted from nuts, cereals, pulses and oilseeds.

Recent findings have shown that some *Aspergillus niger* isolates are able to produce fumonisins in high quantities on agar media with a low water activity. Several agricultural products fit this criterion, including dried vine fruits, dates and figs. In a recent report the mycobiota and fumonisin contamination of various dried vine fruit samples collected from different countries were examined to clarify the role of black Aspergilli in fumonisin contamination of such products. All except two of the examined samples were contaminated with black Aspergilli. Among the 30 *A. niger/A. awamori* isolates identified, 20 were found to be able to produce fumonisins (average contamination: 5.16mg/kg; range: 0.017-19.6mg/kg). The average fumonisin content of the 7 dried vine fruit samples which were found to be contaminated by potential fumonisin producing black Aspergilli was 7.22mg/kg (range: 4.55-35.49mg/kg). The isolates produced several fumonisin isomers also present in the dried vine fruit samples, including fumonisins B1-4, 3-epi-FB3, 3-epi-FB4, iso-FB1, and two iso-FB2,3 forms. Fumonisin B1 was detected for the first time in *A. niger* cultures[24].

Sixty-six A. niger, 4 A. tubingensis, and 16 *A. acidus* strains isolated from raisins were tested for fumonisin production on laboratory media. Neither *A. tubingensis* nor *A. acidus* strains produced fumonisins, but 77% of A. niger strains did. None of the strains produced ochratoxin A. Ten selected fumonisin producing A. niger strains were further able to produce fumonisin B2 and fumonisin B4 on grapes in the range 171-7841 μg fumonisin B2/kg and 14-1157 μg fumonisin B4/kg. Four selected strains were able to produce fumonisin B2 (5-6476 μg/kg) and fumonisin B4 (12-672 μg/kg) on raisins[25].

A new fumonisin, fumonisin B6(1), has been isolated together with fumonisin B2 (2), from stationary cultures of the fungus *Aspergillus niger*[26].

The potential risk of contamination by fumonisin B2 (FB2), although at low levels, has been demonstrated in must and wine. Black aspergilli in general and *Aspergillus niger* in particular are considered to be the major responsible agents of FB2 contamination in grape and its by-products[28].

In addition workers in Italy have identified FB2 in wine. The occurrence of these two fumonisins in wine was investigated by LC/MS/MS in 51 market samples (45 red, five white and one rose wine) produced in various Italian regions. Nine samples of red wine were found to be contaminated by fumonisin B2 at levels ranging from 0.4 to 2.4 ng/ml while FB4 was not detected in any of the tested samples[29].

In a further study, a total of 77 wine samples from 13 countries were tested, 18 (23%) were found to contain fumonisin B2 in the range of 1-25 µg/L[30].

### 5.5.2 Asparagus

*Fusarium oxysporum* and/or *F. proliferatum* were isolated from all asparagus spears with brown spots (which indicate an infection) and from almost all spears without spots. The presence of *Fusarium spp.* and their toxins in the basal parts of asparagus spears was analyzed. Fumonisin B1 (FB1) and moniliformin (MON) were found in spears with brown spots and those without disease symptoms. FB1 was determined in the concentration range 0.16-152.68 ng g-1 (mean 7.52), while moniliformin was detected in the range 15.30-585.00 ng g-1. Only in 10% analyzed spears were metabolites not detected[31].

These results are in contrast to a report from China, in this study, the presence of fumonisins and fumonisin-producing fungi in asparagus spear samples from Zhejiang Province, the major asparagus production province in China was examined. The asparagus did not contain a detectable level of fumonisins. However, the recovery of Fusarium in asparagus was 72.7%, including *F. proliferatum* (40.9%), *F. oxysporum* (22.7%), *F. acuminatum* (4.55%) and *F. equesti* (4.55%)[32].

### 5.5.3 Tea

In a study 91 different tea and herbal infusion samples were analyzed. Only in one sample, Ceylon melange, 76 µg/kg fumonisin B1 was detected[33].

### 5.5.4 Figs

In a survey carried out on 87 rotted fig fruits samples collected in the Apulia region of Italy, the authors isolated 126 Fusarium strains identified as *F. ramigenum* (69 strains), *F. solani* (49), *F. proliferatum* (five) and three not identified. When Fusarium species were analysed for their toxigenicity, 37/69 strains of *F. ramigenum* produced fusaric acid (FA) up to 525 mg kg-1; 30 strains produced beauvericin (BEA) up to 190 mgkg-1; 60 strains produced fumonisin B1 (FB1) and fumonisin B2 (FB2) up to 1575 mgkg-1 of total FBs; and two strains produced fusaproliferin (FUP) up to 345 mg kg-1; all five strains of *F. proliferatum* produced FA at low levels; two strains produced BEA up to 205 mgkg-1; one strain produced FB1 and FB2, 1100 and 470mg kg-1, respectively; and one strain produced FUP, 820 mgkg-1; *F. solani* (30 strains) produced FA, 13 strains up to 215 mgkg-1. These data report for the first time the production of BEA and FB1/FB2 by *F. ramigenum* and show that it is a main agent of fig endosepsis in Apulia and can contribute to fumonisin contamination of fresh and dried figs[34].

### 5.5.5 Other producing fungi

*Tolypocladium inflatum* is known primarily for its production of the cyclosporines that are used as an immunosuppressive drug. However, we report here the production of the carcinogenic fumonisins B2 and B4 by this biotechnologically relevant fungal genus. These mycotoxins were detected in 11 strains tested from three species: *Tolypocladium inflatum*, *T. cylindrosporum*, and *T. geodes*[35].

### 5.5.6 Range of fumonisins

In addition the identification of further fumonisins continues to expand, though to date the production levels of these newly identified compounds is low in comparison to FB1 production. Twenty eight isomers of FB1 were isolated from a

solid rice culture of *F.verticilliodes*. Detection and characterization of twenty-eight isomers of fumonisin B1 (FB1) mycotoxin in a solid rice culture infected with *Fusarium verticillioides* by reversed-phase high-performance liquid chromatography/electrospray ionization time-of-flight and ion trap mass spectrometry[36].
The first fumonisin mycotoxins with three acyl groups have been identified, by ESI-ITMS and ESI-TOFMS following RP-HPLC separation[37].

# 6 Summary

## 6.1 Investigation of the Presence of the Breakdown/Masking of Parent Fumonisins in Retail Food Products

### 6.1.1 Cornflakes

- Hydrolysed fumonisins were detected in all but one cornflake sample, the range being 0-36% of conventionally extractable fumonisins.

- Bound fumonisins were found in all samples, protein bound material, accounted for most of the bound toxins. The total bound fumonisin as a % of total fumonisin equivalents ranged from 7-25%.

- The total fumonisin equivalent for all samples has been calculated, the additional fumonisin recovered with the analysis of both hydrolysed and bound toxins ranged from 13% (extruded cornflakes) to 46% (frosted flakes).

### 6.1.2 Extruded Snacks

- Hydrolysed fumonisins were not detected in any of the extruded snacks.

- Bound fumonisins were found in one sample, protein bound material accounted for most of the bound toxins. The total bound fumonisin as a % of total fumonisin was 7%.

### 6.1.3 Tortilla Chips

- Hydrolysed fumonisins were found in 2 of the 3 tortilla samples, the range being 32-36% of conventionally extractable fumonisins. The third sample had a very low level of parent fumonisins.

- Bound fumonisins were found in the two samples, protein bound material accounted for some of the bound toxins. The total bound fumonisin as a % of total fumonisin equivalents ranged from 12-18%.

- The total fumonisin equivalent for all samples has been calculated, the additional fumonisin recovered with the analysis of both hydrolysed and bound toxins ranged from 40-48%.

**Conclusions**

Parent fumonisins were detected in the maize based food products at levels ranging from 4 to 208µg/kg and hydrolysed fumonisins were detected in the cornflakes and tortilla chips at levels ranging from 6 to 98µg/kg.

Bound fumonisins were determined as the hydrolysed fumonisins. The results showed in cornflakes a 7-25% increase in fumonisins detected as the bound form, 7% in extruded snacks and an increase of 12-18% for tortilla chips.

### 6.2 Simple Model Systems

#### 6.2.1 Maize

Hydrolysed and bound fumonisins were determined in the ground maize sample, the total fumonisin equivalents being 36% higher than the conventionally analysed parent fumonisins. This finding is in line with the results of other workers who have determined bound fumonisins in non-thermally treated maize[39]. Dall'Asta *et al* hypothesised that plant metabolism might be responsible for the transformation of the fumonisin formed by the contaminating fungi into bound conjugates due to the effect of chemical compartmentalisation exerted by the plant towards xenobiotics produced by the contaminating fungi.

#### 6.2.2 Spiked Wheat Flour

The wheat flour contained no fumonisins.

After extraction all added fumonisin was recovered (within limits of analytical uncertainty) though a very low level of hydrolysed material was detected. Determination of bound fumonisins showed a range of 7-15% of the added fumonisins had become bound, primarily to the protein fractions of the wheat.

#### 6.2.3 Simple Sugars

Significant reductions were obtained when salt and sucrose were added into ground maize (9-75% reduction) whereas salt alone caused a reduction of 19-40%, glucose and fructose did not significantly affect the FB1 (4-26% reduction). FB1 bound to the sucrose.
The data showed that using naturally contaminated ground maize treated with various salt/sucrose combinations a significant drop in the levels of parent fumonisins was noted in some cases, however when the total fumonisin equivalent were analysed the majority of the fumonisins present were accounted for. In some combinations the degree of hydrolysis and binding was much increased.

#### 6.2.4 Heat

The heating experiments described above showed that:

- Parent fumonisins decreased after both 10mins (36% decrease) and 4hours (66%)
- No increase in hydrolysed fumonisins
- Total bound fumonisin increased after 4 hours at 120°C.
- Overall decrease in total fumonisin equivalents of between 23-31%.

#### 6.2.5 Corn Pasta

The production of a simple corn pasta resulted in a decrease in parent fumonisins, but when total fumonisins equivalents were determined all the naturally occurring fumonisins were accounted for, the levels of both hydrolysed and bound having increased.

## 6.3 Food Processes

### 6.3.1 Cornflakes

The results showed that:

- Corn grits contained parent, hydrolysed and bound fumonisins, the overall fumonisins levels being significantly higher than the conventionally analysed parent level
- Corn flakes contained only very low level of parent, hydrolysed and bound fumonisins (loss of between 87-95%)
- The cooked grits that had been supplied in some runs also showed much reduced total fumonisin equivalents (in line with levels in cornflakes) indicating that this step gives rise to the reduction.

### 6.3.2 Extruded Snacks

For extruded snacks based on maize grits the results for the analysis of extruded snacks showed:

- Greater variability than cornflakes
- Grits used for the process had a low proportion of bound toxins (0-20% of total fumonisin equivalents)
- Levels of total fumonisin equivalents drop at the extrusion step of the process
- The post drying intermediate and the final product have a much higher proportion of bound fumonisins (between 27-79% of total fumonisin equivalents)

For extruded snacks based on maize flour and pellet processing:

- Maize flour (in most cases) had a higher total fumonisin content than the maize grits used in other food products, in maize flour the proportion of bound fumonisin was 10-13% of the total fumonisin equivalents.
- The pellet had a much reduced total fumonisin equivalent
- Both the pellet and the products have much higher proportion of bound fumonisin (42-75% of total fumonisin equivalents for the pellet and 44-79% for the snack product).
- Conventional analysis of fumonisins in the snack product would under determine the total fumonisins present.

### 6.3.3 Tortilla Chips

The fate of fumonisins during the manufacture of fried tortilla chips was studied.

- FB concentrations in the chips were reduced significantly from 33-76% in the chips, compared to that in the maize flour.
- Hydrolysed fumonisins and parent fumonisins were detected in the raw materials and final products but were not fully accounted for in thermally treated processes.
- The change of total fumonisin equivalents during total production is much less that in the two extrusion processes.

It was recognised soon after the discovery of fumonisins in maize, that certain processing techniques resulted in a decrease in fumonisin levels in the final products, for example the production of tortilla by nixtamilisation with reductions as high at 70%.

Degradation products of fumonisins post thermal processing have been identified as N-carboxymethyl fumonisin (with the N-(1-deoxy-D-fructose-1yl) FB1 occurring a much lower levels.

Studies to characterise the breakdown products have been conducted, it has shown that fumonisins can be hydrolysed and or bind to proteins and to other food matrix components [7, 39].

The nature of the binding has been investigated:
Hydroxy groups of sugars or the thiol group of certain amino acids could react to form a linkage between the fumonisins and the polysaccharide or protein.

Shier *et al* conducted an experiment adding radiolabelled FB1 to corn meal dough and processing. Only 37% of the radioactivity was recovered as parent fumonisins, and a further 46% recovered after disruption of protein binding[13].

