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**Fate of the fusarium mycotoxins fumonisins B1, B2 and B3, deoxynivalenol and zearalenone in maize flour and maize grits during extrusion cooking.**

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3 **Fate of the *fusarium* mycotoxins fumonisins B<sub>1</sub>, B<sub>2</sub> and B<sub>3</sub>,**  
4 **deoxynivalenol and zearalenone in maize flour and maize grits**  
5 **during extrusion cooking.**  
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30  
31 **Abstract**  
32

33 Extrusion technology is used widely to manufacture a range of breakfast cereals and  
34 snacks for human consumption and animal feeds. Deoxynivalenol (DON) and  
35 zearalenone (ZON) in cereals and cereal products and fumonisins B<sub>1</sub> and B<sub>2</sub> (FB<sub>1</sub> and  
36  
37 FB<sub>2</sub>) in maize are controlled in the European Union by legislation with the objective  
38  
39 of minimising consumer exposure to these mycotoxins. Relatively few studies have  
40  
41 examined the losses of *fusarium* mycotoxins during processing. The behaviour of FB<sub>1</sub>,  
42  
43 FB<sub>2</sub> and fumonisin B<sub>3</sub> (FB<sub>3</sub>), DON and ZON during extrusion of naturally  
44  
45 contaminated maize flour and maize grits has been examined here using pilot scale  
46  
47 equipment. Studies of these ingredients show that DON and ZON are mostly stable  
48  
49 during extrusion cooking and that the fumonisins are lost to varying degrees. There  
50  
51 does however appear to be some loss of ZON when present in low concentration and  
52  
53 extruded at higher moistures. The presence of additives such as reducing sugars and  
54  
55 sodium chloride can also affect mycotoxin levels. Moisture content of the cereal feed  
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3 during extrusion is an important factor and has a greater effect than temperature,  
4 particularly on the loss of fumonisins at the lower moistures. The effects appear  
5 complex and not always easy to explain although more energy input to the extruder is  
6 required for drier materials. However, on the basis of these studies, the relationship  
7 between the concentration of *fusarium* toxins in the raw product and the finished  
8 product vary depending on the toxin present and the process undertaken.  
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20 **Keywords:** *Fumonisin, deoxynivalenol, zearalenone, fusarium mycotoxins, maize,*  
21 *flour, grits, processing, extrusion*  
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## 27 Introduction

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32 Extrusion cooking technologies are used to manufacture many forms of foodstuff  
33 from cereals and other ingredients. The range of products includes breakfast cereals,  
34 snack foods, pregels (modified starches used in food products) and breading crumbs  
35 and also animal feeds. Ingredients such as maize flour and grits, wheat flour and other  
36 food components are passed through an extrusion cooker under pressure, mechanical  
37 shearing stresses and elevated temperature, and expand rapidly as they are forced  
38 through the outlet die (Riaz 2000, Guy 2001). During this process, mechanical energy  
39 is transformed into heat by viscous dissipation in the starch paste. This causes  
40 chemical bonds in the polymers to break and free radicals to form. Mycotoxins are  
41 naturally occurring contaminants and compounds such as FB<sub>1</sub>, FB<sub>2</sub> and FB<sub>3</sub>, DON  
42 and ZON are subjected both to high temperatures and pressures and to chemical  
43 reactions by free radical and other mechanisms and might thus be susceptible to  
44 breakdown or interaction with other components in the mixture. Temperatures inside  
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3 the barrel of an extruder can range from 100°C to 200°C and moisture content can be  
4  
5 used from 13 % to 30 % depending on the characteristics of the end product required.  
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8 Extrudates can be designed with different physical properties and  
9  
10 characteristics ranging from highly expanded products, which are used without further  
11  
12 processing, to high-density pellets that are subsequently fried in oil for a very short  
13  
14 time during which they expand rapidly. Extrudates are used as the basis for a wide  
15  
16 range of retail products  
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20 Results reported in the literature are limited and sometimes conflicting but this  
21  
22 may be because all the conditions used are either not reported or not carefully  
23  
24 controlled. For example, if chemical breakdown occurs during the extrusion process  
25  
26 the extent of any loss will be related to time, so that the reduction in mycotoxin  
27  
28 content will depend on the residence time of the material in the extruder, in turn  
29  
30 dependant on the material feed rate. The presence of minor ingredients, or additives,  
31  
32 may also have a significant effect (Accerbi et al. 1999). When sugars such as fructose  
33  
34 and glucose are present in corn-based foods they may react with the amino group of  
35  
36 FB<sub>1</sub> during cooking to form the N-(deoxy-D-fructos-yl-) FB<sub>1</sub> (Lu et al. 1997; Howard  
37  
38 et al. 1998). These details are often missing from reports.  
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44 Extrusion cooking has been shown to have variable effects on mycotoxins and  
45  
46 this has been reviewed recently (Castells et al. 2005, Scudamore et al. 2007).  
47  
48 Approximately 15 % of aflatoxins B<sub>1</sub> and B<sub>2</sub> survived extrusion at 150°C when spiked  
49  
50 corn dough was treated (Martinez and Monsalve, 1989). However, in a study by  
51  
52 Cazzaniga, et al. (2001) in which the effects of flour moisture content, temperature  
53  
54 and addition of sodium metabisulphite were examined, the reduction of aflatoxin B<sub>1</sub>  
55  
56 was only between 10 and 25 % for samples of corn flour experimentally contaminated  
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58 with aflatoxin B<sub>1</sub> at 50 µg kg<sup>-1</sup>. Some loss of ochratoxin A, a mycotoxin formed in  
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3 grain after harvest, was found during pilot scale extrusion, although loss below 150°C  
4 was relatively small (Scudamore et al. 2004). Saunders et al. (2001) showed that  
5 extrusion of dry-milled maize products reduced fumonisin concentrations by 30–90 %  
6 for mixing type extruders and by 20–50 % for non-mixing extruders. Katta et al.  
7 (1998) examined the extrusion of corn grits on the stability of FB<sub>1</sub> using different  
8 temperatures and screw speeds. Both the barrel temperature and screw speed affected  
9 the loss of fumonisin in the extruded product with losses ranging from 34 to 95 %.  
10 However, the loss ranged from 46–76 % in those products with expansion and colour  
11 acceptable for commercial use. Wolf-Hall et al. (1999) showed DON to be quite  
12 stable in heat-treated foods when the effects of high temperature and high pressure  
13 processing of foods spiked with DON were examined.  
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29 Reports from other workers have shown that the *fusarium* mycotoxins can also  
30 degrade at high temperatures. The review by Castells et al. (2005) suggested that FB<sub>1</sub>  
31 was degraded with increasing temperatures from 100°C to give losses of 50 to 92 % at  
32 temperatures above 160°C. The temperatures were only approximate measures of the  
33 barrel temperatures in most cases and the effects of moisture were not separated from  
34 the frictional effects. FB<sub>2</sub> was degraded in a similar manner to FB<sub>1</sub> with high losses at  
35 160°C and up to 100 % at 180°C. Experiments with DON were more extensive, and  
36 results complicated by the use of metabisulphite and problems with the analytical  
37 procedures.  
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50 The current study continues work by the authors examining the fate of  
51 *fusarium* mycotoxins during milling and selected food processing over a wide range  
52 of possible extrusion cooking conditions. The fate of DON, NIV and ZON in wheat  
53 flour during extrusion showed earlier that temperature and moisture were important  
54 variables for degrading mycotoxins (Scudamore et al. 2008). Here we examine the  
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3 loss of mycotoxins from maize grits when extruded to form directly expanded  
4 products and in maize flour either directly expanded or under conditions that produce  
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6 solid pellets that are then subsequently fried in oil when they rapidly expand. This  
7  
8 study concentrates on snack food-like products (Guy 2001) made from a basic  
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10 feedstock of maize grits, and maize flour.  
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## 16 17 **Materials and methods**