In model systems Seefelder (2001, 2003) obtained bound FB1 by reaction (with heat) with sucrose, methyl α-D-glucopyranoside (as a model starch) and amino acid derivatives. It was noted that performing the same experiments with hydrolysed FB1 did not give rise to any bound compounds and the authors conclude that the binding occurred via the two tricarballyic acid side chains. The postulated mechanism is only valid for heat treated products [14,39].

N-fatty acylated derivate of fumonisins have been found in tortilla chips, again determined by use of radiolabelled markers.

As discussed above bound fumonisins have in this study and in work by other workers been found in raw maize. Clearly such matrices have not been heat treated. In studies on the association between fumonisin in raw maize and plant components the authors have conclude that the mechanism is association rather that covalent. In a study to identify which macromolecular components preferentially bind fumonisin Dall' Asta *et al* separated the carbohydrate and proteins of maize. The globulin and prolamins proteins contained the mist significant amount of HFB1 (after hydrolysis) [38].

The determination of total fumonisins in any food sample is complex and challenging, as discussed in previous reports the determination of parent fumonisins is complicated by instabilities during analysis an apparent loss of added (spiked) fumonisins. In addition the presence of hidden fumonisin in samples can give rise to varying recovery due to the conditions used that might over or underestimate the total fumonisin content.

# 7 Conclusions

- A full literature review was on-going throughout the project showing an increased amount of published information on the occurrence of bound toxins.

- Bound and hydrolysed fumonisins were investigated in both simple model systems and food processes. The presence of the breakdown/masking of parent fumonisins in commercial retail food products was also investigated. In some cases the total fumonisin equivalents were found to be higher than the parent fumonisin determined by conventional analysis.

- Hydrolysed and bound fumonisin occurrence is not restricted to thermally treated products, as they were also detected in raw maize, intermediate and final products showing their occurrence at levels ranging from 15 to 78% higher than those found for parent fumonisins. Hence the sum of free and "hidden" toxins could exceed the EU legal limits for total fumonisins.

- In some thermally heated processes (i.e. cornflakes and extruded snacks) there was an apparent loss of fumonisins not accounted for by hydrolysis, protein binding or other binding.

- Limited and sometimes ambiguous toxicological information is available but there are still gaps in the literature. An external activity by ILSI Europe evaluating masked mycotoxins may lead to a workshop in 2011/12 focussing on the toxicological aspects and we are a member of this expert group.

  Reports on the ability of *Aspergillus niger* species to produce fumonisins have continued to appear.
  Recent findings have show*n* that some *Aspergillus niger* isolates are able to produce fumonisins in high quantities on agar media with a low water activity. Several agricultural products fit this criterion, including dried vine fruits, dates and figs. No UK food products have been included in these surveys.

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# 9 Appendix 1 – Literature Review

### 9.1.1 Earlier FSA Funded Work Relevant to the Choice of Processes for Study

**The Fate of Fusarium Mycotoxin during Commercial Food Processing Project**

This project had been investigating the fate of several groups of Fusarium mycotoxin (trichothecenes, zearalenone and fumonisins) that can occur in cereals used to make food products in the UK. The project had sought to identify the fate of each toxin during commercial scale food processes and produce a mass balance for each toxin across the process streams. For most processes the food manufacturing steps have not resulted in any major losses of mycotoxin, largely the toxins have been re-distributed into different process streams. However one class of mycotoxins, the fumonisins, had shown significant losses in certain processes. The data generated on fumonisin loss (defined as not detectable using conventional analytical techniques for fumonisins) is in line with that reported in the scientific literature. Fumonisins levels drop during cornflake production (particularly using the traditional method of production), during extrusion and during snack production.

***Details of Findings***

*Maize: Cornflakes*[6.]

A study was carried out of how Fusarium mycotoxins present in maize at intake changed during the processing of commercial grain samples into cornflakes using a traditional cooking process used for most cornflakes consumed in the UK. Natural concentrations of Fusarium mycotoxins in commercial maize flaking grits are much lower than in raw maize. During processing to manufacture cornflakes, concentrations of fumonisins are reduced further by approximately 95% although there is no apparent loss of DON during this second stage. The consistent high loss of fumonisins found appears to be greater than that suggested by a few samples found during surveys that contained much higher levels. On the basis of milling and extrusion studies carried out during this project, it is suggested that cornflakes produced by extrusion are responsible for these occasional higher levels. This alternative process is known to use maize flour (usually contains higher mycotoxin levels than those in the related grit stream) and extrusion (which has little effect on DON concentrations and causes much less degradation of fumonisins). The market for cornflakes made by this method is relatively small in the UK.

*Maize- based snacks* [6,7.]

The fate of DON, ZON and FB1 and FB2 were examined in three representative snack food production methods. In the tortilla chip, the amount of FB1 + FB2 remaining in the retail product was reduced on average by 59% (very similar to the 60% reduction expected by the legislation for flour to retail snack product). Thus the use of maize containing fumonisins in maize flour at levels just within legal limits would present some risk that a proportion of retail products might fail to meet legislation when the run-to-run variability inherent in sampling and analysis is considered. However in a tortilla chip-like product, the amount of FB1+ FB2 in the retail product was only reduced by a mean value of 41% so that use of maize containing these mycotoxins at levels anywhere near to the legal limits leaves little margin for error especially taking into account the variability inherent in sampling and analysis. Thus whilst overall average reductions for fumonisins looks good there are clearly results that show that the changes through processing cannot be predicted with any certainty.

***Extrusion*[8.]**

Extrusion cooking technology can affect mycotoxin concentrations in different ways. Some factors can produce opposite effects for different mycotoxins. Operation of the extruder with low moisture ingredients requires a higher energy input and tended to result in higher losses of fumonisins at all temperatures although the opposite effect was found for ZON when loss was greater with higher moisture content. Temperature alone does not appear to significantly degrade fumonisins, DON or ZON under the extrusion conditions studied and is related to the amount of water present to a large extent.

**Improved Methodologies for the Detection of Hydrolysed and Bound Fumonisin**

The FSA had recently funded the development of robust analytical methods for the determination of parent fumonisins, hydrolysed fumonisins and masked fumonisins in processed food product. The details of the analytical methodology are given in Section 4 of this report. In a limited study an investigation on processed food test materials showed that the conventional extraction of fumonisins in processed food products does not extract all the fumonisin species present. To recover the total fumonisin a food product would require the hydrolysis of the whole sample and the subsequent determination of hydrolysed fumonisins.

The literature review has been divided into the following sections:-

- **Fumonisin occurrence**
  Raw materials
  Food products
    Cornflakes
  Dry milling

- **Breakdown products**
  Sugars
  Microbial transformations
  Others

- **Processing**
  Thermostability
  Cooking
  Extrusion

- **Fumonisin toxicity/metabolism (in relation to breakdown products)**

- **Analytical methodology**

Each section is preceded with a bullet point summary of the main points.

## 1. Fumonisin Occurrence (Presence and Distribution)

| **Key points** |
|---|
| • Maize and maize products are the major source of fumonisins, produced by Fusarium species.<br>• Fumonisin $B_2$ production by Aspergillus niger reported.<br>• Figs reported to contain $FB_1$ and $FB_2$.<br>• Fumonisin carryover into cow's milk reported.<br>• Single report of occurrence of $FB_1$ in wheat (one sample from Japan).<br>• Corn flakes papers included as this is one area of food production that binding/processing may occur. |

### 1.1 Raw Materials (excluding maize)

**Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment Volume 26, Issue 1, 2009, Pages 94-100**
**Fumonisin $B_2$ production by Aspergillus niger in Thai coffee beans.**
**Noonim, P. Mahakarnchanakul, W., Nielsen, K.F., Frisvad, J.C., Samson, R.A.**

Abstract
During 2006 and 2007, a total of 64 Thai dried coffee bean samples (Coffea arabica) from two growing sites in Chiangmai Province and 32 Thai dried coffee bean samples (Coffea canephora) from two growing sites in Chumporn Province, Thailand, were collected and assessed for fumonisin contamination by black Aspergilli. No Fusarium species known to produce fumonisin were detected, but black Aspergilli had high incidences on both Arabica and Robusta Thai coffee beans. Liquid chromatography (LC) with high-resolution mass spectrometric (HRMS) detection showed that 67% of Aspergillus niger isolates from coffee beans were capable of producing fumonisins B2 (FB2) and B4 when grown on Czapek Yeast Agar with 5% NaCl. Small amounts (1-9.7 ng g-1) of FB2 were detected in seven of 12 selected coffee samples after ion-exchange purification and LC-MS/MS detection. Two samples also contained FB4. This is the first record of freshly isolated A. niger strains producing fumonisins and the first report on the natural occurrence of $FB_2$ and $FB_4$ in coffee.

**Journal of Agricultural and Food Chemistry - Volume 55, Issue 23, 14 November 2007, Pages 9727-9732**
**Fumonisin $B_2$ production by Aspergillus niger**
**Frisvad, J.C. Smedsgaard, J. Samson, R.A. Larsen, T.O. Thrane, U.**

Abstract
The carcinogenic mycotoxin fumonisin $B_2$ was detected for the first time in the industrially important Aspergillus niger. Fumonisin $B_2$, known from Fusarium verticillioides and other Fusaria, was detected in cultures of three full genome sequenced strains of A. niger, in the ex type culture and in a culture of F. verticillioides by electrospray LC-MS analysis of methanolic extracts from agar plugs of cultures grown on several substrates. Whereas F. verticillioides produced fumonisin $B_1$, $B_2$, and $B_3$ on agar media based on plant extracts, such as barley malt, oat, rice, potatoes, and carrots, A. niger produced fumonisin $B_2$ best on agar media with a low water activity, including Czapek yeast autolysate agar with 5% NaCl. Of the media tested, only rice corn steep agar supported fumonisin

production by both F. verticillioides and A. niger. However, A. niger had a different regulation of fumonisin production and a different quantitative profile of fumonisins, producing only $B_2$ as compared to F. verticillioides. Fumonisin production by A. niger, which is a widely occurring species and an extremely important industrial organism, will have very important implications for biotechnology and especially food safety. A. niger is used for the production of citric acid and as producer of extracellular enzymes, and also as a transformation host for the expression of heterologous proteins. Certain strains of A. niger produce both ochratoxin A and fumonisins, so some foods and feeds may potentially contain two types of carcinogenic mycotoxins from this species.

**Food Analytical Methods 2 (2), pp. 128-140**
**Determination of B1 in bovine milk by LC-MS/MS**
**Gazzotti, T., Lugoboni, B., Zironi, E., Barbarossa, A., Serraino, A., Pagliuca, G.**

Abstract
The aim of the present work was to develop a sensitive and selective method for identification and quantification of fumonisin B1 (FB1) in bovine milk. FB1 was isolated by immunoaffinity column and was detected using liquid chromatography coupled with tandem mass spectrometry in positive electrospray ionisation (ESI+). The LOQ of the method was 0.1 μg/kg that was lower than the others reported in the literature. The high coefficient of determination ($R^2 > 0.99$) obtained in the range of 0.1-10.0 μg/kg, the good recovery (84%) and relative standard deviation (7%) of the proposed method ensure correct fumonisin detection in milk even at relatively low concentrations. The developed method was applied on different commercial samples in order to test its efficacy. FB1 was found above the LOQ in eight out of 10 samples analysed and the average level of contamination found was 0.26 μg/kg.

**Food Control - Volume 20, Issue 3, March 2009, Pages 239-249**
**Exposure assessment of mycotoxins in dairy milk**
**Coffey, R., Cummins, E., Ward, S.**
**School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland**

Abstract
The objective of this study was to develop a quantitative Monte Carlo exposure assessment model for mycotoxins in dairy milk and to assess the potential human exposure levels. Mean concentrations of mycotoxins in milk were estimated using the simulation model (Aflatoxin M1 = 0.0161 μg/kg, Ochratoxin A = 0.0002 μg/kg, Deoxynivalenol = 1 μg/kg, fumonisin B1 = 0.36 μg/kg, Zearalenone = 0.39 μg/kg, T-2 = 0.0722 μg/kg) while the simulated tolerable daily intakes (TDIs) from milk for males and females all fell below European Union guidelines. Aflatoxin M1 was the toxin of greatest concern as it had potential to exceed the EU limit of 0.05 μg/kg in milk. The sensitivity analysis identified the concentration of toxins in maize as the area which needs most attention in relation to crop management and agricultural practice. The sensitivity analysis assessed also identified the carry over rate as a factor closely related to risk and as a factor which required further research. 