### 18 19 *Maize grits and maize flour containing mycotoxins*

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22 Two commercial consignments identified as containing mycotoxins suitable for  
23  
24 carrying out the current studies were obtained from UK maize mills. One consisted of  
25  
26 250 kg of maize grits and the other 400 kg of maize flour. The grits had been milled  
27  
28 from Argentine maize and consisted of a product with mean particle size between 500  
29  
30 and 600  $\mu\text{m}$  (micron). The maize flour had been milled from French maize giving a  
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32 product with approximately 70 % of the particles between 150 and 500  $\mu\text{m}$  and about  
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34 30% finer particles.  
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41 Mycotoxin concentrations found in maize grits are usually low in comparison  
42  
43 to those in the whole maize because they are formed in the starchy endosperm of the  
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45 maize seed that make up the grits on milling. Several consignments of grits from  
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47 commercial mills were examined before a suitable 1 tonne consignment was found to  
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49 contain more than 100  $\mu\text{g kg}^{-1}$  DON and 250  $\mu\text{g kg}^{-1}$  of fumonisins, but no ZON. In  
50  
51 the same way a number of consignments of maize flour were examined before one  
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53 with a relatively high level of fumonisins ( $\sim 3000 \mu\text{g kg}^{-1}$  of  $\text{FB}_1 + \text{FB}_2$ ), a low level of  
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55 ZON and no DON was intercepted.  
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3 Because the quantity of the naturally contaminated test materials exceeded the  
4 capacity of the blender available, each sample was divided into several lots, blended  
5 for two hours and placed in a series of polythene bags each containing about 20 kg as  
6 described previously for a study on milling and baking (Scudamore et al. 2003). The  
7 maize ingredients in these bags were individually mixed again and a series of random  
8 samples of about 250 g were taken for mycotoxin analysis for FB<sub>1</sub>, DON and ZON.  
9 The contents of all the individual bags were finally recombined and re-mixed (in  
10 several lots) and then stored in batches of 65-70 kg for convenient handling and  
11 eventual transfer to the extruder during the study. Individual batches of maize were  
12 re-blended and sampled as necessary. After a final blending, samples from each batch  
13 of either grits or flour were analysed again for mycotoxin content to determine the  
14 values for % standard deviation (SD) of the replicate samples.  
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32 The moisture content of the grits was about 13.2 and 13.5 % for maize flour.  
33 Additional mixes were prepared to examine the effect of common food additives on  
34 mycotoxin stability. These were 2 % added salt (sodium chloride) and 2 % sugar  
35 (sucrose) added to the maize grits and 1 % or 2 % salt and 5 % and 10 % dextrose  
36 (glucose) added to the maize flour.  
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#### 46 *Extruder and processing conditions*

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48 A pilot-scale twin-screw extrusion cooker model MPF 50D (supplied by Baker  
49 Perkins, Peterborough, UK) was prepared to reproduce conditions simulating  
50 commercial manufacturing processes. Within the extruder body, an arrangement of  
51 screws comprised of small elements formed sections for conveying cereal, mixing in  
52 water to achieve the required moisture conditions, reverse pumping in a shear zone  
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3 and final high pressure pumping. The extruder was calibrated for flow rate and  
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5 moisture prior to setting up each condition required for extrusion cooking.  
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8 There are a number of possible different combinations of parameters that can  
9  
10 be examined during extrusion cooking. To reduce the number of these, the feed rate of  
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12 the material was standardised to  $700 \text{ g min}^{-1}$ . except when examining pellet formation  
13  
14 in which case it was  $550 \text{ g min}^{-1}$ . Conditions using combinations of up to 3  
15  
16 temperatures and 4 moisture conditions were examined. In an initial trial, the effect of  
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18 screw speed was determined and this then subsequently standardised to 300 rpm so  
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20 limiting the number of combinations required for study.  
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24 Each extrusion run involved a sequential change in extruder conditions, The  
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26 extruder was run up to a steady state at each change over and held for 2 minutes to  
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28 stabilise the extrusion before a sample was collected over 2.5 minutes thus  
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30 representing about 1.5 to 2 kg of extrudate. This was allowed to cool before packing.  
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32 Logging data was taken to confirm the target settings. Data were recorded (but not all  
33  
34 shown here) for temperature,  $^{\circ}\text{C}$ , feed rate,  $\text{g min}^{-1}$ , SME (specific mechanical  
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36 energy),  $\text{kJ kg}^{-1}$ , pressure, MPa, extrudate moisture content, %, extrudate specific  
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44  $\text{L kg}^{-1}$ .  
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#### 48 *Direct extrusion cooking*