**Journal of Food Protection - Volume 71, Issue 7, July 2008, Pages 1500-1504**

**Identification of fumonisin $B_2$, HT-2 toxin, patulin, and zearalenone in dried figs by liquid chromatography-time-of-flight mass spectrometry and liquid chromatography-mass spectrometry**

**Şenyuva, H.Z. , Gilbert, J**

Abstract

Dried figs from Turkey that were visibly mouldy (or fluorescent under UV light) and thus rejected as unsuitable for human food were screened for the presence of fungal metabolites. Crude solvent extracts from individual figs were directly analysed by liquid chromatography combined with time-of-flight mass spectrometry to generate accurate mass data for all detectable components. A comparison of these data with a metabolite database indicated the presence of fumonisins $B_2$ and $B_4$, patulin, HT-2 toxin, and zearalenone among various other metabolites. Portions of the same figs were reextracted and then analysed by conventional liquid chromatography-mass spectrometry. On the basis of coincident retention times and by matching selected ion monitoring for coincident ions with that of authentic standards, the identification of fumonisin $B_2$, HT-2 toxin, patulin, and zearalenone was confirmed.

**Food and Chemical Toxicology - Volume 47, Issue 2, February 2009, Pages 289-292**

**Natural occurrence of fumonisin $B_1$ in dried figs as an unexpected hazard**

**Karbancioglu-Güler, F. , Heperkan, D.**

Abstract

Occurrence of fumonisin $B_1$ ($FB_1$) in dried figs was determined using liquid chromatography with fluorescence detection after extraction with methanol-water and clean-up. One hundred and fifty five dried fig samples were taken while the figs were drying in 7 different districts in the Aegean Region in 2003 and 2004. $FB_1$ contamination were determined in 86 of 115 samples at detectable levels. Average $FB_1$ level was 0.369 and 0.466 in 2003 and 2004, respectively. Overall mean for $FB_1$ was calculated as 0.315 μg/g; overall median was 0.080 μg/g. The $FB_1$ content of positive samples ranged from 0.046 and 3.649 μg/g, while only 9.6% of the samples contained $FB_1$ above 1 μg/g. $FB_1$ contamination in the dried fig samples in 2004 (79.6%) was higher than in 2003 (71.8%). Selcuk (0.565 μg/g) was ranked first among means; however the highest incidences of $FB_1$ contamination was determined in dried figs from Incirliova (84.6%). This is the first report of occurrence of $FB_1$ in dried figs.

**Journal of Food Protection - Volume 72, Issue 6, June 2009, Pages 1327-1331**

**Limited surveillance of fumonisins in brown rice and wheat harvested in Japan**

**Kushiro, M., Zheng, Y., Nagata, R., Nakagawa, H., Nagashima, H.**

Abstract

Fumonisins are mycotoxins mainly produced by Fusarium verticillioid.es, which is a major contaminant of corn. However, there are sporadic reports of fumonisin contamination in wheat worldwide. The rice adherent fungus Gibberella fujikuroi is taxonomically closely related to F. verticillioides. Therefore, the potential risk of fumonisin contamination in rice and wheat is significant. Previously, a sensitive detection method utilizing liquid chromatography with tandem electrospray mass spectrometry (LC-ESI-MS-MS) was developed for the determination of fumonisins in brown rice. In the present study, the incidence of fumonisins in brown rice and wheat harvested in Japan was investigated using LC-ESI-MS-MS. Forty-eight rice samples and 47 wheat samples were screened and analyzed for the major B-type fumonisins: fumonisin $B_1$ ($FB_1$) and fumonisin $B_2$ ($FB_2$). About 1 kg of rice or wheat seed was divided into three subsamples, and 10 g from each subsample was used for the analysis. The limits of detection were 0.012 and 0.011 mg/kg for FB, and $FB_2$, respectively, in rice samples and 0.010 and 0.008 mg/kg for $FB_1$ and $FB_2$, respectively, in wheat samples. The mean (standard deviation) recoveries of FB, spiked at 0.50 mg/kg into toxin-free rice and wheat samples were 77.6 (4.2)% and 84.5 (3.1)%, respectively. One of the wheat samples was positive for $FB_1$ with a value greater than the limit of detection, but no fumonisin was found in any of the rice samples. This is the first report of fumonisins detected in Japanese wheat.

## 1.2 Food Products

**Food Additives and Contaminants - Volume 25, Issue 3, March 2008, Pages 347-355**

**Mycotoxins in breakfast cereals from the Canadian retail market: a 3-year survey.**

**Roscoe, V., Lombaert, G.A., Huzel, V., Neumann, G., Melietio, J., Kitchen, D., Kotello, S., Krakalovich, T., Trelka, R., Scott, P.M.**

Abstract

One hundred and fifty-six samples of breakfast cereals were collected from the Canadian retail marketplace over a 3-year period. The samples were analysed for the mycotoxins deoxynivalenol, nivalenol, HT-2 toxin, zearalenone, ochratoxin A, and **fumonisins** B1 and B2 to contribute to dietary exposure estimates in support of the development of Canadian guidelines for selected mycotoxins in foods. The samples included corn-, oat-, wheat- and rice-based cereals, as well as mixed-grain cereals, and were primarily from North American processors. Overall, deoxynivalenol was the most frequently detected mycotoxin--it was detected in over 40% of all samples analysed. **Fumonisins** and ochratoxin A were each detected in over 30% of all samples. Zearalenone was detected in over 20% of all samples. Nivalenol and HT-2 toxin were each detected in only one sample. The survey clearly demonstrated regular occurrence of low levels of multiple mycotoxins in breakfast cereals on the Canadian market.

**Berichte uber Landwirtschaft - Volume 82, Issue 3, October 2004, Pages 446-470**

**Fumonisin intake of the German consumer [Fumonisinaufnahme des Deutschen verbrauchers]**

**Zimmer, I., Dietrich, R., Märtlbauer, E., Usleber, E., Klaffke, H., Tiebach, R., Weber, R., Majerus, P. Otteneder, H.**

Abstract
In order to calculate the average fumonisin intake of the German consumer, a large survey was performed on a variety of potentially contaminated products in the period between December 1998 and July 2001. A total of 1960 samples was analysed for fumonisins. Furthermore, 272 of these samples were also analysed for hydrolysed fumonisins. Enzyme immunoassays were used for routine analysis and confirmatory and control analyses were performed using HPLC and LC-MS/MS. The daily intake of fumonisins was calculated by combining fumonisin contamination data obtained in this study with available food intake data. It was found that in general there is no increased risk for the German consumer of exceeding the recommended tolerable daily intake of fumonisins. However, certain products (or certain batches of products) were repeatedly found to contain elevated fumonisin levels, which in an extreme case could represent a potential risk for the consumer, in particular if foods for infants and young children are concerned. This could be solved by eliminating these peak contamination levels from the human diet through the introduction of maximum tolerable levels for fumonisins

**Molecular Nutrition and Food Research - Volume 53, Issue 4, April 2009, Pages 492-499**

**Free and bound fumonisins in gluten-free food products**

**Dall'Asta, C., Galaverna, G. Mangia, M., Sforza, S., Dossena, A., Marchelli, R.**

Abstract
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.

### 1.2.1 Cornflakes

**Food Additives and Contaminants - Volume 20, Issue 2, 1 February 2003, Pages 161-169**
**Hidden fumonisin in corn flakes**
**Kim, E.K., Scott, P.M. , Lau, B.P.-Y.**

Abstract
Twenty-five samples of retail corn flakes (from 15 lots) were analysed for fumonisin $B_1$ ($FB_1$) and fumonisin $B_2$ ($FB_2$). They were detected in 22 and 12 samples, respectively, at respective mean concentrations 68 and 8 ng $g^{-1}$. Samples were extracted with methanol-acetonitrile-water (25:25:50) and there was an excellent correlation for $FB_1$ between results obtained with $C_{18}$ clean-up and those obtained with the immunoaffinity column (IAC) clean-up. After extraction of the corn flakes' residue with 1% sodium dodecyl sulphate (SDS) solution and hydrolysis with 2 N potassium hydroxide, hidden (protein bound) fumonisin was determined as $HFB_1$, which was found in residues from all the corn flakes samples, even those containing no detectable $FB_1$; the average concentration of $HFB_1$ was 101 ng $g^{-1}$, equivalent to 180 ng $FB_1$ $g^{-1}$. Thus, our results showed an average of 2.6 times more $FB_1$ present in bound form as was determined by conventional analysis. We found a correlation coefficient of -0.5034 for a logarithmic relationship between the $FB_1$ ($C_{18}$ clean-up) and $HFB_1$ concentrations The highest concentration of $HFB_1$ formed was 288 ng $g^{-1}$ from a sample containing only 12-15 ng $FB_1 g^{-1}$, while the lowest concentration of $HFB_1$ was 26 ng $g^{-1}$ from a sample with 152-155 ng $FB_1$ $g^{-1}$. This low degree of correlation should be taken into account by food safety authorities in estimates of human exposure to protein bound fumonisin.

**International Journal of Food Microbiology - Volume 123, Issue 1-2, 31 March 2008, Pages 81-87**
**Distribution of fumonisins and aflatoxins in corn fractions during industrial cornflake processing**
**Castells, M., Marín, S., Sanchis, V., Ramos, A.J.**

Abstract
The aim of this study was to investigate the distribution of fumonisins ($B_1$ $B_{2,}$ and $B_3$) and total aflatoxins ($B_1$, $B_2$, $G_1$, and $G_2$) in various corn processed fractions. 92 batches of whole corn and derived dry-milled fractions (animal feed flour, flaking grits, corn flour and corn meal) and cooked and roasted cornflakes fractions were industrially obtained. Samples were analysed for both groups of mycotoxins by enzyme-linked immunosorbent assay (ELISA). Dry milling of corn led to a heterogeneous distribution of the two groups of mycotoxins in the different parts of the grain, with increased levels in fractions processed from outer layers (animal feed flour and corn flour) and decreased levels in fractions processed from inner portions, such as corn meal and flaking grits. Levels of fumonisins in cornflakes were lower than 400 μg/kg, the maximum tolerable limit set by the EU. By contrast, three samples of final product were found to exceed the aflatoxin maximum tolerable limit of 4 μg/kg. Animal feed flour showed concentration factors of 317 and 288% for fumonisins and aflatoxins, respectively. Food traceability system was used by the industrial companies which processed corn into breakfast cereals. Nevertheless, even though the use of food traceability, which is defined as the ability to trace any food, feed, food-producing animal or

substance that will be used for consumption through all stages of production, processing and distribution, only initial fumonisin contamination of whole corn and contamination of animal feed flour and corn flour were found to be correlated.

**Journal of Food Protection - Volume 64, Issue 5, 2001, Pages 701-705**
**Effect of processing on fumonisin concentration in corn flakes**
**De Girolamo, A., Solfrizzo, M., Visconti, A.**

Abstract
The stability of fumonisin $B_1$ and fumonisin $B_2$ during processing of corn flakes was investigated with three different methods for analysis of the naturally contaminated raw material (corn flour), intermediate product (extruded, but not roasted corn flakes), and final product (roasted corn flakes). Only one method, using immunoaffinity column clean-up, provided reliable results in the determination of fumonisins in corn flake samples at the intermediate and final steps of processing. About 60 to 70% of the initial amount of fumonisins were lost during the entire cycle of corn flake processing, with less than 30% losses occurring during the intermediate extrusion step (70 to 170°C for 2 to 5 min). The effect of different additives commonly present in commercial products (sodium chloride, sucrose, and ferrous sulfate heptahydrate) on the reliability of fumonisin analysis has also been investigated. The presence of sodium chloride strongly reduced fumonisin recovery when strong anion-exchange (SAX) columns were used for the clean-up step, whereas the other additives appeared to have little or no effect on the accuracy of fumonisin analysis. The use of reliable analytical methods that are effective for both raw materials and processed products is of paramount relevance for studying the effect of food processing on mycotoxin-contaminated commodities. Despite the fact that some effective fumonisin decontamination occurring during corn flake processing has been shown, more work is needed to identify the thermal breakdown products of fumonisins and their relevant toxicity.

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## 1.3 Dry Milling of Maize

**Food Control - 20 (3), pp. 235-238 (2009)**

**Fate of fumonisin $B_1$ in the processing of whole maize kernels during dry-milling**

**Vanara, F., Reyneri, A., Blandino, M.**

Abstract

The aim of this research was to evaluate how the amount of fumonisins in a kernel is re-distributed over the different processing products. The study focused on the description of the dry-milling process, with details on the products and by-products, milling yield, and the granulometric and chemical composition. Maize kernels and four derived milling fractions from twenty-four lots were sampled from 2002 and 2006. The main results were: (a) the animal meal and germ had higher fumonisin content than the unprocessed grain, while human meals were less contaminated; (b) there is an inverse relationship between the particle size and fumonisin contents in meals; (c) toxin tends to concentrate in the bran and germ, while the endosperm is only partially contaminated.