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50 The arrangement of the screw elements for direct extrusion cooking has been given  
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52 previously (Scudamore et al. 2007). There is a section of feed screws conveying the  
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54 maize feedstock into a short mixing section of paddles where water is injected. A  
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56 second conveying section forces the mixture into a reversing paddles section.  
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58 Resistance from the reversing paddles causes the section to fill and generate a  
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3 pressure via the twin start screws. This in turn generates heat and melts the crystalline  
4 structures in the starch granule so that a melt fluid forms. In the final screw section the  
5 hot fluid is sealed under pressure and the water superheated to  $> 140^{\circ}\text{C}$  and as it  
6 forced through a die it returns to atmospheric pressure outside the barrel when the  
7 water vaporises rapidly with the immediate expansion of the extrudate.  
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15 In this work, the die plate contained two circular dies of 4 mm diameter and 4  
16 mm land length. At the start of the extrusion cooking run the barrel heaters were set at  
17 0/50/70/120/140 $^{\circ}\text{C}$  and adjusted to obtain a mass temperature of  $140 \pm 5^{\circ}\text{C}$  in the shear  
18 zone and final pumping section. Subsequently the barrel temperature was raised in the  
19 last two sections to obtain mass temperatures of  $160^{\circ}\text{C}$  and then  $180 \pm 5^{\circ}\text{C}$ . The  
20 powder feed rates and water were adjusted to the computed settings required to run at  
21  $700 \pm 5$  g/min and moisture levels from that of the raw material up to  $21 \pm 1$  % w/w. In  
22 an initial trial the screw speed was adjusted from 200 to  $400 \pm 1$  rpm but subsequently  
23 standardised at 300 rpm.  
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### 39 *Pellet extrusion cooking*

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41 A different configuration was used for extrusion cooking with pellet formation. This  
42 comprised a conveying section of feed screws leading to a water injection point and a  
43 short cooking section with reversing paddles to heat the flour to  $110\text{-}120^{\circ}\text{C}$  and to  
44 melt the crystalline structures in the starch. After this heating section, cooling was  
45 applied gradually to reduce the mass temperature to  $90\text{-}95^{\circ}\text{C}$  before extrusion through  
46 the 6mm dies. The extrudate was cut with a 4-blade knife at 1100 rpm into disc of  
47 approximately 1.5mm thickness. After drying at  $60^{\circ}\text{C}$  for 1-2h, samples (500 g) were  
48 ground for analysis. A second portion (500 g) of dried extrudate was fried in  
49 vegetable oil at  $185 \pm 5^{\circ}\text{C}$  for 20 seconds, drained and ground for analysis.  
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3 Here, die plates contained two circular dies of 6mm diameter and 4mm land  
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5 length. At the start of the extrusion cooking run the barrel heaters were set at  
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7 20/90/120/50/30°C and adjusted to heat and then cool the feedstock using the six  
8  
9 sections and circulating water set at 10°C. The exit temperatures were varied from 90  
10  
11  $\pm 2$  to 95  $\pm 2$  %. The powder and water feed rates were adjusted to the computed  
12  
13 settings on the plan to run at 550  $\pm 5$  % g/min for 28  $\pm 1$  % moisture w/w. The screw  
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15 speed was held at 250  $\pm 1$  rpm for all runs.  
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### 22 *Analysis*

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24 The methods of analysis used were as described for studies on the extrusion of  
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26 wholemeal wheat flour (Scudamore et al 2007) with the addition of a method for the  
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28 determination of fumonisins. The methods used are UKAS (ISO 17025) accredited,  
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30 inhouse validated, published in AOAC, being processed as CEN standards and follow  
31  
32 the UK Food Standards Agency Requirements of External Contracts (Thompson and  
33  
34 Wood, 1995). The methods for DON and ZON are summarised below. All reagents  
35  
36 were purchased from commercial sources and were of analytical grade. Solvents were  
37  
38 of HPLC grade. FUMONIPREP® columns, TRICHOTHECENE P columns and  
39  
40 EASI-EXTRACT® Zearalenone columns were supplied by R-Biopharm Rhone  
41  
42 (Glasgow, Scotland). Mycotoxin standards of DON and ZON were purchased from  
43  
44 Sigma-Aldrich Company Ltd., Gillingham, Dorset, UK. DON and ZON were  
45  
46 quantified prior to use by UV spectrometry, prepared 3-monthly. FB<sub>1</sub>, FB<sub>2</sub> and FB<sub>3</sub>  
47  
48 were obtained from PROMEC Unit, MRC Cape Town, PO Box 19070, Tygerberg  
49  
50 7505, Cape Town. Prior to analysis, all samples were ground to 1µm particle size in a  
51  
52 laboratory mill and mixed for 1 hour on a Winkworth cone blender and Winkworth  
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54 paddle mixer.  
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*Fumonisin*

25 g of the ground sample were extracted with 125 ml of acetonitrile:methanol:water 25:25:50 (v/v/v) by homogenising using an Ultra Turrax on slow speed for 30 seconds, to ensure the sample was mixed with the solvent, and then at high speed for 5 minutes. The sample was filtered and 10 ml of filtrate was transferred into a flask containing 40 ml of phosphate buffered saline solution (PBS). This was mixed well and then filtered through micro fibre filter paper. 10 ml of the filtered aqueous extract was transferred onto a fumonisin immunoaffinity column. The extract was passed completely through the column at a rate of about 1 drop per second under gravity. The column was washed with 10 ml of PBS and dried to remove all excess PBS by pushing approximately 5 - 10 ml of air through it. The fumonisins were eluted from the column with 1.5 ml of methanol at a rate of no more than 1 drop per second and collected in a 4 ml amber vial. The column was washed with 1.5 ml of water and added to the methanol eluate.

Automated pre-column derivatisation was carried out using ortho phthaldialdehyde (OPA) derivatisation reagent. This was prepared by dissolving 0.12 g OPA in 3 ml of methanol. 15 ml of 0.1 M disodium tetraborate solution and 150  $\mu$ l of 2-mercaptoethanol was added and the vial was capped and shaken and kept in the dark. The HPLC system transferred 50  $\mu$ l sample/standard to a clean vial, added 450  $\mu$ l OPA reagent, mixed the solutions, and then injected 50  $\mu$ l of the derivatised solution onto the HPLC column. HPLC used an automatic sample injector (Gilson 231) with dilutor (Gilson 401). A Phenomenex ODS3-Prodigy (15 cm x 4.6 mm i.d.), 5  $\mu$ m particle size, 250 A pore size HPLC column and guard column, C18 (Anachem, C752), 2.5 cm x 4.6 mm i.d. heated at 32 °C was used with a mobile phase -

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3 methanol:0.1 M sodium dihydrogen phosphate solution (76:24, v/v) adjusted to pH  
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5  
6 3.35 by addition of phosphoric acid. Detection was by fluorescence (Jasco FP-1520)  
7  
8 set at excitation 335 nm and emission 440 nm.  
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### 10 11 12 *DON and other trichothecenes*

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15 In summary, a ground sample (20 g) was extracted with 100 ml acetonitrile/water  
16  
17 84:16 by shaking for 2 hours on a wrist action shaker. A 5 ml aliquot of the extract  
18  
19 was applied to a pre-washed charcoal/alumina column and 2 ml of clean-ed up extract  
20  
21 was transferred to a vial and evaporated to dryness. The residue was derivatised to  
22  
23 form the trichothecene -trimethyl silyl (TMS) derivatives and determined by GC/MS  
24  
25 operating in selected ion mode, using 4 ions for confirmation. Trichothecenes detected  
26  
27 by this method are DON, 3AcDON, 15AcDON, nivalenol, diacetoxyscirpenol,  
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29 fusarenon-X, T2 toxin and HT2 toxin.  
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33

### 34 *ZON*

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36 The method used was that of MacDonald et al. (2005) and is summarised here. A  
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38 ground sample (25 g) was extracted with 125 ml of acetonitrile/water 75:25 by  
39  
40 blending for 3-5 minutes and the extract was filtered. 15 ml of the extract was diluted  
41  
42 with 85 ml of PBS. 50 ml of the diluted extract was passed through an EASI-  
43  
44 EXTRACT ZON immunoaffinity column under gravity and the column was washed  
45  
46 with 20 ml water. ZON was eluted under gravity with 1.5 ml of acetonitrile. 2 ml of  
47  
48 water was added to 1 ml of eluate and mixed well. ZON was detected by HPLC. Liquid  
49  
50 chromatography was performed on a Phenomenex ODS3-Prodigy 150 x 4.6 mm, 5  $\mu$   
51  
52 particle size, operated at 1 ml min<sup>-1</sup> with acetonitrile: water 55:45 as mobile phase. The  
53  
54 excitation wavelength of the fluorescence detector was set at 274 nm and the emission  
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56 wavelength was 440 nm. Sample volumes of 400  $\mu$ l were injected.  
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### *Determination, Recovery and Limit of Detection of mycotoxins*

Samples were analysed in batches and each batch included a blank maize flour sample spiked with a known amount of mycotoxin to allow assessment of recovery, with 70-110 % being the acceptable range. The spiking levels were 250  $\mu\text{g kg}^{-1}$  FB<sub>1</sub>, 125  $\mu\text{g kg}^{-1}$  FB<sub>2</sub> and FB<sub>3</sub>, 200  $\mu\text{g kg}^{-1}$  DON and 50  $\mu\text{g kg}^{-1}$  ZON. The recovery and % SD for 10 replicate determinations are given in table 1

LODs were typically 4, 10 and 9  $\mu\text{g kg}^{-1}$  for FB<sub>1</sub>, FB<sub>2</sub> and FB<sub>3</sub> respectively (based on 3 x signal to noise of the lowest concentration calibration standard of 100/50/50  $\mu\text{g kg}^{-1}$ ) while LOQ were 14, 34 and 31  $\mu\text{g kg}^{-1}$  (based on 10 x signal to noise of the lowest concentration calibration standard), 5  $\mu\text{g kg}^{-1}$  and 10  $\mu\text{g kg}^{-1}$  respectively for each trichothecene and 3 and 6  $\mu\text{g kg}^{-1}$  for ZON. For DON and ZON, the limit of detection is defined as 3 times the electronic baseline noise and the limit of determination as 6 times baseline noise. After analysis samples were retained and stored at -20°C.

### *Results from check trials*

Acceptable results have been achieved in the FAPAS Scheme ([www.fapas.co.uk/fapas.cfm](http://www.fapas.co.uk/fapas.cfm)) for fumonisins, DON and ZON in cereals during the period August 2000 to April 2007 and were consistently within the limits for Z scores of  $\pm 2$  considered acceptable.

### *Moisture content determination*

The moisture content of grits, flour and each extrudate was measured by taking a 5 g sample and heating in an oven at 105°C for 16 hours.

## Results and Discussion

All analytical results were corrected for recovery and all extrudates were corrected for changes in moisture.