**Journal of Food Protection - Volume 67, Issue 6, June 2004, Pages 1261-1266**

**Effect of industrial processing on the distribution of fumonisin $B_1$ in dry milling corn fractions**

**Brera, C. , Debegnach, F., Grossi, S., Miraglia, M.**

Abstract

The aim of this study was to investigate the distribution of fumonisin $B_1$ in various corn milling fractions processed by an industrial plant. Corn kernels and six derived milling fractions (germ, bran, large and small grits, animal feed flour, and flour) were sampled. In addition, in order to evaluate the effect of cooking, samples of polenta were prepared starting from naturally contaminated flour obtained from the industrial processing cycle. The industrial plant worked continuously at a rate of 60 tons per day. Two sublots of 5 tons each were investigated with samples of derived products taken at regular time intervals. Due to a similar heterogeneous distribution of fumonisin $B_1$ with other mycotoxins, such as aflatoxins, the sampling scheme was derived from the European Directive 98/53 for aflatoxins. Both lots of kernels showed fumonisin contamination at 4.54 and 5.09 mg/kg, respectively. Germ, bran, and animal feed flour showed contamination levels, namely 8.92 mg/kg (lot 1) and 9.56 mg/kg (lot 2), 7.08 mg/kg (lot 1) and 8.08 mg/kg (lot 2), and 9.36 mg/kg (lot 1) and 6.86 mg/kg (lot 2) higher than large and small grits and flour (0.39 mg/kg [lot 1] and 0.42 mg/kg [lot 2], 0.60 mg/kg [lot 1] and 1.01 mg/kg [lot 2], and 0.40 mg/kg [lot 1] and 0.45 mg/kg [lot 2], respectively). These results seem to account both for the industrial yields of the derived products and the distribution of fumonisin contamination in a kernel. The cooking of polenta in a domestic pressure cooker did not affect fumonisin

contamination because the mycotoxin concentrations were similar to those of the starting flour (0.40 and 0.45 mg/kg).

## 2. Reaction, Binding and Breakdown Products

| **Key points** |
|---|
| • Presence of sugars has significant effects on parent fumonisins, binding occurs and the bound moieties are not detected by conventional techniques. Reported reduction in toxicity.<br>• Increasing number of reports on the sugar-fumonisin reaction products.<br>• Fumonisin bound by lactic acid bacteria and possibly yeast. |

### 2.1 Sugars

**Journal of Agricultural and Food Chemistry - Volume 47, Issue 10, October 1999, Pages 4291-4296**
**Excretion of $^{14}C$-fumonisin $B_1$, $^{14}C$-hydrolyzed fumonisin $B_1$, and $^{14}C$-fumonisin $B_1$-fructose in rats**
**Dantzer, W.R. Hopper, J. Mullin, K. Hendrich, S. Murphy, P.A.**

Abstract
$^{14}C$-Fumonisin $B_1$ ($FB_1$) was produced by Fusarium proliferatum M-5991 in modified Myro liquid medium and purified to > 95% purity with a specific activity of 1.7 mCi/mmol. Nine male and nine female F344/N rats were each dosed by gavage with 0.69 μmol of $^{14}C$-$FB_1$, $^{14}C$-hydrolyzed $FB_1$, or $^{14}C$-$FB_1$-fructose/kg body weight. Urinary excretion of $^{14}C$-$FB_1$ and $^{14}C$-$FB_1$-fructose was 0.5% and 4.4% of the total dose, respectively, and was similar between male and female rats. Urinary excretion of $^{14}C$- hydrolyzed $HFB_1$ was significantly greater ($P > 0.05$) in female rats as compared with male rats (17.3% vs 12.8% of the total dose, respectively). There were no significant ($P > 0.05$) differences in biliary excretion of the three fumonisin compounds with a mean of 1.4% of the dose excreted at 4 h after dosing. Lesser amounts continued to be excreted up to 9.25 h after dosing. Although biliary excretion of the $^{14}C$-$FB_1$, $^{14}C$-hydrolyzed $FB_1$, and $^{14}C$-$FB_1$-fructose was similar, increased urinary excretion of the $^{14}C$-hydrolyzed $FB_1$ as compared to $^{14}C$-$FB_1$ and $^{14}C$-$FB_1$-fructose indicated a greater absorption of the hydrolyzed form.

**Journal of Agricultural and Food Chemistry - Volume 53, Issue 10, 18 May 2005, Pages 4264-4271**
**Fumonisin B-glucose reaction products are less toxic when fed to swine**
**Fernández-Surumay, G. Osweiler, G.D. Yaeger, M.J. Rottinghaus, G.E. Hendrich, S.' Buckley, L.K. Murphy, P.A.**

Abstract
The effects of fumonisin B-glucose reaction products in swine diets was examined. Pigs were fed diets containing 528 μmol of total fumonisin B/kg (FB), 528 μmol of total FB-glucose adducts/kg (FB-G, 122 μmol of unreacted FB/kg), or 0 μmol of total FB/kg for 15 days to test the efficacy of the FB-G reaction products in detoxifying FB. Weight gain in FB pigs was lower than in FB-G or controls, which was correlated with feed intake reduction in FB pigs. Serum aspartate aminotransferase, γ-glutamyltransferase, and total bilirubin in FB pigs were higher than in FB-G or control pigs. Serum sphinganine/shingosine ratios in FB pigs were

higher than in FB-G or control pigs. Microscopic examination of tissues from FB pigs showed generalized liver necrosis and apoptosis with marked cellular pleomorphism and disorganized hepatic cords. The liver and kidneys in the FB-G group appeared to be normal. Tissues of controls were free of lesions. Results suggest that dietary FB-G products are less toxic to swine and may provide an detoxification approach in instances of widespread FB grain contamination ($p < 0.05$).

**Journal of Agricultural and Food Chemistry - Volume 45, Issue 3, March 1997, Pages 803-809**

**Reaction with Fructose Detoxifies Fumonisin $B_1$ while Stimulating Liver-Associated Natural Killer Cell Activity in Rats**

**Lu, Z. Dantzer, W.R. Hopmans, E.C. Prisk, V. Cunnick, J.E. Murphy, P.A. Hendrich, S.**

Abstract

Fumonisin $B_1$ ($FB_1$) was reacted with fructose in an attempt to detoxify this mycotoxin. Fischer 344/N rats were initiated with diethylnitrosamine (15 mg/kg body weight) and then fed 69.3 μmol $FB_1$/kg diet or 69.3 μmol $FB_1$ reacted with fructose ($FB_1$-fructose)/kg diet for 4 weeks. In comparison with the rats fed basal diet or $FB_1$-fructose, the $FB_1$-fed rats had significantly increased plasma cholesterol ($P < 0.01$), plasma alanine aminotransferase activity ($P < 0.05$), and endogenous hepatic prostaglandin production ($P < 0.05$). Placental glutathione S-transferase-positive and γ-glutamyl transferase-positive altered hepatic foci occurred only in the $FB_1$-fed rats. Liver-associated natural killer (NK) cell activity was significantly decreased in the $FB_1$-fed rats and increased in the group fed $FB_1$-fructose, as compared with the basal group ($P < 0.03$). Therefore, modifying $FB_1$ with fructose seems to prevent $FB_1$-induced hepatotoxicity and promotion of hepatocarcinogenesis while stimulating liver-associated NK cell activity in rats.

**Journal of Food Protection - Volume 71, Issue 10, October 2008, Pages 2036-2041**

**Reduced toxicity of fumonisin $B_1$ in corn grits by single-screw extrusion**

**Voss, K.A. Bullerman, L.B. Bianchini, A. Hanna, M.A. Ryu, D.**

Abstract

Corn grits spiked with 30 μg/g fumonisin $B_1$ and two batches of grits fermented with Fusarium verticillioides (batch 1 contained 33 μg/g, and batch 2 contained 48 μg/g fumonisin $B_1$), which were extruded by a single-screw extruder with and without glucose (10%, dry weight basis) supplementation were fed to rats. Control groups were fed uncontaminated grits. Extrusion with glucose more effectively reduced fumonisin $B_1$ concentrations of the grits (75 to 85%) than did extrusion alone (10 to 28%). With one exception, the fumonisin $B_1$-spiked and fermented extrusion products caused moderately severe kidney lesions and reduced kidney weights, effects typically found in fumonisin-exposed rats. Lesions in rats fed the least contaminated grits (batch 1) after extrusion with 10% glucose were, however, significantly less severe and not accompanied by kidney weight changes. Therefore, extrusion with glucose supplementation is potentially useful for safely reducing the toxicity of fumonisins in corn-based products and studies to determine the optimal conditions for its use are warranted.

**Journal of Agricultural and Food Chemistry - Volume 51, Issue 18, 27 August 2003, Pages 5567-5573**

**Bound fumonisin $B_1$: Analysis of fumonisin-$B_1$ glyco and amino acid conjugates by liquid chromatography-electrospray ionization-tandem mass spectrometry**

**Seefelder, W., Knecht, A., Humpf, H.-U.**

Abstract

To study the formation of fumonisin artifacts and the binding of fumonisins to matrix components (e.g., saccharides and proteins) in thermal-treated food, model experiments were performed. Fumonisin $B_1$ and hydrolyzed fumonisin $B_1$ were incubated with α-D-glucose and sucrose (mono- and disaccharide models), with methyl α-D-glucopyranoside (starch model), and with the amino acid derivatives N-α-acetyl-L-lysine methyl ester and BOC-L-cysteine methyl ester (protein models). The reaction products formed were analyzed by liquid chromatography-electrospray ionization-tandem mass spectrometry. The incubation of D-glucose with fumonisin $B_1$ or hydrolyzed fumonisin $B_1$ resulted in the formation of Amadori rearrangement products. Whereas conjugates were found following the reaction of sucrose, methyl α-D-glucopyranoside, and the amino acid derivatives with fumonisin $B_1$, the heating with hydrolyzed fumonisin $B_1$ yielded no artifacts. For structural determination, the stable reaction product formed by heating of methyl α-D-glucopyranoside (as starch model) with fumonisin $B_1$ was purified and identified by nuclear magnetic resonance spectroscopy as the diester of the fumonisin tricarballylic acid side chains with methyl α-D-glucopyranoside. These model experiments demonstrate that fumonisins are able to bind to polysaccharides and proteins via their two tricarballylic acid side chains.

**Journal of Agricultural and Food Chemistry - Volume 50, Issue 16, 31 July 2002, Pages 4726-4733**

**Characterization of fumonisin $B_1$-glucose reaction kinetics and products**

**Lu, Y., Clifford, L., Hauck, C.C., Hendrich, S., Osweiler, G., Murphy, P.A.**

Abstract

The reaction of fumonisin $B_1$ with the reducing sugar D-glucose can block the primary amine group of fumonisin $B_1$ and may detoxify this mycotoxin. A method to separate hundred milligram quantities of fumonisin $B_1$-glucose reaction products from the excess D-glucose with a reversed-phase $C_{18}$ cartridge was developed. Mass spectrometry revealed that there were four primary products in this chain reaction when fumonisin $B_1$ was heated with D-glucose at 65°C for 48 h: N-methyl-fumonisin $B_1$, N-carboxymethyl-fumonisin $B_1$, N-(3-hydroxyacetonyl)-fumonisin $B_1$, and N-(2-hydroxy, 2-carboxymethyl)-fumonisin $B_1$. The N-(1-deoxy-D-fructos-1-yl) fumonisin $B_1$ (fumonisin $B_1$-glucose Schiff's base) was detected by mass spectrometry when fumonisin $B_1$ was heated with D-glucose at 60°C. The nonenzymatic browning reaction of fumonisin $B_1$ with excess D-glucose followed apparent first-order kinetics. The activation energy, $E_a$, was 105.7 kJ/mol. Fumonisin $B_1$ in contaminated corn could precipitate the nonenzymatic browning reaction with 0.1 M D-glucose at 60 and 80°C.

**Journal of Food Science - Volume 66, Issue 3, 2001, Pages 416-421**
**Loss of fuminosin $B_1$ in extruded and baked corn-based foods with sugars**
**Castelo, M.M, Jackson, L.S., Hanna, M.A Reynolds, B.H., Bullerman, L.B.**

Abstract
The objective of this work was to determine the effect of added sugars on fumonisin $B_1$ ($FB_1$) levels in baked corn muffins and extruded corn grits. Muffins containing added glucose had significantly lower $FB_1$ levels than muffins with sucrose, fructose, or no added sugar. Extrusion cooking of the grits resulted in significant ($p< 0.05$) reductions of $FB_1$ in all treatments relative to unextruded controls, but use of glucose resulted in greater reductions of $FB_1$ (45.3 to 71%) than did the use of fructose (29.5 to 53%) or sucrose (19.2 to 39%). When extrusion conditions were optimized, 92.1% loss of $FB_1$ was found when grits were extruded with glucose. Adding glucose to thermally processed food can result in a substantial reduction in FB, levels.