### *Homogeneity of maize grits and maize flour*

After initial mixing and blending the % SD between samples from individual bags of maize grits was 17.3 % for FB<sub>1</sub> and 8.3 % for DON using replicate pairs of samples from up to 10 bags. After further mixing prior to extrusion, the bags were re-sampled and the % SD improved to 8.8 % for FB<sub>1</sub> but not for DON (12.1 %). Mean concentrations of mycotoxins in the maize grits were then 274 µg kg<sup>-1</sup> for FB<sub>1</sub>, 66 µg kg<sup>-1</sup> for FB<sub>2</sub>, 22 µg kg<sup>-1</sup> for FB<sub>3</sub> and 143 µg kg<sup>-1</sup> for DON.

After thorough mixing of the maize flour, 10 pairs of random samples from the bags gave % SD values of 13.5 %, 14.3 %, 14.0 % and 14.1 % for FB<sub>1</sub>, FB<sub>2</sub>, FB<sub>3</sub> and ZON respectively. Mean concentrations were then 2009 µg kg<sup>-1</sup> for FB<sub>1</sub>, 409 µg kg<sup>-1</sup> for FB<sub>2</sub>, 285 µg kg<sup>-1</sup> for FB<sub>3</sub> and 22.0 µg kg<sup>-1</sup> for ZON. The precision of the replicate samples which is a combination of a sampling variability factor and analytical precision is quite similar to that for the analytical methods alone, table 1, so that most of the variability is probably due to the analysis and rather than inhomogeneity

In addition, in carrying out each study, a sample was taken from the feed hopper for analysis immediately before processing and compared with the corresponding extrudate sample after extrusion. This was carried out by timing the flow of material through the extruder. The result for every set of conditions examined in this work was the combined result from separate runs thus based on two replicates.

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3 The mean % SD for all the replicate pairs of results is given at the foot of each table  
4 of results. This represents the combined variability due to sample in-homogeneity and  
5 to the analysis. The values for grits and flour are mostly between 1% and 4% that  
6 compares very favourably with values usually expected from trace analysis alone at  
7 these levels, table 1. A wide range of conditions have been examined, not all of which  
8 may be used in commercial operations. Thus, in assessing the likely behaviour of  
9 mycotoxins in a particular process the manufacturer should ensure that the appropriate  
10 conditions in this study are referred to.  
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### 25 *Maize Grits*

#### 26 *Effect of extruder screw speed*

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28 An initial study was carried out to determine whether the screw speed of the extruder  
29 feed shaft affected the results obtained. To do this the barrel temperature was set to  
30 160°C and moisture contents of 17 % and 21 % were used. The results obtained for  
31 FB<sub>1</sub> and DON are shown in table 2. Once each extrudate condition had stabilised, a  
32 sample of grits was taken from the feed hopper and the corresponding sample of  
33 extrudate as described earlier. The mean values from two separate runs are given.  
34 Extrusion of maize grits of 17 % moisture content showed that there was a loss of FB<sub>1</sub>  
35 that increased as screw speed increased from 200 to 400 rpm. A similar higher loss  
36 occurred at 21 % moisture content as the screw speed increased from 300 to 400 rpm.  
37 Results show that losses of the fumonisins are consistently greater at lower moistures.  
38 More energy is applied to the extrusion system under drier conditions and this may be  
39 the reason for an increased breakdown of the thermally labile fumonisins. However,  
40 there was no significant effect on DON concentrations as the screw speed was  
41 changed. These findings are consistent with the relative thermal stability of the two  
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3 compounds. As a result of this initial study, it was decided thereafter to standardise  
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5 screw speed at 300 rpm and thus reduce the number of variables.  
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#### 10 *Effect of extruder temperature and maize moisture content*

11  
12 Table 3 shows the loss of FB<sub>1</sub> during direct extrusion of maize grits at different  
13  
14 temperatures and extruder moistures. Results for FB<sub>2</sub> and FB<sub>3</sub> (not shown here) were  
15  
16 very similar but subject to greater variability probably because their concentrations  
17  
18 were lower. The loss of fumonisins was greater at 17 % moisture content than at 21  
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20 %. The loss also increased slightly as the temperature was raised as might be expected  
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22 with a thermally labile compound although the moisture of the extruded product had a  
23  
24 more significant effect than temperature. In contrast, temperature had little or no  
25  
26 effect on the survival of DON suggesting it is heat stable under the conditions used.  
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28 However there was a small loss under all conditions that might be due to reduced  
29  
30 extractability during analysis or to some binding with the extrudate material. The %  
31  
32 SD for each pair of results was between 3 and 4 % that showed that the variability in  
33  
34 analysis and sampling was very small and that the effects shown are significant. The  
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36 Specific Volume of the products (s.v) are given and this indicates how greatly the  
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38 density of the products formed can change depending on the conditions of extrusion.  
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46 The presence of small amounts of certain other ingredients added to the cereal  
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48 extruder feed has been shown previously to affect the stability of some mycotoxins  
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50 during processing. The effect of two commonly used additives were examined here, 2  
51  
52 % w/w sodium chloride (salt) and 2 % w/w sucrose. These additives were each added  
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54 to a separate batches of the maize grit feed and examined in separate extruder runs.  
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#### *Salt*

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3 Table 4 shows the result on concentrations of FB<sub>1</sub> and DON of adding 2 % NaCl to  
4 the maize grits. The loss of fumonisin B<sub>1</sub> is less in the presence of sodium chloride at  
5  
6 both temperatures although the overall loss is less at the higher moisture content. The  
7  
8 reason for this is unclear but higher extraction efficiency is sometimes obtained when  
9  
10 salt is present in analytical extraction solvent mixtures and suggests that it may be an  
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12 extractability effect. Less pronounced results were obtained for DON which is less  
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14 susceptible to breakdown. .  
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### 22 *Sucrose*

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24 Table 5 shows that the addition of 2 % sucrose has no effect on the levels of FB<sub>1</sub> or  
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26 DON when compared with samples without sucrose processed under similar  
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28 conditions although losses were consistently greater under the drier conditions as  
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30 discussed earlier..  
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### 36 *Maize Flour*