**Food Additives & Contaminants: Part A**
**Reduction of fumonisin B1 in extruded corn breakfast cereals with salt, malt and sugar in their formulation**
Authors: **M. Castells A. J. Ramos; V. Sanchis S. Marín**

Abstract
The objective was to determine the effect of added sodium chloride, barley malt and sucrose on the stability of fumonisin $B_1$ ($FB_1$) present in corn flour. Two levels of both sodium chloride (0.4% and 2%) and barley malt (0.8% and 5%) were added to the unextruded corn flour, and six levels of sucrose (3-10%) were used. The addition of sucrose at the lowest salt content (0.4%) as well as salt, either at 0.4% or at 2%, led to a significant decrease of $FB_1$ levels in extruded samples, whereas malt, either at 0.8% or at 5%, did not significantly affect $FB_1$ stability. Decontamination rates depended on the concentrations of added ingredients and ranged from 2% to 92%. The greatest reductions in $FB_1$ content were achieved with extrusion cooking with a high salt content, whilst the lowest reductions were the result of processing corn flour with low contents of both salt and sucrose. Salt at 2% was the most effective ingredient in reducing $FB_1$ content of the final extruded food.

**Journal of Agricultural and Food Chemistry - Volume 50, Issue 5, 27 February 2002, Pages 1318-1324**
**N-(1-Deoxy-D-fructos-1-yl) fumonisin $B_1$ the initial reaction product of fumonisin $B_1$ and D-glucose**
**Poling, S.M., Plattner, R.D., Weisleder, D.**

Abstract
Incubation of fumonisin $B_1$ and D-glucose in aqueous solutions resulted in the formation of N-(1-deoxy-D-fructos-1-yl) fumonisin $B_1$ in addition to the previously reported N-(carboxymethyl) fumonisin $B_1$. N-(1-Deoxy-D-fructos-1-yl) fumonisin $B_1$ is the first stable product formed after the Amadori rearrangement of the Schiff base formed by the reaction of the primary amine of fumonisin $B_1$ and the aldehyde group of D-glucose. N-(1-Deoxy-D-fructos-1-yl) fumonisin $B_1$ was synthesized by reacting fumonisin $B_1$ with an excess of D-glucose in methanol and heating for 6 h at 64°C. It was purified using $C_{18}$ and strong cation exchange solid-phase extraction cartridges and characterized by nuclear magnetic

resonance and liquid chromatography-mass spectrometry. Subsequently, N,N-dimethyl-formamide was found to be a better reaction solvent, requiring reaction for only 2-3 h at 64°C and eliminating the formation of methyl esters. Alkaline hydrolysis of N-(1-deoxy-D-fructos-1-yl) fumonisin $B_1$ gave a mixture of hydrolyzed fumonisin B1 and hydrolyzed N-(carboxymethyl) fumonisin $B_1$.

**Journal of Agricultural and Food Chemistry - Volume 46, Issue 9, September 1998, Pages 3546-3557**
**Formation of N-(Carboxymethyl) fumonisin $B_1$, Following the Reaction of Fumonisin $B_1$ with Reducing Sugars**
**Howard, P.C., Churchwell, M.I., Couch, L.H, Marques, M.M., Doerge, D.R.**

Abstract
The fumonisins are mycotoxins produced by fungi that contaminate primarily corn and are toxic through interruption of intracellular sphingolipid synthesis. Several reports have indicated that fumonisin $B_1$ concentrations decreased when heated in aqueous solutions of reducing sugars. The incubation of fumonisin $B_1$ with D-glucose resulted in the formation of N-(carboxymethyl) fumonisin $B_1$, which was characterized by NMR and electrospray mass spectroscopy. We determined the methylene carbon of the carboxymethyl group is derived from C1 on glucose, while the carbonyl carbon is derived from the C2 of glucose, using $^{13}C$ glucose. Apparently N-(carboxymethyl) fumonisin $B_1$ arises from Schiffs base formation, Amadori rearrangement to a β-ketoamine, and oxidation with molecular oxygen. N-(Carboxymethyl)fumonisin $B_1$ formation is favored by alkaline conditions (pH >7), requires molecular oxygen, and is catalyzed by several reducing sugars. N-(carboxymethyl)-fumonisin $B_1$ was detected in raw corn samples that contained fumonisin $B_1$ (0.5-1.4 ppm) at an average of 4% of the fumonisin $B_1$ levels.

## 2.2 Microbial transformations

**Journal of Agricultural and Food Chemistry - Volume 56, Issue 12, 25 June 2008, Pages 4523-4528**
**Reduction in Fusarium toxin levels in corn silage with low dry matter and storage time**
**Boudra, H., Morgavi, D.P.**

Abstract
Under unfavourable climatic conditions, Fusarium spp. can contaminate corn plants in the field and produce toxins that are present at the time of ensiling. The stability of deoxynivalenol, fumonisins B1 and B2, and zearalenone in corn silage was tested over two consecutive years. Variables studied were corn dry matter (DM) and storage length and temperature. The concentration of all Fusarium toxins decreased upon ensiling ($P < 0.001$). Increasing the length of storage and ensiling with low DM resulted in a higher rate of toxin disappearance, particularly for the water soluble toxins deoxynivalenol and fumonisin B1. Toxin disappearance ranged from 50% for zearalenone to 100% for deoxynivalenol. In contrast, temperature did not have any effect on stability ($P > 0.05$). These results indicate that low DM at ensiling as well as a prolonged storage could be a practical way to reduce or eliminate some Fusarium toxins in contaminated silages.

## Lactic Acid Bacteria

**Journal of Applied Microbiology - Volume 106, Issue 3, March 2009, Pages 977-985**

**Cell wall component and mycotoxin moieties involved in the binding of fumonisin $B_1$ and $B_2$ by lactic acid bacteria**

**Niderkorn, V.˙ Morgavi, D.P., Aboab, B., Lemaire, M., Boudra, H.**

Abstract

The ability of lactic acid bacteria (LAB) to bind fumonisins $B_1$ and $B_2$ ($FB_1$, $FB_2$) in fermented foods and feeds and in the gastrointestinal tract could contribute to decrease their bioavailability and toxic effects on farm animals and humans. The aim of this work was to identify the bacterial cell wall component(s) and the functional group(s) of FB involved in the LAB-FB interaction. Methods and Results: The effect of physicochemical, enzymatic and genetic treatments of bacteria and the removal/inactivation of the functional groups of FB on toxin binding were evaluated. Treatments affecting the bacterial wall polysaccharides, lipids and proteins increased binding, while those degrading peptidoglycan (PG) partially decreased it. In addition, purified PG from Gram-positive bacteria bound FB in a manner analogue to that of intact LAB. For FB, tricarballylic acid (TCA) chains play a significant role in binding as hydrolysed FB had less affinity for LAB. Conclusions: Peptidoglycan and TCA are important components of LAB and FB, respectively, involved in the binding interaction. Significance and Impact of the Study: Lactic acid bacteria binding efficiency seems related to the peptide moiety structure of the PG. This information can be used to select probiotics with increased FB binding efficiency.

## Fermentation

**Applied and Environmental Microbiology - Volume 58, Issue 1, 1992, Pages 233-236**

**Fate of fumonisin $B_1$ in naturally contaminated corn during ethanol fermentation**

**Bothast, R.J., Bennett, G.A., Vancauwenberge, J.E., Richard, J.L.**

Abstract

Two lots of corn naturally contaminated with fumonisin $B_1$ (15 and 36 ppm) and a control lot (no fumonisin $B_1$ detected) were used as substrates for ethanol production in replicate 8.5-liter yeast fermentations. Ethanol yields were 8.8% for both the control and low-fumonisin corn, while the high- fumonisin corn contained less starch and produced 7.2% ethanol. Little degradation of fumonisin occurred during fermentation, and most was recovered in the distillers' grains, thin stillage, and distillers' solubles fractions. No toxin was detected in the distilled alcohol or centrifuge solids. Ethanol fermentation of fumonisin-contaminated corn coupled with effective detoxification of distillers' grains and aqueous stillage is suggested as a practical process strategy for salvaging contaminated corn.

## 3. Processing

| Key points |
| --- |
| • Largely thermostable<br>• Reductions during processing occur:<br>  ○ Cornflakes (see papers above)<br>  ○ Extrusion<br>  ○ Nixtamilsation<br>  It some cases bound and hydrolysed analysed for and detected. |

**International Journal of Food Microbiology - Volume 119, Issue 1-2, 20 October 2007, Pages 140-146**
**Stability of mycotoxins during food processing**
**Bullerman, L.B. , Bianchini, A.**

Abstract
The mycotoxins that commonly occur in cereal grains and other products are not completely destroyed during food processing operations and can contaminate finished processed foods. The mycotoxins most commonly associated with cereal grains are aflatoxins, ochratoxin A, fumonisins, deoxynivalenol and zearalenone. The various food processes that may have effects on mycotoxins include sorting, trimming, cleaning, milling, brewing, cooking, baking, frying, roasting, canning, flaking, alkaline cooking, nixtamalization, and extrusion. Most of the food processes have variable effects on mycotoxins, with those that utilize the highest temperatures having greatest effects. In general the processes reduce mycotoxin concentrations significantly, but do not eliminate them completely. However, roasting and extrusion processing show promise for lowering mycotoxin concentrations, though very high temperatures are needed to bring about much of a reduction in mycotoxin concentrations. Extrusion processing at temperatures greater than 150 °C are needed to give good reduction of zearalenone, moderate reduction of aflatoxins, variable to low reduction of deoxynivalenol and good reduction of fumonisins. The greatest reductions of fumonisins occur at extrusion temperatures of 160 °C or higher and in the presence of glucose. Extrusion of fumonisin contaminated corn grits with 10% added glucose resulted in 75-85% reduction in Fumonisin $B_1$ levels. Some fumonisin degradation products are formed during extrusion, including small amounts of hydrolyzed Fumonisin $B_1$ and N-(Carboxymethyl) - Fumonisin $B_1$ and somewhat higher amounts of N-(1-deoxy-d-fructos-1-yl) Fumonisin $B_1$ in extruded grits containing added glucose. Feeding trial toxicity tests in rats with extruded fumonisin contaminated corn grits show some reduction in toxicity of grits extruded with glucose.

**Stewart Postharvest Review - Volume 4, Issue 6, December 2008**
**Effects of processing on mycotoxins**
**Ryu, D., Bianchini, A., Bullerman, L.B.**

Abstract
Purpose of the review: This review summarises the effects of common food processes on several important mycotoxins. Findings: Aflatoxins, ochratoxin A, fumonisins, deoxynivalenol and zearalenone are commonly occurring secondary fungal metabolites known to be toxic to animals and humans. These mycotoxins are fairly heat stable and some level tends to remain in processed food products. While varying degrees of reduction have been documented during some food

processes, including sorting, cleaning, milling, baking, canning, frying, roasting, brewing, nixtamalisation and extrusion, removal or destruction is not complete. The reduction of mycotoxins is generally correlated with the degree of heat employed in the process; however, heat energy alone may not cause complete elimination of mycotoxins during food processing. Extrusion cooking as a process has been shown to be effective in reducing most mycotoxins at temperatures above 150°C Fumonisins, in particular, may be reduced significantly in the presence of a reducing sugar such as glucose, but the degradation or reaction mechanism is not fully understood. Directions for future research: Additional future research is needed to delineate the chemical and toxicological fate of mycotoxins and their degradation products during food processing to ensure the safety of processed foods.

### 3.1 Thermostability

**Journal of Agricultural and Food Chemistry - Volume 44, Issue 3, March 1996, Pages 906-912**
**Effects of time, temperature, and pH on the stability of fumonisin B1 in an aqueous model system**
**Jackson, L.S. Hlywka, J.J., Senthil, K. Bullerman, L.B., Musser, S.M.**

Abstract
Fumonisins, mycotoxins produced by Fusarium moniliforme in corn, have been implicated in several animal and human diseases. The effects of processing time and temperature on fumonisin $B_1$ ($FB_1$) stability (5 ppm) in aqueous solutions at pH 4, 7, and 10 were determined. Analysis of the thermally processed solutions by liquid chromatography/mass spectrometry indicated the predominant presence of hydrolysis products of $FB_1$. The rate and extent of $FB_1$ decomposition increased with processing temperature. After processing at ≤125 °C for 60 min, <27% of $FB_1$ was lost; after 60 min at 150 °C, 18-90% was lost, depending on buffer pH. Overall, $FB_1$ was least stable at pH 4 followed by pH 10 and 7, respectively. At >175 °C, >90% of $FB_1$ was lost after processing for 60 min, regardless of pH. $FB_1$ levels may be substantially reduced in foods that reach ≥150 °C during processing.

**Applied and Environmental Microbiology - Volume 59, Issue 9, 1993, Pages 2864-2867**
**Thermostability of fumonisin $B_1$, a mycotoxin from Fusarium moniliforme, in corn**
**Dupuy, J. Le Bars, P. Boudra, H. Le Bars, J.**

Abstract
Fumonisin $B_1$ ($FB_1$) is a mycotoxin from Fusarium moniliforme that is frequently associated with corn. Thermal treatments are used in many processes concerning this cereal and its derivatives. The thermostability of this toxin in dry contaminated corn, resulting from F. moniliforme culture, was studied in different time-temperature combinations. $FB_1$ was quantified by instrumentalized thin-layer chromatography after a two-step sequential development and postchromatographic derivatization by p-anisaldehyde. The identity of $FB_1$ in extracts, before and after heat treatments, was confirmed by high-pressure liquid chromatography. For each temperature, the natural logarithm of the ratio of resulting $FB_1$ on initial content (In $C/C_0$) is linearly correlated to exposure time.

The calculated half-lives ($L_{50}$), corresponding to the 50% value, were 10 min, 38 min, 175 min, and 8 h at 150, 125, 100, and 75°C, respectively. There is a linear relationship between calculated $L_{50}$s on a logarithmic scale and temperature. Therefore $FB_1$ is not significantly destroyed by the main drying processes of corn or thermal treatments used for its derivatives. Other associated means are required for detoxification.

**Journal of Food Protection - Volume 61, Issue 8, August 1998, Pages 1030-1033**

**Stability of fumonisins in thermally processed corn products**

**Castelo, M.M. Sumner, S.S. Bullerman, L.B.**

Abstract

Little is known about the stability of fumonisins in corn-based foods during heating. This study investigated the effects of canning, baking, and roasting (dry heating) processes on the stability of fumonisins in artificially contaminated and naturally contaminated corn-based foods. All samples were analyzed for fumonisin levels by both a commercial enzyme-linked immunosorbent assay (ELISA) and a high-performance liquid chromatographic (HPLC) method. Canned whole-kernel corn showed a significant ($P \leq 0.05$) decrease in fumonisins by both ELISA (15%) and HPLC (11%) analyses. Canned cream-style corn and baked corn bread showed significant ($P \leq 0.05$) decreases in fumonisin levels at an average rate of 9% and 48%, respectively, as analyzed by ELISA. Corn-muffin mix artificially contaminated with 5 µg of fumonisin $B_1$ ($FB_1$) per g and naturally contaminated corn-muffin mix showed no significant ($P \leq 0.05$) losses of fumonisins upon baking. Roasting cornmeal samples artificially contaminated with 5 µg of $FB_1$ per g and naturally contaminated cornmeal samples at 218°C for 15 min resulted in almost complete loss of fumonisins.

**European Food Research and Technology - Volume 213, Issue 3, 2001, Pages 187-193**

**Investigations on the change of fumonisin content of maize during hydrothermal treatment of maize. Analysis by means of HPLC methods and ELISA**

**Meister, U.**

Abstract

The study was subjected to the investigation of the effects of extrusion cooking, gelatinization, and cornflaking on the stability of fumonisins in artificially contaminated maize grits, spiked with fumonisin $B_1$ and $B_2$ at levels of 2 mg/kg and 0.6 mg/kg, respectively. All the processed samples were analyzed according to the AOAC-HPLC method, and some selected samples were analyzed additionally by a commercial enzyme-linked immunosorbent assay (ELISA) and after alkaline hydrolysis. All the samples showed significant decreases of the fumonisin levels. If analyzed according to AOAC-HPLC method, cooking extrusion and gelatinization reduced fumonisin levels to approximately 30-55%, cooking the grits for flaking to approximately 20-65%, and roasting the flakes to approximately 6-35% (depending on the selected technological parameters). With ELISA the fumonisin contents were 15-50% and after alkaline hydrolysis 19-380% higher than with the AOAC-HPLC method. However, the fumonisin amount added before the technological tests could not be recovered in any of the samples.

### 3.2 Nixamilisation

**Journal of Nutrition - Volume 133, Issue 10, 1 October 2003, Pages 3200-3203**
**Total fumonisins are reduced in tortillas using the traditional nixtamalization method of Mayan communities**
**Palencia, E., Torres,O., Hagler, W., Meredith, F.I., Williams, L.D, Riley, R.T.**

Abstract
Fumonisin $B_1$ ($FB_1$) is a maize mycotoxin. In tortilla preparation, maize is treated with lime (nixtamalization), producing hydrolyzed $FB_1$ ($HFB_1$) due to loss of the tricarballylic acid side chains. This study determined the following: 1) whether nixtamalization by Mayan communities reduces total fumonisins, and 2) the steps in the process at which reduction occurs. Tortillas prepared by the traditional process contained $FB_1$, $FB_2$ and $FB_3$ and their hydrolyzed counterparts. There were equimolar amounts of $FB_1$ and HFB $_1$ in the tortillas, but the total fumonisins were reduced 50%. The total $FB_1$ plus $HFB_1$ in the residual lime water and water washes of the nixtamal accounted for 50% of the total $FB_1$ in the uncooked maize. $HFB_1$ and $FB_1$ were present in a 1:1 mol/L ratio in the water washes of the nixtamal, the masa dough and the cooked tortillas, whereas the ratio of $HFB_1$:$FB_1$ in lime water after steeping was 21. Water washes contained 11% of the $FB_1$ that was in the uncooked maize. The results show that the traditional method reduced the total fumonisins in tortillas and reduced the sphinganine elevation (a biomarker closely correlated with fumonisin toxicity) in cells treated with extracts of tortillas compared with cells treated with extracts of contaminated maize.

**Bulletin of Environmental Contamination and Toxicology - 2002 (October), 69 (4), 471-478**
**Cortez-Rocha M.O., Trigo-Stockli D.M., Wetzel D.L., Reed C.R**.

Abstract
Because the mycotoxin fumonisin B1 (FB1) has been found in tortilla chips and masa, there is a need to evaluate the extrusion processing of alkali-cooked corn. The effects of moisture content and die configuration during extrusion processing on the occurrence of FB1 and hydrolysed FB1 (HFB1) in alkali-cooked corn were investigated. The amounts of moisture during processing significantly affected the levels of FB1 and HFB1 in the extruded product. Extrusion processing reduced the recoverable FB1 and HFB1 in alkali-cooked contaminated corn flour.

### 3.3 Cooking (Baking/Frying)

**Journal of Agricultural and Food Chemistry - Volume 49, Issue 6, 2001, Pages 3120-3126**
**Fate of fumonisins during the production of fried tortilla chips**
**Voss, K.A., Poling, S.M., Meredith, F.I., Bacon, C.W., Saunders, D.S.**

Abstract
The fate of fumonisin $B_1$ ($FB_1$), a mycotoxin found in corn, during the commercial manufacture of fried tortilla chips was studied. $FB_1$ and hydrolyzed $FB_1$ ($HFB_1$) concentrations in four lots of corn and in the masa, other intermediates, liquid and waste by-products, and fried chips were determined by HPLC. $FB_1$ concentrations in the masa and chips were reduced significantly, up to 80% in the fried chips, compared to that in the raw corn. $HFB_1$ was also found in the masa and chips, but

at low concentrations compared to $FB_1$. LC-MS analyses corroborated HPLC findings and further showed the presence of partially hydrolyzed $FB_1$ ($PHFB_1$), which, like $HFB_1$, was formed during the nixtamalization (cooking/steeping the corn in alkaline water to make masa) step and found predominantly in the cooking/steeping liquid and solid waste. No significant amounts of N-(carboxymethyl)-$FB_1$ or N-(1-deoxy-D-fructos-1-yl)-$FB_1$, indicative of fumonisin - sugar adduct formation, were found. Thus, $FB_1$ is removed from corn and diverted into liquid and waste by-products during the commercial production of fried tortilla chips. Nixtamalization and rinsing are the critical steps, whereas grinding, sheeting, baking, and frying the masa had little effect.

**Journal of Agricultural and Food Chemistry - Volume 45, Issue 12, December 1997, Pages 4800-4805**

**Effects of Baking and Frying on the Fumonisin $B_1$ Content of Corn-Based Foods**

**Jackson, L.S. Katta, S.K. Fingerhut, D.D. DeVries, J.W. Bullerman, L.B.**

Abstract
Fumonisins are mycotoxins produced primarily by Fusarium moniliforme and Fusarium proliferatum in corn. Fumonisins have been implicated as the causal agents in a variety of animal diseases and are epidemiologically linked to the high incidence of human oesophageal cancer in some regions of the world. Little is known about the effects of common processing methods on the fumonisin content of food. The objective of this study was to determine the effects of baking and frying on the stability of fumonisin $B_1$ ($FB_1$) spiked into corn-based foods. Baking corn muffins spiked with 5 µg/g (dry weight basis) $FB_1$ at 175 and 200 °C for 20 min resulted in 83.7 ± 3.5% and 72.4 ± 5.9% retention of $FB_1$, respectively. At both temperatures, losses of $FB_1$ were significantly ($p < 0.05$) greater at the surface than at the core of the muffins. No significant losses of $FB_1$ were found when spiked corn masa was fried at 140-170 °C for 0-6 min. $FB_1$ began to degrade at frying temperatures ≥180 °C and times ≥8 min. Frying chips for 15 min at 190 °C resulted in 67% loss of $FB_1$. These processing studies suggest that fumonisins are heat stable compounds that survive under most conditions used during baking or frying.

## 3.4 Extrusion

**Journal of Food Science - Volume 63, Issue 4, 1998, Pages 696-698**

**Extrusion cooking reduces recoverability of fumonisin $B_1$ from extruded corn grits**

**Castelo, M.M. Katta, S.K. Sumner, S.S., Hanna, M.A., Bullerman, L.B**

Abstract
A split-split plot design was used to determine the effects of extrusion cooking on the recoverability of the mycotoxin Fumonisin $B_1$ ($FB_1$). Unextruded and extruded samples of spiked corn grits were analyzed for $FB_1$ by two methods, commercial enzyme linked immunosorbant assay (ELISA) and HPLC. Extrusion cooking resulted in more apparent loss of $FB_1$ with mixing screws than nonmixing screws. Losses of recoverable $FB_1$ ($p \leq 0.05$) were observed at 120°C and 160°C with the mixing screws. A linear increase in loss of recoverable $FB_1$ was observed (with the nonmixing screws) as the moisture content increased.

**Cereal Chemistry - Volume 76, Issue 1, January 1999, Pages 16-20**
**Effect of temperature and screw speed on stability of fumonisin $B_1$ in extrusion-cooked corn grits**
**Katta, S.K. Jackson, L.S. Sumner, S.S. Hanna, M.A. Bullerman, L.B.**

Abstract
Corn grits spiked with fumonisin $B_1$ ($FB_1$) at a level of 5 µg/g were extrusion cooked in a corotating twin-screw extruder at different temperatures (140, 160, 180, and 200°C) and screw speeds (40, 80, 120, and 160 rpm). Good recoveries of $FB_1$ were obtained from the nonextruded as well as the extruded grits by using high-performance liquid chromatography. Both the barrel temperature and the screw speed significantly ($P \leq 0.05$) affected the extent of fumonisin reduction in extruded grits. As expected, the $FB_1$ recovered decreased with an increase in temperature and a decrease in screw speed. The amount of $FB_1$ lost from cooking grits at the different extrusion parameters used in this study ranged from 34 to 95%. About 46-76% of the spiked $FB_1$ was lost when the grits were cooked at temperatures and screw speeds that resulted in acceptable product expansion and colour.

## 4. Fumonisin toxicity/metabolism (in relation to breakdown products)

| **Key points** |
| --- |
| Toxicity of hydrolysed fumonisins studied.<br>Fumonisin-matrix, and hydrolysed fumonisin-matrix binding affects on toxicity, reduction in bioavailability and toxicity. |

**Molecular Nutrition and Food Research - Volume 51, Issue 9, September 2007, Pages 1120-1130**
**Hydrolyzed fumonisins $HFB_1$ and $HFB_2$ are acylated in vitro and in vivo by ceramide synthase to form cytotoxic N-acyl-metabolites**
**Seiferlein, M. Humpf, H.-U. Voss, K.A., Sullards, M.C., Allegood, J.C., Wang, E., Merrill Jr., A.H.**

Abstract
Fumonisins $B_1$ and $B_2$($FB_1$ and $FB_2$) are the most abundant members of the fumonisins - mycotoxins that are produced by Fusarium verticillioides and are natural inhibitors of ceramide synthase. Their hydrolyzed forms, $HFB_1$ and $HFB_2$ (also called $AP_1$ and $AP_2$) are found in some foods, and they are not only inhibitors of ceramide synthase but also undergo acylation by this enzyme. This study characterized the conversion of $HFB_1$ and $HFB_2$ by ceramide synthase to their respective N-acylated metabolites using rat liver microsomes and palmitoyl-CoA or nervonoyl-CoA as cosubstrates, and examined animals that had been dosed with hydrolyzed fumonisins to ascertain if acylation occurs in vivo. Using an HPLC-MS/MS method that allowed the sensitive and selective detection of the acylation products, both $HFB_1$ and $HFB_2$ were found to be metabolized in vitro to nervonoyl- or palmitoyl$HFB_1$ and -$HFB_2$ (i. e. $C_{24:1}$-$HFB_{1/2}$ and $C_{16}$-$HFB_{1/2}$, respectively). The apparent $v_{max}$ was considerably higher for formation of $C_{24:1}HFB_1$ (157 pmol/mm/mg protein) than for formation of $C_{16}HFB_1$ (8.7 pmol/min/mg protein). The acylation products also inhibited ceramide synthase and significantly reduced the number of viable cells in an in vitro [3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT)] assay using a human colonic cell line (HT29). Furthermore, HPLC-MS/MS analysis of tissues

from rats given intraperitoneal doses of HFBi confirmed that formation of N-acyl-HFB, occurs in vivo to produce metabolites with fatty acids of various chain lengths. The contribution of acylated $HFB_1$ and $HFB_2$ metabolites to fumonisin toxicity in vivo warrants further investigation.