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38 The flour fraction from maize milling is composed of smaller particles than those  
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40 comprising the grits and has a higher oil content of about 2-3 %. There are  
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42 commercial processes that use both direct expansion in which the extruder operates  
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44 with lower moisture content material and higher temperatures and pellet formation  
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46 that is used with higher moisture extrudate (28-30 %) and lower temperatures  
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48 (~100°C). The dense pellets that are formed are then fried in oil at high temperature  
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50 for about 20 seconds and as a result they expand rapidly to form the end product. Both  
51  
52 processes were examined here.  
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3 The mycotoxin concentrations in the maize flour were 2009  $\mu\text{g kg}^{-1}$  for FB<sub>1</sub>,  
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5 409  $\mu\text{g kg}^{-1}$  for FB<sub>2</sub>, 285  $\mu\text{g kg}^{-1}$  for FB<sub>3</sub>, 22  $\mu\text{g kg}^{-1}$  for ZON. The DON concentration  
6  
7 was too low to obtain quantitative results.  
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### 10 11 12 *Direct extrusion*

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14 The losses of FB<sub>1</sub>, FB<sub>2</sub> and FB<sub>3</sub> shown in table 6 were all >39 % under all conditions  
15  
16 studied. Loss also increased as temperature was raised but this effect was much less  
17  
18 than that caused by changes in moisture content. However, the effect of temperature  
19  
20 became more marked at lower moisture contents. Each fumonisin was affected in the  
21  
22 same way. The loss of ZON was unexpected as it is usually regarded as a very  
23  
24 thermally stable mycotoxin and moisture content had the opposite effect when  
25  
26 compared to the fumonisins. This is difficult to explain although the concentration of  
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28 ZON was low (22  $\mu\text{g kg}^{-1}$ ) and this might play a role.  
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34 Unfortunately no study of the effect on ZON in maize grits was possible in the  
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36 absence of this mycotoxin from that ingredient. However, in a previous study of ZON  
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38 in wholemeal wheat, a much higher concentration was present (300  $\mu\text{g kg}^{-1}$ ). In that  
39  
40 study, there was a tendency for more than 100 % of ZON to be measured although  
41  
42 this was less marked at the higher moisture contents. Limited studies of the effects of  
43  
44 extrusion have been carried out but some loss of ZON has been reported previously  
45  
46 (Ryu et al. 1999).  
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50 Our study with maize grits showed that the addition of sodium chloride  
51  
52 produced higher recovery of fumonisins. Here in table 7, the addition of 1 % sodium  
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54 chloride also similarly reduced the apparent loss of fumonisin under each of the  
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56 conditions examined. We reported a similar result with wholemeal wheat in which the  
57  
58 presence of added salt also reduced the levels of mycotoxin lost. Food manufacturers  
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3 are under pressure to reduce salt levels in food so it is important that the effect of salt  
4 and other ingredients is noted. It was not possible to decide whether this is an  
5 analytical artefact due to better extractability or whether thermal breakdown or  
6 binding to the maize structure is reduced. This study was repeated with maize flour  
7 and the results in table 7 confirm the effect of salt. Higher values for mycotoxins are  
8 found consistently in the presence of salt.  
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Studies with maize grits showed that the addition of sucrose had no effect on  
either fumonisins or DON so that it was decided to examine whether reducing sugars  
affect concentrations of some mycotoxins. Table 8 shows the effect of adding 5 % and  
10 % of dextrose (glucose) to maize flour under the same conditions as tested for salt.  
Levels of FB<sub>1</sub> recovered were much reduced under all conditions by the addition of  
only 5 % of dextrose. The higher 10 % addition further increased the loss but clearly  
even a small addition of reducing sugar has a significant effect. However, fumonisins  
are known to bind with glucose.

It was observed that extrudate expansion of grits was similar to normal snack  
products at 17 % moisture but denser and more like breakfast cereals at 21 %  
moisture.

The results for ZON, Table 8 shows that its loss does not appear to be affected  
by the presence of dextrose at either moisture at 180°C although there was some loss  
under all concentrations. However, the loss appears to be reduced by the presence of  
dextrose at 160°C. ZON is much less polar than DON and this may be a factor. It is  
difficult to explain some of the results for both of these *fusarium* mycotoxins.  
However, they are both relatively resistant to loss during extrusion compared with the  
fumonisins. The apparent increase under some conditions requires further  
confirmatory studies. The trend for residual ZON values to increase going from 140°C

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3 to 180°C in table 7 is contrary what would be expected from chemical degradation.  
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5 The answer to these changes may lie with the efficiency of the extraction solvent used  
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7 for removing mycotoxins in the extruded materials or that bound mycotoxins are  
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9 being released by the extrusion conditions- or a combination of both.  
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13 It has already been established that DON is very stable under the conditions  
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15 studied but the presence of 2 % sodium chloride seems to result in an even higher  
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17 survival. This may be that sodium chloride assists in the extraction, as has been noted  
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19 and used in some analytical extraction procedures for several mycotoxins. This effect  
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21 also seems to occur for FB<sub>1</sub> although the overall survival is less than for DON. There  
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23 appears to be no such effect with sucrose, where the presence of 2 % does not appear  
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25 to cause any significant difference under any conditions tested.  
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29 Reduction of salt content by processors in line with current requirements may  
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31 apparently reduce fumonisins concentrations because recovery is affected. The extent  
32  
33 of this will much depend on the physical properties of the extrudate. This factor needs  
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35 to be examined in detail. On the other hand, the addition of sucrose under these  
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37 conditions did not significantly lower fumonisin concentrations.  
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#### 43 *Extruded pellets*