## 4.1 Nixtamilisation

**Food and Chemical Toxicology - Volume 46, Issue 8, August 2008, Pages 2841-2848**

**Fumonisin concentrations and in vivo toxicity of nixtamalized Fusarium verticillioides culture material: Evidence for fumonisin-matrix interactions**

**Burns, T.D., Snook, M.E. Riley, R.T. Voss, K.A.**

Abstract

The toxic potential of nixtamalized foods can be underestimated if, during cooking, reversible fumonisin-food matrix interactions reduce the amount of mycotoxin that is detected but not the amount that is bioavailable. Fusarium verticillioides culture material (CM) was nixtamalized as is (NCM) or after mixing with ground corn (NCMC). Additional portions were sham nixtamalized without (SCM) or with corn (SCMC). Nixtamalization and sham nixtamalization reduced $FB_1$; CM, NCM, and SCM diets contained 9.08, 2.08, and 1.19 ppm, respectively. $FB_1$ was further reduced in the NCMC (0.49 ppm) but not the SCMC (1.01 ppm) diets compared to their NCM and SCM counterparts. Equivalent weights of the cooked products, uncooked CM, corn (UC) or nixtamalized UC (NUC) were fed to rats for up to three weeks. Kidney lesions in the NCM-fed group were less severe than in the CM-fed, positive control group and no lesions were found in the NCMC and other groups. Group kidney sphinganine (biomarker of fumonisin exposure) concentrations decreased in the order: CM (absolute concentration (nmol/g) = 600-800) > NCM (400-600) > SCM and SCMC (30-90) > NCMC, UC and NUC (<8). Together, these results suggest that mycotoxin-corn matrix interactions during nixtamalization reduce the bioavailability and toxicity of $FB_1$.

**Journal of Agricultural and Food Chemistry - Volume 48, Issue 11, 2000, Pages 5781-5787**

**Effect of nixtamalization (alkaline cooking) on fumonisin-contaminated corn for production of masa and tortillas**

**Dombrink-Kurtzman, M.A. , Dvorak, T.J. Barron, M.E., Rooney, L.W.**

Abstract

Studies were undertaken to determine the fate of the mycotoxins, fumonisins, during the process of alkaline cooking (nixtamalization), using normal-appearing corn that was naturally contaminated with fumonisin $B_1$ ($FB_1$) at 8.79 ppm. Corn was processed into tortillas, starting with raw corn that was cooked with lime and allowed to steep overnight; the steeped corn (nixtamal) was washed and ground into masa, which was used to make tortillas. Calculations to determine how much of the original fumonisn remained in the finished products took into consideration $FB_1$ will be converted to hydrolyzed fumonisin $B_1$ ($HFB_1$) by the process of alkaline cooking. All fractions, including steeping and washing water, were weighted, and percent moisture and fumonisin content were determined. Tortillas contained approximately 0.50 ppm of $FB_1$, plus 0.36 ppm of $HFB_1$, which represented 18.5% of the initial $FB_1$ concentration. Three-fourths of the original amount of fumonisin

was present in the liquid fractions, primarily as $HFB_1$. Nixtamalization significantly reduced the amount of fumonisin in maize.

**Molecular Nutrition and Food Research - Volume 48, Issue 4, September 2004, Pages 255-269**
**Effects of thermal food processing on the chemical structure and toxicity of fumonisin mycotoxins**
**Humpf, H.-U., Voss, K.A.**

Abstract
Fumonisins are Fusarium mycotoxins that occur in corn and corn-based foods. They are toxic to animals and at least one analogue, fumonisin $B_1$, is carcinogenic to rodents. Their effect on human health is unclear, however, fumonisins are considered to be risk factors for cancer and possibly neural tube defects in some heavily exposed populations. It is therefore important to minimize exposures in these populations. Cleaning corn to remove damaged or mouldy kernels reduces fumonisins in foods while milling increases their concentration in some and reduces their concentration in other products. Fumonisins are water-soluble and nixtamalization (cooking in alkaline water) lowers the fumonisin content of food products if the cooking liquid is discarded. Baking, frying, and extrusion cooking of corn at high temperatures (≥190°C) also reduces fumonisin concentrations in foods, with the amount of reduction achieved depending on cooking time, temperature, recipe, and other factors. However, the chemical fate of fumonisins in baked, fried, and extruded foods is not well understood and it is not known if the reduced concentrations result from thermal decomposition of fumonisins or from their binding to proteins, sugars or other compounds in food matrices. These possibilities might or might not be beneficial depending upon the bioavailability and inherent toxicity of decomposition products or the degree to which bound fumonisins are released in the gastrointestinal tract. In this review the affects of cooking and processing on the concentration and chemical structure of fumonisins as well as the toxicological consequences of known and likely fumonisin reaction products are discussed.

**Food and Chemical Toxicology - Volume 44, Issue 2, February 2006, Pages 161-169**
**Effects of aminopentol on in utero development in rats**
**Collins, T.F.X., Sprando, R.L., Black, T.N., Olejnik, N., Eppley, R.M., Shackelford, M.E., Howard, P.C., Rorie, J.I., Bryant, M., Ruggles, D.I.**

Abstract
Aminopentol (AP1), the backbone and main hydrolysis product of the mycotoxin fumonisin B1 (FB1), is present in corn-based foods which are consumed daily as a substantial part of the diet in some areas of the world. The toxicity of FB1 has been attributed to altered sphingolipid metabolism, but the toxicity of AP1 is less certain. Epidemiological correlations and in vitro studies have suggested that AP1 can increase neural tube defects (NTDs), but no in vivo developmental study of AP1 was done prior to this study. AP1 was given once daily to rats by gavage on gestation days (GD) 3-16 at doses of 0, 15, 30, 60, or 120 mg/kg. Reproductive and developmental parameters were measured at GD 17, one day after the last dose, and on GD 20. In addition, on GD 17, maternal and fetal tissues were analyzed for sphingolipid content. Conclusions: AP1 reduced dam body weight gain, but was less toxic than FB1. AP1 was not teratogenic, did not affect tissue

sphingolipid ratios, did not alter reproduction or development of foetuses, and produced no dose-related histopathological effects in dams.

## 5. Analytical methodology
## (note not all multi toxin methods included)

| **Key points** |
| --- |
| • Further reports on the instability of fumonisins during analysis.<br>• Simultaneous analysis of parent and hydrolysed fumonisins. |

**The Journal of AOAC International - 1994 Mar-Apr; 77(2):541-5.**
**Stability and problems in recovery of fumonisins added to corn-based foods.**
**Scott, P.M., Lawrence, G.A.**

Abstract
Because the natural occurrence of fumonisins is so far known almost exclusively in corn, we have limited our investigations on their stability to corn-based foods. In these studies, distinction must be made between real losses, binding, and any matrix-related method problems. Fumonisins B1 (FB1) and B2 (FB2) were about 40% recovered when heated in corn meal at 190 degrees C, about 20-30% recovered when heated in moist corn meal at 190 degrees C, and completely unstable in corn meal at 220 degrees C. Average recoveries of FB1 and FB2 added to blank heated matrixes were 69-107% in control experiments. Baking corn meal muffins spiked with 2.5 micrograms FB1 and FB2/g corn meal at 220 degrees C also resulted in losses of fumonisins. Little or no fumonisins were recovered from corn bran flour when methanol-water (3 + 1) was used as extraction solvent. However, when methanol-borate buffer (pH 9.2) (3 + 1) was used, recoveries averaged 91 +/- 17 and 84 +/- 9%, respectively, for FB1 and FB2; and natural contamination of the corn bran flour with FB1 and FB2 at levels of 1.9 and 0.95 microgram/g, respectively, was revealed. Comparable recoveries were observed for 1 brand of a corn bran breakfast cereal, but the binding effect was not seen with a second brand, for which methanol-water (3 + 1) alone was a good extraction solvent. Recoveries of FB1 and FB2 from a mixed cereal for babies were only about 50% with either extraction solvent mixture.

**Analytical and Bioanalytical Chemistry - 2009, Pages 1-11**
**Difficulties in fumonisin determination: the issue of hidden fumonisins**
**Dall'Asta, C., Mangia, M., Berthiller, F., Molinelli, A., Sulyok, M., Schuhmacher, R., Krska, R., Galaverna, G., Dossena, A., Marchelli, R.**

Abstract
In this paper, the results obtained by five independent methods for the quantification of fumonisins $B_1$, $B_2$, and $B_3$ in raw maize are reported. Five naturally contaminated maize samples and a reference material were analyzed in three different laboratories. Although each method was validated and common calibrants were used, a poor agreement about fumonisin contamination levels was obtained. In order to investigate the interactions among analyte and matrix leading to this lack of consistency, the occurrence of fumonisin derivatives was checked. Significant amounts of hidden fumonisins were detected for all the considered samples. Furthermore, the application of an in vitro digestion protocol to raw maize allowed for a higher recovery of native fumonisins, suggesting that the

interaction occurring among analytes and matrix macromolecules is associative rather than covalent. Depending on the analytical method as well as the maize sample, only 37-68% of the total fumonisin concentrations were found to be extractable from the samples. These results are particularly impressive and significant in the case of the certified reference material, underlying the actual difficulties in ascertaining the trueness of a method for fumonisin determination, opening thus an important issue for risk assessment.

**Food Chemistry 112 (4), pp. 1031-1037 (2009)**
**Analysis of fumonisin in corn-based food by liquid chromatography with fluorescence and mass spectrometry detectors**
**Silva, L., Fernández-Franzón, M. , Font, G., Pena, A. Silveira, I. Lino, C., Mañes, J.**

Abstract
The presented procedure involves an extraction with methanol-water, centrifugation and cleanup with immunoaffinity columns. A comparison study between fluorescence detector, mass spectrometry, and tandem mass spectrometry with a triple quadrupole (QqQ) analyzer using an electrospray ionisation interface for the determination of fumonisin $B_1$ and $B_2$ in corn-based products has been performed. Limits of quantification obtained by the three detectors were lower than the maximum levels established by European Commission. Liquid chromatography coupled to tandem mass spectrometry provides higher sensitivity (12 µg kg-1for fumonisins B1 and B2) when compared to mass spectrometry (40 µg kg-1for both fumonisins), and fluorescence detection (20 µg kg-1 for fumonisin B1 and 15 µg kg-1 for B2), and also showed to be more precise. At 150 and 250 µg kg-1 spiking levels, the recovery rates for fumonisin B1 and B2 in corn products varied from 79% to 102%, with a relative standard deviation ranging from 9% to 17%. A critical assessment including advantages and drawbacks of each technique is presented. A total of 41 organic and non-organic corn-based food samples from Valencia markets were analyzed. Seven samples were contaminated with levels ranging from 68 µg kg-1 to 922 µg kg-1of fumonisin B1 and 42 µg kg-1 to 640 µg kg-1of fumonisin B2. Only one sample exceeded the maximum level for the sum of fumonisin B1 and B2, proposed for corn products in a recent EU regulation. The contamination frequency of organic corn samples (40%) was higher than non-organic ones (3.7%), and contained higher levels of fumonisin B1 and B2.

**Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences** - **Volume 819, Issue 1, 5 May 2005, Pages 97-103**
**Simple method for the simultaneous isolation and determination of fumonisin $B_1$ and its metabolite aminopentol-1 in swine liver by liquid chromatography-fluorescence detection**
**Pagliuca, G., Zironi, E. Ceccolini, A., Matera, R., Serrazanetti, G.P., Piva, A.**

Abstract
An analytical method based on high-performance liquid chromatography (HPLC) combined with fluorescence detection (FL) has been developed for the simultaneous determination of fumonisin $B_1$ ($FB_1$) and its totally hydrolized metabolite aminopentol-1 ($AP_1$) in pig liver. The sample preparation is based on a single solid phase extraction (SPE). o-Phthalaldehyde (OPA) was used for pre-column derivatization before the programmed reversed-phase analysis on

phenylhexyl column. The developed method shows good repeatibility for inter- and intra-day precision as well as adequate linearity of calibration curves ($r^2$ was 0.9855 for $FB_1$ and 0.9831 for $AP_1$). Average recoveries from the matrix were 93.6% for $FB_1$ and 95.3% for $AP_1$. The limit of quantification (LOQ) in swine liver was 75 µg/kg for $FB_1$ and 42 µg/kg for $AP_1$.

**Journal of Chromatography A - Volume 1203, Issue 1, 29 August 2008, Pages 88-93**

**Development of a new analytical method for the determination of fumonisins $B_1$ and $B_2$ in food products based on high performance liquid chromatography and fluorimetric detection with post-column derivatization**

**Muscarella, M., Magro, S.L. Nardiello, D, Palermo, C., Centonze, D.**

Abstract
A sensitive and selective analytical method was developed for the quantitative determination of fumonisins $B_1$ and $B_2$ in maize-based foods for direct human consumption. The method, based on high-performance liquid chromatography and fluorescence detection, presents a rapid and automated on-line post-column derivatization, performed with o-phtalaldehyde and N,N-dimethyl-2-mercaptoethylamine. Several factors affecting the separation and detection of fumonisins were investigated, including mobile phase composition, column features, derivatization agent flow-rate and both the excitation and the emission wavelengths. Optimal fluorescence detection was obtained by using a $\lambda_{exc}$ of 343 nm and a $\lambda_{em}$ of 445 nm. Under the optimized experimental conditions, a complete separation of fumonisins was obtained in less than 13 min by using a $C_{18}$ column and a gradient elution at 0.8 mL/min with methanol and 0.1 M phosphate buffer at pH 3.15. The limits of detection for $FB_1$ and $FB_2$ were 4 and 5 µg/L corresponding to 5 and 6 µg/kg in matrix. Each fumonisin was determined in the range 40-320 µg/L that corresponds to 50-400 µg/kg in matrix. The necessary requirements for accuracy, reproducibility and sensitivity were fulfilled and recovery values ranged from 87 to 94% for $FB_1$ and from 70 to 75% for $FB_2$ in cornflake samples at three fortification levels in the range 100-300 µg/kg. The potential of this method, combined with a simple clean-up procedure, was assessed by the measurements of $FB_1$ and $FB_2$ in maize-based products, such as maize flour, "polenta", tortillas and cookies.

**Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment Volume 25, Issue 4, April 2008, Pages 472-489**

**LC-MS/MS multi-method for mycotoxins after single extraction, with validation data for peanut, pistachio, wheat, maize, cornflakes, raisins and figs**

**Spanjer, M.C., Rensen, P.M., Scholten, J.M.**

Abstract
Mycotoxin analysis is usually carried out by high performance liquid chromatography after immunoaffinity column cleanup or in enzyme-linked immunosorbent assay tests. These methods normally involve determination of single compounds only. EU legislation already exists for the aflatoxins, ochratoxin A and patulin in food, and legislation will come into force for deoxynivalenol, zearalenone and the fumonisins in 2007. To enforce the various legal limits, it would be preferable to determine all mycotoxins by routine analysis in different

types of matrices in one single extract. This would also be advantageous for HACCP control purposes. For this reason, a multi-method was developed with which 33 mycotoxins in various products could be analysed simultaneously. The mycotoxins were extracted with an acetonitrile/water mixture, diluted with water and then directly injected into a LC-MS/MS system. The mycotoxins were separated by reversed-phase HPLC and detected using an electrospray ionisation interface (ESI) and tandem MS, using MRM in the positive ion mode, to increase specificity for quality control. The following mycotoxins could be analysed in a single 30-min run: Aflatoxins $B_1$, $B_2$, $G_1$ and $G_2$, ochratoxin A, deoxynivalenol, zearalenone, T-2 toxin, HT-2 toxin, α-zearalenol, α-zearalanol, β-zearalanol, sterigmatocystin, cyclopiazonic acid, penicillic acid, fumonisins $B_1$ $B_2$ and $B_3$, iacetoxyscirpenol, 3- and 15-acetyl-deoxynivalenol, zearalanone, ergotamin, ergocornin, ergocristin, α-ergocryptin, citrinin, roquefortin C, fusarenone X, nivalenol, mycophenolic acid, alternariol and alternariol monomethyl ether. The limit of quantification for the aflatoxins and ochratoxin A was 1.0 μg $kg^{-1}$ and for deoxynivalenol 50 μg $kg^{-1}$. The quantification limits for the other mycotoxins were in the range 10-200 μg $kg^{-1}$. The matrix effect and validation data are presented for between 13 and 24 mycotoxins in peanuts, pistachios, wheat, maize, cornflakes, raisins and figs. The method has been compared with the official EU method for the determination of aflatoxins in food and relevant FAPAS rounds. The multi-mycotoxin method has been proven by the detection of more than one mycotoxin in maize, buckwheat, figs and nuts. The LC-MS/MS technique has also been applied to baby food, which is subject to lower limits for aflatoxin $B_1$ and ochratoxin A, ergot alkaloids in naturally contaminated rye and freeze-dried silage samples.

**Journal of Chromatography A - Volume 1209, Issue 1-2, 31 October 2008, Pages 188-194**

**Analysis of fumonisins $B_1$, $B_2$ and $B_3$ in corn-based baby food by pressurized liquid extraction and liquid chromatography/tandem mass spectrometry**

**D'Arco, G. Fernández-Franzón, M.[a], Font, G., Damiani, P. Mañes, J.**

Abstract

A sensitive and reliable method using pressurized liquid extraction (PLE) and liquid chromatography (LC)/electrospray ionization (ESI) tandem mass spectrometry with a triple quadrupole (QqQ) analyzer has been developed for the analysis of fumonisin $B_1$ ($FB_1$), fumonisin $B_2$ ($FB_2$) and fumonisin $B_3$ ($FB_3$) in corn-based baby foods. Influence of several extraction parameters that affect PLE efficiency such as temperature, pressure, solvent extraction, number of cycles and dispersant/clean-up agents were studied. The selected PLE operating method was: 3 g of sample was packed into 11 ml stainless-steel cell and fumonisins were extracted with methanol at 40 °C, 34 atm in one cycle of 5 min at 60% flush. The analytes were ionized in ESI operating with positive ion mode and identified by selecting two monitoring transitions, permitting quantification and confirmation in a single injection. Recoveries ranged from 68% to 83% at fortification levels of 200 μg $kg^{-1}$ with relative standard deviation (RSD) from 4% to 12%. The limits of quantification were from 2 μg $kg^{-1}$ for $FB_1$ and $FB_2$, and 5 μg $kg^{-1}$ for $FB_3$, which are below the maximum residue level established by the European Union legislation in infant formulas. The proposed method was successfully applied to the analysis of twenty seven samples of baby food products collected from different markets, and one positive sample with a content of 15.9 μg $kg^{-1}$ for $FB_1$, 9.2 μg $kg^{-1}$for $FB_2$ and 5.8 μg $kg^{-1}$ for $FB_3$ was obtained. Given the simplicity and potential of the proposed procedure, its application for safety control is recommended. 

**Journal of Agricultural and Food Chemistry - Volume 41, Issue 10, 1993, Pages 1655-1658**
**Detection of fumonisins $B_1$, $B_2$, and $B_3$ and hydrolyzed fumonisin $B_1$ in corn-containing foods**
**Hopmans, E.C., Murphy, P.A.**

Abstract
Selected corn-containing foods were analyzed for the presence of fumonisins $B_1$, $B_2$, and $B_3$ ($FB_1$, $FB_2$, and $FB_3$) and hydrolyzed fumonisin $B_1$ ($HFB_1$). Samples were extracted with $H_2O/CH_3CN$ (1:1). Following a cleanup procedure using a $C_{18}$ SPE cartridge, analytical reversed-phase HPLC and fluorometric detection of the o-phthaldialdehyde derivatives were performed. Detection limits were 25 and 50 ng/mL for the $FB_1$ and $FB_2$ standards, respectively. $FB_1$ recovery for three tested levels, ranging from 250 to 1000 ng/g, was 115%. $FB_1$ and $FB_2$ contents ranged from 17 to 1410 ppb and from 0 to 414 ppb in foods, respectively. $FB_3$ was detected in 10 of 13 foods. $HFB_1$ was detected in tortilla chips, masa, and canned yellow corn.

**Food Additives and Contaminants - Volume 21, Issue 12, December 2004, Pages 1168-1178**
**Analysis of heat-processed corn foods for fumonisins and bound fumonisins**
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Abstract
Thirty retail samples of heat-processed corn foods, i.e. corn flakes, corn-based breakfast cereals, tortilla chips and corn chips, were analysed for fumonisins - fumonisin $B_1$ ($FB_1$), fumonisin $B_2$ ($FB_2$) and hydrolysed $FB_1$ ($HFB_1$) - as well as for protein- and total-bound $FB_1$. Bound (hidden) fumonisins cannot be detected by conventional analysis. Improved methods for the determination of bound $FB_1$ were developed. The protein-bound $FB_1$ was extracted with 1% sodium dodecylsulfate (SDS) solution. The SDS, which interfered with high-performance liquid chromatography (HPLC) analysis, was then separated from protein-bound $FB_1$ by complexing with methylene blue followed by solvent extraction and hydrolysis with 2 N KOH. To measure total-bound $FB_1$, the sample itself was hydrolysed with KOH. In both cases, clean-up was accomplished on an OASIS polymeric solid-phase extraction column and the bound fumonisins were determined by HPLC measurement of $HFB_1$. Fourteen of 15 samples of corn flakes and other corn-based breakfast cereals analysed contained detectable levels of $FB_1$ with a mean in positive samples of 67 ng $g^{-1}$ (13-237 ng $g^{-1}$). Two samples also had detectable levels of $FB_2$ (21-23 ng $g^{-1}$). Bound $FB_1$ was found in all samples; the mean protein-bound $FB_1$ measured was 58 ng $g^{-1}$ (22-176 ng $g^{-1}$) and the mean total-bound $FB_1$ measured was 106 ng $g^{-1}$ (28-418 ng $g^{-1}$), reported as $FB_1$ equivalents after correction for recoveries of $HFB_1$. There was an average of about 1.3 times more $FB_1$ in the bound form compared with extractable $FB_1$, and this was about twice as much as protein-bound $FB_1$. Seven of the 15 samples of alkali-processed corn-based foods, such as tortilla chips and corn chips, contained $FB_1$ and three contained $HFB_1$ with means in measurable positive samples of 78 (48-134) and

29 (13-47) ng $g^{-1}$, respectively. Five of these alkali-processed corn foods contained bound $FB_1$; the mean measurable protein-bound $FB_1$ was 42 ng $g^{-1}$ (39-46 ng $g^{-1}$) and the mean measurable total-bound $FB_1$ was 100 ng $g^{-1}$ (54-209 ng $g^{-1}$). $HFB_1$ derived from bound $FB_1$ in selected samples was confirmed by HPLC with mass spectrometry (MS).

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**Bound fumonisin B1: Analysis of fumonisin-B1 glyco and amino acid conjugates by liquid chromatography-electrospray ionization-tandem mass spectrometry**

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Abstract

To study the formation of fumonisin artefacts and the binding of fumonisins to matrix components (e.g., saccharides and proteins) in thermal-treated food, model experiments were performed. Fumonisin $B_1$ and hydrolyzed fumonisin $B_1$ were incubated with α-D-glucose and sucrose (mono- and disaccharide models), with methyl α-D-glucopyranoside (starch model), and with the amino acid derivatives N-α-acetyl-L-lysine methyl ester and BOC-L-cysteine methyl ester (protein models). The reaction products formed were analyzed by liquid chromatography-electrospray ionization-tandem mass spectrometry. The incubation of D-glucose with fumonisin B1 or hydrolyzed fumonisin B1 resulted in the formation of Amadori rearrangement products. Whereas conjugates were found following the reaction of sucrose, methyl α-D-glucopyranoside, and the amino acid derivatives with fumonisin B1, the heating with hydrolyzed fumonisin B1 yielded no artifacts. For structural determination, the stable reaction product formed by heating of methyl α-D-glucopyranoside (as starch model) with fumonisin B1 was purified and identified by nuclear magnetic resonance spectroscopy as the diester of the fumonisin tricarballylic acid side chains with methyl α-D-glucopyranoside. These model experiments demonstrate that fumonisins are able to bind to polysaccharides and proteins via their two tricarballylic acid side chains.