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45 Conditions used to produce extruded pellets are very different from those needed for  
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47 direct expansion. The moisture of the material in the extruder is about 30 % and only  
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49 a little lower at the point when forced through the die. Barrel temperature was set to  
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51 110°C or 120°C but this fell to 90°C and 95°C respectively at the die face. The pellets  
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53 were then dried to about 9-10 % and stored until required. In the final frying stage the  
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55 pellets are dropped into hot cooking oil for a short time during which they expand  
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3 rapidly. At the same time the moisture content falls to between 2 and 4 % depending  
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5 on the pellet, table 9.  
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8 The results in table 9 show that there appears to be an increase in FB<sub>1</sub> level  
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10 during pellet formation after correction for the change in moisture content. This was  
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12 the same for pellets formed at both 110°C and 120°C. However in the same samples  
13  
14 there was more loss for ZON at the lower temperature. Throughout this study each  
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16 value is the mean of 2 samples taken from different runs. The variability is small for  
17  
18 the fumonisin results but rather larger for the lower levels of ZON. During the  
19  
20 subsequent frying, there was a substantial reduction in FB<sub>1</sub>, particularly in the higher  
21  
22 temperature pellets. In contrast the loss of ZON was much less. Changes over the  
23  
24 whole process are very similar for FB<sub>1</sub>, FB<sub>2</sub> and FB<sub>3</sub> and this further suggests that  
25  
26 these differences are real and not artefacts or analytical problems. The degree of  
27  
28 expansion of the pellets from the 110°C and 120°C material is quite different giving  
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30 products of specific volume of 2.1 and 3.2 respectively and this suggests that the  
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32 composition of the pellets plays a significant part in determining the loss of  
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34 mycotoxins.  
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41 The loss of each fumonisin from the pellet to the fried product might be due to  
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43 dissolution of the mycotoxin in the frying oil. However this seems unlikely as little  
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45 loss of ZON occurred during the frying. This compound is less polar and would be  
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47 expected to dissolve more readily in the oil. It thus suggests that the fumonisins, but  
48  
49 not ZON, were partially degraded in the oil.  
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53 The effect of salt was examined again in this process by adding 1 % and 2 %  
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55 to the maize feed recipe. In contrast to studies on direct expansion, salt did not  
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57 produce any clear effect during pellet formation although the high loss of fumonisins  
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59 from the 120°C pellets was subsequently reduced during frying and for the overall  
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3 process recovery. Recoveries of ZON were also improved overall in the expanded  
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5 product in the presence of salt.  
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## 10 **Conclusions**

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12 Extrusion cooking technology is complex and can affect mycotoxin concentrations in  
13 different ways. Some factors can produce apparently opposite effects in different  
14 mycotoxins. Moisture content has been found to be a more important factor than  
15 temperature. Operation of the extruder with low moisture ingredients requires a higher  
16 energy input and tends to result in higher losses of fumonisins at all temperatures  
17 although the opposite effect has been found for ZON. Temperature alone does not  
18 appear to significantly degrade any of the fumonisins, DON or ZON under the  
19 extrusion conditions studied.  
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32 Because loss is less affected by temperature than by moisture content it seems  
33 likely that binding to cereal components and/or solvent extractability are also  
34 significant factors. Current EC legislation (EC, 2007) sets limits for FB<sub>1</sub> + FB<sub>2</sub>  
35 concentrations of 2000 µg kg<sup>-1</sup> in flour (milling fraction with particles ≤500 µ) or  
36 1400 µg kg<sup>-1</sup> (particles >500 µ) and 1000 µg kg<sup>-1</sup> in maize-based foods for direct  
37 human consumption or 800 µg kg<sup>-1</sup> in breakfast cereals and 200 µg kg<sup>-1</sup> in processed  
38 maize-based foods and baby foods for infants and young children. Similar categories  
39 apply to limits for DON and ZON (EC, 206). Thus processes using extrusion need to  
40 be carefully considered to ensure that mycotoxins in the appropriate consumer end  
41 product will meet legislative limits.  
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7  
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9 Association, Chipping Campden, UK.  
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Table 1. Analytical recovery and performance data (n=10)

Parameter	Mycotoxin, $\mu\text{g}/\text{kg}$			
	DON	ZON	FB1	FB2
Spike level	500	50	250	125
Mean recovery	104	95	104	99
RSD, %	7	16	9	7

Table 2. The effect of screw speed on FB<sub>1</sub> (mean concentration 274  $\mu\text{g kg}^{-1}$ ) and DON (mean concentration 143  $\mu\text{g kg}^{-1}$ ) in maize grits at constant feed rate and two temperatures

screw speed, rpm	mc, %	FB <sub>1</sub>			DON		
		values	mean	loss, %	values	mean	loss, %
200	17	189			132		
			199	27.3		137	4.3
200	17	209			142		
300	17	*			128		
			189	31.2		128	10.7
300	17	189			128		
400	17	178			130		
			179	34.8		135	5.5
400	17	180			141		
300	21	234			141		
			235	14.4		137	4.7
300	21	235			132		
400	21	217			125		
			209	23.8		128	10.7
400	21	201			130		
% SD			3.37		3.75		

\* outlier value excluded

Table 3. Effect of extruder temperature and maize moisture content on FB<sub>1</sub> (mean concentration 274  $\mu\text{g kg}^{-1}$ ) and DON (mean concentration 143  $\mu\text{g kg}^{-1}$ ) during extrusion of maize grits

T, °C	mc, %		sp. v		FB <sub>1</sub>		DON		
	grits	ext	L kg <sup>-1</sup>	value	mean	loss, %	values	mean	loss, %
140	17			219			128		
		7.88	7.60		203	26.1	131	130	9.6
160	17			*			128		
		7.78	9.12		189	31.2	128	128	10.7
180	17			177			129		
		7.70	8.21		174	36.5	119	124	13.4
140	21	12.4	2.51	246			146		
				243	11.2	132	139	3.3	
140	21	3		240			141		
160	21	12.1	2.24	234			132		
				235	14.4	136	4.8		
160	21	8		235			134		
180	21	11.9	3.06	230			139		
				233	15.1	144	2.9		
180	21	6		236					
% SD for the sample pairs					4.13		3.94		

Table 4. Effect on FB<sub>1</sub> (mean concentration 274  $\mu\text{g kg}^{-1}$ ) and DON (mean concentration 143  $\mu\text{g kg}^{-1}$ ) concentrations of adding NaCl to maize grits

NaCl	T, °C	mc, %		FB <sub>1</sub> , $\mu\text{g kg}^{-1}$			DON, $\mu\text{g kg}^{-1}$		
		grits	Ext.	value	mean	loss, %	value	mean	loss, %
0	180	17		1767			129		
	180	17	7.70	172	174	36.5	119	124	13.4
+ 2 %	180	17		210			137		
	180	17	7.80	200	205	25.2	144	140	2.0
0	180	21	11.9	230			134		
	180	21	6	236	233	15.1	144	139	2.9
+ 2 %	180	21	12.1	231			143		
	180	21	3	257	244	10.9	139	141	1.7
% SD for the sample pairs					3.67			4.14	

Table 5. Effect on FB<sub>1</sub> (mean concentration 274  $\mu\text{g kg}^{-1}$ ) and DON (mean concentration 143  $\mu\text{g kg}^{-1}$ ) concentrations of adding sucrose to maize grits

sucrose	T, °C	mc, %		FB <sub>1</sub> , $\mu\text{g kg}^{-1}$			DON, $\mu\text{g kg}^{-1}$		
		grits	ext.	value	mean	loss, %	value	mean	loss, %
0	180	17	7.70	177	174	36.5	129	124	13.4
	180	17		172			119		
+ 2 %	180	17	7.26	177	177	35.5	129	122	15.0
	180	17		177			115		
0	180	21	11.96	230	233	15.1	134	139	2.9
	180	21		236			144		
+ 2 %	180	21	11.61	217	221	19.2	131	135	5.8
	180	21		226			139		
% SD for the sample pairs				1.63			5.73		

Table 6: Reduction in FB<sub>1</sub>, FB<sub>2</sub> and FB<sub>3</sub> (mean concentrations 2009, 409 and 285 µg kg<sup>-1</sup> respectively) and ZON (22 µg kg<sup>-1</sup>) content during direct extrusion of maize flour at constant screw speed and feed rate

flour	mc, %		sp.v. L kg <sup>-1</sup>	barrel T., °C	loss of mycotoxin, %			
	extrudate				FB <sub>1</sub>	FB <sub>2</sub>	FB <sub>3</sub>	ZON
21	10.1		4.0	140	39	46	44	54
21	9.7		5.8	160	42	46	45	45
21	8.9		6.7	180	43	45	47	42
17	7.5		7.5	140	45	48	51	46
17	6.2		8.0	160	55	56	60	40
17	5.6		8.8	180	60	57	60	23
15	5.3		8.7	140	56	55	61	40
15	5.8		8.8	160	54	52	60	34
15	4.4		9.2	180	71	68	70	16
13.5	3.2		9.5	140	75	70	74	15
13.5	3.4		8.9	160	78	73	76	21
13.5	3.2		9.0	180	85	81	85	6
% SD for the sample pairs					1.45	2.03	5.10	2.76



Table 7. Effect of NaCl on the loss of FB<sub>1</sub> (2009 µg kg<sup>-1</sup>) and ZON (22 µg kg<sup>-1</sup>) content during direct extrusion of maize flour.

flour	mc, % extrudate	Barrel T, °C	NaCl, %	
			0	2
FB <sub>1</sub>			loss, %	
17	6.20/5.25	160	51	41
17	5.60/6.33	180	58	49
15	5.80/4.93	160	53	48
15	4.40/5.79	180	68	40
% SD for the sample pairs				2.03
ZON			loss, %	
17	6.20/5.25	160	40	24
17	5.60/6.33	180	23	10
15	5.80/4.93	160	34	15
15	4.40/5.79	180	16	16
% SD for the sample pairs				1.27

Table 8. Change in FB<sub>1</sub> (2009 µg kg<sup>-1</sup>) and ZON (22 µg kg<sup>-1</sup>) content during direct extrusion of maize flour, effect of dextrose

mc, %		barrel T, °C	Dextrose, %		
flour	extrudate*		0	5	10
FB <sub>1</sub>			loss, %		
17	6.20/4.57	160	51	80	88
17	5.60/4.62	180	58	84	88
15	5.80/4.80	160	53	87	93
15	4.40/4.59	180	68	90	93
% SD for the sample pairs				1.45	
ZON			loss, %		
17	6.20/4.57	160	40	23	18
17	5.60/4.62	180	23	19	26
15	5.80/4.80	160	34	20	18
15	4.40/4.59	180	16	12	20
% SD for the sample pairs				3.71	

\* = 0/10 % dextrose

Table 9. Change in FB<sub>1</sub>, FB<sub>2</sub> and FB<sub>3</sub> (mean concentrations 2009, 409 and 285 µg kg<sup>-1</sup> respectively) and ZON (22 µg kg<sup>-1</sup>) during pellet production and frying of maize flour and effect of NaCl.

T, °C	NaCl, %	loss of mycotoxin, %					mc, %, after frying,
		flour: pellet	pellet: fried	flour :fried	flour :fried	FB <sub>1</sub>	
						FB <sub>2</sub>	FB <sub>3</sub>
110	0	13 <sup>+</sup>	22	12	11	12	4.00
120	0	13 <sup>+</sup>	60	46	46	50	2.41
110	1	7 <sup>+</sup>	20	14	20	12	3.73
120	1	8 <sup>+</sup>	33	28	34	24	2.53
110	2	11 <sup>+</sup>	25	17	31	21	3.12
120	2	0	21	21	31	25	2.89
% SD			4.43	3.93			
ZON							
110	0	31	5	34			
120	0	8	13	18			
110	1	14	10	9			
120	1	5	+1	5			
110	2	12	+3	10			
120	2	14	4	18			
% SD			8.84	6.01			

<sup>+</sup> Apparent increase

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